An observational study investigating the effect of time elapsed from the initiation of semen thaw until insemination of the cow upon 24-day non-return rates

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Gippsdairy

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Summary

An observational study of 24-day non-return rates for 5,992 inseminations obtained from 778 visits conducted on 113 farms at four herd improvement centres and involving 36 professional AI technicians was undertaken in the 2000 mating season. The effect of time delay from initiation of semen thaw until insemination of the cow was examined to determine if there was an important effect upon 24-day non-return rates. Analysis was conducted using logistic regression. The overall 24-day non-return rate for inseminations within the study was 71.7%.

Significant variables identified from univariate analysis were farm, farm size, AI centre, technician, number of days calved at insemination, and thaw time (time elapsed from the initiation of semen thaw until insemination of the cow). Significant variables included within the final multivariate model were farm, number of days calved at insemination, thaw time, ambient temperature and the interaction between thaw time and ambient temperature. The effect of technician was not significant within the final model.

The effect of thaw time was significant, but the magnitude of the effect was small. The reduction in 24-day non-return rate was less than 3.0% over the first ten minute thaw time period. The effect of temperature was also significant, but again the magnitude of the effect was small. A reduction in 24-day non-return rate of less than 1.2% was observed when ambient temperature at insemination was above 17° Celsius compared to inseminations performed when the ambient temperature at insemination was below 17° Celsius. There was a significant interaction between thaw time and ambient temperature, however, again the magnitude of the effect was small. The negative effect of thaw time upon 24-day non-return rate is enhanced when the ambient temperature at insemination was above 17° Celsius.

It is concluded that use of professional AI technicians is to be encouraged. This recommendation is based upon the small variation between the professional AI

technicians that was observed when the effects of cow and farm were controlled in analysis.

Professional AI technicians are encouraged to thaw no more straws of semen at any one time than can be easily inseminated into cows within a ten-minute period between the onset of the semen thaw until the insemination of a cow with the last straw of the thaw batch.

Farm visits for insemination of cows should be programmed to occur at times of day when ambient temperatures are likely to be below 17^{o} Celsius. This is necessary to minimise the detrimental effect of temperature upon 24-day non-return rates. It is recommended that all cows must be inseminated within ten minutes of the straw of semen being thawed when the ambient temperature is above 17^{o} Celsius. This is necessary to minimise the combined effects of high ambient temperature and prolonged delay from initiation of semen thaw until insemination of the cow.

Introduction

An observational study of the effect of time from initiation of semen thaw to insemination of the cow upon non return rates for professional AI technicians was conducted during the 2000 mating period at four Victorian AI centres. This study was considered necessary following a series of reports of a negative effect of time taken from thawing of a straw to insemination of the cows upon pregnancy rate (eg Hoard's Dairyman, March 2000). A controlled study conducted in Australia was considered an important and necessary undertaking. The Dairy Herd Improvement Fund, GippsDairy, Genetics Australia, and the four herd improvement centres involved jointly funded this study. These herd improvement centres were Northern Herd Development, Maffra Herd Improvement, Colac Herd Improvement and Timboon Herd Improvement.

Materials and method

Professional AI technicians were recruited into the study at each centre. These technicians were asked to record extra data at suitable insemination visits for a selection of the farms that they serviced. The farms that were enrolled into the study had to meet certain requirements; namely good cow identification, recording of heat data on the herd recording centre database, the regular provision of more than one cow for insemination and a willingness to participate in the study. The technicians were instructed to collect data from visits involving more than one cow. Data was not collected from every visit to the selected farms when more than one cow was presented for insemination. Data collection was not expected when the technician was too busy or when collection was not convenient. Data was not collected when more than one technician was present on the farm, with one technician thawing straws and the other technician inseminating cows.

The following data was collected at each visit; farm, date, technician, time of arrival, cow, number of semen thaws performed, number of straws of semen thawed at each thaw, cow insemination order and duration of time from initiation of the first thaw to insemination of the last cow. The farmer provided the following information; address, herd size, start date for AI and end date for AI. Cow event data was obtained from the herd recording centre database at the end of the study period. This data provided information on cow age, last calving date and heat dates. From this information the duration of effective heat detection within each herd was identified. The last date of effective heat detection was identified from cow event records and the date 24 days prior to this identified for each farm. This date was defined as the last date for eligible inseminations for the farm. Non-return rate was calculated using a 24-day period to allow for cycle length variation within cows. Any cow that had trial data recorded for an insemination and inseminated prior to the last date for eligible inseminations was included in the study. Cows with more than one eligible insemination were identified and the second and subsequent inseminations eliminated from the data. This was undertaken to ensure that all observations within the final database were independent.

Daily three-hour temperature data was collected from the Bureau of Meteorology for each centre. The Cape Otway weather station was used to collect data for the Colac region, the East Sale weather station was used for Maffra region, the Deniliquin Post Office weather station was used for the Northern irrigation district, and the Cape Nelson Lighthouse weather station was used for the Timboon district. The approximate ambient temperature at each was estimated from the time of the visit and using interpolation of the weather station data.

Data was centralised and incorporated into a database. Extensive data checking and data validation was undertaken prior to analysis. Descriptive data analysis was conducted using Access ™ and Excel™. Data was sent forward for statistical analysis. Logistic regression (LR) was used to quantify the effects of each variable upon the risk of a cow returning to heat within 24 days of insemination. Univariate analysis with a liberal p value ($P<0.25$) was used for each variable in order to identify variables to be sent forward to the multivariate analysis stage. Univariate analysis was undertaken using SPSS 10.0.5™.

Multivariate analysis was conducted using SPSS 10.0.5™. All variables identified as significant at the univariate stage, as well as those variables that were not significant at the univariate stage but were of interest or had been identified as influential in other studies were included in the multivariate analysis. Group level variables such as herd and technician were included as fixed effects at the multilevel stage. This technique was employed to assist control their potential confounding effect upon the main variables of interest; time elapsed from initiation of thaw to insemination of the cow (TTime). The multivariate model was constructed using two criteria; firstly a significant change ($P<0.05$) in deviance (D) was required for a variable to be kept within the model using a forward stepwise approach. Secondly, a variable was kept within the model if inclusion resulted in a considerable change in coefficient for the time variables (TTime). A change in the coefficient of 0.1 was calculated to result in a change in non-return rate of 2% when the non-return rate was around 70% (ie change from 70% to 72%), thus any variable that produced a change in beta coefficients for a time variable of more than 0.1 was included in the final model irrespective of the effect of term inclusion upon change in model deviance.

Results

Descriptive analysis

A total of 113 farms provided inseminations for analysis from the four centres (Table 1). These farms provided 5,992 inseminations for analysis (Table 2).

Table 1: Number of farms per centre

Centre	Number of farms
Colac	13
Maffra	46
Northern	52
Timboon	2
Total	113

Table 2: Total inseminations per centre

Centre	Inseminations
Colac	937
Maffra	2441
Northern	2357
Timboon	257
Total	5992

These inseminations were obtained from 778 farm visits involving 38 technicians. Farm size was estimated from the total number of cows joined from the herd recording centre events database. Farms were categorized into three groups; small, medium and large for farms with less than 250 cows joined, 251 to 500 cows joined and more than 501 cows joined respectively per year. The distribution of herd sizes per centre is given in Table 3.

Table 3: Herd size strata distribution for each centre

Centre	Herd size strata	Number of farms
Colac	0-250	8
	251-500	1
	501+	4
Maffra	0-250	29
	251-500	8
	501+	9
Northern	0-250	36
	251-500	3
	501+	13
Timboon	0-250	1
	251-500	1
	501+	0
TOTAL	0-250	74
	251-500	13
	501+	26

The distribution of inseminations and 24-day non-return rates within herd size strata and within centre is given in Table 4.

Table 4: Total inseminations and number of cows returning to service and non-return rate versus herd size strata versus centre

Centre	Herd size strata	Total Inseminations	No. of cows returning	Non return rate
Colac	0-250	529	156	70.5%
	251-500	346	105	69.7%
	501+	62	18	71.0%
	Total	937	279	70.2%
Maffra	0-250	1050	306	70.9%
	251-500	951	236	75.2%
	501+	440	127	71.1%
	Total	2441	669	72.6%
Northern	0-250	1779	557	68.7%
	251-500	248	47	81.0%
	501+	330	93	71.8%
	Total	2357	697	70.4%
Timboon	0-250	170	46	72.9%
	251-500	87	3	96.6%
	Total	257	49	80.9%
TOTAL	0-250	3528	1065	69.8%
	251-500	1632	391	76.0%
	501+	832	238	71.4%
	TOTAL	5992	1694	71.7%

The non-return rate for all cows within the study was 71.7%. The distribution of 24-day non-return rates for the different herd size strata is given in Figure 1 below

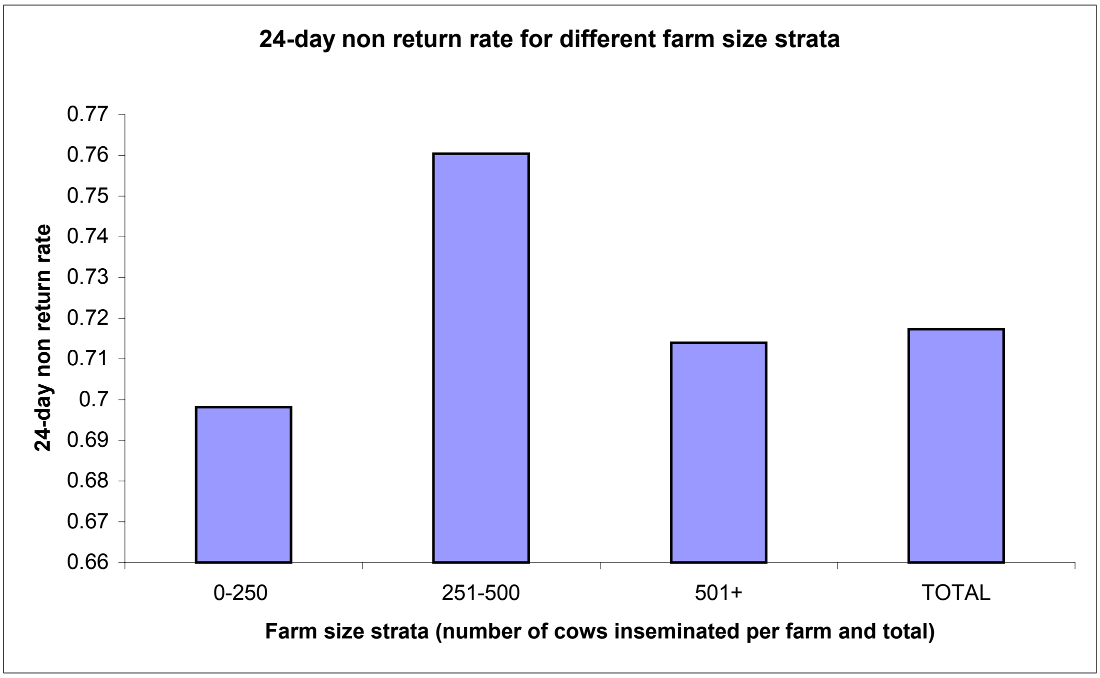


Figure 1

The age distribution for cows included within the study is given in Figure 2

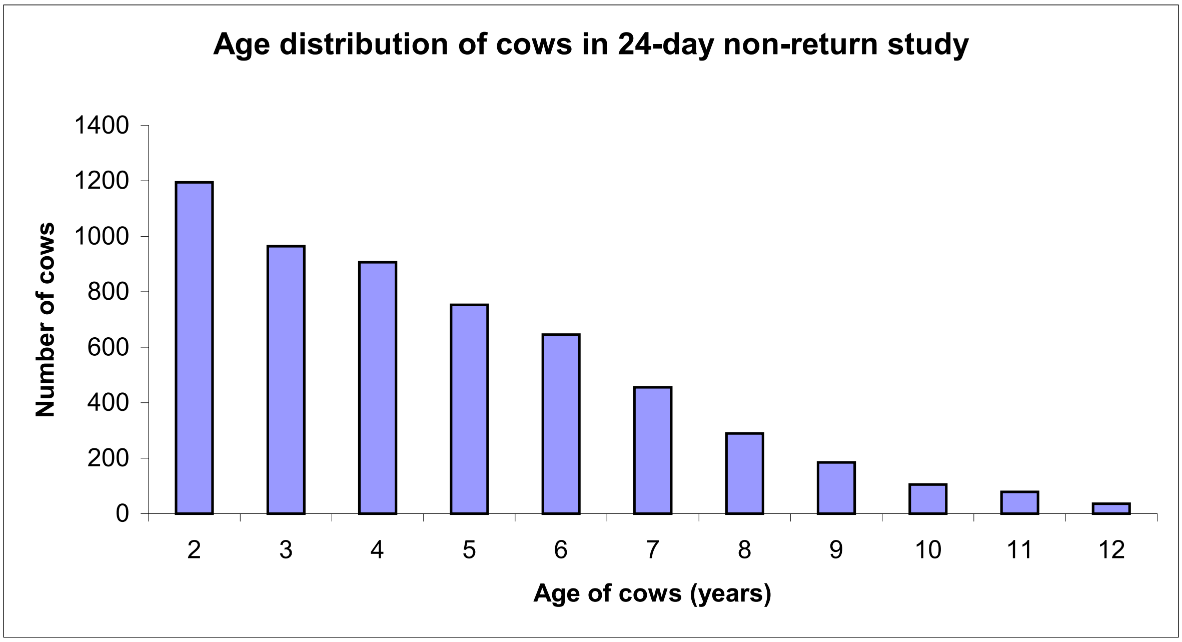


Figure 2

The distribution of age-strata 24-day non-return rates is given in Figure 3. The non-return rate was approximately the same for ages 2-11 years.

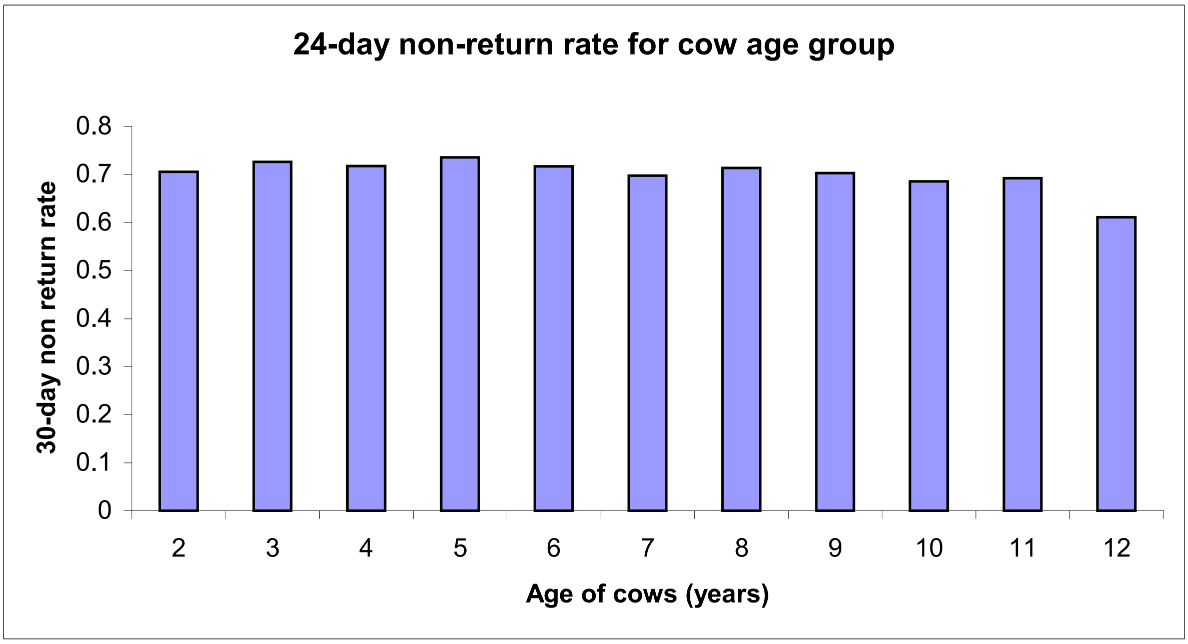
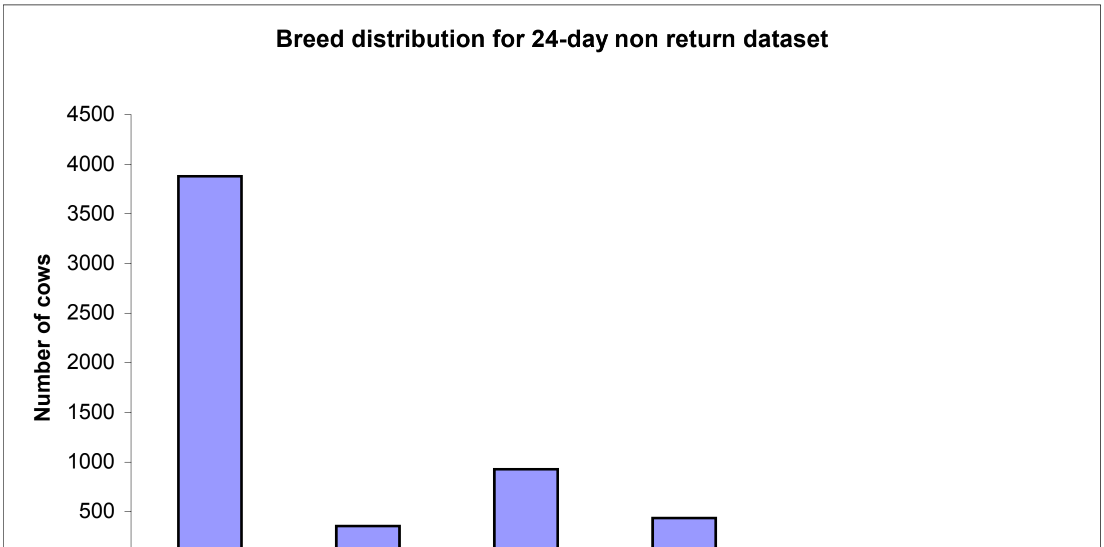


Figure 3

The breed distribution for cows included within the study is given in Figure 4.



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The distribution of number of days calved at insemination is given in Figure 6. The majority of cows were more than 60 days calved at insemination.

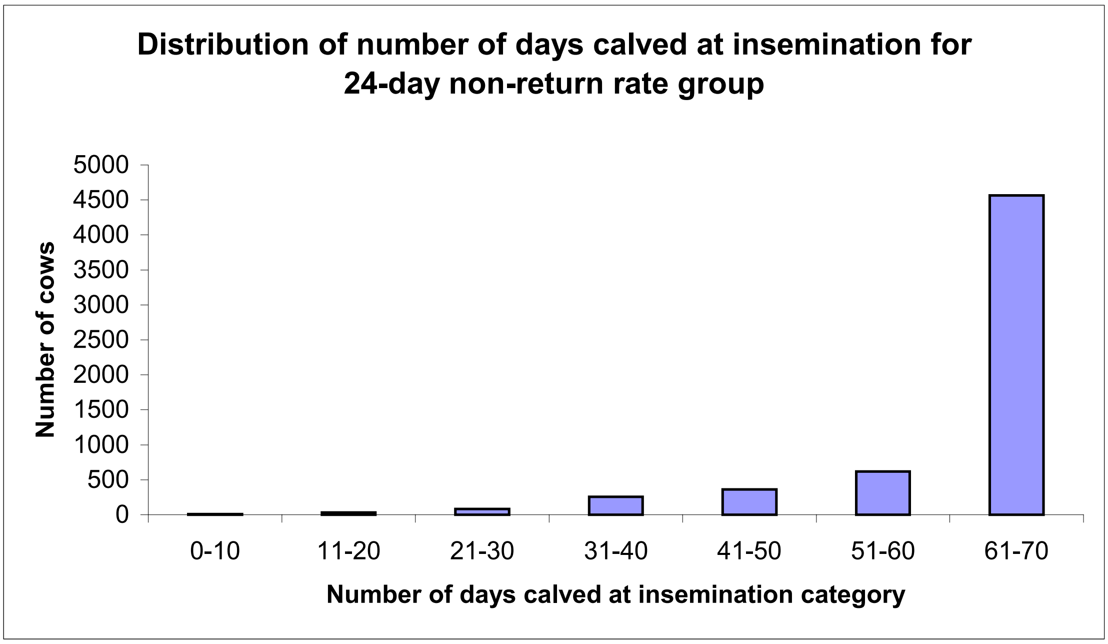


Figure 6

The 24-day non-return rates for each number of days calved at insemination strata is given in Figure 7. Cows calved 60-70 days before insemination had higher 24-day non-return rates than cows calved for a lesser period of time before insemination.

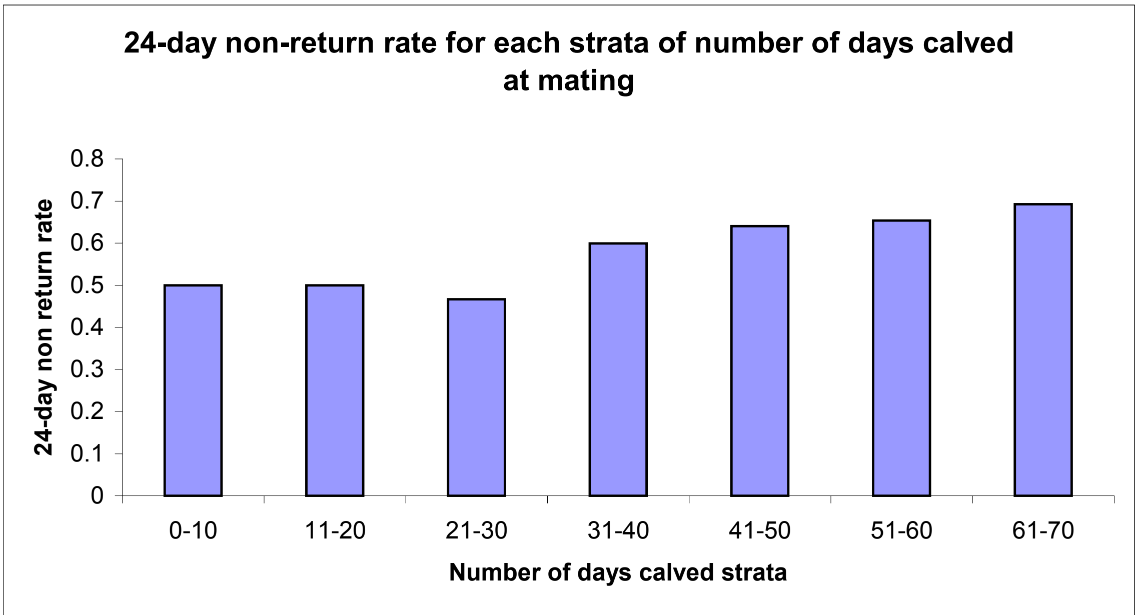


Figure 7

The 24-day non-return rate distribution for technicians is given in Figure 8.

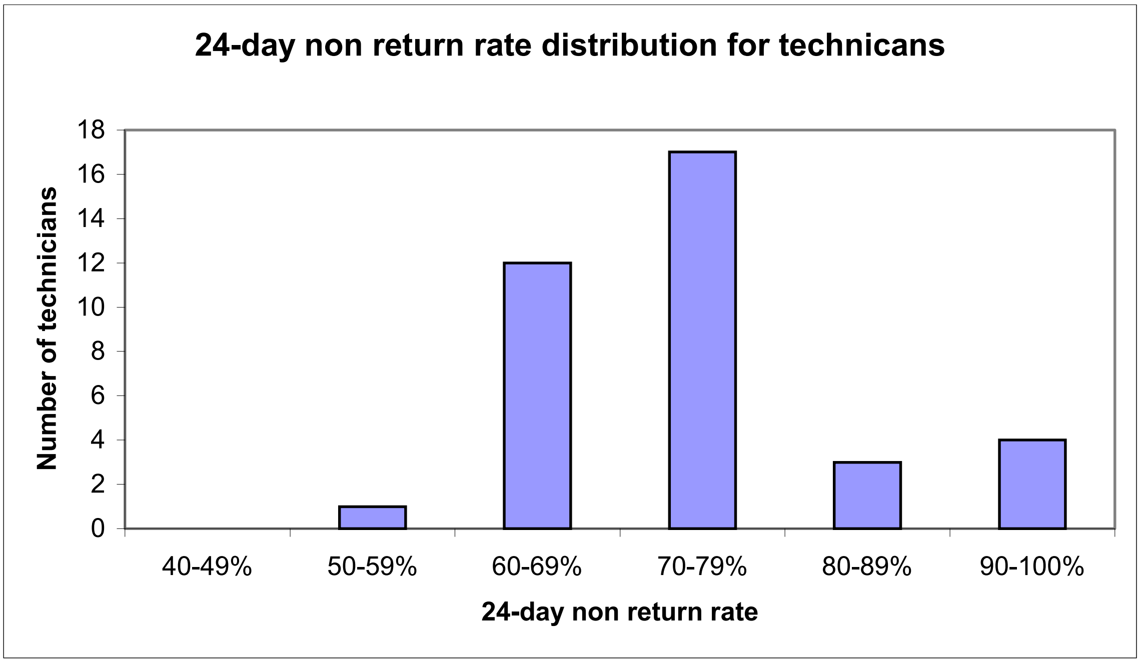
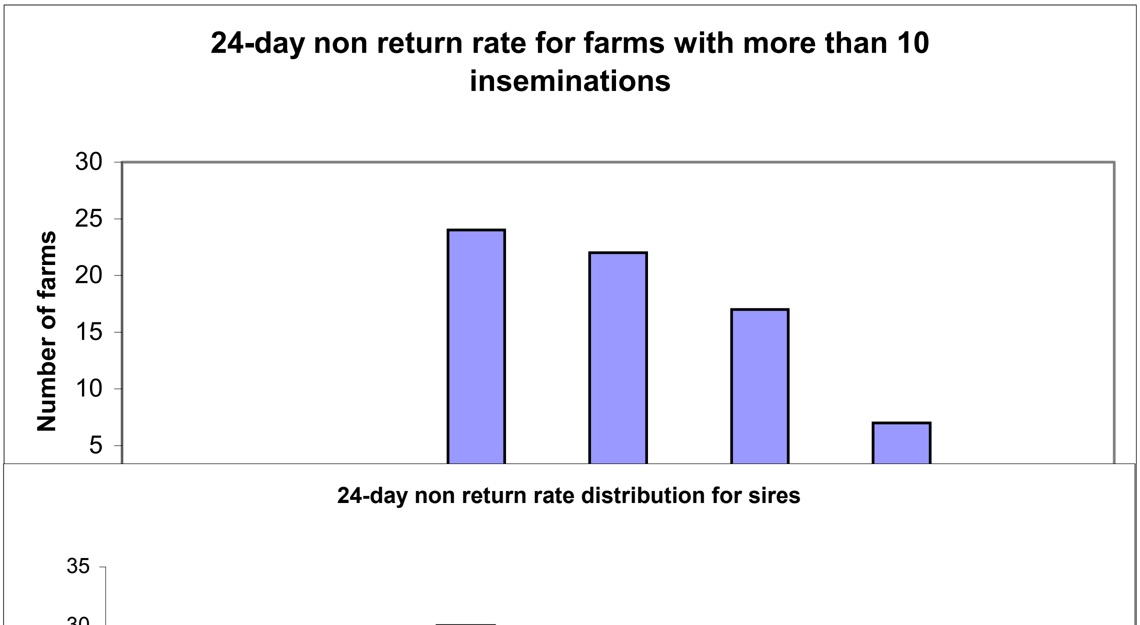
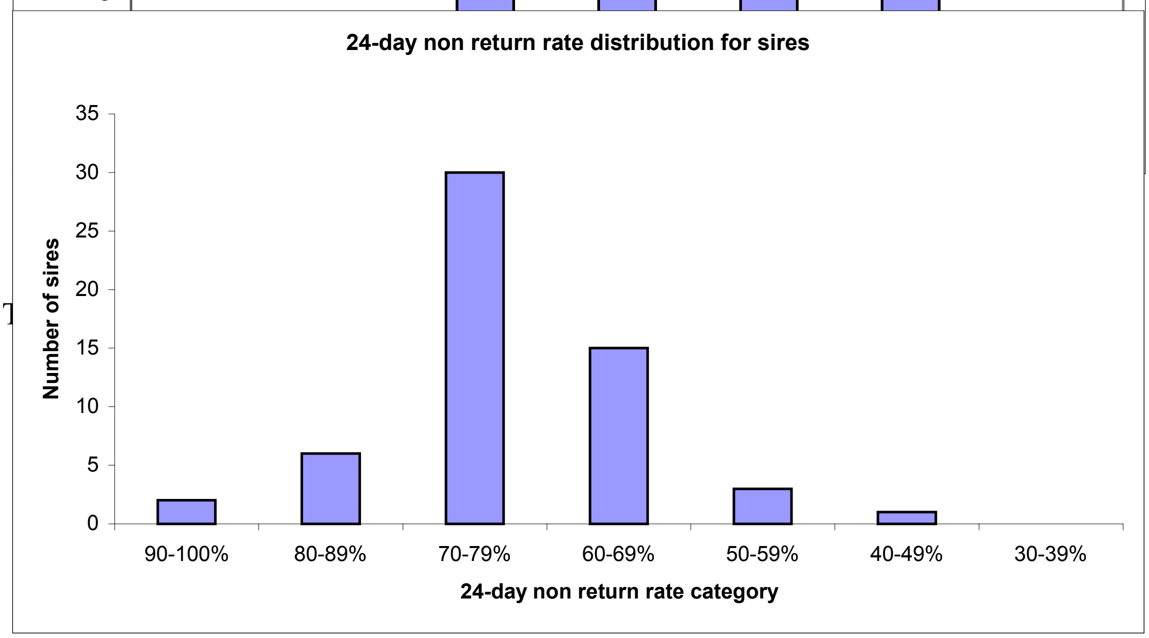


Figure 8

The 24-day non-return rate distribution for farms that provided more than 10 inseminations is given in Figure 9.





The distribution of thaw time (total minutes from initiation of thaw until insemination of the cow) is given in Figure 11.

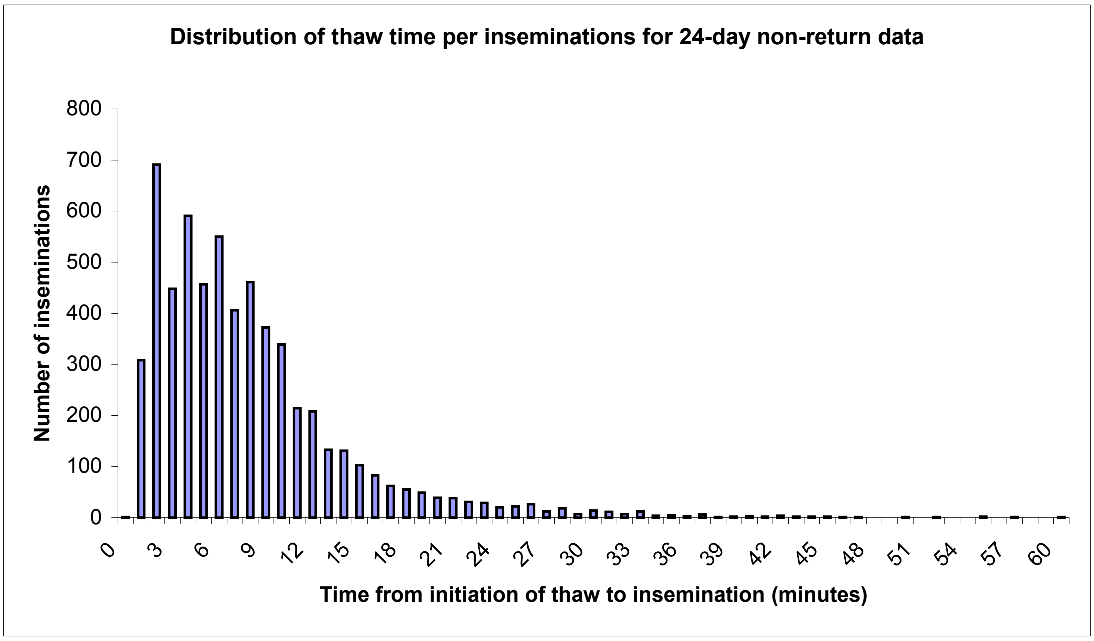


Figure 11

The 24-day non-return rate for cows inseminated at different straw thaw times is given in Figure 12

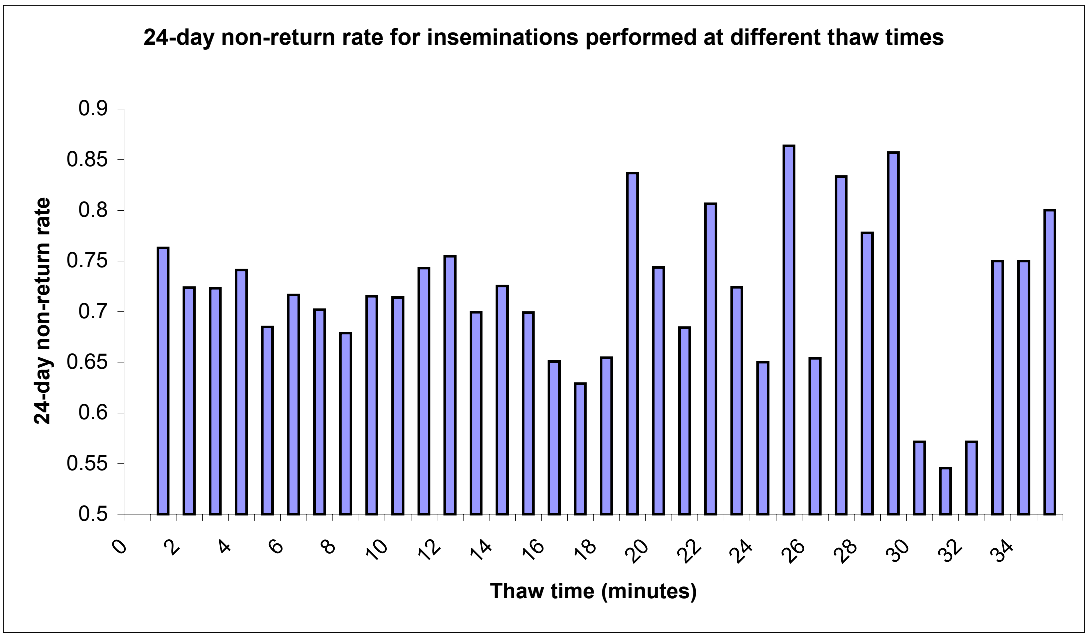


Figure 12

Univariate analysis

Univariate analysis was conducted using logistic regression with a liberal p value (P<0.25).

The amount of time in minutes from initiation of semen thaw to insemination of the cow was recorded in a variable called 'thaw time'. The effect of thaw time upon 24-day non-return rates was examined to determine if the relationship was linear in the logits. Thaw time was used to produce a new time variable called 'time Q'. Time Q was coded with the thaw time quartile membership (ie integer values from 1 to 4). Time Q was then analysed as a categorical variable in logistic regression with quartile 1 as the reference category. The logit for each quartile was plotted against the quartile median thaw time and the graph examined for linearity. Results are presented in Figure 13

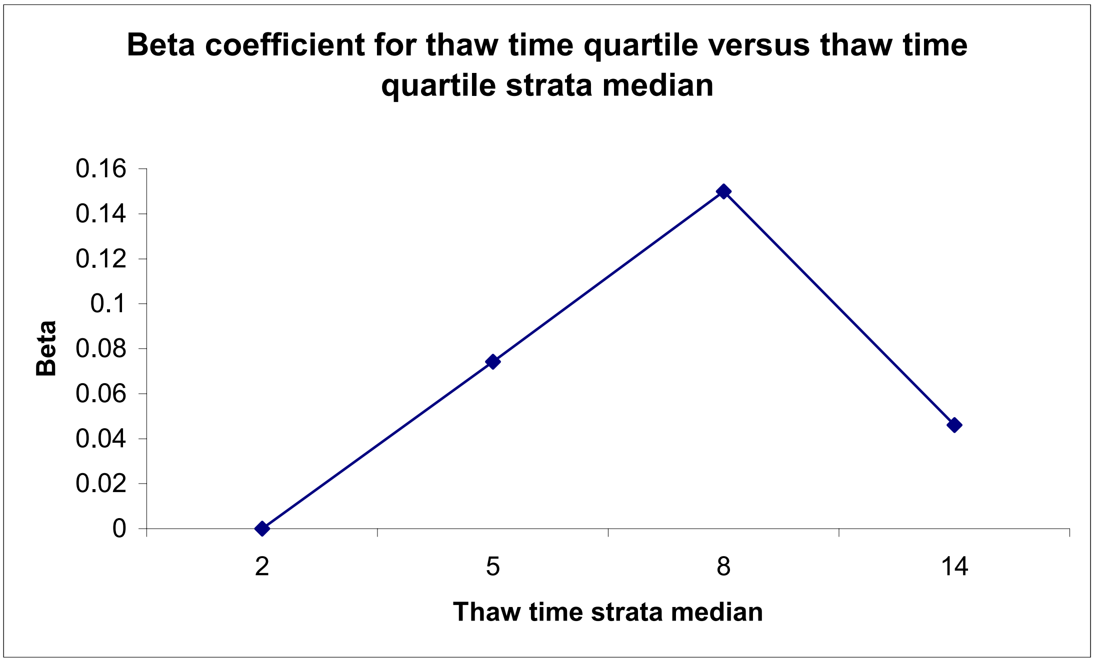


Figure 13

Results indicated that the effect of thaw time is not linear in the logit. Thus thaw time was analysed as a categorical variable using thaw time quartile to define class status. These classes are defined in Table 5. The crude 24-day non-return rates for each thaw time quartile are given in Table 6

Table 5: Thaw time quartile parameters

Thaw time quartile	Thaw time range (minutes)	Median thaw time
1	0-3	2
2	4-6	5
3	7-10	8
4	11-60	14

The final quartile is markedly rightly skewed. This quartile contains the thaw time outliers. The presence of outliers within this category indicates that inferences regarding this category must be made with care. Inferences for the first three categories can be made with greater surety.

Table 6: Thaw time quartile crude 24-day non-return rates

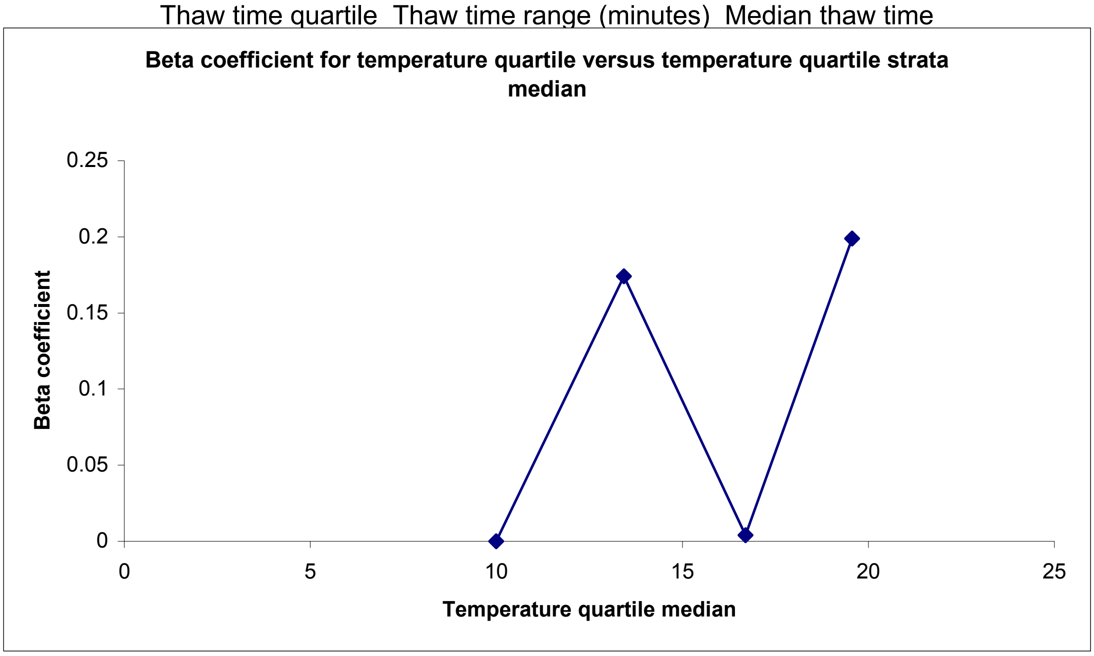
Thaw time quartile	1	2	3	4
Total inseminations	1448	1598	1578	1368
Total returns	389	453	472	380
24-day non-return rate	73.1%	71.7%	70.1%	72.2%

There appears to be a decrease in 24-day non-return rate of around 3.0% over the first ten-minute period from initiation of semen thaw to insemination of the cow (thaw time quartiles 1-3).

Ambient temperature at semen thaw was also non linear in the logit. Results are given in Figure 14 and Table 7 below

Table 7: Temperature quartile parameters

Thaw time quartile	Thaw time range (minutes)	Median thaw time



Temperature was dichotomised prior to multivariate analysis. The cut point was 17.1 degrees. The crude 24-day non-return rates for thaw time and ambient temperature categories are given in Table 8 and Table 9.

Table 8: Crude 24-day non-return rates for ambient temperature categories

	Temperature < 17.2	Temperature > 17.2
Total inseminations	3994	1998
Total returns	1113	581
24-day non-return rate	72.1%	70.9%

It appears that the 24-day non-return rate decreases by around 1.2% for inseminations performed when the ambient temperature is above 17.1^{o} C at the time of insemination compared to inseminations performed when the ambient temperature was below 17.1^{o} C.

Table 9: Crude 24-day non-return rates for different thaw time and ambient temperature categories

		Temperature category (degrees celsius)	
		1	2
Time category	Range (minutes)	0-17.1	17.13-31.2
1	0-3	72.0%	75.5%
2	4-6	72.1%	70.7%
3	7-10	69.9%	70.5%
4	11-60	74.9%	67.2%

Significant variables (P<0.25) at the univariate stage are given in Table 10

Table 10: Variables significantly associated with 24-day non-return probability at liberal p (P<0.25)

Variable name	Variable description
Days C / 10	Number of days calved at insemination divided by 10
Size strata	Herd size strata (0-250 cows, 251-500 cows, 500+ cows)
Centre	AI centre
Farm	Farm
TechNo	Technician
TQ3	Thaw time quartile 3
TI4TE2	Time quartile 4 by temperature category 2 (interaction)

Variables not identified as significant at the univariate stage are given in Table 11

Table 11: Variables not significantly associated with 24-day non-return probability at liberal p (P>0.25)

Variable name	Variable description
Arrival hour	Hour of arrival on farm
Insemination order	Order of insemination at visit
Age	Cow age in years
Breed	Breed of cow
Sire	Sire identification
Days C / 10 Sq	(Number of days calved at insemination / 10)^2
TQ2	Thaw time quartile 2
TQ4	Thaw time quartile 4
TE2	Temperature category 2
TI2TE2	Thaw time quartile 2 Temperature category 2 interaction
TI3TE2	Thaw time quartile 3 Temperature category 2 interaction

All variables identified as significantly associated with probability of 24-day non-return were sent forward for multivariate analysis except the 'Centre' variable. 'Farm' and 'Centre' were collinear variables ('Farm' is nested within 'Centre'). The inclusion of only one term was considered appropriate. The variable 'Farm' was sent forward.

Variables identified as non-significant at the univariate stage, but who were part of a dummy coded variable that was identified to have at least one significant interaction term at the univariate level were sent forward in their entirety for multivariate analysis. Temperature category variable (TE2) and the interaction terms (TI2TE2 and TI3TE2) were included because of the significant interaction term (TI3TE2). 'Days C / 10 Sq' was also included because previous studies have identified that number of days calved at insemination has a statistically significant polynomial relationship with pregnancy risk. These variables are given in Table 12

Table 12: Variables sent forward for multivariate analysis

Variable name	Variable description
Days C / 10	Number of days calved at insemination / 10
Days C / 10 Sq	(Number of days calved at insemination / 10)^2
Size strata	Herd size strata
Farm	Farm
TechNo	Technician
TE2	Ambient temperature at time of insemination category 2 (13.0 – 17.1 degrees C)
TQ2	Thaw time quartile 2 (4-6 minutes)
TQ3	Thaw time quartile 3 (7-10 minutes)
TQ4	Thaw time quartile 4 (11-60 minutes)
TI2TE2	Interaction term between time quartile 2 and temperature category 2
TI3TE2	Interaction term between time quartile 3 and temperature category 2
TI4TE2	Interaction term between time quartile 4 and temperature category 2

Multivariate analysis

Mutivariate analysis was conducted using a backwards stepwise approach. The least statistically significant variable was removed from the model if its beta coefficient was not significant (P>0.05), removal did not significantly alter the model deviance from the model that included the term, and removal did not alter the beta coefficients associated with all the ‘time quartile’ terms.

Time quartile and temperature quartile interaction terms were tested in the final model by examining the change in deviance from the model without interaction terms to the model with all interaction terms included. If the deviance change was not significant following addition of the interaction terms, all interaction terms were excluded from the final model. If the deviance change was significant, all dummy coded interaction terms and all constituent raw variables were left within the model. The process of variable removal proceeded until all unimportant variables were eliminated.

The final model is presented in Table 13

Table 13: Final mulitvariate 24-day non-return logistic regression model - beta coefficients and standard errors

Variable	B	S.E.	Wald	df	Sig.	Exp(B) (odds ratio)	Lower 95% CI	Upper 95% CI
TQ2	0.0172	0.1020	0.0284	1	0.8661	0.9830	0.8330	1.2425
TQ3	-0.1129	0.1025	1.2130	1	0.2707	1.1195	0.7306	1.0920
TQ4	0.2349	0.1202	3.8232	1	0.0505	0.7906	0.9994	1.6007
TE17.1	0.2482	0.1455	2.9089	1	0.0881	0.7802	0.9637	1.7047
TI2TE17	-0.2555	0.1809	1.9948	1	0.1578	1.2911	0.5433	1.1041
TI3TE17	-0.1383	0.1806	0.5862	1	0.4439	1.1483	0.6112	1.2407
TI4TE17	-0.5388	0.1957	7.5774	1	0.0059	1.7139	0.3976	0.8563
FARM*	0.0000	0.0000	182.5603	112				
TECHNO*#	0.0000	0.0000	19.2949	20				
DAYSC10	0.0145	0.0039	14.1413	1	0.0002	0.9856	1.0070	1.0223
Constant	6.8447	25.9207	0.0697	1	0.7917	0.0011	0.00	1E+25

*- Beta coefficients and standard errors for individual farm and technician have been removed

- Included in final model because removal resulted in large change in 'Time Quartile' and 'Temperature by Time Quartile' coefficients

The effect of ambient temperature and thaw time upon β coefficients for 24-day non-return rates are summarised in Table 14

Table 14: Beta coefficients for ambient temperature and thaw time categories

	Temperature (<17.1°C)	Temperature (>17.1° C)
TQ1 (0-3 mins)	0	0.2482
TQ2 (4-7 mins)	0.0172	0.0099
TQ3 (8-10 mins)	-0.1129	-0.0030
TQ4 (11-60 mins)	0.2349	-0.0556

The β coefficients were converted into odds ratios by exponentiation. Results are given in Table 15

Table 15: Odds ratios for ambient temperature and thaw time categories

	Temperature (<17.1°C)	Temperature (>17.1° C)
TQ1 (0-3 mins)	1.000	1.282
TQ2 (4-7 mins)	1.017	1.010
TQ3 (8-10 mins)	0.893	0.997
TQ4 (11-60 mins)	1.265	0.946

The 24-day non-return rate for the referent group (thaw time three minutes or less and ambient temperature at insemination less than 17.1° Celsius) was 72.0%. The odds ratios were converted to average 24-day non-return rates for each thaw time and ambient temperature category. Results are given in Table 16.

Table 16: 24-day non-return rates for ambient temperature and thaw time categories

	Temperature (<17.1°C)	Temperature (>17.1° C)
TQ1 (0-3 mins)	72.0%	76.7%
TQ2 (4-7 mins)	72.3%	72.2%
TQ3 (8-10 mins)	69.7%	71.9%
TQ4 (11-60 mins)	76.5%	70.9%

Conclusions

The effects of time from initiation of semen thaw until insemination upon 24-day non-return rates is statistically significant (P<0.05). However, the effect of time is small. It is likely that the reduction in 24-day non-return rates is less than 3.0% for the first ten minute period from initiation of semen thaw to insemination of the cow (ie a reduction in 24-day non-return rate from 72.0% for cows inseminated within one minute of initiation of semen thaw to 69.0% for cows inseminated ten minutes after initiation of semen thaw).

The effect of temperature is also statistically significant (P<0.05). However, the magnitude of the effect of temperature upon 24-day non-return rates is also small. It is likely that the reduction in 24-day non-return rates is less than 1.2% for inseminations conducted when the ambient temperature was above 17.1^{o} C compared to inseminations conducted when the ambient temperature was below 17.1^{o} C.

There was a statistically significant interaction between time from initiation of semen thaw and ambient temperature at insemination upon 24-day non-return rates. The only significant time and temperature interaction term in the final model was for inseminations performed at ambient temperatures above 17.1^{o} C when the duration of time from initiation of semen thaw until insemination of the cow was greater than 10 minutes (quartile four). Once again the magnitude of this effect is small; the reduction in 24-day non-return rates for cows inseminated within this (statistically significant) category was 1.2% when compared to the referent group.

There was no statistically significant effect of technician upon 24-day non-return rates. Technician was left within the final model due to the confounding effect that this variable had upon temperature and thaw time variables. Whilst the removal of technician from the final model did not affect the fit of the model, the removal resulted in a large change in beta coefficients for the temperature and thaw time category variables. Technician was thus left within the model in order to improve the accuracy of the estimates obtained for the effect of time and temperature upon 24-day non-return rate. This confounding effect is likely to have occurred due to the multilevel nature of the data in this study. Individual cows are nested within farms and individual farms are nested within centre. Also, most farms had all inseminations performed by the same technician, thus the final model was unable to differentiate the effect of technician from the effects of centre or farm.

A valid interpretation of a lack of statistical significance for the variable 'technician' is there is little discernible variation in 24-day non-return rates between professional AI technicians when the effects of cow, herd and environment have been controlled. These findings are in contrast to the results of InCalf studies, which demonstrated a statistically significant effect of DIY inseminator upon conception rates. The results from the current study and the InCalf finding of variable pregnancy rates for DIY inseminators both support the use of professional AI technicians within the industry.

Farm and number of days calved were a statistically significant variables in the final model. These findings also support recent results reported from InCalf studies. Farm and number of days calved explained a significant proportion on conception rate variation in InCalf studies.

Recommendations and findings from this study are:

1. The use of professional AI technicians is encouraged. Professional AI technicians tend to perform within a narrow range of 24-day non-return rates when the effects of cow and farm are controlled.

2. There is a small effect of time delay from initiation of semen thaw until insemination of the cows. The magnitude of this effect is a reduction in 24-day non-return rates of less than 3.0% over the first ten-minute period. It is recommended that no more straws of semen be thawed by professional AI

technicians at any one time than can be easily inseminated into cows within a ten-minute period between the onset of the semen thaw until the insemination of a cow with the last straw of the thaw batch.

3. There is a small effect of ambient temperature upon 24-day non-return rates. The magnitude of this effect is a reduction in 24-day non-return rate of less than 1.2% for ambient temperatures above 17.1^{o} Celsius at the time of insemination. It is recommended that farm visits for insemination of cows be timed to occur when ambient temperatures are below 17^{o} Celsius whenever possible.

4. The magnitude of the reduction in 24-day non-return rate due to time delay from initiation of semen thaw until insemination of the cow is enhanced when the ambient temperature is above 17.1^{o} C. It is recommended that all cows must be inseminated within ten minutes of the straw of semen being thawed when the ambient temperature is above 17^{o} Celsius.

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