Modeling Reproductive Intervention Treatment Responses in Seasonally-Calving Dairy Cows

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Summary
A field trial was undertaken to compare the effects of different treatment programmes against prolonged anovulatory anoestrous (AA) in seasonally-calving dairy cows in New Zealand. Statistical analysis with different regression models was performed to aid in definition of treatment and confounding variable effects over differing time periods, and to increase statistical efficiency.

Introduction
Insemination of cows in the seasonal-calving New Zealand dairy system is restricted to about a 3 to 4 month period. It is important that cows conceive early in this period and that herds achieve a low final non-pregnancy rate. Cows with extended AA are a major problem in New Zealand. Anovulatory anoestrous cows have a higher risk of being inseminated later, calving late the following season, or being culled for reproductive failure. Current progesterone and oestradiol treatment protocols still result in lower conception rates than in cycling cows, so improvements are still required (Rhodes et al, 2003).

Objectives
To assess multiple modeling approaches for reproductive data, examining responses over time in an intervention study carried out to determine the effect of different treatments for AA.

Materials and Methods
A prospective intervention study was undertaken involving 17 convenience-chosen dairy herds in the Waikato region of the north island of New Zealand. Cows (n =1883) not detected in oestrus by 10 days before the planned start of seasonal breeding programme (PSM) and calved at least 22 days were submitted for veterinary examination by rectal palpation. Cows (n=1555) without a palpable corpus luteum were blocked by age (2 yrs and > 2yrs) in a 2 by 2 factorial design: two doses of intravaginal progesterone (P4)-releasing device (1Q vs. 3Q- “Cuemate”, Pfizer Animal Health, N.Z Ltd.) and two durations of treatment (6 vs. 8 days).

Logistic regression was used to quantify the effect of treatment and other confounding variables on the probability of conceiving at the first service within 7 days of PSM. Odds ratios from the logistic regression output were converted to relative risk using the method of Beaudeau and Fourichon (1998). Kaplan-Meier survival curves were plotted for categorical variables, and differences tested by log-rank test. Cox proportional hazards regression was used to estimate the hazard ratios of the treatment strata based on time to pregnancy throughout the mating period, including the same confounding variables as in logistic regression. Ties were handled using the Efron

method, as this is more accurate when there are many tied event times (Hosmer and Lemeshow, 1999) as in this study. The proportionality of hazards assumption was tested by visual assessment of a plot of scaled Schoenfeld residual versus time for each variable (Hosmer and Lemeshow, 1999), with a loess-smoothed curve.

It was possible that clustering of responses at herd level may have occurred. Various design and statistical techniques have been proposed for dealing with this (McDermott, 1994). Four analytical methods were compared to consider a possible correlation of the treatment response within herd: fixed effect and generalized estimating equation (GEE) in logistic regression, and fixed effect and robust variance estimate in the Cox proportional hazards regression. Data analysis was performed using R 1.7.1- A Language and Environment (http://www.r-project.org/). Statistical significance was declared at p<= 0.05.

Results

In the final logistic regression model with herd as a fixed-effect, there was no statistical difference in 1st service conception rate between 3 and 1 Q (p = 0.75, RR 1.04, 95% CI 0.89- 1.17). Cows treated for 8 days vs 6 days had a 21% increased risk of conception at the first insemination within 7 days of PSM (p = 0.01, RR = 1.21, 95% confidence interval 6 – 39%) after adjusting for herd and age category effects. The mean estimated conception rate for 8 day treatment was 42.3% (95% CI 38.7- 46.0%), and for 6 day treatment 34.9% (95% CI 31.4- 38.6%). The interaction term for days by treatment was not significant (p = 0.93). GEE logistic regression coefficient and standard error estimates were virtually identical to those of the first model. Intracluster correlation was estimated at 0.006 (s.e = 0.005).

Kaplan-Meier survival curves were estimated for each treatment combination (Figure 1.) and display the effect of increased conception rate with both 8 day treatments over days 1-7, but diminishing differences over the remainder of the mating period. Survival curves did not differ by treatment (χ-square = 5.5, df = 3, p = 0.14). With herd fitted as a fixed effect in the Cox model, neither treatment duration (8 day vs 6 day, p = 0.42, HR = 1.05, 95% CI 0.94 – 1.17) nor treatment dosage (3Q vs 1 Q, p = 0.79, HR = 0.96, 95% CI 0.89 – 1.10) had a significant effect on the daily hazard of conception, after adjustment for days from calving to PSM and milk protein percentage. The interaction term for the 6 day 3 Q treatment compared to 8 day 3 Q treatment approached significance (p= 0.09, HR = 0.87, 95% CI 0.74 – 1.02). Use of robust variance estimates with herd as the cluster term found the daily hazard of conception for the 6 day 3 Q treatment interaction term to be significantly different from 8 day 3 Q (p = 0.01, HR = 0.87, 95% CI 0.78 – 0.97).

Discussion

Both ordinary and generalized estimating equation logistic regression gave equivalent results. This is in part due to the low intra-cluster correlation coefficient of this response variable. In the final fixed-effects Cox model, neither treatment duration or dosage, or their interaction terms, were significant. However, the robust Cox model gave an increase in efficiency in estimates of treatment effects over the fixed effects

model, as measured by smaller standard errors (reduced by 25%). This led to a new inference, that 6 day 3 Q treated cows had a significantly lower daily hazard of conception compared to 8 day 3 Q treated cows. Considering all model outcomes, results support the use of the 8 day 1 Q treatment programme.

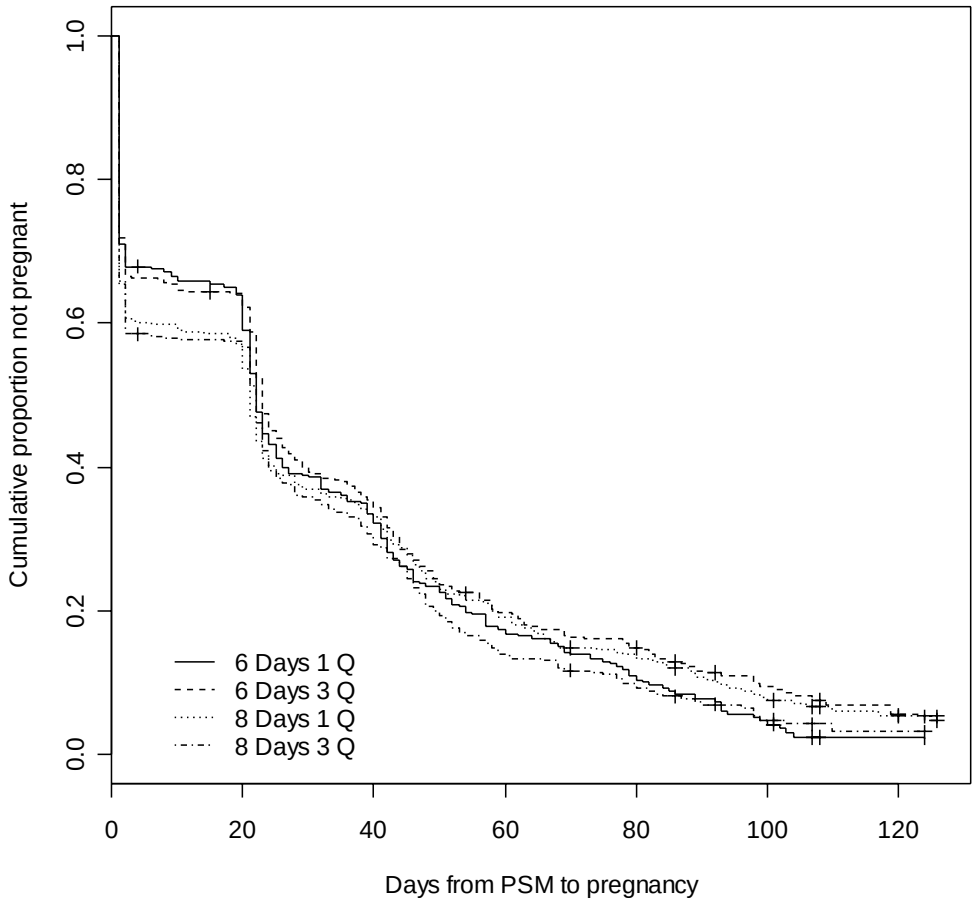

Figure 1. Kaplan-Meier survival curves plotted for each treatment combination.

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