EFFECTS OF FRESH-COW DISEASES ON REPRODUCTION IN A LARGE COMMERCIAL DAIRY HERD

by

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Abstract

The 2007 NAHMS (National Animal Health Monitoring System) survey indicated that early lactation health issues are major factors influencing reproduction and culling on U.S. dairy herds. The objective of this study was to evaluate fresh-cow health during the first 30 days in milk, and its association with days to pregnancy in the concurrent lactation. Data were collected on cattle that calved over a two month period (July and August 2009) on a dairy farm located in the Upper Midwest region of the U.S. Health and production data were collected daily for each cow from the beginning of lactation until the majority of the study population was confirmed pregnant. Both a competing risk analysis and a semi-parametric Cox regression model were used to test the association between specific health-related events and days to pregnancy and the outcomes of the two models were compared. These analyses showed metritis and dystocia in the first 30 days of lactation were associated with greater days to pregnancy. The only difference noted between parities was that lactation-five and greater cows were significantly associated with greater days to pregnancy. The two analyses showed conflicting significance of association between retained placenta, ketosis, twinning, lameness, and other non-specific illnesses with days to pregnancy. This study found that a competing risk analysis and a semi-parametric regression model were appropriate methods to analyze time sensitive data such as reproductive efficiency. This study supports the evidence that parity, metritis, retained placenta, ketosis, dystocia, twinning, lameness, and other non-specific illnesses can have an impact on reproductive efficiency.

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Chapter 1 - Literature Review

Introduction of postpartum diseases effects on reproduction

The 2007 NAHMS reports a 4.6% annual incidence of reproductive problems (e.g. dystocia, metritis) as a percent of total dairy cows in the United States, it also reports an incidence of 7.8% for retained placenta, 4.9% for milk fever, 3.5% for displaced abomasum, 2.5% for diarrhea problems, 3.3% for respiratory problems, 16.5% for mastitis issues, and a 14% incidence for lameness [1]. Numerous studies in the literature also show comparable results for reproductive and postpartum disorders. Collard et al., in a study looking at dairies in Canada found a lactational incidence risk for all udder problems, mastitis, all locomotion problems, laminitis, all digestive problems, and reproductive problems to be 37.1%, 35%, 35%, 25.7%, 19.3%, and 16.4%, respectively [2]. Gröhn et al. found an incidence risk for dystocia to be 2.1%, 3.1% for retained fetal membranes at parturition, 3.2% for metritis; he also found the incidence risk of reproductive disorders for silent heat, ovarian cysts, and other infertility problems to be 8.1%, 7.3%, and 1.9%, respectively [3]. Curtis et al. found similar results in a study looking at the first 30 days postpartum, they found an incidence risk of veterinary-assisted dystocia of 1.5%, retained fetal membranes of 11.4%, milk fever of 6.6%, metritis of 7.8%, left displaced abomasum (LDA) of 1.4%, ketosis of 3.5%, and mastitis to be 2.8% [4]. Over the last 40 years, the first service conception risk has declined from about 65% to 38%, and the percent of cows standing to be mounted and the amount of time cattle spent in standing estrus has declined [5]. The 2007 NAHMS reports 12.9% of cows are declared open past 150 Days in Milk (DIM), and 26.3% of the cows culled from herds (excluding cows that died) were culled due to reproductive problems [1]. Many authors point to increased milk production as the primary selection criteria for breeding decisions as being associated with the described reduction in fertility parameters [5].

Many other authors remain unclear as to whether milk production directly affects fertility. What is more universally accepted is that disease parameters like ketosis, metritis, retained placenta, and cystic ovarian disease have a greater effect on reproductive parameters than non-disease parameters like milk production and body condition score [3, 5-7]. One study found that cows with postpartum disorders (e.g. dystocia, metritis, etc.) spent on average an additional 30 days open compared to cows that were healthy in the postpartum period when using a multiple regression model [8]. Another study found that cows with these disorders were open on average 14-17 more days than cows without postpartum disorders [9].

Dystocia

Very few studies have looked at the effects of dystocia on reproductive parameters. One study found the conception risk for cows with dystocia to be 0.88 compared to the referent population of cows without dystocia [9]. In contrast, other researchers found a dystocia event at parturition was not significantly associated with the time to pregnancy and was removed from their Cox regression model [10, 11]. Numerous studies did implicate dystocia for increasing the occurrence of other peri-parturient diseases. One study showed that cows with dystocia were 4.1 times more likely to have a retained placenta, and 3 times more likely to have a metritis event in the postpartum period [3]. Other researchers have found an increased risk for metritis but not for a retained placenta for cows with a dystocia at parturition [4, 8, 12]. Numerous researchers found cows with retained fetal membranes were strongly correlated with the incidence of a metritis event in the postpartum period [3, 4, 8, 12-14].

Metritis

Metritis negatively affected reproductive parameters in most studies. Coleman et al. found that cows with metritis had an additional 7 days to first service and 0.31 more services per conception when compared to cows that did not have metritis [8]. Fourichon et al. found similar negative results in all of the studies in their meta-analysis. They found that a metritis event in the first 30 DIM resulted in an increase of 8.2 days to first service, 0.1 more services per conception, and an average increase of 27.7 days open compared to cows that did not have metritis [9]. Loeffler et al. analyzed their data with a multiple logistic regression model and found cows with a metritis event anytime during the lactation had a decreased odds ratio of 0.74 for the first service pregnancy risk when compared to cows without metritis [15]. LeBlanc et al. analyzed their data with a Cox proportional hazard model and found, on average, cows with endometritis diagnosed between 20 and 33 days postpartum had a 27% (Hazard Ratio (HR)= 0.73) reduction in the relative pregnancy risk when adjusted for all other variables, as compared to cows that did not have endometritis. In this same study they also found cows with endometritis were 73% more likely to be culled for a reproductive reason [16]. Dubuc et al. found very similar results when using a Cox proportional hazard model [17]. Gröhn et al. saw a mildly decreased effect of metritis in the postpartum period with the relative pregnancy percentage decreased by 15% (HR=0.85) compared to the referent population when using a Cox proportional hazard model [3]. On the other hand, other researchers found that a metritis event in the postpartum period was not significantly associated with the time to pregnancy [10, 11].

Numerous other researchers not only found metritis in the first 30 DIM to negatively affect the pregnancy rate and time to pregnancy, but also milk production suffered when compared to the referent population [12, 13, 18]. Numerous researchers have tried to investigate why cows with metritis exhibit poor milk production and reproductive performance. Huzzey et al. found that cows with metritis consumed less feed in the week prior to parturition and the three weeks following parturition compared to healthy cows [12], and another researcher found similar results [17]. Other researchers have further analyzed why metritis causes drastic effects on reproductive parameters. Crowe et al. reviewed the current literature and found cows with metritis during the postpartum period had suppressed pituitary hormone secretions, decreased ovarian steroidogenesis, and abnormal luteal phases that resulted in the first postpartum ovarian follicle to be smaller and produce less estradiol than clinically healthy cows [5].

Retained Placenta

Cows with retained fetal membranes at parturition exhibited negatively affected reproductive performance in most studies. Fourichon et al. found that cows with a retained placenta 12 hours after parturition had an average of 27.6 more days to conception, 0.36 more services per conception, and a 16% (HR= 0.84) decreased daily probability of conception [9]. Gröhn et al. found similar effects, with a retained placenta decreasing the relative pregnancy percentage by 14% (HR=0.86) compared to the referent population when using a Cox proportional hazard model [3]. Dubuc et al. found that cows with a retained placenta decreased their pregnancy risk by 7.5% at 300 DIM when compared to referent population when analyzed using a univariable logistic regression model [18]. On the other hand, one researcher found that a retained placenta in the postpartum period was not significantly associated with the time to pregnancy [10].

Ketosis

Cows affected with ketosis during the postpartum period were negatively associated with reproductive performance in numerous studies. Walsh et al. using logistic regression modeling found that cows diagnosed with subclinical ketosis using beta-hydroxybutyrate (BHBA) concentrations in the first two weeks postpartum had a 20% reduction in their first service pregnancy risk, and cows diagnosed with clinical ketosis in the same time period had a 50% reduction in their first service pregnancy risk when compared to cows that did not have increased levels of BHBA [19]. In the same study, they also found that cows with subclinical ketosis in the first two weeks postpartum negatively affected the probability of pregnancy until about 140 DIM, after which the probability of pregnancy was the same as cows that did not have increased levels of BHBA [19]. Numerous researchers have found having a negative energy balance in the early postpartum period results in a prolonged period of a disrupted hypothalamic-pituitary-ovarian axis which causes a delay in normal luteal activity and thus a delayed pregnancy [19]. Fourichon et al. found the effects of ketosis to vary greatly between studies, with the main reason of variability being the qualifications for an animal to be diagnosed with clinical ketosis [9]. Cows diagnosed with clinical ketosis in the first 50 DIM had a decreased first service conception risk of 3.8%, 5.9 more days open for cows that became pregnant, and a 13% (Hazard ratio= 0.87) reduction in conception risk from 56 to 120 days postpartum when compared to cows that were not diagnosed with clinical ketosis. But when analyzing the conception risk over the entire lactation, the effect of clinical ketosis was found to be non-significant [9]. Another study found that ketosis events were a non-significant factor towards the first service pregnancy risk when analyzed using a logistic regression model [15]. Numerous other researchers have found that a negative energy balance at and around freshening compromised and delayed reproductive performance later in lactation [2, 14, 17].

Lactation

The effect of parity group on reproductive parameters varied widely in the literature. Numerous studies found no significant effect of parity group on reproductive parameters [10, 11, 14, 20]. Numerous other studies either did not analyze parity group for numerous reasons or used parity group as an adjustment factor [16, 18, 21], whereas Barker et al. found that cows greater than their 5th lactation required significantly more services per conception than other parity groups

[22]. Ray et al. found that second through fifth lactation cows had less services per conception and a shorter calving interval compared to first lactation and sixth and greater lactation cows, with lactation one having the worst reproductive parameters using a least square means method of analysis [23]. Another researcher found similar results [15].

Twins

Very few articles analyzed the effect of twining on the dam's reproductive performance. Beerepoot et al. found that dams that have twins at parturition spent more days open, had a decreased milk production, were culled earlier in lactation, had more abortions, and an increased cost of drug therapy when compared to cows that did not have twins using an economic model [21]. They also found that most cows that had twins were multiparous cows, and interestingly, they found twinning at parturition did not influence the risk of postpartum disease in the first 30 DIM [21].

Lameness

Lameness events varied widely in their effect on reproductive performance. One study by Lee et al. showed lameness events during the first 150 days of lactation increased the days from calving to conception by 28 days as compared to cows that did not have a lameness event [24]. Suriyasathaporn et al. found that cows that were lame in the first 45 days in milk had an impaired conception risk (HR = 0.88) compared to healthy cows, but a lameness event later in lactation did not significantly affect conception risk [25]. Fourichon et al. did a meta-analysis of numerous studies and found the effects of lameness on conception to be mixed, with an overall daily probability of conception for cows with a lameness event anytime during the lactation to have an HR=0.69 when compared to cows that did not have a lameness event [9]. Numerous other studies have found varying effects of lameness events on reproductive parameters [10, 14, 15]. The main difference in all of these studies is when lameness events were recorded in relation to the lactation cycle.

Mastitis

The effects of mastitis on reproductive performance varied greatly depending on when the mastitic event occurred. Barker et al. found that cows that had a mastitis event prior to their first AI event (around the first 60 DIM) had more days to first service and more days open compared to all other cows in the study [22]. Fourichon et al. found similar results with cows that had a mastitis event in the first 45 DIM experiencing an extended days to first service and days open when compared to the referent population [9]. Loeffler et al. on the other hand found that cows bred 21 days after a mastitis event negatively affected their risk of becoming pregnant when compared to the rest of the population using a logistic regression model [14].

Milk fever and LDA

Only one study using logistic regression modeling reported that a left displaced abomasum event any time before the first service significantly affected the odds ratio of becoming pregnant compared to the referent population (OR=0.25) [14]. Other authors concluded a milk fever or left displaced abomasum event in the postpartum period had no significant impact on reproductive performance [4, 9, 15].

Culling Effects

Open cows, especially in late lactation with low milk production, are at an increased risk of being culled. But disorders in the postpartum period also have an effect on being culled from the population. In one study, cows with metritis in the postpartum period or cows with dystocia at parturition had a significantly increased likelihood of being culled not only at the time of occurrence but also at the end of the lactation as compared to cows that did not have these problems [3]. Another study found cows with dystocia, metritis, ketosis, mastitis, or an abortion any time during lactation had an increased risk of being culled later in lactation [9]. While another study found postpartum disorders had no significant effect on the risk of being culled later in lactation when analyzed with logistic regression modeling and Cox regression modeling [18]. And one study found that cows treated for mastitis in the first 30 DIM were twice as likely to be culled from the herd than cows that did not have mastitis early in lactation [26].

In summary, diseases in the postpartum period affect reproductive performance throughout the lactation. The first objective of this study was to determine the effect postpartum diseases in the first 30 days in milk have on days to pregnancy in the subsequent lactation.

Statistics Overview

Epidemiology is defined by the Merriam-Webster dictionary as a branch of medical science that deals with the incidence, distribution, and control of disease in a population [27]. In order to use epidemiology to determine the incidence, distribution, and control of diseases in a population the use of statistics must be implemented. Statistics as defined by the Merriam-Webster dictionary is a branch of mathematics dealing with the collection, analysis, interpretation, and presentation of masses of numerical data [27].

There are many different types of statistical models that can be used to analyze epidemiologic data. The first is a linear regression model, which can be used when the independent variable is measured on a continuous scale [28]. This of course would include many veterinary studies, but there are certain assumptions that must be true in order to use a linear regression model. These assumptions include homoscedasticity, a normal distribution, linearity, and independence [28]. Homoscedasticity as defined by Dohoo et al. is the assumption that the variance of the outcome is the same at all levels of the predictor variable [28]. Meaning if you have an independent variable like days to first breeding and a dependent variable such as days to pregnancy, then the variance for days to pregnancy must be the same at 55 days to first breeding and at 155 days to first breeding [28]. If this is not linear and constant then a linear regression model should not be used [28]. Normal distribution is the assumption that the standard errors are normally distributed at all values of the dependent variable [28]. Meaning the standard errors of a dependent variable like days to pregnancy are not skewed to one side or the other and they are not bi-modal or quadratic. Linearity is the assumption that the independent variable and the dependent variable have a relationship that can be described with a straight line [28]. Independence is the assumption that the values of the dependent variable are statistically independent of one another [28]. Meaning the days to pregnancy for one animal is independent of the days to pregnancy for another animal. One area of research where the assumption of independence may be lost is in time series analyses [28]. Since the value of an outcome on one day is likely to be correlated to the value of the outcome on the previous day [28]. Another type of statistical modeling is logistic regression modeling, it is very similar to linear regression modeling but now the outcome of the study (the dependent variable) is not continuous but a dichotomous variable [28]. The assumptions of independence and linearity must also be met in order to use a logistic regression model [28]. Both of these assumptions are already previously described. The one benefit of using a logistic regression over a linear regression model is with a logistic regression model the data can have heteroscedasticity and the data no longer needs to be homoscedastic [28]. Another vastly different approach to modeling is the use of a Poisson regression model. A simplified explanation is that a Poisson model tracks the incidence of new cases (i.e. mastitis, lameness, squamous cell carcinomas) while adjusting for the amount of time an animal is at risk [28]. A much deeper explanation of all of these model types can be found in Epidemiology textbooks such as Dohoo [28].

Survival analysis can be used to analyze time-to-event data when the assumptions necessary for linear regression and logistic regression are in serious doubt. There are three common situations involving time-to-event data (e.g. time-to-first breeding, time-to-pregnancy) that makes survival analysis preferable to linear or logistic regression. The first issue that arises is that time to an event is a continuous variable, making logistic regression impossible to use [28]. The second issue is that time-to-event data is not normally distributed, in fact it is frequently skewed to one side or the other or it may be bimodal or quadratic [28]. This violates the assumption of normality for a linear regression model [28]. In many instances, it is possible to transform the data in order to make a linear regression model work fairly accurately. Unfortunately before computer software programs with survival analysis packages became available, a linear regression model, even after transforming the data, was much easier computationally than doing the calculations of a survival analysis by hand [28]. The third issue is censoring. Censoring is defined by Dohoo et al. as the occurrence (or possible occurrence) of a failure when the animal is not observed [28]. In linear regression modeling all animals in the study must have the event of interest or they need to be dropped from the study. This creates bias in the analysis by over- or under-estimating the effects of an independent variable [28]. Take for example a data set looking at the effects of postpartum diseases on the days to pregnancy, if a linear regression model was used, all the cows that were either culled or did not become pregnant would have to be dropped from the study. This would result in the effects of diseases to be underestimated because all of the cows remaining in the study became pregnant. Whereas a survival analysis can use the information obtained from the cows that were culled or remained open and these cows become factored into the risk of becoming pregnant up until they are either culled or leave the study [28]. These animals would be termed to be right censored in a survival analysis since they did not have the event of interest before either the study ended or the animals were lost due to other reasons [28]. There is also interval censoring, which occurs when an individual is observed periodically and the event of interest occurs during a time the animal is not being observed [28]. This occurs more in human medicine when the event of interest occurs in-between a visit to the doctor, and it makes the precise time an event occurred unknown, resulting in a less accurate survival analysis [28]. There is also left censoring, which occurs when the event of interest occurs before the subject begins the observation period forcing these subjects to be dropped from the study [28].

Survival Analysis

Survival analysis modeling is basically the analysis of the time to the occurrence of an event [29]. As described above, a survival analysis model is much like a linear regression model except it substitutes the normality assumption for a formula that fits the data more appropriately and it retains information from censored data [29]. There are three general approaches to analyzing survival analysis data: non-parametric modeling, semi-parametric modeling, and parametric modeling [28]. A non-parametric survival analysis model makes no assumptions about the distributions of the survival times, or the functional relationship between an independent variable and the survival times [28]. This means no information can be obtained for the time to an event of interest, and continuous independent variables should not be evaluated with a non-parametric model [28]. A semi-parametric survival analysis model makes no assumptions about how the survival times are distributed, but it uses the survival times to make a chronological order of the survival times to predict the probability of the event of interest occurring [28]. This will be discussed in further detail later in this study. A parametric survival analysis model replaces the assumption of normal distribution with a more appropriate distribution that correctly reflects the pattern of survival time in the data [28]. Another way to explain this is; a parametric analysis takes a combination of several separate binary-outcome analyses for all possible failure times, making a particular interval of time with no failures occurring informative and adjusts the rates accordingly [29]. Whereas a semi-parametric analysis takes a combination of analyses like with a parametric model, but only when a failure of the event of interest occurs making periods when no failures occur non-informative [29].

The most common type of semi-parametric survival analysis modeling is the use of a multivariable Cox regression model, also known as a proportional hazard model, as it allows the researcher to simultaneously evaluate the effects of numerous independent variables [28]. In order to better understand this concept, the functions a Cox regression model need to be defined. The survival function is the probability an individual's time to the occurrence of the event of interest will be greater than some defined time [28]. This leads to the failure function, which is the probability of not "surviving" past a defined point in time and is simply one minus the survivor function [28]. The probability density function is the slope of the failure function and it represents the instantaneous rate at which individuals are having the event of interest in the study population at a given point in time [28]. This leads to the hazard function which is the probability density function divided by the survivor function, and it represents the probability of the event of interest will occur for a study population within a given period of time, assuming that the individuals in the study population have survived up to the beginning of the period of time in question [29]. This leads to the hazard ratio which is the effect of a one unit change in the independent variable on the frequency of the event of interest [28]. For example, if one did a

Cox regression model to analyze the time to pregnancy and found that giving a drug of some kind resulted in a Hazard Ratio (HR) of 1.10, then cows that were given that drug had a 10% higher rate of pregnancy than cows that were not given this drug [28]. Another way to illustrate hazard ratios is to think about them like the revolutions per minute (RPM) of a car. If one group of cars have the RPM set at 100 RPM for a predetermined amount of time, and another group of cars have it set at 110 RPM for the same period of time. Then the group of cars with the faster RPM would have a HR of 1.10 and the engines would be going at a 10% faster rate [29]. Another concept is the cumulative hazard, which is basically the accumulation of hazard over time and it represents the expected number of outcomes of interest that would occur over a period of time (e.g. the entire study period, or a certain portion of the study period) [28]. A good way to illustrate this is with the RPM example and if you use the same 100 RPM, then the cumulative hazard over two minutes would be 200 revolutions [29].

A Cox regression model works by determining a baseline hazard and then determines an exponential hazard function for each of the independent variables in the model to determine the effect on the dependent variable [28]. Since it is a semi-parametric model, there is no assumption as to the shape of the baseline hazard, and the model has no y-intercept [28]. This baseline hazard is calculated and formed by all the independent variables in the model being equal to zero [28]. This permits the estimation of parameters without making assumptions about the distribution form of the baseline hazard and the distribution can more closely match what is given in the data [26]. The model then makes the appropriate corrections in the calculations by factoring in the baseline hazard and factoring the effect other independent variables have on the outcome to determine each independent variable's hazard ratio on the outcome of interest [29]. This type of analysis has been used widely in the human medical field to assess morbidity and mortality factors. It has also been used widely for genetic trait evaluations and to study fertility traits in dairy cattle, since the researcher is able to retain information from cows that were either culled or remained open in the study and as a result obtain a more accurate estimate [26]. In one study on genetic trait testing, correlations were predicted between the true breeding value and the sire breeding value using a survival analysis model and a linear regression model; they found a much higher correlation in the survival analysis model as compared to the linear regression model [26].

Competing Risks

Competing risk regression models are very similar to Cox regression models, with the main difference being how subjects are censored in the analysis. As discussed previously, a Cox regression model is able to use information from subjects that are right censored or have not had the event of interest, and their information is used in the formation of the hazard ratio up until they leave the study [29]. As defined previously, subjects in a study are right censored anytime something else happens to the subject before they have had the event of interest [28]. And a fundamental assumption of a Cox regression model is that censoring is not dependent on the event of interest, meaning the subjects that are censored are assumed to have the same survival distribution as the animals that were not censored in the study [28]. For subjects that are still in the study at the end of the study period and have not had any outcome, it could be assumed that these subjects would have a similar survival distribution as those subjects that did fail at the end of the study period. For example, in a study looking at the time to pregnancy, the cows that remain open at the end of a study period are expected to become pregnant at the same rate as the cows that did become pregnant near the end of the study period. But animals that have died or were culled from the herd obviously do not have the same risk of becoming pregnant as cows that are still open [30]. This problem occurs more frequently in the study of human medicine and especially in cancer research. For example, end-stage leukemia patients many times undergo a very high dose of radiation and chemotherapy in order to eradicate the leukemia and prepare the patient for a bone marrow transplant. This hopefully results in resolution of the leukemia and a thriving population of new bone marrow cells. But researchers many times want to look at indicator variables to analyze their effect on the recurrence of leukemia, and patients may succumb to a graft versus host disease or other diseases before they have a recurrence of leukemia [31]. If a typical Cox regression model was used to analyze the recurrence of leukemia you would bias the relationship between prognostic factors (independent variables) and the recurrence of leukemia, fail to analyze an interrelationship between the recurrence of leukemia and a graft versus host disease, and overestimate the failure rate of the recurrence of leukemia by not controlling for the competing risk of a graft versus host disease [31]. The reason a Cox regression model overestimates the failure rate and creates a bias in the relationships is because it censors the competing risk (i.e. death due to a graft versus host disease) observations [32]. The magnitude of error that arises is dependent on the incidence of the competing risk [32]. Table 1.1 illustrates work done by Kim et al. [32] illustrating why a survival analysis method like a Cox regression model will overestimate the cumulative incidence of the event of interest. Table 1.1 is a fictional Cox regression model and a Competing risk model calculation table. The event of interest in table 1.1 was a local relapse of a cancer, and the competing risk was a distant metastasis that existed in this fictional study of 10 patients: 4 patients had a local relapse, 3 patients had a distant metastasis, and 3 patients were healthy throughout the study period. The reason this occurs is because the total sum of the proportion of subjects surviving and the proportion of animals failing at any one point in time must be equal to one [32]. With a survival analysis like a Cox regression model there are only two possible categories for a subject to fall into, the subject has either failed to the event of interest or that subject is censored and still being factored into the pool of survived individuals [32]. With a competing risk model there are three possible categories for a subject to fall into, either the subject has failed to the event of interest, the subject has failed due to the competing event, or the subject is censored and being factored into the pool of survived individuals [32]. Numerous other researchers agree that a survival analysis will overestimate the risk in the presence of a competing risk [30, 33-35]. This problem of competing risks have been a struggle for researchers in the past, and numerous researchers have developed formulas to correctly handle competing risks [34, 35]. Recently, statistical software has been released that allows researchers to correctly handle competing risks in a much more user-friendly method. This has allowed numerous research articles in human medicine to obtain a more in-depth and accurate analysis [36-38]. There are also numerous competing events that occur in veterinary medicine; for example, when studying reproductive parameters, cows that are culled from the herd is a competing risk to the effects of nearly all reproductive parameters [26]. Currently in the veterinary literature there is only one study of lamb mortality that utilized a competing risk analysis [39]. That is why the second objective of this study was to compare and contrast the results from a survival analysis and a competing risk analysis.

Chapter 1 Table

Table 1.1: Illustration of Cox regression modeling versus competing risk modeling

				Cox Regression		Competing Risk		Cox regression		Competing Risk		Competing Risk
Patient #	Follow-up time	Event	# at risk	Local relapse free survival		Local relapse and distant metastasis free survival		Cox regression cumulative incidence of local relapse		Competing risk cumulative incidence of local relapse		Competing risk cumulative incidence of distant
1	10	Local Relapse	10	1 x (9/10) =	0.90	1 x (9/10) =	0.90	0 + (1 x (1/10))=	0.1	0 + (1 x (1/10))=	0.1	0
2	20	Alive	9	0.9 x (9/9) =	0.90	0.9 x (9/9) =	0.90	0.1 + (0.9 x (0/9))=	0.1	0.1 + (0.9 x (0/9))=	0.1	0
3	30	Distant Metastasis	8	0.9 x (8/8) =	0.90	0.90 x (7/8) =	0.78	0.1 + (0.9 x (0/8))=	0.1	0.1 + (0.9 x (0/8))=	0.1	0.11
4	40	Local Relapse	7	0.9 x (6/7) =	0.77	0.78 X (6/7) =	0.66	0.1 + (0.9 x (1/7))=	0.23	0.1+(0.78x(1/7))=	0.21	0.11
5	50	Alive	6	0.77 x (6/6) =	0.77	0.66 x (6/6) =	0.66	0.23+(0.77 x(0/6))=	0.23	0.21+(0.66x(0/6))=	0.21	0.11
6	60	Distant Metastasis	5	0.77 x (5/5) =	0.77	0.66 x (4/5) =	0.53	0.23+(0.77x(0/5))=	0.23	0.21+(0.66x(0/5)=	0.21	0.24
7	70	Local Relapse	4	0.77 x (3/4) =	0.58	0.53 x (3/4) =	0.40	0.23+(0.77x(1/4))=	0.42	0.21+(0.53x(1/4))=	0.34	0.24
8	80	Local Relapse	3	0.58 x (2/3) =	0.38	0.40 x (2/3) =	0.27	0.42+(0.58x(1/3))=	0.61	0.34+(0.4x(1/3))=	0.47	0.24
9	90	Distant Metastasis	2	0.38 x (2/2) =	0.38	0.27 x (1/2) =	0.14	0.61+(0.38x(0/2))=	0.61	0.47+(0.27x(0/2)=	0.47	0.38
10	100	Alive	1	0.38 x (1/1) =	0.38	0.14 x (1/1) =	0.14	0.61+(0.38x(0/1))=	0.61	0.47+(0.14x(0/1)=	0.47	0.38

Chapter 2 - Materials and Methods

Data were collected on cattle that freshened over a two month period (July and August 2009) on a 6,000-cow dairy farm located in the Upper Midwest region of the U.S. The housing consisted of three cross-ventilated barns. All cows received a Total Mixed Ration (TMR) fed twice a day and consisting of a dry cow, fresh cow, or lactating cow ration, depending on stage of lactation. The ration was formulated to meet NRC requirements and consisted of corn silage, dry hay, straw, ground corn, soybean meal, and a mineral mix. The majority of the cows were milked in a large carousel style parlor, with newly freshened cows and cows receiving therapeutic treatment being milked in a parallel hospital parlor.

Study Animals

All heifers were brought to the farm 60 days before their expected parturition and housed in one of two dry cow pens until the onset of parturition. Late gestation lactating cows were dried off with intramammary infusions of cloxacillin benzathine (500 mg cloxacillin, Dry Clox®-Fort Dodge Animal Health, Iowa) 45 days before their expected parturition date and housed in one of the two dry cow pens until the onset of parturition. Once the beginning of parturition was noticed by a farm employee, the animal was moved to a calving pen and monitored for dystocia. Once parturition was complete, the cow's record was updated, the calf removed, and the cow moved to a maternity pen for approximately four days. Four days after parturition cows were moved to the fresh cow lactating pen. After freshening, farm employees would monitor and identify any cow that appeared physically ill, that did not expel the fetal membrane, or had low milk production. These cows were then examined more closely to determine rectal temperature, heart rate, respiration rate, and rumen motility status. In addition, the reproductive tract was palpated per rectum for determination of uterine tone and the presence of malodorous uterine discharge, the udder was examined for evidence of mastitis, and the abdomen was auscultated during percussion for evidence of a displaced abomasum (DA). From this examination a presumptive diagnosis was made by the farm employee and the cow was classified as having dystocia, retained placenta, metritis, pneumonia, diarrhea, ketosis, milk fever, DA, acidosis, mastitis, lameness, or having an undifferentiated illness. The cow would then be treated according to a prescribed treatment protocol and placed in the appropriate hospital pen. Once a cow reached approximately 55 DIM, an injection of prostaglandin F2α (25mg dinoprost tromethamine, Lutalyse®- Pfizer Animal Health, New York) was given and the cow was monitored for signs of estrus by farm employees. If a cow showed signs of estrus, she would be artificially inseminated approximately 12 hours later. Inseminated cows would be monitored daily for signs of estrus, or checked for pregnancy status via uterine palpation per rectum approximately 35 days after being bred. If a cow was not bred by 70 DIM or was confirmed open while being checked for a pregnancy, she would then be enrolled into a time insemination synchronization protocol utilizing prostaglandin F2α and gonadotropin-releasing hormone. Once the cow was confirmed pregnant she was rechecked at 90 DIM and 220 DIM to ensure she maintained the pregnancy.

Data

Data were collected on all cows that calved from the time period of June 22, 2009 through August 31, 2009. Dairy Comp305® (An on-farm dairy management software program, DairyComp305®- Valley Agricultural Software, California) and DairyPlan C21® (An integrated monitoring and control of parlor systems, DairyPlan C21®- GEA Farm Technologies, Germany) data were collected daily from the day each cow freshened until the end of the study, or until she was culled from the herd. Each cow was monitored and her identification number, current pen number, current days in milk, freshening date, current reproductive status, lactation number, calving ease, the length of the preceding dry period, the number of calves born in the current lactation, the Days in Milk (DIM) to first breeding, the number of times bred, the DIM to pregnancy, and daily milk production data were recorded. In addition, any presumptive diagnosis of disease that occurred in the first 30 DIM, and all culling data were recorded by hand. Although all farm employees evaluating the cattle for health status received the same training, it is likely that case definitions were not completely consistent between employees. Table 2.1 lists each disease and the case definition for each disease. Once a presumptive diagnosis was made, the cow was recorded with her identification (ID) number, disease classification, and treatment received.

The Dairy Comp and DairyPlan C21 data were collected daily for all cows, and the health related events were recorded by a person who was blinded to all production decisions in this study. Data were collected on a total of 1,098 individual cows. Ten cows failed to begin data collection at the beginning of their lactation, two cows disappeared in the records during the study, and twenty cows had errors in their DairyComp305 record. As a result thirty-two cows were dropped from the study, and 1,066 individual cows with a complete data set were used in the analysis of this study. Table 2.2 shows the number of cows and the reason they were not included in this study.

Once the data collection period was completed on February 28, 2010, each individual cows' data was summarized by reporting:

- Cow ID number
- Lactation number
- Freshening date
- End of study date

- The number of days the animal was on trial or until culled from the herd
- Calving ease score
- The number of calves delivered at parturition
- The number of days spent in the dry cow pen
- Days to first breeding
- DairyComp305® assigned reproduction code at the end of the trial
- Whether the cow ever became pregnant during the trial period
- The number of days in milk it took for her to become pregnant
- Whether or not the cow aborted
- The number of times bred
- The total number of illnesses she had during her first 30 DIM
- The specific presumptive diagnosis (i.e. metritis, retained placenta, pneumonia, diarrhea, ketosis, milk fever, DA, acidosis, bacterial infection, dystocia, mastitis, lameness)
- If the cow died or was culled during the study

A new variable was formed and titled "illness other", which included illnesses that by themselves had a poor case definition. Illness other included the designations: diarrhea, acidosis, and undifferentiated illness. If a cow was confirmed pregnant via rectal palpation, the number of days to pregnancy for that cow was determined by the days in milk of her last artificial insemination before being confirmed pregnant.

This information was analyzed in STATA 11 using the competing risk analysis component and the Cox regression analysis component. Cows that were not pregnant at the end of the study period were right censored. Because of a low sample size in some strata, some classifications were collapsed. There were few cows in their sixth or greater lactation, therefore, all cows in their fifth, sixth, or seventh lactation were combined into one category called fifth or greater lactation cows. In addition, there were only a small number of cows with a calving ease score of four or five. As a result, cows with calving ease scores of three, four, and five were collapsed into a single category of calving ease score, three or greater. To classify the number of illness episodes during the first 30 DIM, cows with three, four, or five recorded illnesses were combined into one category, three or greater illnesses. Also, all cows bred more than four times during the study were classified as being bred four or greater times.

Statistical Analysis

Using the summary data, a bi-variable analysis was preformed, looking at each independent variable on how it was associated with days to becoming pregnant. Then a casual web analysis was performed in order to ensure only variables with biologic significance to becoming pregnant were included in the multivariate analysis. A pairwise correlation and a spearman correlation analysis were performed with all variables to determine collinearity between variables. As a result of that analysis, the number of days to first breeding, and the number of services to become pregnant were determined to be collinear with the outcome of becoming pregnant and the days to pregnancy and as a result were not analyzed in the study. Also the total number of illness a cow had in the first 30 DIM was determined to be collinear with the individual illness categories, and since this study was more concerned about specific illnesses and not illness in general, this category was dropped from the analysis. The daily milk production data collected was found to be too inconsistently recorded, and was dropped from the analysis. Abortions were not analyzed in this study, due to our primary concern of days to pregnancy in this study.

A competing risk analysis study was chosen as the gold standard of analysis for this study because there were animals that were culled during the study, since these animals were not censored and they did not drop out of the study but instead were competing with the risk to become pregnant. A Cox regression model was also completed, and results were compared with the competing risk model. Both models used a semi-parametric analysis. The multivariable model analysis began by including all variables in the model, except those variables removed as explained. Once all variables were placed into the multivariate analysis, each variable with a p value greater than 0.05 globally was removed in a step-wise fashion and the model was rerun. The Breslow method for ties was used in both studies. For all analyses, values of $P < 0.05$ were considered significant. All variables removed in the previous step were placed back into the model individually to check for confounding. No variables were found to be a confounder at the 25% level. Interaction terms for twins were analyzed in the competing risk model. All interaction terms were analyzed in the Cox regression model.

Chapter 2 Tables

Table 2.1: Case definition for disease classification by farm employees

Disease	Classification Criteria
Metritis	Rectal Temperature > 104°F, detection of a malodorous or discharge from the vagina, or a retained placenta of greater than 2 days duration.
Retained Placenta	The placenta is still retained 12 hours after parturition.
Pneumonia	Rectal Temperature > 104°F, and the lungs sound raspy via auscultation with a stethoscope, and the cow has a depressed attitude or is reluctant to rise.
Chronic Pneumonia	Treated > 3 times for pneumonia, and designated to be a possible cull candidate
Diarrhea	Manure score of 3 or less on a 1 to 5 scale, with 1 being similar to water in consistency and 5 having virtually no water in the manure.
Ketosis	Classified using a Ketostix® (Bayer Corporation, Indiana) indication of >40 mg/dl of Acetoacetic acid via a voided urine sample. If a cow appeared overweight or lame then these cows would be classified ketotic if they measured > 15 mg/dl of Acetoacetic acid via the same method.
Milk Fever	Having cold ears and being reluctant to rise.
Left or Right DA	A DA ping on the appropriate side and location via auscultation of a stethoscope.
Acidosis	Manure that smelled or looked abnormal, but not classified as diarrhea, and the cow appeared physically ill.
Bacterial Infection	Manually palpating subcutaneous emphysema along the cows' dorsum with manure that smelled abnormal, and the cow appeared physically ill.
Dystocia	Needing outside intervention during parturition.
Lame Cow	Difficulty walking when observed from the side or back of the animal. (lameness score greater than 1- Zinpro Corp., Minnesota)
Mastitis	Abnormal flakes in the milk after 3 to 4 pre-strips as the cow was being prepped to be milked.

Table 2.2: Cows not included in study due to specified reasons

Number of cows dropped from the study	Reason not in study
10	Missing data
2	Disappeared from the record
20	Irreconcilable errors in record

Chapter 3 - Results

A total of 1,098 individual cow records were collected, and usable records from 1,066 cows were analyzed (Table 2.2). The study population had a seven month daily tank average of 63.2 (+/-2.9) lbs/cow/day. There were 302 first lactation cows, 585 second lactation cows, 67 third lactation cows, 67 fourth lactation cows, and 45 fifth or greater lactation cows analyzed in this study. Other herd parameters are outlined in table 3.1.

Statistical Analysis

Table 3.2 outlines the variables that were analyzed in the bi-variable analysis. There were 524 animals that did not have a recorded illness in the first 30 DIM, of which 454 animals became pregnant resulting in a pregnancy success in the study period of 86.6%. There were 542 animals with a recorded illness in the first 30 DIM, of which 366 animals became pregnant resulting in 67.5% becoming pregnant during the study period. As a result, the unadjusted risk of becoming pregnant differed ($P<0.05$) between those with a recorded illness and those without a recorded illness in the first 30 DIM.

Multivariable Competing Risk Analysis

The multivariable competing risk analysis model revealed that lactation, metritis, retained placenta, dystocia, other illnesses, and the interaction of twins and ketosis were significant factors associated with days to become pregnant (Table 3.3).

Lactation number was significantly associated ($P<0.01$) with the number of days it took for a cow to become pregnant. When the referent population was first lactation cows, then second, third, and fourth lactation cows were not significantly different ($P>0.05$) from the referent population and collapsed into one single variable. But the fifth and greater lactation cows were

56% (SHR=0.44) slower to become pregnant as compared to first through fourth lactation cows. In figure 3.1, the referent population is first through fourth lactation cows and it can be seen that for lactation fifth and greater cows the lines do not cross and diverge early in the breeding period. Also, in all of these figures there are two dramatic steps in the graph; one step at about 60 DIM which coincides with the end of the voluntary waiting period when the cows were be bred based on detection of estrus. Then there is another step that occurs at about 80 DIM at that time the cows that have not been bred are enrolled in a timed-AI estrous synchronization protocol. The risk for becoming pregnant for cows in lactations fifth and greater was less than cows in their first through fourth lactation over the entire breeding period.

Being classified as having metritis was negatively associated (P<0.01) with time to become pregnant (SHR=0.74). Indicating that cows classified as having metritis were 26% slower to become pregnant than cows that did not have metritis. Figure 3.2 shows the cumulative incidence curves for the variable metritis, and it shows the main difference found in the analysis to occur at about 60 DIM only with all other time periods being similar and parallel to each other.

Cows classified as having a retained placenta were associated with (P=0.05) greater days to become pregnant (SHR=0.76). Indicating that cows that had a retained placenta were 24% slower to become pregnant than cows that did not have a retained placenta. Figure 3.3 shows the cumulative incidence curves for the variable retained placenta, the main difference found in the analysis occurred at about 60 DIM with all other time periods being similar and parallel to each other.

Cows classified as having dystocia were also associated with (P<0.01) an increase in days to becoming pregnant (SHR=0.22). Dystocic cows were 78% slower to become pregnant than cows that did not experience dystocia. Figure 3.4 shows the cumulative incidence curves for the variable dystocia, the difference found in the analysis occurred at about 60 DIM and continued to reveal a difference throughout the breeding period.

The classification of 'illness other' included all of the cows that were classified as having diarrhea, acidosis, or an undifferentiated illness event during the postpartum period. Cows classified with any of these presumptive diagnoses were associated with (P<0.01) with an increase in days to becoming pregnant (SHR=0.67) when all other factors in the model were accounted for. Indicating that cows that had one of the 4 illnesses listed above were 33% slower to become pregnant than those cows that did not contract another illness. Figure 3.5 illustrates the cumulative incidence curves for the variable 'illness other', and the difference found in the analysis occurred at about 60 DIM with all other time periods being parallel to each other.

The interaction model decreased the effects of twinning and increased the effect of ketosis when the interaction term twins and ketosis was significantly associated (P=0.03) with days to becoming pregnant (SHR=3.17). Indicating that cows with a twin and not having ketosis at and around parturition were 217% faster to become pregnant than cows that had ketosis and did not have a twin at parturition. Cows twinning was no longer significantly associated (P=0.10) with a greater risk of becoming pregnant (SHR=1.32), but forced into the model since it was significant in the main effects model and part of the interaction. Cows classified as having ketosis were still associated (P<0.01) with increased days to becoming pregnant (SHR=0.48). Figure 3.6 shows the cumulative incidence curves for the variable twins and ketosis, and it shows cows with twins always had shorter days to pregnancy as compared to cows with no twinning at parturition and cows with ketosis around parturition always had longer days to pregnancy as compared to cows that did not have ketosis around parturition. But cows with a twinning and ketosis had more days to pregnancy than the referent population.

Cox Regression Analysis

The multivariable Cox regression model revealed that lactation, metritis, dystocia, and lameness were significant factors associated with days to become pregnant (Table 3.4). Interactions were tested for the Cox Regression model and found to be non-significant.

Lactation five and greater was still significantly associated (P=0.04) with greater days to pregnancy (HR= 0.64). When compared to the same referent population as described above lactation five and greater cows were 36% slower to become pregnant. When compared to the competing risk analysis above, lactation had less of an effect on days to pregnancy using the Cox regression model. In figure 3.7 it can be seen that Lactations 1-4 had an improved pregnancy rate early in lactation at around 60 DIM, and they had continued improvement throughout the lactation as the graph below show divergence in numerous areas of the chart.

Cows with metritis was still significantly associated (P<0.01) with greater days to become pregnant (HR= 0.70). Cows with metritis were 30% slower to become pregnant than cows that did not have metritis in the first thirty days in milk. When compared to the competing risk model, cows with metritis had a greater effect on days to pregnancy when using the Cox Regression model. In figure 3.8 cows with metritis showed divergence away from the referent population at around 60 DIM, with all other time periods being similar and parallel to each other.

Dystocia was also still significantly associated (P=0.03) with greater days to become pregnant (HR=0.38). Dystocic cows were 62% slower to become pregnant than cows without dystocia.

When compared to the competing risk analysis above dystocia during parturition had less of an effect on days to pregnancy when using the Cox Regression model. In figure 3.9 cows with dystocia during parturition showed a vast degree of divergence away from the referent population throughout the lactation starting at around 60 DIM.

The Cox Regression model showed lameness was significantly associated (P=0.04) with fewer days to pregnancy (HR= 1.58). Meaning cows that had a lameness event in the first thirty days of lactation were 58% faster to become pregnant than cows that did not have a lameness event in this time period. When compared to the competing risk analysis, lameness was found to be a non-significant factor for days to become pregnant. In figure 3.10 cows with lameness had continued improvement in days to pregnancy early in the breeding period with other time periods being parallel to each other.

The Cox Regression model did not show a significant association to days to becoming pregnant for cows that experienced a retained placenta, ketosis, another illness, or a twinning event in the first 30 DIM.

Chapter 3 Figures and Tables

Table 3.1: Results of production parameters during the study period on this farm

Variables	Average	SD	Minimum	Maximum	
Calving Ease	1.3	0.65	1	3	
Days Dry	48.9	11.7	5	97	Days
Days to First Breeding	69.4	10.7	48	182	Days
Days to Conception	90.6	30.3			Days
# Times Bred	2.07	1.3	1	8	Times
# Milk/Cow/Day	63.2	2.9			Pounds
Days to Pregnancy	90.6	30.3	55	196	Days

Table 3.2: Bivariate analysis of variables as it relates to becoming pregnant and time to becoming pregnant

Variable [1]	Covariate Levels	Total # of animals out of 1066 (%)	# animals that became pregnant during study period	% Pregnant	P value [2]
Lactation	1	302 (28.3)	234	77.5%	0.23
	2	585 (54.9)	472	80.7%	
	3	67 (6.3)	47	70.1%	
	4	67 (6.3)	45	67.2%	
	5	45 (4.2)	22	48.9%	
Calving Ease	1	863 (81.0)	676	78.3%	0.19
	2	87 (8.2)	71	81.6%	
	3	116 (10.9)	73	62.9%	
Twins	No	1025 (96.2)	787	76.8%	0.63
	Yes	41 (3.8)	33	80.5%	
Recorded Illness	No	524 (49.2)	454	86.6%	<.01
	Yes	542 (50.8)	366	67.5%	
Metritis	No	867 (81.3)	692	79.8%	<.01
	Yes	199 (18.7)	128	64.3%	
Retained Placenta	No	975 (91.5)	764	78.4%	<.01
	Yes	91 (8.5)	56	61.5%	
Pneumonia	No	800 (75.0)	631	78.9%	0.10
	Yes	266 (25.0)	189	71.1%	
Diarrhea	No	1010 (94.7)	786	77.8%	0.87
	Yes	56 (5.3)	34	60.7%	
Ketosis	No	1007 (94.5)	791	78.6%	0.03
	Yes	59 (5.5)	29	49.2%	
Milk Fever	No	1046 (98.1)	809	77.3%	0.46
	Yes	20 (1.9)	11	55.0%	
LDA	No	1024 (96.1)	796	77.7%	0.20
	Yes	42 (3.9)	24	57.1%	
Acidosis	No	1028 (96.4)	799	77.7%	0.10
	Yes	38 (3.6)	21	55.3%	
Bacterial Infection	No	1052 (98.7)	812	77.2%	0.75
	Yes	14 (1.3)	8	57.1%	
Dystocia	No	1048 (98.3)	815	77.8%	0.01
	Yes	18 (1.7)	5	27.8%	
Sick Cow	No	1050 (98.5)	811	77.2%	0.92
	Yes	16 (1.5)	9	56.3%	
Mastitis	No	933 (87.5)	734	78.7%	0.50
	Yes	133 (12.5)	86	64.7%	
Lame Cow	No	1037 (97.3)	800	77.1%	0.08
	Yes	29 (2.7)	20	69.0%	
Illness Other	No	951 (89.2)	753	79.2%	0.28
	Yes	115 (10.8)	67	58.3%	

[1] Variables of interest analyzed in this study.
[2] P values were derived from a Pearson $\chi2$ 2-sided test of association (unadjusted) comparing cows in each subset of the population.

Table 3.3: Final multivariable competing risk analysis as it relates to time to become pregnant

Variable [1]	Covariate Level	x [4]	SE (x) [6]	P Value [3]	SHR [5]	95% CI
Lactation	1-4 [2]					
	5+	-0.81	0.21	< 0.01	0.44	0.29, 0.67
Twins	No					
	Yes	0.27	0.16	0.10	1.32	0.95, 1.81
Metritis	No					
	Yes	-0.30	0.10	< 0.01	0.74	0.61, 0.91
Retained Placenta	No					
	Yes	-0.27	0.14	0.05	0.76	0.58, 1.00
Ketosis	No					
	Yes	-0.74	0.22	< 0.01	0.48	0.31, 0.73
Dystocia	No					
	Yes	-1.53	0.45	< 0.01	0.22	0.09, 0.52
Illness Other	No					
	Yes	-0.41	0.13	< 0.01	0.67	0.51, 0.86
Twins & Ketosis	No					
	Yes	1.15	0.52	0.03	3.17	1.14, 8.80

[1] Variables found to be significant ($p<0.05$).

[2] Lactation 5 and greater cows are compared to cows in lactation 1,2,3,4.

[3] Global test of significance of all levels of affiliated indicator variables.

[4] The logit of probability of outcome change as the predictor is changed by one unit.

[5] Sub-Hazard Ratio (SHR) =the percentage difference from the control for each variable.

[6] SE(x) =Robust Standard Error of X.

Figure 3.1: Parity group cumulative incidence of pregnancy graph

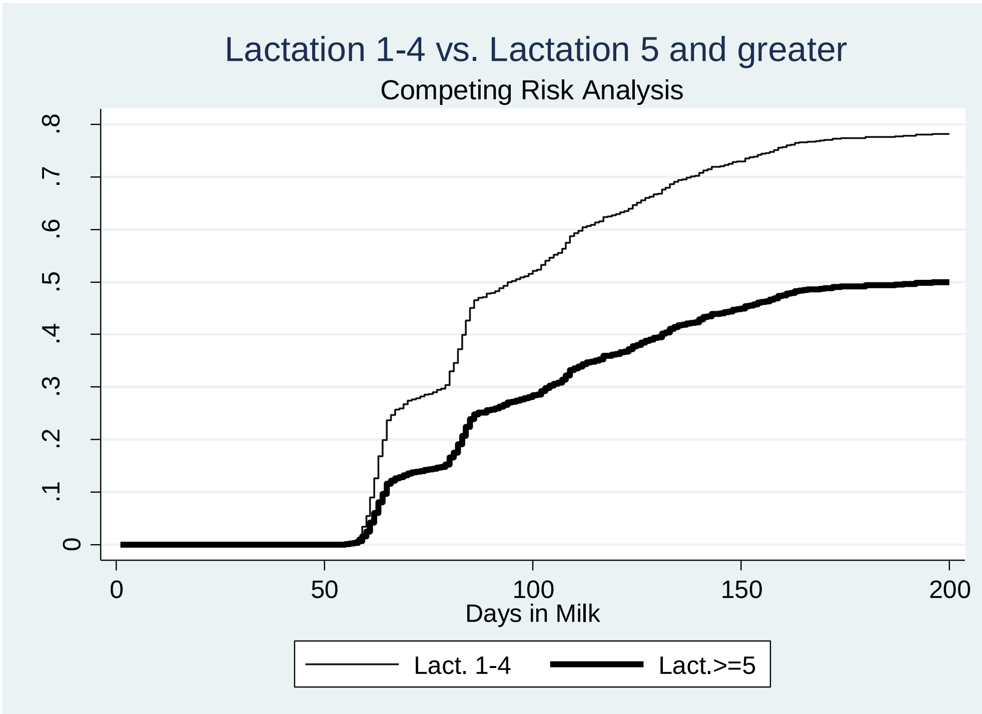


Figure 3.2: Metritis cumulative incidence of pregnancy graph

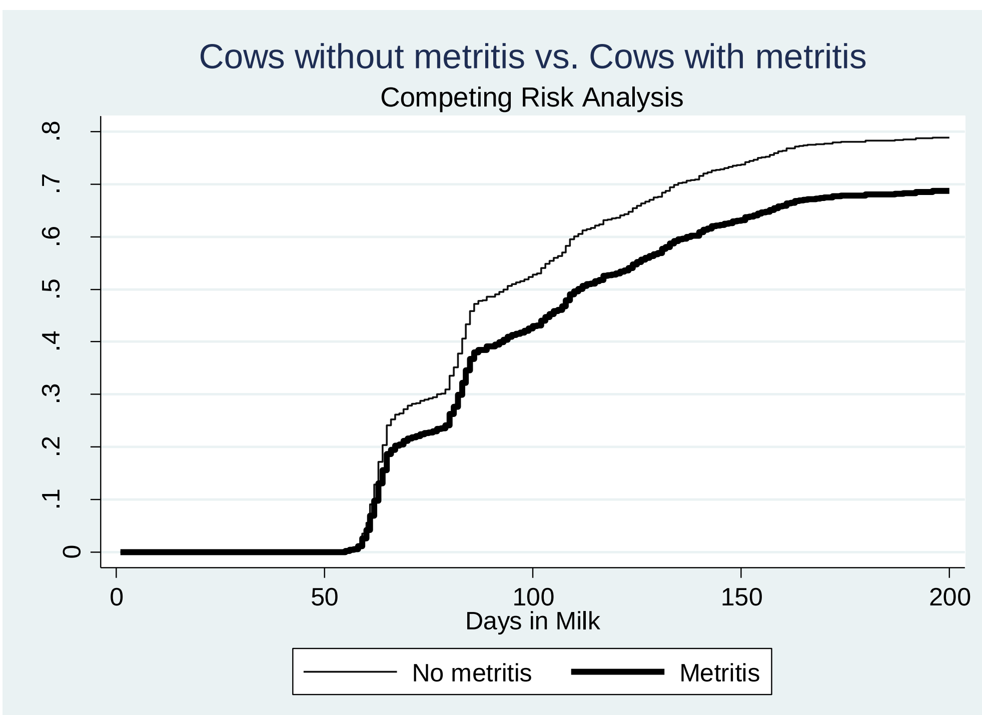


Figure 3.3: Retained placenta cumulative incidence of pregnancy graph

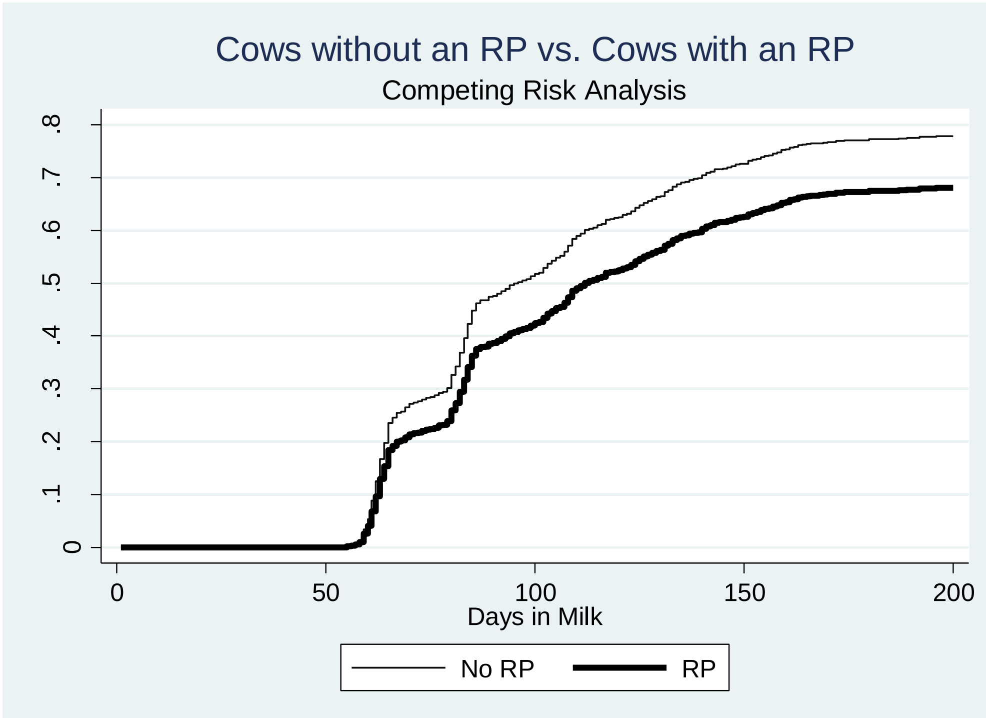


Figure 3.4: Dystocia cumulative incidence of pregnancy graph

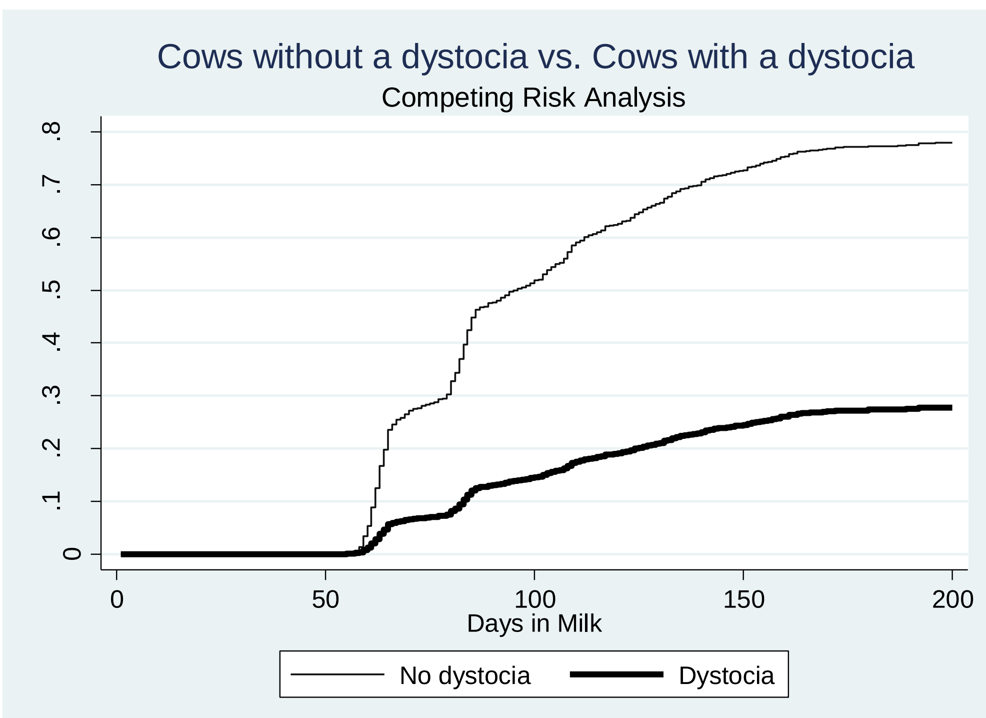


Figure 3.5: Illness other cumulative incidence of pregnancy graph

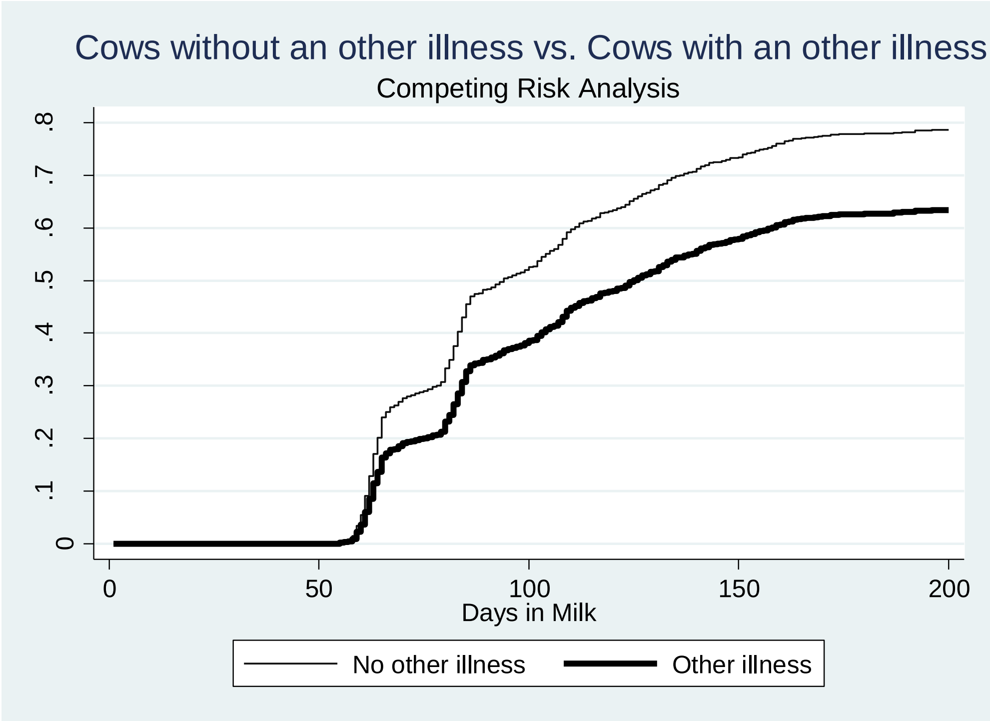


Figure 3.6: Cumulative incidence of pregnancy graph for the interaction term twins and ketosis

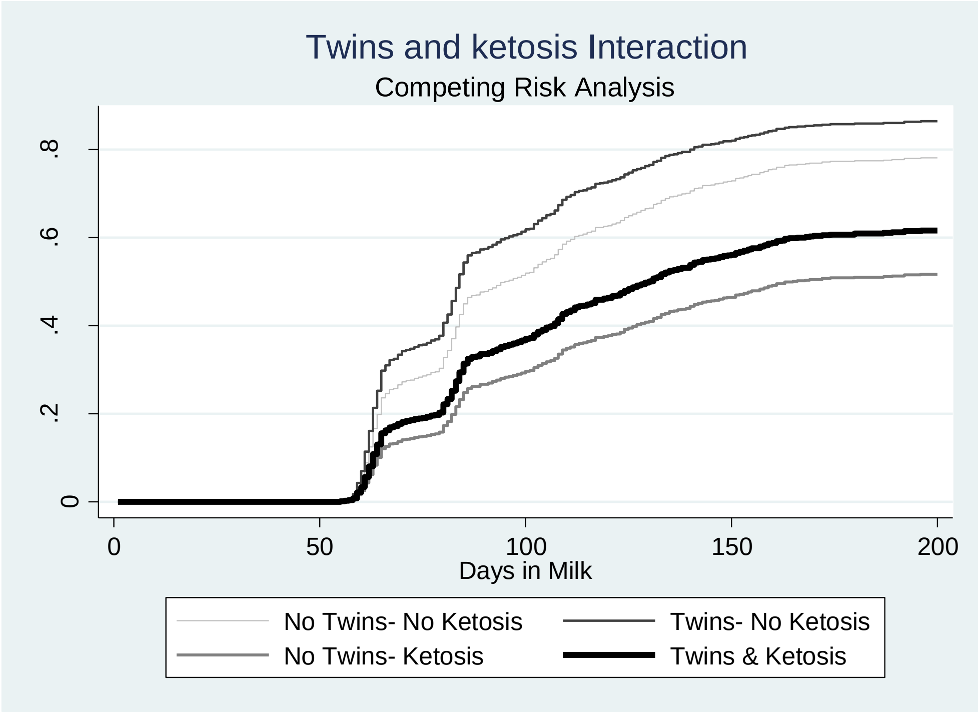


Table 3.4: Final multivariable Cox regression analysis as it relates to time to become pregnant.

Variable [1]	Covariate Level	x [4]	SE (x) [6]	P Value [3]	HR [5]	95% CI
Lactation	1-4 [2]					
	5+	-0.44	0.22	0.04	0.64	0.42, 0.98
Metritis	No					
	Yes	-0.36	0.10	< 0.01	0.70	0.58, 0.84
Dystocia	No					
	Yes	-0.96	0.45	0.03	0.38	0.16, 0.92
Lameness	No					
	Yes	0.46	0.23	0.04	1.58	1.01, 2.48

[1] Variables found to be significant ($p<0.05$).
[2] Lactation 5 and greater cows are compared to cows in lactation 1,2,3,4.
[3] Global test of significance of all levels of affiliated indicator variables.
[4] The logit of probability of outcome change as the predictor is changed by one unit.
[5] Hazard Ratio (HR) = the percentage difference from the control for each variable.
[6] SE(x) =Standard Error of X.

Figure 3.7: Parity group K-M cumulative incidence of pregnancy graph

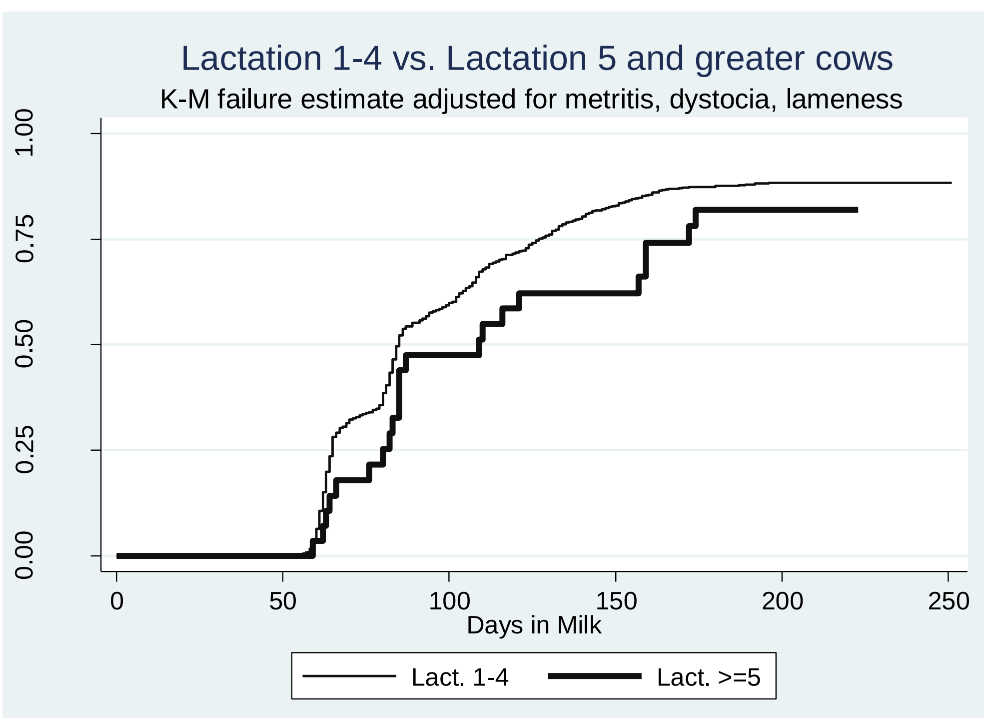


Figure 3.8: Metritis K-M cumulative incidence of pregnancy graph

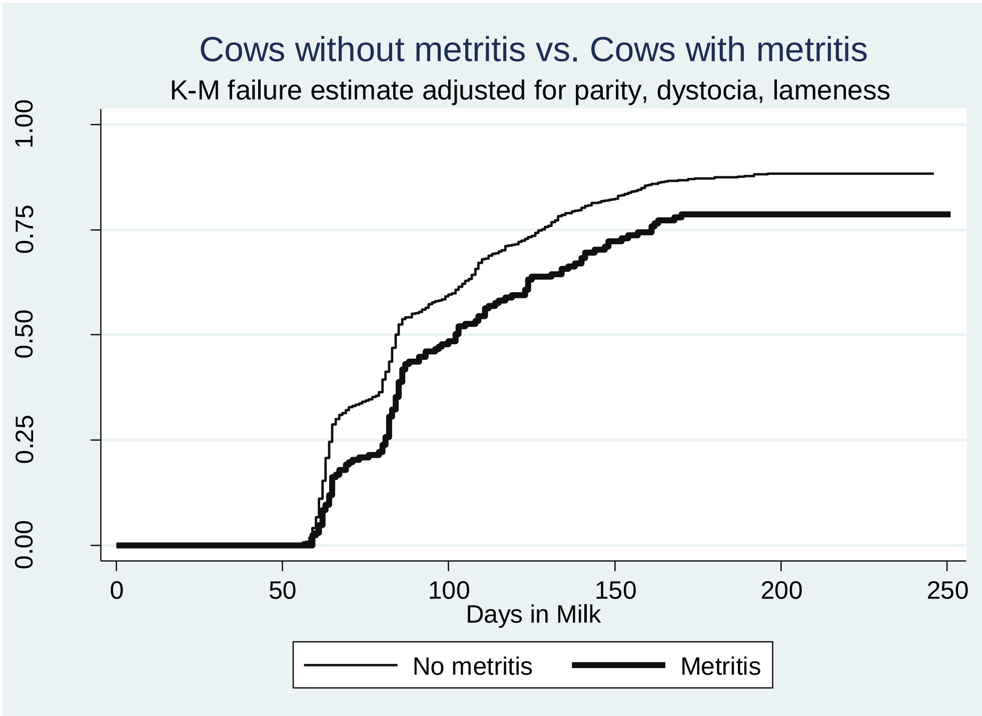


Figure 3.9: Dystocia K-M cumulative incidence of pregnancy graph

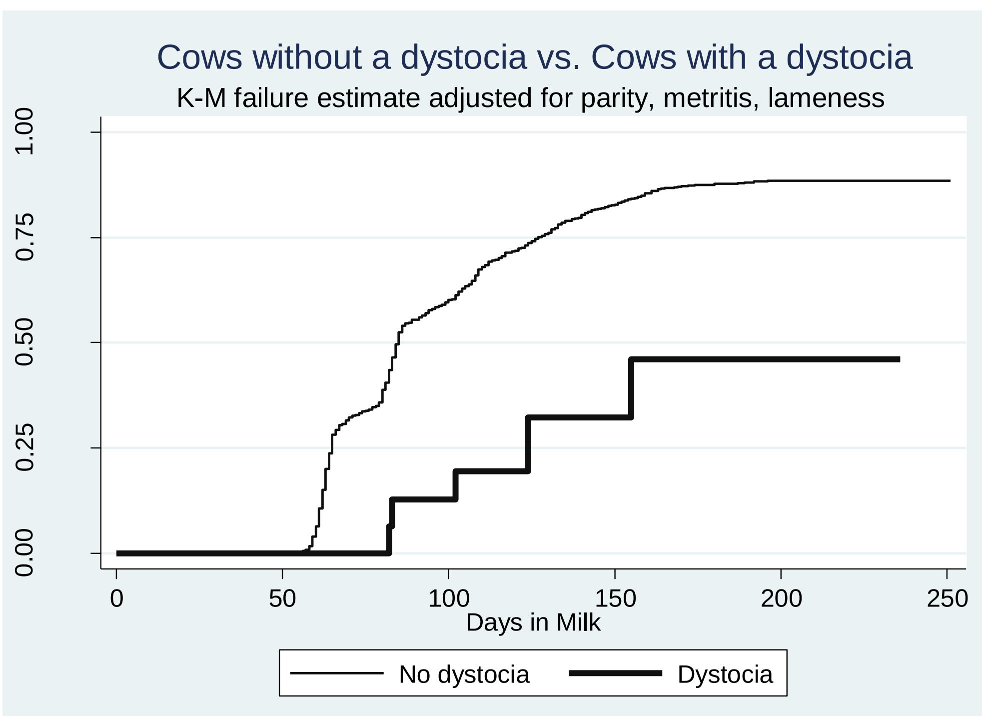
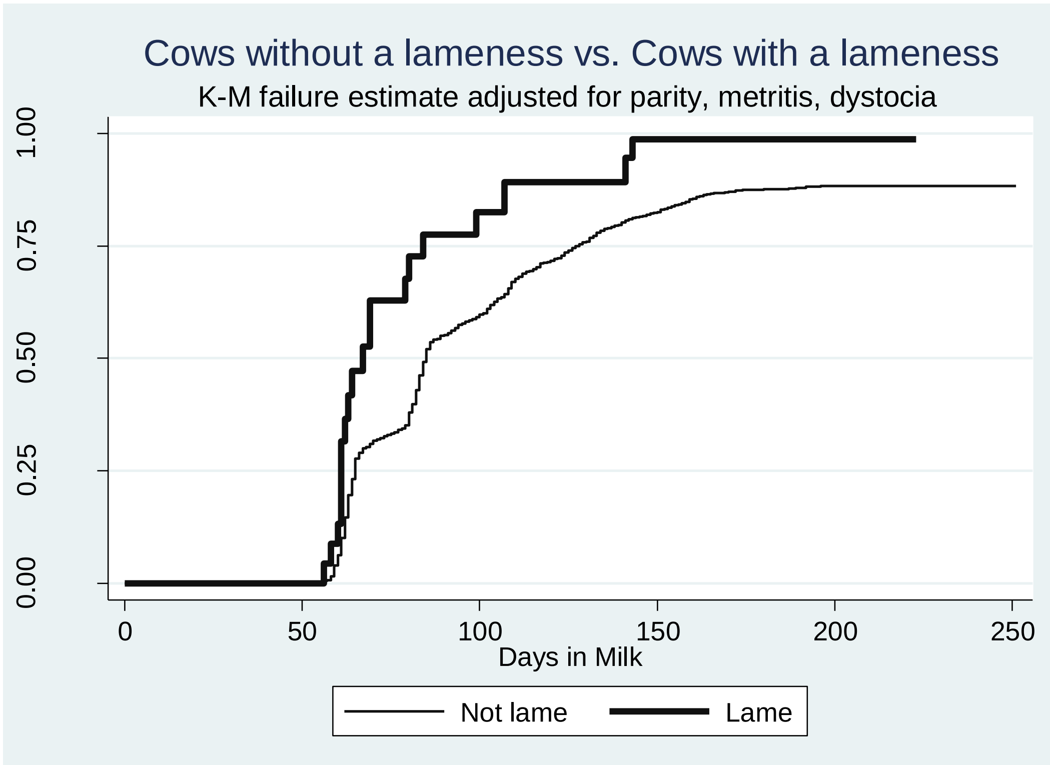


Figure 3.10: Lameness K-M cumulative incidence of pregnancy graph



Chapter 4 - Discussion

In this study, the recorded incidences of postpartum diseases (i.e. twins, metritis, retained placentas, pneumonia, ketosis, milk fever, displaced abomasum, dystocia, and mastitis) were similar to what has been reported in the literature, with a few exceptions. This study had a recorded cumulative incidence risk of metritis of 18.7%. Three studies had a very similar incidence of metritis [8, 14, 18], but Gröhn et al. had an incident risk of 3.2% [3], Curtis et al. had an incidence of 7.8% [4], and Cobo-Abreu et al. had an incidence risk of 7.9% [40]. This is probably explained by the case definition used in each study for metritis, some studies used a very specific case definition for metritis whereas other studies, including this one, used a broad case definition based on clinical signs.

The incidence risk in this study for retained fetal membranes was 8.5%. Two studies had a similar incidence risk [1, 10]; however, Gröhn et al. and Loeffler et al. had much lower incident risks of retained placentas at 3.1% and 5.1%, respectively [3, 14].

The cumulative incidence risk in this study for a ketosis event in the first 30 DIM was 5.5%. Few studies in the literature reported the incidence of ketosis but three studies were identified that reported lower incidence risks for ketosis than observed in this study. Cobo-Abreu et al. reported a cumulative incidence for ketosis of 4.5% [40], Curtis et al. reported an incidence of 3.5% [4], and Loeffler reported an incidence of 1% [14]. This difference is probably also explained by different case definitions for ketosis used in each study.

The incidence risk for milk fever in this study was 1.9%; four other studies reported a higher incidence with a range of 4.9% to 6.6% [1, 4, 14, 40]. This is possibly explained by improved prepartum diets in the last decade, with more widespread use of a properly balanced cation-anion difference (DCAD) diet for prepartum cows as discussed by Overton et al. in 2004 [41].

The incidence risk of pneumonia in this study was much higher at 25% than what was reported in two other studies. The 2007 NAHMS survey reported a lactational incidence risk for pneumonia of 3.3% [1], and Cobo-Abreu et al. reported a lactational incidence risk of 4.9% [40]. This is probably also explained by the case definition used in each study, with this study using a very broad case definition for pneumonia.

Competing Risk Analysis

The present study had a well distributed population of cows with and without a recorded illness in the first thirty days in milk, with 50.8% of the study population having a recorded illness. The study began with a bivariate analysis, which modeled each individual variable (e.g. metritis, ketosis, DA, etc.) and determined its significance on the time to pregnancy for the population. The bivariate analysis did find metritis, retained placenta, ketosis, and dystocia to have a significant impact on the time to pregnancy. But a bivariate analysis does not control for the effects of other variables, and it can only assist to describe the data. Whereas a multivariable model, for example, a Cox regression model or a competing risk model, controls for all variables in the model and it removes the confounding effects between variables to illustrate the effect each specific variable (e.g. metritis, ketosis, etc.) places on the model. A Cox regression model, as described in the beginning of this study is an appropriate multi-variable model to analyze time-to-event data such as reproductive data on the dairy when the incidence of a competing risk is low. When the incidence of a competing risk is high, such as cows that either died or were culled from a study, a Cox regression model will overestimate the risks in the model [32]. That is why the competing risk model is considered to be the most appropriate method to evaluate the effect of post-partum disease on days to pregnancy in dairy cows.

As expected, cows with metritis were 26% slower to become pregnant than cows that did not have metritis up to 250 DIM. Similar effects to pregnancy have been reported in other studies [3, 16, 17], with this study falling within the range previously reported in the literature. The present study further analyzed this problem and found the main detriment metritis placed on the time to pregnancy was early in the breeding period, right at the end of the voluntary waiting period (VWP) at about 60 DIM. While during the rest of the breeding period, cows with metritis became pregnant at relatively the same rate as cows without metritis. Though we cannot calculate the exact amount of days open for cows with metritis in this study, we can conclude cows with metritis had more days open than cows without metritis. And work done by Meadows et al. in 2005 found that for each additional day open a cow spent past 160 days in milk there was a cost to the dairymen of $1.37 per cow [42]. In conclusion, a cow with metritis not only has an upfront cost of treatment and labor and decreased milk production, but it also has an additional cost of increased days open and ultimately increased cost of culling and replacement.

Cows with a retained placenta were 24% slower to become pregnant than cows that did not have a retained placenta. This study showed a greater negative effect than previously reported in the literature, which ranged from a 7.5% to 16% decrease in pregnancy risk for cows with a retained placenta [3, 9, 18]. The effect of a retained placenta was similar to that seen with metritis, with most of the effect on time to pregnancy being early in the breeding period at around 60 DIM.

Cows with other illnesses as defined in this study were 33% slower to become pregnant than cows that did not experience these other illnesses. The main detriment these diseases placed on the days to pregnancy was early in the breeding period at around 60 DIM. The reason cows with diarrhea, acidosis, bacterial infections, and undifferentiated illness were included as a group in this study was each of these diseases have an imprecise case definition. There are no other studies found in the literature that evaluated the effect of these minor diseases on dairy cow reproduction, and their overall effect on the herd level is minimal. But as shown in this study they should not be forgotten, as they do play a role in negatively affecting days to pregnancy.

Cows with dystocia were severely affected by being 78% slower to become pregnant than cows without experiencing dystocia at calving. Fourichon et al. found a much different hazard ratio of 0.88 on conception risk of cows with dystocia [9]. Other studies in the literature either did not analyze dystocia's effect on reproductive parameters, or found the effect to be non-significant. The present study showed the effect of dystocia started right at the beginning of the breeding season, and the effects of dystocia remained throughout the breeding period to around 200 DIM. Though we did not directly analyze this in the study, cows with dystocia could have experienced other postpartum disease as a result of having a dystocia.

Fifth lactation cows and greater were 56% slower to become pregnant than cows in their first, second, third, or fourth lactations. This is similar to what has been reported in the literature, though no studies in the literature used a survival analysis to measure the effect lactation had on reproductive parameters. Three researchers found cows that were greater than their fifth lactation required more services per conception [15, 22, 23]. Two researchers found this group of cows spent more days open than their younger referent population [15, 23]. The present study showed the effect of these older animals started at the beginning of the breeding period around 60 DIM, and the negative effect on days to pregnancy continued throughout the breeding period to around 200 DIM. This pattern of when the effect occurs in the breeding period as was seen with dystocia makes biological sense. As one would not expect older animals to suddenly improve their reproductive performance later in lactation, whereas animals that experience metritis, or a retained placenta in the postpartum period are likely to have diminished negative effects as these animals progress in days in milk and the affected cows would perform more like their unaffected herd-mates as time passes, as demonstrated in this study.

Interaction of twinning with other variables was tested in an attempt to help explain the positive effect of twinning on days to pregnancy seen in the main effects model. The interaction of twins and ketosis was found to be significant in the final competing risk model. When compared to the effects of the main competing risk analysis model this interaction term decreased the effect of twinning and increased the effect of having ketosis as it relates to days to pregnancy. Indicating cows that had a twin but no ketosis in the postpartum period were 217% (SHR=3.17) faster at becoming pregnant than cows that had ketosis but did not have a twin at parturition. Cows that had a twin were 32% faster at becoming pregnant than cows that did not have twins. Cows that had a ketosis event were 52% slower at becoming pregnant than cows that did not have ketosis. This positive effect of twinning does not agree with the previously published literature [9, 21]. This may have resulted due to increased treatment pressure placed by the farm employees on cows that had twins, such that cows with a twin were given extra care and treatment. As a result these cows rebounded faster after calving and resulted in a group of cows that outperformed the average in reproductive performance. Since this was a small population of animals, further research should be conducted in this area to further analyze this paradigm. All other interactions tested in this model were found to be non-significant towards days to pregnancy.

Cox Regression Analysis

Lactation five and greater cows were found to be significant in both the competing risk model and the Cox regression model. In the competing risk model, cows in their fifth or greater lactation were 56% (SHR=0.44) slower to become pregnant than the referent population. In the Cox regression model, cows in their fifth or greater lactation were 36% (HR=0.64) slower to become pregnant than the referent population. The cumulative incidence of pregnancy around 200 DIM for cows in their fifth or greater lactation was about 0.5 in the competing risk model, and the Cox regression model showed the cumulative incidence to be greater than 0.75. The incidence risk of pregnancy in this group of animals using raw data was 48.9%. The cumulative incidence of pregnancy around 200 DIM for cows in their first through the fourth lactations were less than 0.8 in the competing risk model, and the Cox regression model showed the cumulative incidence to be greater than 0.8. The incidence risk of pregnancy in this group of animals using raw data was 78.1%.

The effect of metritis on days to pregnancy was found to be significant in both the competing risk model and the Cox regression model. In the competing risk model, cows with metritis were 26% (SHR=0.74) slower to become pregnant than the referent population. In the Cox regression model, cows with metritis were 30% (HR=0.70) slower to become pregnant than the referent population. The cumulative incidence of pregnancy around 200 DIM for cows with metritis was less than 0.7 in the competing risk model, and the Cox regression model estimated the cumulative incidence to be greater than 0.75. The incidence risk of pregnancy for cows with metritis using raw data in this study was 64.3%. The cumulative incidences of pregnancy in cows without metritis in both models are similar to what was reported in lactation one through four cows. The raw data incidence risk of pregnancy for cows without metritis was 79.8%. It is interesting to note in this group the competing risk and the Cox regression models were both very close in their cumulative incidence calculations, which may indicate the incidence of the competing risk (cows culled) was much lower for cows with metritis than the other variables in the Cox regression model.

Dystocia was found to be significantly associated with days to pregnancy in both the competing risk model and the Cox regression model. In the competing risk model, cows with a dystocia were 78% (SHR=0.22) slower to become pregnant than the referent population. In the Cox regression model, cows with a dystocia were 62% (HR=0.38) slower to become pregnant than the referent population. The cumulative incidence of pregnancy for cows with dystocia around 200 DIM was less than 0.3 in the competing risk model, and the Cox regression model estimated the cumulative incidence of pregnancy at the same time period to be less than 0.5. The incidence risk of pregnancy for cows with dystocia was 27.8% using raw data. The cumulative incidences of pregnancy for cows without dystocia in both models were similar to what was reported with the lactation one through four cows. The incidence risk in cows without dystocia using raw data was 77.8%.

Associations between twinning, retained placenta, a ketosis event, or another illness in the postpartum period and days to pregnancy were found to be non-significant in the Cox regression model. The exact cause as to why this occurred cannot be determined in this study. One would suspect these disorders to have a lesser impact on culling, since they were found to be significant in the competing risk model and non-significant in the Cox regression model. But further analysis would have to be done to analyze the effects of culling on these diseases.

Presence of a lameness event was found to be non-significantly associated with days to pregnancy in the competing risk model, but it was significant in the Cox regression model. Cows with a lameness event were 58% (HR=1.58) faster to become pregnant than the referent population. All other research in the literature found a lameness event early in the lactation cycle to have a negative effect on days to pregnancy [9, 25]. The incidence risk of pregnancy at the end of the study for cows with and without a lameness event was 69.0% and 77.1%, respectively using raw data. From the raw data cows with a lameness event had a lower incidence of pregnancy compared to cows without a lameness event. And the competing risk model found cows with a lameness event to be a non-significant factor towards days to pregnancy when controlling for the effects of cows being culled throughout the study period. Indicating lameness was highly affected by culling. Had only a Cox regression model been done in this study it would have been determined that lameness has a positive effect on days to pregnancy, when in fact lameness affects cows to be culled and is non-significant towards days to pregnancy. But again further analysis would have to be done to analyze this effect on culling. Another possible explanation is that cows in the present study were diagnosed with a minor lameness more frequently than cows in other studies, and this level of lameness did not affect their risk of becoming pregnant for the cows that survived the entire study period; and since this is a small subset of the population, these cows just happened to outperform the average. Also since these lameness events were recorded in the first thirty days in milk; since this study was concerned about the effect postpartum diseases had on reproductive performance, and this study was not concerned about diseases that occurred throughout lactation. It could be the lameness event was caught early in the pathogenesis of disease and these cows were able to recover before the start of the breeding period.

The Cox regression model found similar results as compared to the competing risk model. The Cox regression model found lactation five and greater cows, metritis, and dystocic cows to significantly have a negative effect on time to pregnancy. This model also found lameness to have a significant improvement in time to pregnancy. Since the two models did not find the exact same independent variables to be significant towards the time to pregnancy, the models cannot be directly compared. This is because each variable in a specific model will alter the remaining hazard or sub-hazard ratios in the model. Some generalizations can be made about the two models though. It is interesting to note when the cumulative incidence graphs for lactation group, metritis, and dystocia after being adjusted for the remaining variables in each model are compared between the competing risk model and the Cox regression model, it can be seen at around 200 DIM the cumulative incidences for the Cox regression model are greater than the cumulative incidences for the competing risk model. Suggesting the Cox regression model overestimated the cumulative incidence of all the variables in the model. Also when the variables found to be significant in the Cox regression model are placed into a competing risk model, some of the variables are non-significant and the hazard ratios in the Cox regression model are larger than the reported sub-hazard ratios in the competing risk model. Numerous researchers and scholars have modeled and described how and why a Cox regression model will overestimate the hazard ratios and the cumulative incidence of days to pregnancy [29, 32].

Summary

In summary, there are many factors that are associated with a dairy cow becoming pregnant. This study showed that having metritis, retained placenta, ketosis, dystocia, and other illness events in the first 30 days of the postpartum period were negative factors towards a cow becoming pregnant. This study also showed having twins or a lameness event in the first 30 DIM were positively associated with days to become pregnant. And that lactation number was also associated with days to becoming pregnant. The competing risk analysis model and the Cox regression model showed similar but slightly different results. In conclusion, cows affected with metritis, retained placenta, ketosis, dystocia, and other illness event have an increased cost to the dairy, both in an increase in labor and treatment cost, but also an increased cost of days spent open and ultimately an increase in replacement costs. The dairy industry and veterinary profession needs to continue to work to decrease the incidence of these diseases, to improve reproductive dairy herd performance and profitability.

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