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Dinoprost Versus Cloprostenol: Does Route of Injection Modulate their Efficacy in Dairy Cattle?

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Abstract

This factorial study was conducted to determine the reproductive performance of 402 dairy cattle treated with either dinoprost (25 mg) or cloprostenol (500 µg), intravenously (IV) or intramuscularly (IM), for synchronization of estrus in a randomized clinical trial. Breeding, conception and pregnancy rates were analyzed with multivariate logistic regression. The model included the treatments (2 types of prostaglandins and 2 routes of injection) as well as eight clinically-relevant covariables. Interactions between treatments were not significant and comparisons were made on the main effect. Comparisons between types of prostaglandins ($PGF2_{\alpha}$) showed similar breeding rates (82.6 vs 83.0%; P=0.93), but lower conception rate (38.6 vs 46.6%; P=0.13) and pregnancy rate (31.4 vs 39.2%; P=0.14) for dinoprost versus cloprostenol. When comparing routes of injection, a similar breeding rate (84.1 vs 81.4%; P=0.45), but lower conception rate (38.7 vs 46.7%; P=0.13) and pregnancy rate (32.5 vs 38.1%; P=0.23) were noted for IM versus IV injections. The statistical power of the study was 19%, which allowed detection (P<0.05) of a 5% difference or more in pregnancy rate between the main effect of treatments (200 animals per sample).

Gender of calves was recorded on 117 calves and analyzed with univariate logistic regression. Overall, the male calf rate was not different from the expected population value (57.3 vs 53.0%; P=0.36). There was a greater proportion of male calves when using dinoprost compared to cloprostenol (72.3 vs 47.1%; P<0.01), when using IV rather than IM injections (66.1 vs 49.2%; P=0.07) and when inseminating cattle 4 days or later after $PGF2_{\alpha}$ injection instead of earlier (69.4 vs 51.9%; P=0.08). It is concluded that the type of $PGF2_{\alpha}$ used and the route of injection might affect conception rate and hence, pregnancy rate, and that the statistical power of the study was too weak to detect any significant differences. It is also concluded that the interval (days) from $PGF2_{\alpha}$ injection to breeding, the type of $PGF2_{\alpha}$ and the route of injection might influence the sex ratio of calves.

Résumé

Cette étude a été menée pour déterminer la performance en reproduction de 402 vaches laitières traitées avec du dinoprost (25 mg) ou avec du cloprostenol (500 ug), en injection intraveineuse (IV) ou intramusculaire (IM), pour la synchronisation des oestrus dans le cadre d'un essai clinique randomisé. Les taux de saillie, de conception et de gestation ont été analysés avec une régression logistique multivariée. Le modèle incluait les différents traitements (deux types de prostaglandines et deux voies d'injection) de même que huit covariables cliniquement pertinentes. Les interactions entre les traitements n'étaient pas significatives et les comparaisons portaient donc sur les effets principaux. Les comparaisons au niveau du type de prostaglandine ($PGF2_{\alpha}$) n'ont montré aucune différence entre les taux de saillie (82.6% versus 83.0%; P = 0.93) mais ont révélé des taux de conception (38.6% versus 46.6%; P = 0.13) et de gestation (31.4% versus 39.2%; P = 0.14) plus faibles pour le traitement avec le dinoprost que pour le traitement avec le cloprostenol. Les comparaisons au niveau des voies d'injection n'ont pas montré de différence entre les taux de saillie (84.1% versus 81.4%; P = 0.45) bien que les taux de conception (38.7% versus 46.7%; P = 0.13) et de gestation (32.5% versus 38.1%; P = 0.23) étaient plus faibles pour le traitement IM que pour le traitement IV. Le pouvoir statistique de l'analyse était de 19% ce qui permettait la détection (P < 0.05) d'une différence de l'ordre de 5% ou plus dans les taux de gestation associés aux différents traitements (200 animaux par groupe).

Le sexe a été noté chez 117 veaux et analysé avec une régression logistique univariée. Dans l'ensemble, la proportion de veaux mâles n'était pas différente de celle attendue dans la population (57.3% versus 53.0%; P = 0.36). Il y avait une plus grande proportion de mâles chez les vaches traitées avec le dinoprost qu'avec le cloprostenol (72.3% versus 47.1%; P < 0.01), chez les vaches recevant l'injection IV plutôt que l'injection IM (66.1% versus 49.2%; P = 0.07) de même que chez les

vaches inséminées quatre jours ou plus après l'injection de prostaglandine plutôt qu'inséminées avant (69.4% versus 51.9%; P = 0.08). On conclut que le type de prostaglandine utilisé de même que la voie d'injection peuvent influencer le taux de conception, et par conséquent le taux de gestation, mais que le pouvoir statistique de l'étude était trop faible pour détecter des différences significatives. On conclut aussi que l'intervalle de temps entre l'injection de prostaglandine et la saillie de même que le type de prostaglandine et la voie d'injection ont une influence sur le rapport des sexes des veaux.

Introduction

In Canada, two types of prostaglandins ($PGF2_{\alpha}$) are popular: dinoprost, a tromethamine salt (THAM) of the natural $PGF2_{\alpha}$, and cloprostenol, a synthetic analogue. Both products can be injected intramuscularly (IM) or intravenously (IV), but the IM route is recommended by manufacturers and is more desirable from a standpoint of commercial application than the IV injection.[6,12] Cloprostenol has a longer biological half-life and is a much more potent luteolytic agent than dinoprost since it is not degraded by 15-hydroxydehydrogenase and 13,14-reductase.[1] The IV injected $PGF2_{\alpha}$ is metabolized during the first few passages through the lungs, resulting in a shorter peripheral exposure than the IM injected $PGF2_{\alpha}$, which is released more slowly from the injection site.[13] Very limited research has been conducted to compare the reproductive efficiency obtained with these two $PGF2_{\alpha}$ products and no clinical trial adressing the route of injection issue was found in the literature.

A recent review of the literature pertaining to the effect of time of insemination on sex ratio indicates that treatments used for synchronization of estrus or ovulation in cattle may influence the sex ratio.[15] Yet no information was found on the effects of type of $PGF2_{\alpha}$ used and route of injection on the sex ratio of calves.

Until 1992, veterinarians from the Coaticook Veterinary Clinic (Coaticook, Québec, Canada) were exclusively using cloprostenol IV. In 1992, they switched from cloprostenol IV to dinoprost IM. Many dairy producers were unsatisfied with the new $PGF2_{\alpha}$ and the new route of injection. This prompted the initiation of a field study to compare the reproductive efficiency of animals treated with each of the two types of $PGF2_{\alpha}$ given IM or IV. It was hypothesized that both treatments would yield similar results and have no effect on the sex ratio of calves.

Material and Methods

Design

Lactating dairy cows and heifers past their voluntary waiting period and having a corpus luteum (CL), as determined by rectal palpation, were assigned randomly to a 2 x 2 factorial design that included a dinoprost[a] (25 mg or 5 ml) and a cloprostenol[b] (500 µg or 2 ml) group injected either IM[c] (gluteal muscle or croup) or IV (coccygeal vein). All records were maintained in DSA software[d] (Dossier de Santé Animale) from ASTLQ.[e] Sample size was not calculated before the experiment, but a goal of 100 animals in each treatment group was considered realistic.

Animal Selection

The study was conducted from May 1, 1993 to June 1, 1994 on dairy cattle from 152 farms participating in a monthly herd health program. The final data set included 402 valid cases after 10 animals were culled subsequent to synchronization of estrus, but prior to pregnancy diagnosis. Owner permission was requested before including an animal in the study. Most participants (n=69) had a tie stall operation and cows were allowed to graze on pasture in the summer. The average herd had 54 (range 26-123) Holstein cows with a 305-day mature milk production of approximately 17,600 lb (8,000 kg). Artificial insemination (AI) was performed following an observed estrus, although some producers occasionally used fixed-time breeding 3 and 4 days post-treatment. Inseminations were done by a team of experienced AI technicians. Pregnancy was determined *via* rectal palpation 35 to 60 days following AI. Sex of calves was not determined prior to calving.

Procedure

A technical assistant prepared 480 sealed and numbered envelopes containing two syringes (2 ml and 5 ml), one filled with one of the two commercial $PGF2_{\alpha}$ products under study (dinoprost or cloprostenol) and the other with sterile physiological saline. Each envelope contained a note indicating the route of injection to be used and was randomly allocated to a participating animal. Both syringes were injected by the same route to the same animal in order to avoid selection bias and to blind both the producer and the veterinarian injecting the $PGF2_{\alpha}$.

Data Collection and Definitions

The following information was gathered at the farm and included in the database: date of injection, herd and animal identification, and the name of the veterinarian injecting the $PGF2_{\alpha}$ product. The following information was also included in the database: birth date, lactation number, calving date, treatment, days in milk (DIM), breeding dates, cystic ovarian disease (COD) diagnosis, pregnancy examination date and pregnancy outcome. Since heat signs were not routinely recorded by all producers, they were not included in the analysis. Only the breedings made within 7 days after the $PGF2_{\alpha}$ injection

were considered in the study. When multiple inseminations were performed during the same estrous cycle, only the first breeding was considered in the analysis (15 cases overall, 7 cases in herd 11; Table 3).

Three relevant fertility rates as well as the male calf rate were considered as dependent variables. Breeding rate was defined as the number of cattle inseminated within 7 days post-injection divided by the number of cattle assigned. Conception and pregnancy rates were derived from pregnancy status at first transrectal examination following the induced estrus. Pregnancy loss was not considered. Conception rate was defined as the number of pregnant cattle divided by the number of inseminated cattle, and pregnancy rate was the number of pregnant cattle divided by the number of cattle assigned. The male calf rate was the number of male calves divided by the number of calves born with recorded gender. Each twin counted as a single calf for the male calf rate analysis.

Statistical Analysis

Chi-Square tests of independence and logistic regression analysis were conducted with Statistix.[f] Significance was declared at $P<0.05$. Retrospective determination of sample size was obtained from Episcope.[g] Comparisons on the interval from injection to day of breeding were done with the two-sample T Test. Residual analysis revealed no aberrant data.

Multivariate logistic regression was conducted for fertility rates and included the following independent covariables: $PGF2_{\alpha}$, ROUTE, LACTA, GROUP, AI, SEASON, COD, DAY, VET, and HERD (Table 1). Interactions needed to attain a 0.05 significance level to be retained in the model. Transformation of odds ratios (OR) into rates (%) was achieved by the following equations adapted from Hosmer and Lemeshow:[8]

$$P1\ (\%) = 100 \times OR \times P0 / [\ (OR \times P0) + (100 - P0)\ ]$$

$$\text{Calculated average rate}\ (\%) = [\ (P1 \times n1) + (P0 \times n0)\ ] / (n1 + n0)$$

where OR is the odds ratio, P represents the rate (%) and n represents the number of cattle, with "0" and "1" referring to treatments as coded in Table 1. For a given odds ratio, P1 is derived from P0 until the calculated average rate equals the observed average rate in the data set. Logistic regression was conducted on the male calf rates and included an univariate model (only one independent variable) and a more complete model: $PGF2_{\alpha}$, ROUTE, and $PGF2_{\alpha}$ x ROUTE. The male calf rates were further compared to an expected population value of 53.04% (n=22, 209 calves) with the Chi-Square test of independence.

Results

Descriptive data in Table 2 revealed no significant differences between the four treatment groups. Fertility rates associated with nine clinically-relevant covariables are presented in Table 3. Continuous covariables (lactation number, grouping of injections, number of AI before the treatment during the same lactation and stage of lac-

Table 1. Variables and codification used in univariate and multivariate logistic regression analyses.

Variable	Definition
BR	Breeding rate (number of cattle inseminated within 7 days after $PGF2_{\alpha}$ divided by number of cattle injected with $PGF2_{\alpha}$; 1 = bred, 0 = not bred)
CR	Conception rate (number of cattle pregnant at induced estrus divided by number of cattle inseminated following $PGF2_{\alpha}$ injection; 1 = pregnant, 0 = not pregnant)
PR	Pregnancy rate (number of cattle pregnant at induced estrus divided by number of cattle injected with $PGF2_{\alpha}$; 1 = pregnant, 0 = not pregnant)
MCR	Male calf rate (number of male calves divided by number of calves with a recorded gender; 1 = male, 0 = female)
$PGF2_{\alpha}$	Type of prostaglandins (1 = cloprostenol, 0 = dinoprost)
ROUTE	Route of injection (1 = intravenously, 0 = intramuscularly)
LACTA	Lactation number; 4 categories: 0, 1, 2-3-4, > 4
GROUP	Grouping of injections, i.e., number of cattle injected during the same visit (1 = > 3, 0 = 1-2-3)
AI	Number of AI before treatment during the same lactation; 3 categories: 0, 1-2, >2
SEASON	Season of treatment; 4 categories: December-February, March-May, June-August, September-November
COD	Lactational incidence of cystic ovarian disease before treatment (1 = present, 0 = absent)
DAY	Day of the week when $PGF2_{\alpha}$ injection was administered; 5 categories: Monday, Tuesday, Wednesday, Thursday and Friday
VET	Veterinarian who administered $PGF2_{\alpha}$; 6 categories: Vet No. 1 to Vet No. 6
HERD	Herd in which treatment was administered; 16 categories: herd 1 to herd 15 each with 10 treatments or more and herd 16 includes all other herds
STAGE	Stage of lactation when $PGF2_{\alpha}$ injection was administered; 3 categories: < 100, 100-199 and >199 days in milk

Table 2. Descriptive data on the 4 treatment groups.

	Dinoprost		Cloprostenol		
	Intramuscular	Intravenous	Intramuscular	Intravenous	P[a]
Heifers, n	17	18	18	17	
Cows, n	85	81	83	83	
Lactation number					
-mean (sd)	2.14 (1.88)	2.37 (1.95)	2.36 (2.07)	2.45 (2.01)	0.70
-range	0–9	0–8	0–8	0–9	
Days in milk,[b] days					
-mean (sd)	142 (72.2)	134 (69.4)	133 (58.4)	122 (52.1)	0.24
-range	59–426	55–473	60–374	52–283	
Age of heifers,[b] days					
-mean (sd)	591 (114.7)	624 (123.1)	617 (109.4)	608 (80.0)	0.83
-range	423–847	447–928	430–940	459–766	
AI[c] number					
-mean (sd)	0.88 (1.14)	0.87 (1.38)	0.76 (1.06)	0.64 (1.05)	0.42
-range	0–5	0–7	0–6	0–6	

[a]P-value from One-Way Anova
[b]At $PGF2_{\alpha}$ treatment
[c]Artificial insemination before $PGF2_{\alpha}$ treatment for cows and heifers

tation when $PGF2_{\alpha}$ was injected) needed to be categorized because their association with outcomes was not linear.[8] The breeding rate was significantly associated with the number of cattle injected during the same visit (grouping of injections), lactational incidence of COD, the veterinarian who injected the $PGF2_{\alpha}$ and the herd (Table 3). The conception and the pregnancy rates were significantly associated with lactation number, grouping of injections and stage of lactation. The pregnancy rate was also significantly associated with lactational incidence of COD. The male calf rate was not significantly associated with any of the listed covariables. Mean days from injection to AI was shorter in heifers compared to lactating cows (3.25 days, SE=0.086 vs 3.45 days, SE=0.047; P=0.05) and not different between types of $PGF2_{\alpha}$ (3.42 days, SE=0.061 vs 3.40 days, SE=0.056; P=0.86) and between routes of injection (3.43 days, SE=0.062 vs 3.40 days, SE=0.054; P=0.65).

The significance levels for the interaction between treatments ($PGF2_{\alpha}$ x ROUTE) were 0.37, 0.99 and 0.64 for the breeding rate, the conception rate and the pregnancy rate, respectively. No interactions between the listed covariables and between treatments were retained in the multivariate logistic regression model. Estimated odds ratios and 95% confidence intervals (CI) for the comparison of cloprostenol against dinoprost were 1.03 (0.59, 1.82), 1.42 (0.88, 2.30) and 1.41 (0.90, 2.20), respectively for breeding, conception and pregnancy rates. For the comparison of IV against IM injection, they were 0.83 (0.45, 1.50), 1.39 (0.83, 2.32) and 1.28 (0.80, 2.05), respectively, for breeding, conception and pregnancy rates.

Fertility odds ratios were converted to rates, which are first presented for simple effects of each treatment group (Table 4) and for the main effect of treatments (types of $PGF2_{\alpha}$ and routes of injection), after pooling data (Table 5). Fertility rates were not statiscally different between treatment groups, between types of $PGF2_{\alpha}$ used and between routes of injection. Conception rate was nearly 10% higher for each type of $PGF2_{\alpha}$ when injected IV rather than IM (+9.9%, P=0.21 for dinoprost IV and +9.7%, P=0.19 for cloprostenol IV; Table 4). When using pooled data, conception rate was also 8.0% (P=0.13) higher for cloprostenol than dinoprost and was 8.0% (P=0.13) higher for IV rather than IM injections (Table 5).

The logistic regression on the male calf rate with $PGF2_{\alpha}$, ROUTE, and $PGF2_{\alpha}$ x ROUTE in the model yielded a non-significant interaction term (P=0.27). Results of univariate logistic regression analysis are presented in Tables 6 and 7. As for the fertility rates, the male calf rates are also presented for the simple effects of each treatment group (Table 6) and for the main effect of treatments, after pooling data (Table 7). In each table, results are further stratified according to the interval (days) from $PGF2_{\alpha}$ injection to breeding. A higher male calf rate was observed when dinoprost was injected IV rather than IM (+26.4%, P=0.04 on 47 calves; Table 6). The male calf rate when dinoprost was injected IV was also greater than the expected population value (+33.4%, P<0.01 on 22 calves; Table 6). The overall male calf rate was not different from the expected population value (+4.3%, P=0.36; Table 7). A greater proportion of

Table 4. Fertility rates for each treatment group (multivariate logistic regression analysis).

	Dinoprost			Cloprostenol		
	IM[a]	IV[a]	P	IM[a]	IV[a]	P
Cases, n	102	99		101	100	
Breeding rate, %	85.9	79.3	0.23	82.8	83.4	0.90
Conception rate, %	33.7	43.6	0.21	41.8	51.5	0.19
Pregnancy rate, %	29.3	33.6	0.45	34.9	43.6	0.50

[a]IM=intramuscular and IV=intravenous

Table 5. Fertility rates for the main effects of types of prostaglandins ($PGF2_{\alpha}$) and routes of injection (multivariate logistic regression on pooled data).

	Type of $PGF2_{\alpha}$			Route of injection			
	Dinoprost	Cloprostenol	P	IM[a]	IV[a]	P	Overall
Cases, n	201	201		203	199		402
Breeding rate, %	82.6	83.0	0.93	84.1	81.4	0.45	82.84
Conception rate, %	38.6	46.6	0.13	38.7	46.7	0.13	42.64
Pregnancy rate, %	31.4	39.2	0.14	32.5	38.1	0.23	35.32

[a]IM=intramuscular and IV=intravenous

Table 6. Male calf rates for each treatment group and stratification according to the interval (days) from prostaglandins ($PGF2_{\alpha}$) injection to breeding (univariate analysis).

	Dinoprost			Cloprostenol		
	IM[a]	IV[a]	P	IM[a]	IV[a]	P
Abortions, n	3	2		1	1	
Cows sold before calving, n	3	4		2	1	
Missing gender[b], n	3	3		1	4	
Set of twins, n	1	0		2	0	
Calves with recorded gender, n	25	22		36	34	
Male calf rate, %	60.0	86.4	0.04	41.7	52.9	0.34
P-value[c]	0.49	<0.01		0.17	0.99	
$PGF2_{\alpha}$ - Breeding (days)						
2-3 days						
calves, n	17	15		25	24	
male calf rate, %	52.9	86.7	0.04	24.0	45.8	0.11
P-value[c]	0.99	<0.01		0.09	0.48	
4-5-6 days						
calves, n	8	7		11	10	
male calf rate, %	75.0	85.7	1.00[d]	54.5	70.0	0.66[d]
P-value[c]	0.21	0.08		0.92	0.28	

[a]IM=intramuscular and IV=intravenous
[b]From 9 herds not routinely reporting the gender of calves to the attending veterinarian
[c]Compared to an overall expected population value of 53.04% (n=22,209; singles + twins)
[d]Fisher exact test (two-tailed)

Table 3. Distribution, frequency, fertility rates (univariate analysis) and P-value (Chi-Square test of independence) of all covariables.

VARIABLE	n (%)	Breeding rate		Conception rate		Pregnancy rate	
		%	P	%	P	%	P
Lactation number							
0	70 (17.4)	87.1		57.4		50.0	
1	93 (23.1)	80.7		50.7		40.9	
2 – 3 – 4	184 (45.8)	81.5		38.7		31.5	
> 4	55 (13.7)	85.5	0.63	23.4	<0.01	20.0	<0.01
Grouping of injections							
1 – 2 – 3	254 (63.2)	78.3		37.7		29.5	
> 3	148 (36.8)	90.5	<0.01	50.0	0.03	45.3	< 0.01
AI before injection (n)							
0	219 (54.5)	84.0		42.4		35.6	
1 – 2	153 (38.1)	81.0		46.8		37.9	
> 2	30 (7.5)	83.3	0.75	24.0	0.11	20.0	0.17
Season at injection							
Dec. – Feb.	112 (27.9)	86.6		47.4		41.1	
Mar. – May	94 (23.4)	78.7		43.2		34.0	
June – Aug.	88 (21.9)	83.0		37.0		30.7	
Sept. – Nov.	108 (26.9)	82.4	0.52	41.6	0.59	34.3	0.46
Cystic ovarian							
Present	25 (6.2)	68.0		23.5		16.0	
Absent	377 (93.8)	83.8	0.04	43.7	0.10	36.6	0.04
Injection day							
Monday	80 (19.9)	88.8		49.3		43.8	
Tuesday	62 (15.4)	83.9		38.5		32.3	
Wednesday	115 (28.6)	77.4		42.7		33.0	
Thursday	63 (15.7)	85.7		31.5		27.0	
Friday	82 (20.4)	81.7	0.30	47.8	0.27	39.0	0.25
Veterinarian							
1	33 (8.2)	90.9		43.3		39.4	
2	39 (9.7)	84.6		36.4		30.8	
3	26 (6.5)	80.8		42.9		34.6	
4	30 (7.5)	66.7		35.0		23.3	
5	81 (20.1)	75.3		39.3		29.6	
6	193 (48.0)	87.0	0.02	45.8	0.85	39.9	0.37
Herd							
1	15 (3.7)	66.7		70.0		46.7	
2	10 (2.5)	80.0		62.5		50.0	
3	11 (2.7)	100.0		63.6		63.6	
4	11 (2.7)	81.8		44.4		36.4	
5	10 (2.5)	80.0		37.5		30.0	
6	11 (2.7)	100.0		27.3		27.3	
7	19 (4.7)	84.2		25.0		21.1	
8	23 (5.7)	65.2		46.7		30.4	
9	12 (3.0)	100.0		41.7		41.7	
10	12 (3.0)	83.3		50.0		41.7	
11	18 (4.5)	94.4		41.2		38.9	
12	17 (4.2)	100.0		47.1		47.1	
13	13 (3.2)	69.2		33.3		23.1	
14	11 (2.7)	90.9		30.0		27.3	
15	14 (3.5)	50.0		0.0		0.0	
16-69 (lumped)	195 (48.5)	83.6	<0.01	43.6	0.37	36.4	0.24
Stage of lactation[a]							
<100 DIM	129 (38.9)	82.9		35.5		29.5	
100-199 DIM	157 (47.3)	82.8		46.2		38.2	
>199 DIM	46 (13.9)	76.1	0.54	25.7	0.05	19.6	0.04

[a]Heifers excluded; DIM = days in milk

Table 7. Male calf rates for the main effects of types of prostaglandins ($PGF2_{\alpha}$) and routes of injection and stratification according to interval from $PGF2_{\alpha}$ injection to breeding (univariate analysis on pooled data).

	Type of $PGF2_{\alpha}$			Route of injection			
	Dinoprost	Cloprostenol	P	IM[a]	IV[a]	P	Overall
Calves, n	47	70		61	56		117
Male calf rate, %	72.3	47.1	<0.01	49.2	66.1	0.07	57.3
P-value[b]	<0.01	0.32		0.55	0.05		0.36
$PGF2_{\alpha}$ - Breeding (days)							
2-3 days							
calves, n	32	49		42	39		81
male calf rate, %	68.8	34.7	<0.01	35.7	61.5	0.02	51.9
P-value[b]	0.07	0.01		0.02	0.29		0.83
4-5-6 days							
calves, n	15	21		19	17		36
male calf rate, %	80.0	61.9	0.25	63.3	76.5	0.39	69.4
P-value[b]	0.04	0.41		0.38	0.05		0.05

[a] IM=intramuscular and IV=intravenous
[b] Compared to an overall expected population value of 53.04% (n=22,209; singles + twins)

male calves was recorded using dinoprost rather than cloprostenol (+25.2%, P<0.01; Table 7), injecting IV rather than IM (+16.9%, P=0.07; Table 7) and inseminating cattle 4 days or later after $PGF2_{\alpha}$ injection rather than earlier (+17.5%, P=0.08; Table 7). The male calf rate using dinoprost was different from the expected population value (+19.3%, P<0.01 on 47 calves; Table 7). The male calf rate also differed from the expected population value when $PGF2_{\alpha}$ was injected IV (+13.1% on 56 calves, P=0.05; Table 7).

Discussion

The reproductive efficacy of $PGF2_{\alpha}$ was tested under typical veterinarian/client field settings on dairy farms with heifers and cows ready to breed; some having previously been observed in heat, others having had silent heat. There was a bias toward lower fertility rates resulting from selection of infertile cows for treatment when comparing to other studies done on experimental premises for example. All categories of cattle were admissible in the study, resulting in the selection of 30 animals with 3 previous breedings or more and 46 cows with 200 days in milk or more (Table 3).

Inclusion of a placebo was not retained because it would have decreased voluntary participation to the study and probably diminished breeding rate. Randomization was an important issue since many possible confounding factors were not available for statistical adjustment: milk production, body condition loss after calving, heat stress, dietary protein intake, heat detection quality, sire fertility, AI technician, cow comfort, genetics, status of the reproductive tract, general health, postpartum disease, level of inbreeding, lameness, etc.[2,3,5,7,11] Randomization was likely successfull because the multivariate logistic regression, including all listed covariables (except stage of lactation), yielded fertility rates for both types of $PGF2_{\alpha}$ which were almost identical to those obtained with the univariate analysis (data not presented).

Before 1992, veterinarians from the Coaticook Veterinary Clinic were injecting cloprostenol IV to protect against possible leakage from the IM injection site, considering the small dosage (2 ml). Route of injection was not blinded to the dairy producer and could have introduced a favorable bias for the IV route since many dairy producers were complaining about the new IM route of administration. Nonetheless the breeding rate was 2.7% higher for the IM route (Table 5). Many studies report the estrous response; since this information was not routinely collected, it was replaced by the breeding rate. It is assumed that some breedings were done without a positive estrus observation. This may therefore have increased the breeding rate and decreased the conception rate in comparison to studies where breeding followed an estrous response.

Results obtained in Table 5 clearly show that sample size was not adequate to demonstrate a clinically important difference between treatments. Retrospectively, the sample size needed could be calculated from the following criteria: 1) $\alpha = 5\%$, being the significance level (P<0.05) or the probability of making a type

I error, *i.e.*, declaring that a difference exists when, in fact, there is no such difference; 2) β=20%, being the probability of making a type II error, *i.e.*, declaring that there is no difference when a difference indeed exists; and 3) a minimum predicted difference of 5% in pregnancy rate (*i.e.*, 35 vs 30%) between treatments, which is considered of clinical and economical importance. Sample size needed in each group, using those criteria, was 1,375 animals (Win Episcope version 2.0).

Statistical power of a test equals 1 - β and is equivalent to 80% in the ideal case with 1,375 animals per sample. Statistical power indicates the capacity of a study to detect a predicted difference if it really exists. With approximately 200 animals per sample when the main effect of treatments was considered, the statistical power of the study was 19% instead of 80%. Upgrading α to 10% is one way to increase the statistical power. Using P<0.10 instead of P<0.05 would have increased power to 28%. Thus, the statistical power in the present study was definitely too weak to detect the presence of any reasonable difference even at P<0.10, thereby resulting in a high probability of type II error. In fact, in order to reach the acceptable statistical power of 80% with P<0.05 and 200 animals/sample, the minimum predicted difference in pregnancy rate between treatments needs to be set to at least 13%. Such a difference in pregnancy rate between treatments is improbable in the population and if differences as small as 5% are important to consider from an economical standpoint, then a large sample size is required to detect them. When sample size is very small, such as for the male calf rate analysis (Tables 6 and 7), the probability of type I and II error increases further and results should be interpreted with caution.

Multivariate logistic regression is an appropriate way to analyze data when possible confounders may be present, particularly in field studies. All available clinically-relevant covariables were included regardless of their statistical significance in order to provide as complete a control of confounding as possible within the data set. It is possible for individual variables not to exhibit strong confounding, but when taken collectively, considerable confounding can be present in the data.[8] Stage of lactation was excluded from the model since it would have restricted the analysis to lactating dairy cows. Odds ratios were converted to rates to facilitate interpretation. Pregnancy rate was included in fertility rates because it is a good measure of reproductive efficiency in production medicine. It is essentially a product of heat detection efficiency and conception rate.[4] In this study, the overall pregnancy rate was 35.32% (Table 5). Since it was based on an induced estrus, it is likely to be greater than the pregnancy rate obtained in most dairies where estrus detection is a problem. The differences in conception and pregnancy rates between main effects of treatments (Table 5), though non-significant, are relatively important considering the weak statistical power and the quality of the adjustment to available confounders with multivariate logistic regression analysis.

Male calf rate did not need to be adjusted with multivariate logistic regression because it was not associated with any of the listed covariables. To account for the normally greater incidence of male calves, an additional Chi-Square test of independence was conducted between male calf rate and expected population value (53.04%) obtained from an earlier study with 22,209 calves born to primiparous and multiparous Holstein-Friesian cows, as done in an earlier study.[14] Missing gender of calf was unlikely to have biased the results since cases were well distributed across treatment groups. Only 11 calves were born with unrecorded sex to 9 farms unwilling to report any gender of calf to the attending veterinarian. The number of calves in each treatment group and in pooled data is small, particularly after stratification according to the interval from $PGF2_{\alpha}$ injection to breeding. Thus, results are not strongly conclusive even when statistical significance is achieved.

Many dairy producers have reported a higher incidence of male calves after the use of $PGF2_{\alpha}$. In our study, an overall male calf rate greater, but not statistically different from the expected population value, was obtained (+4.3%, P=0.36; Table 7). However, type of $PGF2_{\alpha}$ (dinoprost), route of injection (IV) and increasing the interval from $PGF2_{\alpha}$ injection to breeding seemed to skew the sex ratio toward males. Roy and Twagiramungu[16] reported a similar trend toward male calves when the interval from $PGF2_{\alpha}$ injection to AI was increased. They conducted a retrospective study on sex ratio of beef calves (n=526) when luteolysis was induced with cloprostenol. Male calf rates were 41.0%, 47.1% and 56.5%, respectively, when AI was done 24 to 60 h (n=178), 60 to 72 h (n=187) and 72 to 120 h (n=161) after cloprostenol injection. It was concluded that the male calf rate was higher when AI was practiced between 72 and 120 h than between 24 and 60 h (P<0.01) after cloprostenol injection.

It is believed that the timing of insemination during estrus influences the sex ratio of offsprings, with early insemination resulting in more females and late insemination in more males.[15] A better synchrony between ovulation and AI would increase the male calf rate because the Y-bearing spermatozoa capacitate before the X-bearing spermatozoa, giving the male spermatozoa a greater ability to fertilize the oocyte by ovulation time.[22] A bias toward males can also result from sex-specific death of female embryos after fertilization.[15] The embryo death rate was not measured in this study, but is likely to be associated with a lower conception rate. Conception rate was 9.9% higher

(P=0.21, Table 4) for dinoprost IV compared to dinoprost IM, yet, male calf rate was 26.4% higher (P=0.04, Table 6). This suggests that sex-specific death of female embryos after fertilization was not a contributing factor to the male calf rate obtained with dinoprost IV.

Odds ratios in Table 8 were derived from published data. Odds ratio is a measure of association which approximates how much more likely (or unlikely) it is for the animal to be bred or pregnant when using cloprostenol rather than dinoprost. Odds ratio higher than 1.00 indicates better results obtained with cloprostenol compared to dinoprost and conversely if OR is smaller than 1.00. Odds ratio allows between-studies comparisons which are independent from the average fertility rate obtained in each study. For example, odds ratios for breeding rate were similar, yet average breeding rates were quite different, being 53.8% and 85.4%, respectively, in Sudweeks *et al*[20] and for the herd C of Salverson *et al*[18] (Table 8). Cloprostenol was about 2.4 times more likely to induce an estrus when compared to dinoprost in both studies, yet breeding rates for cloprostenol vs dinoprost were 65.7% vs 42.7% (+23.0%) and 90.2% vs 80.0% (+10.2%), respectively, in Sudweeks *et al*[20] and for the herd C of Salverson *et al*.[18] Comparison of results between studies is easier using an odds ratio basis (Table 8) but is nonetheless difficult due to differences in protocol, treatment regimen, breed, lactating status, parity of animals, etc. Great variability exists between studies and is likely to result from insufficient sample size and presence of confounding factors.

It is appropriate to compare the efficacy of the two main sources of $PGF2_{\alpha}$, since they are commonly injected alone when a functional corpus luteum is detected, especially in small farms where adequate heat detection is feasible. The practicing veterinarian is often challenged by dairy producers about the equal efficacy of those $PGF2_{\alpha}$ products. Dinoprost and cloprostenol have also been used without distinction in the new protocols for timing of insemination and their equal efficacy remains to be addressed. A larger scale study on high-producing dairy cattle is definitely needed to clarify the effects of type of $PGF2_{\alpha}$, route of injection and time interval from $PGF2_{\alpha}$ injection to breeding on fertility and sex ratio of calves.

Conclusion

In conclusion, type of $PGF2_{\alpha}$ used (cloprostenol) and route of injection (IV) might improve conception rate and, hence, pregnancy rate, yet the statistical power of the study was too weak to detect any significant differences. It can also be concluded that type of $PGF2_{\alpha}$ (dinoprost), route of injection (IV) and increasing the

Table 8. Fertility rates for all cattle and estimated odds ratio for the comparison of cloprostenol intramuscular vs dinoprost intramuscular (univariate analysis).

Study	Class[a]	Total N	Fertility rate[b], %			Odds ratio[c]		
			BR	CR	PR	BR	CR	PR
Sudweeks *et al* (1983)[d]	DH	145	53.8	66.7	35.9	2.55**	1.49	2.41*
Johnson (1984)	DC	52	51.9	59.3	30.8	0.46	0.38	0.32
Seguin *et al* (1985)								
experimental	NLDC	124	92.7	—	—	3.82	—	—
field settings	DC	245	65.7	50.9	33.5	0.96	0.98	0.96
Turner *et al* (1987)	BCH	63	71.3	47.2	33.6	1.50	0.83	1.10
Wenkoff (1987)	NLBC	139	71.2	—	—	1.33	—	—
Kelton (1989)	DC	129	48.8	46.0	22.5	0.63	0.79	0.63
Salverson *et al* (2002)								
herd A	BH	292	80.5	69.4	55.8	1.06	0.82	0.89
herd B	BH	181	95.6	50.9	48.6	1.69	1.42	1.46
herd C	BH	96	85.4	46.3	39.6	2.30	0.77	0.97
herd D	BH	209	88.5	80.5	71.3	0.97	1.68	1.40
herd E	BH	216	88.9	74.5	66.2	1.81	0.95	1.16

[a]D=dairy; B=beef; H=heifer; C=cow; NL=non lactating

[b]BR=breeding rate, CR=conception rate, PR=pregnancy rate

[c]OR higher than 1.00 indicates better results obtained with cloprostenol compared to dinoprost and conversely if OR is smaller than 1.00

[d]Only 1st breeding considered; conception rate for 1st breeding assumed similar to conception rate for 1st and 2nd breeding; sample size assumed to be 72 and 73, respectively for cloprostenol and dinoprost

*P<0.05; ** P<0.01

interval from $PGF2_{\alpha}$ injection to breeding might skew the sex ratio toward males.

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Footnotes

[a] Lutalyse® - Pharmacia et Upjohn Company, Orangeville, Ontario

[b] Estrumate® - Coopers Agropharm, Ajax, Ontario

[c] Beef quality assurance (BQA) guidelines now recommend that all injections be administered only in front of the point of the shoulder.

[d] DSA, version 4.2, ASTLQ inc., Faculté de Médecine Vétérinaire, Université de Montréal, Québec, J2S 7C6

[e] Amélioration de la Santé des Troupeaux Laitiers du Québec

[f] Statistix, version 7.0, Analytical Software, Tallahassee, FL 32317

[g] Win Episcope, version 2.0, developed by K. Frankena and J.O. Goelema, from the Department of Animal Sciences of Wageningen Agricultural University (The Netherlands)

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