Scientific Article

Efficacy of inclusion of equine chorionic gonadotrophin into a treatment protocol for anoestrous dairy cows

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Abstract

AIM: To compare the performance of intravaginal devices containing 1.0 g (DIB) or 1.38 g (CIDR) progesterone and to determine the efficacy of inclusion of equine chorionic gonadotrophin (eCG) into progesterone-based anoestrous cow treatment protocols for New Zealand dairy cows.

METHODS: Anoestrous cows (n = 1,906) from 12 herds were randomly assigned to four treatments: 100 μg gonadorelin (GnRH) at Day -10; 500 μg cloprostenol at Day -3; 100 μg GnRH at Day -1 and fixed time artificial insemination (FTAI) on Day 0 (gonadotrophin-prostaglandin-gonadotrophin [GPG] group, n = 475); GPG with CIDR device (1.38 g progesterone) inserted between Day -10 and Day -3 (CIDR group, n = 477); GPG with DIB device (1.0 g progesterone) inserted between Day -10 and Day -3 (DIB group, n = 477); and DIB with 400 IU eCG at Day -3 (DIB + eCG group, n = 477). Conception rates to FTAI and pregnancy at Day 28 were analysed using generalised estimating equations (GEE). Time to conception and time to return to oestrus were analysed using Kaplan-Meier survival analysis and Cox's proportional hazards regression.

RESULTS: The proportion of cows conceiving to FTAI was 0.34 (95%CI = 0.29–0.37), 0.38 (95%CI = 0.34–0.43), 0.38 (95%CI = 0.33–0.42) and 0.41 (95%CI = 0.37–0.46) for GPG, CIDR, DIB and DIB + eCG groups, respectively. The proportion of cows pregnant by Day 28 was 0.55 (95%CI = 0.51–0.60), 0.57 (95%CI = 0.52–0.61), 0.56 (95%CI = 0.52–0.60) and 0.63 (95%CI = 0.59–0.67) for GPG, CIDR, DIB and DIB + eCG groups, respectively. There was an interaction between treatment and number of days calved ($p < 0.05$). Cows more than 60 days calved and treated with DIB + eCG had higher FTAI conception and 28-day pregnancy rates than cows treated with GPG ($p < 0.001$). Median interval to conception did not differ between treatments ($p > 0.05$). There were no differences between DIB and CIDR groups for any parameter ($p > 0.05$). The range of the relative risk distribution among herds comparing DIB + eCG to DIB groups was greater than that comparing CIDR to DIB groups for conception to FTAI and pregnancy at Day 28.

CONCLUSIONS: The inclusion of eCG into progesterone-based anoestrous cow treatment protocols improves conception to FTAI and 28-day pregnancy rates in cows >60 days calved at treatment compared with a GPG protocol. There was no difference in clinical performance between DIB and CIDR devices.

CLINICAL RELEVANCE: The combination of a low payload (1.0 g) progesterone releasing intravaginal device with eCG treatment at device removal within a GPG treatment is a clinically effective treatment for anoestrous in New Zealand dairy cows.

KEY WORDS: *dairy cattle, anoestrous, treatment, eCG*

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Introduction

The majority of the pasture-based dairy industry of New Zealand uses seasonal calving whereby the herd is mated to calve within a 10–12-week period just before peak spring pasture growth. Achieving a high herd pregnancy rate with a compact conception pattern is becoming increasingly difficult for many New Zealand farms (McDougall and Compton 2006). The mean prevalence of anoestrus – defined as failure to detect cows in oestrus before the start of the breeding period – was estimated at approximately 20% of cows ten years ago in New Zealand (Rhodes *et al.* 2003).

Progesterone-releasing devices have been used extensively in New Zealand to control the oestrus cycle and to stimulate ovarian function in anoestrous cows (Xu *et al.* 1997; Macmillan 2002; McDougall and Compton 2005). It has also been demonstrated that the addition of a progesterone device to a gonadotrophin-prostaglandin-gonadotrophin (GPG) treatment protocol was associated with a 10–20% improvement in reproductive performance (McDougall 2010a).

The amount of progesterone contained within progesterone intravaginal devices varies between devices available in New Zealand. The relationship between device payload and the amount of

BCS	Body condition score
eCG	Equine chorionic gonadotrophin
FTAI	Fixed time artificial insemination
GEE	Generalised estimating equation
GPG	Gonadotrophin-prostaglandin-gonadotrophin (Ovsynch) treatment protocol
GnRH	Gonadotrophin releasing hormone
PSM	Planned start of mating

progesterone absorbed and therefore residual device progesterone is not linear (Macmillan and Petersen 1993). A recent study comparing progesterone profiles in cycling, lactating Holstein cows treated with progesterone devices containing either 1.90 g (CIDR with 1.9 g progesterone; Pfizer Salud Animal S.A., Argentina), 1.56 g (Cue-Mate; Bioniche Animal Health Canada Inc), 1.38 g (CIDR with 1.38 g progesterone; Pfizer Animal Health, USA) or 1.0 g (DIB; Syntex SA, Argentina) progesterone, revealed no difference between treatments in peak or mean concentrations of progesterone in plasma from insertion to Day 7 (Rogan *et al.* 2007). In synchronised cycling Holsteins, mean peak concentrations of progesterone in serum were 3.0 ng/mL for a device containing 1.0 g progesterone (DIB 1.0, Syntex SA) on Day 1 after insertion reducing to 2.3 ng/mL by Day 3, and profiles did not differ in a device containing 0.5 g progesterone (Videla *et al.* 2008).

Exogenous gonadotrophin releasing hormone (GnRH) has been shown to reliably induce ovulation of mature dominant follicles in anovulatory anoestrous cows (McDougall *et al.* 1995). However, few anoestrous cows treated with GnRH alone resumed normal oestrus cycles after the induced ovulation. Therefore the GPG protocol may not induce the resumption of oestrous cycles in all anoestrous cows that fail to become pregnant to the synchronised ovulation of this protocol.

Equine chorionic gonadotrophin (eCG) has a long half-life and dual follicle-stimulating hormone and luteinising hormone effects in the cow. A recent New Zealand study found a positive effect of inclusion of eCG within a progesterone-based anoestrous cow treatment protocol for cows over five years of age (Bryan *et al.* 2010). Other studies have demonstrated a variable effect of eCG on pregnancy rates in cattle (Macmillan and Petersen 1993; Small *et al.* 2010).

Early treatment of anoestrous cows with either a GPG treatment protocol (GnRH administered on Day -10, prostaglandin administered on Day -3, GnRH administered again on Day -1) and fixed time artificial insemination (FTAI) 16–20 hours later on Day 0 or with a GPG protocol plus the addition of a progesterone releasing intravaginal device applied on Day -10 and removed on Day -3 (GPG + P4 protocol) have been demonstrated to be more profitable than not treating anoestrous cows in New Zealand (McDougall 2010b). This cost-benefit analysis demonstrated a return of NZ$47.50 for GPG and $NZ80.40 for GPG + P4 treatments over costs from treatment.

The present study compared the performance of different FTAI treatment protocols on conception rate to FTAI and on subsequent conception patterns in anoestrous seasonally calving dairy cows in New Zealand. The study specifically evaluated the efficacy and inter-herd variability of inclusion of eCG at device removal within a progesterone-based anoestrous cow treatment protocol, and the efficacy of a low payload (1.0 g) progesterone intravaginal device (DIB-V, Syntex SA).

Materials and methods

The AgResearch Ruakura Animal Ethics Committee approved this study (Approval No 12132).

Seven experienced dairy veterinarians from six veterinary practices located in the Waikato and Taupo regions of the North Island and the Otago and Canterbury regions of the South Island of New Zealand were recruited as study investigators. These veterinarians subsequently recruited 12 commercial seasonally calving dairy farms (six in the North Island and six in the South Island). Cows not detected in oestrus in the 28 days prior to the planned start of mating (PSM), that were more than 30 days calved by the PSM and had no clinical disease or reproductive abnormality were eligible for inclusion into the study. Cows were not submitted for veterinary reproductive examination prior to treatment.

The four treatment protocols compared were: 100 μg gonadorelin (Gonasyn, Syntex SA, Buenos Aires, Argentina) administered on Day -10, 500 μg cloprostenol (Cyclase, Syntex SA) on Day -3, 100 μg gonadorelin on Day -1 and FTAI on Day 0 (GPG group); as for GPG with intravaginal insertion of a CIDR device (Pfizer Animal Health, Auckland, NZ) containing 1.34 g progesterone on Day -10 and removal on Day -3 (CIDR group); as for GPG with intravaginal insertion of a DIB device (DIB-V, Syntex SA) containing 1.0 g progesterone on Day -10 and removal on Day -3 (DIB group); as for DIB with 400 IU of eCG (Novormon eCG, Syntex SA) administered on Day -3 (DIB + eCG group). Cows were assigned to treatment group using a stratified random allocation system whereby cows were assigned sequentially to treatment group based on presentation order to the veterinarian within the race on the day of first examination (Day -10). Day 0 was set at PSM.

The Day -1 treatments were administered in the afternoon as this timed FTAI for the morning of Day 0 (16–20 hours after Day -1 treatment). Cows detected in oestrus after the prostaglandin treatment (Day -3) but before the proposed time for FTAI were submitted for artificial insemination at the next opportunity and recorded as receiving an early insemination. All remaining cows were submitted for FTAI on Day 0.

Study veterinarians administered all treatments and professional insemination technicians performed FTAI. Each treatment was recorded at each intervention and the study veterinarians assessed and scored the body condition of each cow using the 1–10 scoring scale recommended by DairyNZ on Day -10 (Anonymous 2012). Oestrus detection aids (e.g. tail paint or commercial oestrus mount detectors) were applied to all cows after FTAI to assist detection of returns to service. All farms continued to detect oestrus and to use artificial insemination for at least 28 days after FTAI. The farmer recorded returns to service and subsequent inseminations. All trial cows were submitted for pregnancy diagnosis with foetal aging (manual or by ultrasonography) by the study investigator between 35 and 84 days after FTAI. Cows not detected pregnant at this examination were submitted for a subsequent pregnancy diagnosis to determine final pregnancy status. Cow breed, age, last calving, mating dates, disease and culling information were obtained from herd records (e.g. MINDA database, LIC, Hamilton, NZ).

One study veterinarian examined 330 cows receiving an intravaginal device at the time of device removal (CIDR: n = 110, DIB: n = 220). Device losses were recorded and vaginitis was assessed using an ordinal scoring system for gross vaginal discharge (0–no purulence; 1–flecks of purulent material; and 2–large amounts of purulent material). The device loss rate and prevalence of vaginitis and associated 95% confidence intervals were calculated and compared between DIB and CIDR groups.

Statistical analysis

Data were entered into Microsoft Excel spread sheet version 14.2.5 (Microsoft Corporation 2010, Redmond, WA, USA) for cleaning and collation. The R language and environment for statistical computing version 2.15.0 (R Core Team, 2012, R Foundation for Statistical Computing, Vienna, Austria) was used for all statistical analysis.

Comparison of conception rates to FTAI, pregnancy rates at Day 28, interval to pregnancy and interval to return to service in cows not pregnant to FTAI between treatment groups was performed using multivariable methods that controlled for the effect of clustering at herd level and for other predictor variables. Significance was set at $p = 0.05$.

Age, body condition score (BCS), breed and days calved were classified into ordinal groups. Class cut points were chosen to reflect biological differences between groups and to ensure adequate distribution of cows across classes. Age was therefore categorised into 2-year-old, 3-year-old, 4–6 year-old and ≥7-year-old classes. Body condition score was categorised into <3.0, 3.0, 3.5, 4.0 and >4.0 classes. Breed was classified into Friesian and non-Friesian classes (there were only 69 purebred Jersey cows recruited). Number of days calved by FTAI was dichotomised into <60 days and ≥60 days classes. There was moderate correlation between days calved, age and BCS. In general 2-year-olds are mated to calve ahead of the cows, late calved cows tend to be in lighter BCS than early calved cows and 2-year-olds tend to be in lighter BCS after calving compared to older cows. Where the correlation between variables resulted in large changes to the coefficient estimates (> 10%) or inability of the model to converge, the least predictive member of the correlated variable pair was removed from the multiple regression models. Individual predictor variables offered to multiple regression models were island (north or south), BCS, age, breed, days calved by FTAI and early mating (defined as a cow detected in oestrus after prostaglandin treatment (Day -3) and before FTAI (Day 0) and inseminated to the detected oestrus).

Cows providing at least one pregnancy result were sent forward for analysis. Generalised estimating equations (GEE), a fitting methodology for a GLM with clustered data, was used to examine binary outcomes of conception rate to FTAI and pregnancy at 28-days controlled for clustering at herd level. Individual predictor variables were examined and where significant at $p < 0.1$ were sent forward for multiple regression along with two-way interaction terms between included predictor variables. Multiple regression models were constructed from cows with complete records for included variables. A backward stepwise approach was used for final model construction. Individual variables were dropped from the model if the overall fit of the reduced model was not significantly different (i.e. $p > 0.05$) compared to the full model. Treatment was forced into all models irrespective of statistical significance. The GPG treatment protocol was the reference treatment and CIDR and DIB treatments were specifically compared using a separate model. Analysis was conducted using the geeglm function from the geepack library in R.

Differences in the interval from FTAI to conception and in the interval from FTAI to return to oestrus (in cows not pregnant to FTAI) were compared between treatments using Kaplan-Meier survival analysis. The effects of individual predictor variables on survival until conception were also examined using Cox's proportional hazards regression controlled for clustering within herd using the coxph function from the survival library of R. Individual predictors were sent forward for multiple regression if statistically significant ($p < 0.1$) along with 2-way interaction terms between included variables. Again, the final model was limited to data from cows with complete records for included variables and the final model was constructed using backward stepwise methods – individual terms were dropped if the difference between the reduced and full model was not significant (i.e. $p > 0.05$). Again, the GPG treatment protocol was the reference treatment. CIDR and DIB treatments were specifically compared using a separate model.

Variability of effect within herd due to the inclusion of eCG within a progesterone-based anoestrous cow treatment protocol was assessed by calculating the relative risks for conception to FTAI and pregnancy at Day 28 for the DIB + eCG group compared to the DIB group within herd, the CIDR compared with DIB group, and the GPG compared with DIB group.

Results

A total of 1,940 cows were recruited from 12 farms (mean per farm = 162, range 95–320). Of these, 1,906 cows (98.2%) provided at least one pregnancy result and were sent forward for analysis. There were no significant differences in the distribution of the predictor variables BCS, age, breed or days calved by FTAI between treatment groups (Table 1).

Progesterone device adverse events

A total of 13 DIB devices were reported missing at device removal (Day -3) from 954 DIB-treated cows. The loss rate was 0.014 (95%CI = 0.007–0.023). Five CIDR devices were reported lost from 477 CIDR-treated cows. This loss rate was 0.010 (95% CI = 0.003–0.024). Some study veterinarians reported occasional rotation of the DIB device in the vagina (resulting in the device tail being drawn into the vagina).

The prevalence of purulence (purulence score of 1 or 2) was 0.55 (SE 0.04) for DIB and 0.87 (SE 0.04) for CIDR groups ($p < 0.001$). Analysis of conception rates to FTAI found no impact of purulence score on risk of conception when analysis was controlled for days in milk, herd and BCS.

Conception to FTAI

The number and proportion of cows conceiving to FTAI is presented in Table 2. The final multiple regression (GEE) model predicting risk of conception to FTAI for anoestrous cows is presented in Table 3. From this model, Friesian cows had lower conception rates to FTAI than non-Friesians, and cows with BCS < 4.0 had lower conception rates than those ≥4.0. Cows inseminated early on detected of oestrus had higher conception rates than those inseminated on Day 0. There was a significant interaction between treatment and days calved. Conception rates were essentially similar between treatments for cows less than 60 days calved. In cows more than 60 days calved the CIDR and DIB groups had similar conception rates to the GPG group, whereas for the DIB + eCG group conception rates were superior to the GPG group. There was no significant difference in conception rates to FTAI between DIB and CIDR

Table 1. Number of cows, mean age (years), mean interval from calving to planned start of mating (days), mode of body condition score (BCS) class and number of pure-bred Friesian cows from 1,906 cows within 12 seasonally calving New Zealand dairy herds recruited into a study comparing four treatments for anoestrus, commencing 10 days before the herd's planned start of mating (Day -10).

Treatment	Number of cows	Mean age ± SD	Mean interval from calving ± SD	Mode of BCS class	Number of purebred Friesians
GPG[a]*	475	4.7 ± 2.4	63.8 ± 16.5	4.0	188
CIDR[b]	477	4.8 ± 2.5	64.0 ± 16.7	4.0	209
DIB[c]	477	4.7 ± 2.4	64.3 ± 16.7	4.0	195
DIB + eCG[d]	477	4.9 ± 2.5	62.8 ± 16.2	4.0	197
Total	1906	4.8 ± 2.4	63.7 ± 16.5	4.0	789

[a]Gonadotrophin-releasing hormone (GnRH) Day -10, cloprostenol Day -3, GnRH Day -1
[b]GPG and intravaginal progesterone-releasing device (CIDR) inserted Day -10, removed Day -3
[c]GPG and intravaginal progesterone-releasing device (DIB) inserted Day -10, removed Day -3
[d]DIB and 400 IU of equine chorionic gonadotrophin on Day -3

Table 2. Number and proportion of cows conceiving to fixed time artificial insemination (FTAI; Day 0) and pregnant by Day 28 for 1,906 cows within 12 seasonally calving New Zealand dairy herds recruited into a study comparing four treatments for anoestrous.

		FTAI			Day 28		
Treatment	Total	Pregnant	Proportion	95%CI	Pregnant	Proportion	95%CI
GPG[a]*	475	160	0.34	(0.29–0.38)	262	0.55	(0.51–0.60)
CIDR[b]	477	183	0.38	(0.34–0.43)	270	0.57	(0.52–0.61)
DIB[c]	477	179	0.38	(0.33–0.42)	267	0.56	(0.52–0.60)
DIB + eCG[d]	477	196	0.41	(0.37–0.46)	301	0.63	(0.59–0.67)
Total	1906	718			1100		

[a]Gonadotrophin-releasing hormone (GnRH) Day -10, cloprostenol Day -3, GnRH Day -1
[b]GPG and intravaginal progesterone-releasing device (CIDR) inserted Day -10, removed Day -3
[c]GPG and intravaginal progesterone-releasing device (DIB) inserted Day -10, removed Day -3
[d]DIB and 400 IU of equine chorionic gonadotrophin on Day -3

Table 3. Final multiple regression model predicting risk of conception to fixed time artificial insemination at Day 0 for 1,906 cows within 12 seasonally calving New Zealand dairy herds recruited into a study comparing four treatments (GPG[a], CIDR[b], DIB[c] and DIB + eCG[d] groups) for anoestrous.

Parameter[e]	Estimate	SE	OR (95%CI)	Wald statistic	Wald p-value
(Intercept)	-1.249	0.572	0.29 (0.09–0.88)	4.780	0.029
BCS = 3.0	0.329	0.376	1.39 (0.67–2.90)	0.770	0.38
BCS = 3.5	0.460	0.371	1.58 (0.77–3.28)	1.540	0.22
BCS = 4.0	0.570	0.324	1.77 (0.94–3.34)	3.100	0.078
BCS = 4 +	1.003	0.305	2.73 (1.50–4.96)	10.790	0.001
Breed = Friesian	-0.315	0.052	0.73 (0.66–0.81)	36.200	<0.001
Days calved = 60 +	-0.005	0.280	0.99 (0.57–1.72)	0.000	0.99
Early mating = TRUE	0.462	0.149	1.59 (1.18–2.13)	9.530	0.002
Group = CIDR[a]	-0.099	0.307	0.91 (0.50–1.65)	0.100	0.75
Group = DIB[b]	0.074	0.282	1.08 (0.62–1.87)	0.070	0.80
Group = DIB + eCG[c]	-0.239	0.278	0.79 (0.46–1.36)	0.740	0.39
Days Calved 60+ : CIDR	0.475	0.375	1.61 (0.77–3.35)	1.610	0.21
Days Calved 60+ : DIB	0.210	0.365	1.23 (0.60–2.52)	0.330	0.57
Days Calved 60+ : DIB + eCG	0.914	0.254	2.49 (1.52–4.10)	12.930	<0.001

[a]Gonadotrophin-releasing hormone (GnRH) Day -10, cloprostenol Day -3, GnRH Day -1
[b]GPG and intravaginal progesterone-releasing device (CIDR) inserted Day -10, removed Day -3
[c]GPG and intravaginal progesterone-releasing device (DIB) inserted Day -10, removed Day -3
[d]DIB and 400 IU of equine chorionic gonadotrophin on Day -3
[e]Reference categories for BCS <3.0, Breed = Non-Friesian, Days calved <60 days calved, Group =GPG
BCS = Body condition score

Table 4. Final multiple regression model predicting risk of pregnancy by Day 28 after fixed time artificial insemination at Day 0 for 1,906 cows within 12 seasonally calving New Zealand dairy herds recruited into a study comparing four treatments (GPG[a], CIDR[b], DIB[c] and DIB + eCG[d] groups) for anoestrous.

Parameter[e]	Estimate	SE	OR (95%CI)	Wald statistic	Wald p-value
(Intercept)	-0.253	0.672	0.78 (0.21–2.90)	0.140	0.71
BCS = 3.0	0.074	0.598	1.08 (0.33–3.48)	0.020	0.90
BCS = 3.5	0.522	0.549	1.69 (0.57–4.95)	0.900	0.34
BCS = 4.0	0.631	0.472	1.88 (0.74–4.74)	1.780	0.18
BCS = 4 +	0.893	0.468	2.44 (0.98–6.12)	3.640	0.057
Breed = Friesian	-0.485	0.071	0.62 (0.54–0.71)	47.110	<0.001
Days calved = 60 +	0.026	0.215	1.03 (0.67–1.56)	0.020	0.90
Early mating = TRUE	0.414	0.159	1.51 (1.11–2.07)	6.810	0.009
Group = CIDR[a]	-0.244	0.270	0.78 (0.46–1.33)	0.820	0.37
Group = DIB[b]	-0.133	0.361	0.88 (0.43–1.77)	0.140	0.71
Group = DIB + eCG[c]	-0.060	0.244	0.94 (0.58–1.52)	0.060	0.81
Days Calved 60+ : CIDR	0.495	0.380	1.64 (0.78–3.45)	1.700	0.19
Days Calved 60+ : DIB	0.331	0.388	1.39 (0.65–2.98)	0.730	0.39
Days Calved 60+ : DIB + eCG	0.768	0.263	2.16 (1.29–3.61)	8.570	0.003

[a]Gonadotrophin-releasing hormone (GnRH) Day -10, cloprostenol Day -3, GnRH Day -1
[b]GPG and intravaginal progesterone-releasing device (CIDR) inserted Day -10, removed Day -3
[c]GPG and intravaginal progesterone-releasing device (DIB) inserted Day -10, removed Day -3
[d]DIB and 400 IU of equine chorionic gonadotrophin on Day -3
[e]Reference categories for BCS <3.0, Breed = Non-Friesian, Days calved <60 days calved, Group = GPG
BCS = Body condition score

treatment groups when these two treatments were compared using a separate model (p > 0.05).

Pregnancy by Day 28

The number and proportion of cows pregnant by 28 days after FTAI is presented in Table 2. The final multiple regression (GEE) model for 28-day pregnancy rate of anoestrous cows is presented in Table 4.

Again, Friesian cows had lower pregnancy rates than non-Friesians, and cows with BCS <4.0 had lower pregnancy rates than those ≥4.0. Cows inseminated early on detected oestrus had higher pregnancy rates than those inseminated on Day 0. The significant interaction between treatment and days calved persisted for 28-day pregnancy rates. The 28-day pregnancy rates were similar between treatments for cows less than 60 days calved. In cows more than 60 days calved pregnancy rates for the CIDR and DIB groups were similar to the GPG group, and the DIB + eCG treatment was superior to the GPG group. There was no difference in 28-day pregnancy rate between DIB and CIDR treatment groups (p > 0.05) when these two treatments were compared using a separate model.

Time to conception

The cumulative proportion of cows conceiving to FTAI between Days 0 and 42 for the four treatment groups are presented in Figure 1. Survival times (as non-pregnant) post FTAI were compared between treatments using univariate Kaplan-Meier survival analysis. Differences in survival times between all four groups were not significant (χ^2 = 7.05, df = 3, p = 0.070).

Cox's proportional hazards regression analysis was performed to assess the impact of various predictor variables on median survival intervals. The summary of this model is provided in Table 5. Interval to conception was longer in Friesian than non-Friesian cows, in cows calved <60 days compared with ≥60 days, for cows outside the age range of 4–6 years and for cows inseminated at FTAI compared with those inseminated on detection of oestrus before Day 0. There was no significant effect of treatment on median time to conception. Specifically there was no significant difference in median time to conception comparing CIDR and DIB treatments when these two treatments were compared using a separate model.

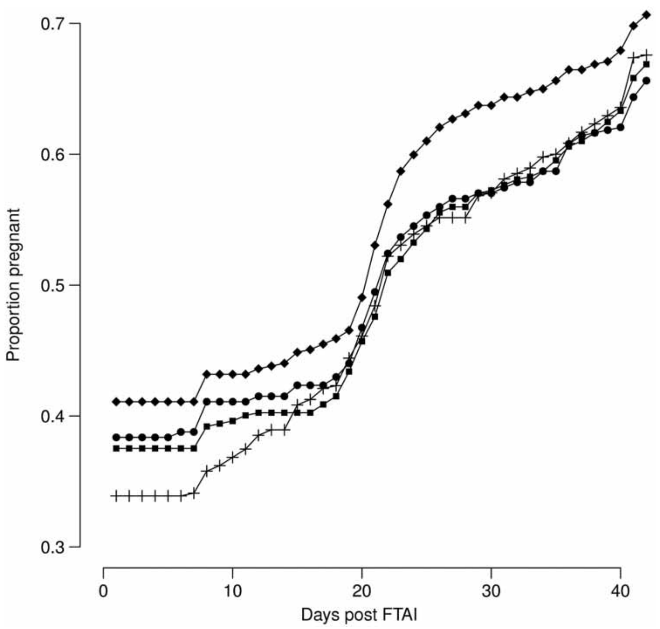

Figure 1. Cumulative proportion of 1,906 anoestrous cows conceiving to fixed time artificial insemination (FTAI) from Day 0 to Day 42 following treatment commencing on Day -10 . Cows were treated with either: gonadotrophin-releasing hormone (GnRH) on Day -10, cloprostenol on Day -3 and GnRH on Day -1 (GPG: —+—); GPG with a CIDR device (CIDR: —●—); GPG with a DIB device (DIB: —■—); or DIB with 400IU of equine chorionic gonadotrophin on Day -3 (DIB + eCG: –◆–).

Table 5. Final Cox's proportional hazards regression model predicting median survival time to pregnancy after fixed time artificial insemination at Day 0 for 1,906 cows within 12 seasonally calving New Zealand dairy herds recruited into a study comparing four treatments (GPG[a], CIDR[b], DIB[c] and DIB + eCG[d] groups) for anoestrous.

Parameter[e]	Estimate	SE	Hazard ratio (95%CI)	z statistic	z statistic p-value
Age = 3 years old	0.122	1.130	1.13 (0.95–1.31)	1.700	0.089
Age = 4–6 years old	0.261	1.299	1.30 (1.15–1.45)	3.910	0.000
Age = 7+ years old	0.157	1.170	1.17 (1.00–1.34)	1.140	0.254
BCS = 3.0	0.438	1.549	1.55 (0.95–2.14)	0.960	0.336
BCS = 3.5	0.486	1.626	1.63 (1.05–2.20)	1.030	0.303
BCS = 4.0	0.442	1.555	1.56 (0.98–2.13)	0.970	0.331
BCS = 4 +	0.610	1.840	1.84 (1.26–2.42)	1.290	0.197
Breed = Friesian	-0.245	0.783	0.78 (0.68–0.89)	-5.220	0.000
Days calved = 60 +	0.284	1.328	1.33 (1.22–1.44)	3.840	0.000
Early mating = TRUE	0.248	1.281	1.28 (1.14–1.42)	2.410	0.016
Group = CIDR[a]	-0.048	0.953	0.95 (0.81–1.10)	-0.550	0.579
Group = DIB[b]	0.010	1.010	1.01 (0.86–1.16)	0.130	0.895
Group = DIB + eCG[c]	0.126	1.134	1.13 (0.99–1.28)	1.410	0.158

[a]Gonadotrophin-releasing hormone (GnRH) Day -10, cloprostenol Day -3, GnRH Day -1
[b]GPG and intravaginal progesterone-releasing device (CIDR) inserted Day -10, removed Day -3
[c]GPG and intravaginal progesterone-releasing device (DIB) inserted Day -10, removed Day -3
[d]DIB and 400 IU of equine chorionic gonadotrophin on Day -3
[e]Reference categories for Age = 2 years old, BCS <3.0, Breed = Non-Friesian, Days calved <60 days calved, Group =GPG
BCS = Body condition score

Time to return to oestrus

Differences in survival time for return to oestrus after FTAI in 1,063 cows not pregnant to FTAI were not significant between treatments (χ^2 = 6.99, df = 3, p = 0.07). However, two-way comparisons between GPG and the following treatments were significant: CIDR (χ^2 = 4.03, df = 1, p = 0.045), DIB + eCG (χ^2 = 5.49, df = 1, p = 0.019) and to progesterone devices (CIDR and DIB) combined (χ^2 = 6.35, df = 1, p = 0.042). There was no significant difference in time to return to oestrus after FTAI in cows not pregnant to FTAI between CIDR and DIB treatments. The cumulative proportion of cows not pregnant that returned to oestrus between Days 0 and 42 is presented in Figure 2.

Variability between herds

The relative risk distribution between herds for risk of conception to FTAI and pregnancy at Day 28 comparing DIB + eCG, CIDR and GPG treatment groups against the DIB treatment group is summarised in Table 6. The range of the relative risk distribution among herds comparing DIB + eCG to DIB was greater than that comparing CIDR to DIB for conception to FTAI and pregnancy at Day 28. The range of the relative risk distribution comparing DIB + eCG to DIB was less than that comparing GPG with DIB for conception to FTAI, but appeared similar for pregnancy at Day 28.

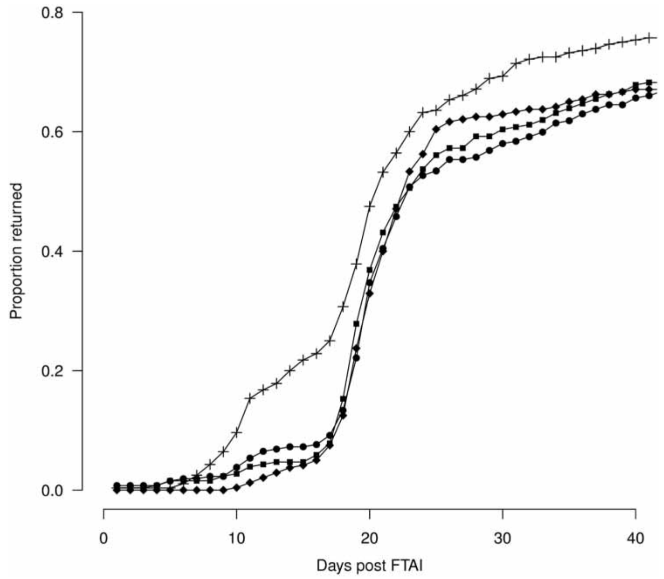

Figure 2. Cumulative proportion of 1,063 anoestrous cows that were not pregnant to fixed time artificial insemination (FTAI) that returned to oestrus from Day 0 to Day 42 following treatment commencing on Day -10. Cows were treated with either: gonadotrophin-releasing hormone (GnRH) on Day -10, cloprostenol on Day -3 and GnRH on Day -1 (GPG: —+—); GPG with a CIDR device (CIDR: —●—); GPG with a DIB device (DIB: —■—); or DIB with 400IU of equine chorionic gonadotrophin on Day -3 (DIB + eCG: —◆—).

Discussion

The results of this study support the addition of eCG to P4 implant-based treatments for anoestrous cows, particularly in earlier calving cows. The greatest improvement in pregnancy rate following addition of eCG was observed in cows calved >60 days before mating. The trophic effect of eCG on follicular and luteal development and hormone production may be more pronounced in this group resulting in reduced incidence of delayed ovulation, luteal insufficiency and/or premature luteal regression. Examination of the cumulative returns to oestrus (Figure 2) suggests that cows in the DIB + eCG group had fewer short returns (i.e. < 17 days after FTAI) especially in comparison with the GPG group. This suggests that fewer cows receiving eCG experienced luteal insufficiency following FTAI.

The return to oestrus characteristics of the GPG group, where a higher proportion of GPG-treated cows were observed to experience short returns compared to the other groups, was also observed in a previous study where approximately 20% of cows

Table 6. Comparison of minimum, maximum and range of relative risk of conception to fixed time artificial insemination (FTAI) on Day 0 and pregnancy at Day 28 for 1,906 anoestrous cows within 12 seasonally calving New Zealand dairy herds treated using GPG[a], CIDR[b], DIB[c] or DIB + eCG[d] protocols.

	Relative Risk		
Parameter	DIB + eCG *vs.* DIB	CIDR *vs.* DIB	GPG *vs.* DIB
FTAI conception rate			
Min	0.76	0.65	0.25
Max	2.20	1.50	1.86
Range	1.44	0.85	1.62
Pregnancy rate at Day 28			
Min	0.83	0.85	0.45
Max	1.90	1.20	1.46
Range	1.07	0.35	1.01

[a]Gonadotrophin-releasing hormone (GnRH) Day -10, cloprostenol Day -3, GnRH Day -1
[b]GPG and intravaginal progesterone-releasing device (CIDR) inserted Day -10, removed Day -3
[c]GPG and intravaginal progesterone-releasing device (DIB) inserted Day -10, removed Day -3
[d]DIB and 400 IU of equine chorionic gonadotrophin on Day -3

not pregnant to FTAI after treatment with a GPG programme returned to oestrus within 18 days of insemination (Shephard 2005). The increased incidence of short returns suggests either a failure of synchrony following the GPG protocol and/or increased levels of luteal insufficiency following FTAI (Walsh *et al.* 2011).

The results for GPG group observed in this study were similar to a recent New Zealand study comparing treatments for anoestrous cows, where the estimated mean conception rate to first insemination was 34% for GPG-treated cows (McDougall 2010a). However, in cows treated with GPG plus progesterone mean conception rate to first insemination was 46%, which was higher than that for the CIDR, DIB and DIB + eCG groups in this study.

This study found evidence of greater variation in pregnancy performance of the DIB + eCG and GPG treatment groups compared to the progesterone-based treatments within herds. Variability in performance of the GPG protocol in treatment of anoestrus cows has been previously reported (Shephard 2005). The variation in pregnancy performance between herds in the DIB + eCG group is consistent with previous observations (Macmillan and Petersen 1993). Therefore, whilst the addition of eCG to progesterone-based treatment protocols for anoestrous cows is supported by this study, results may not be predictable on individual farms.

There was no significant difference in performance between the CIDR and DIB groups in this study. The use of a low payload (1.0 g) progesterone releasing intravaginal device within a treatment protocol for anoestrous cows in New Zealand is supported.

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