Factors affecting ovulation within three weeks postpartum in dairy cows in Florida

M.M.A.A. Vercouteren BSc, Utrecht University
Established at the University of Florida, Gainesville, 2012
Supervisor: P.L.A.M. Vos DVM, PhD, Dipl ECAR & ECBHM, Associate professor
Supervisor abroad: K.N.A. Galvao DVM, PhD, MPVM, DACT

Table of Contents

Abstract

Virtually, all dairy cows have their first follicular wave within two weeks postpartum. However, only 30 - 50% ovulates within three weeks postpartum. The aim of this study was to evaluate factors affecting ovulation within 21 days in milk (DIM) in dairy cows. Cows (n = 768) from 2 herds had their ovaries scanned by ultrasonography twice a week starting at 17±3 DIM for a total of 4 ultrasound examinations (US). Ovulation was characterized by the presence of a corpus luteum (CL) ≥ 20mm in any US or when a CL <20mm appeared in two consecutive US. If a cow received GnRH on her first US, a CL of ≥22mm in the second US represented ovulation. The following information was collected (for up to 14 DIM): Parity, body condition score at enrollment (BCS), calving problems (dystocia, twins, stillbirths (dead on arrival, DOA), abortions, retained fetal membranes (RFM)), metabolic problems (ketosis or hypocalcaemia), infections (metritis or mastitis), digestive problems (indigestion or displaced abomasum (DA)), lameness, calving season (CS; summer or fall vs. winter or spring), dry period length (DPL; ≤70 or >70d), body weight loss (BWL; ≤28 or >28kg) in the first 14 DIM, and average daily milk yield in the first 14 DIM. Data were analyzed with a univariable and a multivariable analysis. For the multivariable analysis, the GLIMMIX procedure of SAS was used. Three models were constructed: 1 – excluding both DPL (not available for primiparous cows) and BWL (only available for 456 cows); 2 – including DPL; 3 – including BWL. Only variables with a *P*≤0.2 were included in each model. Herd was random. In model 1, cows with metabolic problems (20.7 vs. 33.9%; *P*=0.003), digestive problems (19.4 vs. 32.2%; *P*=0.05), or cows that calved in the winter or spring (23.5 vs. 33.1%; *P*=0.02) had a delay in ovulation within 21 DIM. Dystocia tended to decrease (24.1 vs. 32.9%; *P*=0.06) ovulation. In model 2, cows with metabolic problems (22.0 vs. 38.6%; *P*=0.02) and metritis (17.3 vs. 35.8%; *P*=0.05) had a delay in ovulation early postpartum. Cows with >70d DPL tended to have decreased (23.9 vs. 36.2%; *P*=0.07) ovulation. In model 3, cows with metritis (21.2 vs. 34.7%; *P*=0.03), digestive problems (20.0 vs. 33.4%; *P*=0.05), calved in the winter or spring (24.1 vs. 35.2%; *P*=0.01), or lost >28kg BW (27.7 vs. 38.5%; *P*=0.04) experienced a delay in the resumption of ovarian cyclicity. In conclusion, cows that had metabolic problems, digestive problems, dystocia, metritis, long DPL, calved in the winter or spring or lost > 28kg BW in the first 14 DIM had decreased ovulation within 21 DIM.

1. Introduction

Reproductive failure is one of the greatest economic losses in the dairy industry (De Vries 2006). When fertility can be improved, more cows will be in earlier lactational stages, reducing the average days in milk (DIM) of the whole herd and therefore increasing the average milk yield of a herd. One of the key factors of improving fertility is an earlier resumption of ovarian cyclicity postpartum (Santos, Rutigliano & Sa Filho 2009, Galvao et al. 2010), since calving to conception interval should not exceed 85 days in order to acquire a desirable calving interval of 12 months (Opsomer et al. 2000, Gautam et al. 2010).

Overall, the first postpartum follicular wave commences about two weeks postpartum after a rise in plasma FSH in the first five days after calving (Beam, Butler 1998, Beam, Butler 1997). This first follicular wave can either ovulate, regress and a new wave will be started, or the follicles become cystic (Beam, Butler 1997). If the follicle will ovulate, depends on whether the pulsatile secretion of LH can be established after parturition (Butler 2003). Within 21 DIM, about 30-50% of the cows will ovulate their first wave follicles. Around 20 to 40% of cows remains anovulatory by 50 to 60 DIM (McCoy 2006, Dubuc et al. 2012, Beam, Butler 1998, Galvao et al. 2010). Roughly, 30 DIM is the average day cows ovulate for the first time after parturition (McCoy 2006). Galvao et al. already determined that cows that ovulated from the first follicular wave were more fertile than cows that ovulated later postpartum. Cows that were cyclic by 21±3 DIM (Cyc21) had fewer median days to insemination compared to cows that became cyclic by 49 DIM (Cyc49), or did not ovulate at all in their voluntary waiting period (VWP)(NotCyc) (71, 76 and 96 for Cyc21, Cyc49 and NotCyc, resp.). Cyc21 also had greater conception rate to the first artificial insemination (AI) (38.6% vs. 28.1% (Cyc49, P=0.04) and 38.6% vs. 23.6% (NotCyc, P=0.03)). Furthermore, they had an increased hazard of pregnancy up to 300 DIM (HR=1.52 compared with Cyc49 (P=0.002) and HR=1.98 compared with NotCyc (P<0.001)) and less days to pregnancy (103, 147 and 173 for Cyc21, Cyc49 and NotCyc, resp.). Finally, Cyc21 had less prevalence of subclinical endometritis compared to NotCyc (29.9% vs. 43.7%, P=0.04) (Galvao et al. 2010). More information is known about cows being anovulatory around 50 – 60 days postpartum. Cows not being cyclic around this time postpartum had lower conception and pregnancy rates at the end of the VWP and more days to pregnancy (Dubuc et al. 2012, Gumen, Guenther & Wiltbank 2003, Santos, Rutigliano & Sa Filho 2009).

Based on these facts, the aim of this study was to evaluate factors that can affect early ovarian cyclicity after calving in dairy cows. Research has been done on the resumption of ovarian cyclicity later postpartum, but not on the early resumption in less than 21 DIM. The following factors will be evaluated for their effect on early ovarian resumption: Parity, body condition score at enrollment (BCS), dystocia, twins, stillbirth (DOA), abortion, concurrent diseases in the first fourteen days postpartum (retained fetal membranes (RFM), hypocalcaemia, ketosis, metritis, mastitis, displaced abomasums (DA), indigestion, lameness), calving season, length of the dry period (DPL) and loss of body weight (BWL) and the daily milk production during the first fourteen days postpartum.

2. Materials and Methods

2.1 Animals

A sample size of 768 Holstein cows (280 primiparous and 488 multiparous) was obtained for this study from two different commercial dairy farms in North-Florida, varying in herd size from 450 cows in milk (farm 1) to 4500 cows in milk(farm 2), with rolling herd averages of approximately 10500kg of milk/cow/year. On farm 1, cows are milked and fed TMR (total mixed ratio) twice a day, whereas on the other farm, milking and feeding TMR was performed three times a day. The TMR was formulated to meet or exceed the NRC (2001) nutrient requirements for lactating Holstein cows weighing 680 kg and producing 45 kg of 3.5% fat-corrected milk. All the cows were housed in free stall barns. 461 cows were used from Farm 1 and 307 from Farm 2.

2.2 Study design

Data was collected from October 2010 to October 2012. Cows were enrolled on Tuesday and either Friday or Saturday. At the time of enrollment, cows were 17±3 DIM. The BCS was obtained and ultrasound examinations (US) (Easi-Scan, 4.5 – 8.5 MHz linear transducer, BCF Technology Ltd, Livingston, Scotland) were performed twice a week (Tuesday mornings and Friday afternoons or Saturday mornings) until resumption of ovarian cyclicity, up to a maximum of four consecutive US (27±3 DIM) (Fig. 1).

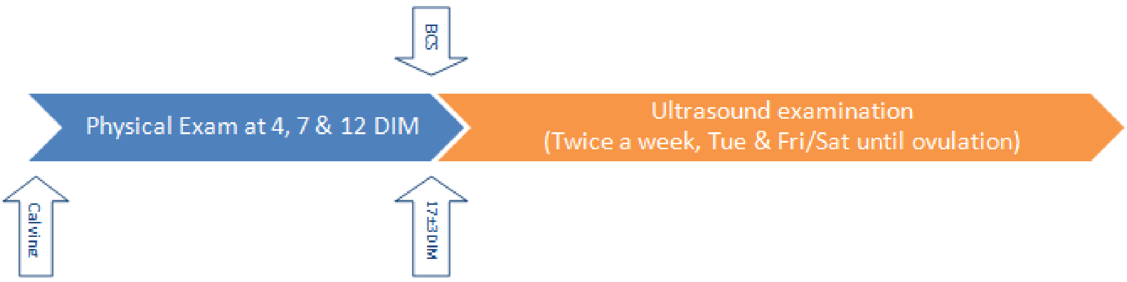

Fig 1. Study design, Farm 1

Cows were part of another clinical trial, in which some cows (n=256) were randomly assigned to receive GnRH on their first US (17±3 DIM) if they did not have a CL at that time. For this study, two definitions for the resumption of early ovarian cyclicity postpartum were made: 1 – for cows that did not receive GnRH on their first US; 2 – for cows that received GnRH. 1:*"The resumption of ovarian cyclicity will require the detection of a CL ≥ 22mm in one US or a CL <22mm in two consecutive US. Resumption was called early if detected ≤21±3DIM."* 2: *"If a cow has a CL of <22mm on her second US (20±3DIM), that ovulation was induced by the injection of GnRH. The detection of a CL ≥ 22mm on the second US means that GnRH had no significant effect and the cow was assigned to have ovulated* *spontaneously within 21±3DIM "*. Since the formation of a CL usually takes about 3 days, a CL could be detected up to 24 DIM (ovulation would still be within 21 DIM). The cutoff point of 22 mm was based on a previous study (Bicalho et al., 2008), in which they found that the specificity and sensitivity for finding a functional CL on an US were optimized using this cutoff diameter.

For the BCS at enrollment cows were scored using a five-point scale with 0.25 point-increments (Ferguson, Galligan & Thomsen 1994) and classified into two groups: Low (BCS ≤2.75) or Medium (BCS ≥3.00). Assessment of the general health of fresh cows after calving was performed by farm personnel during the routine physical examination at days 4, 7, and 12 after calving on farm 1 and at days 4, 6 and 10 on farm 2, according to the farm's Standard Operating Procedures (SOP) (see references). Further information was then obtained from the farm management software program (AfiFarm© (v. 3.076, SAE Afikim Kibbutz Afikim, Israel) for farm 1 and PC Dart© (v. 7.15.0.2, Dairy Records Management Systems, Raleigh, NC) for the other farm). The information consisted of their parity, if cows needed assistance during calving, if they had twins, stillbirths, abortions or concurrent diseases in the first fourteen days postpartum (retained fetal membranes, hypocalcaemia, ketosis, metritis, mastitis, displaced abomasum, indigestion, lameness), the calving season, the length of the dry period and the average daily milk production and the loss of body weight in the first fourteen days postpartum.

Calving difficulty was recorded by the farm personnel as calving-ease scores (CES) according to the National Association of Animal Breeders. Scores ranged from 1 to 5. 1: no assistance needed, 2: Slight problem, 3: Needed assistance, 4: considerable force, 5: Caesarian section or foetotomy. A score of 3 or more was listed as 'dystocia'. Cows were checked for RFM (fetal membranes that retained longer than 24 hours) within the first twelve hours after calving, on 24 hours after calving, and according to the fresh cow checks mentioned before. If affected, cows were treated with antibiotics recommended by a veterinarian. Hypocalcaemia was treated based on clinical signs (down cow or cow unsteady prior to calving to 1 or 2 days after calving and no other abnormal physical exam findings).They were treated with Calcium injectable product IV or with Calcium gel orally. Keton bodies were measured at every fresh cow check, using a urine test strip (Ketostix ®, Bayer). The treatment for ketosis was Vitamin B Complex (10-20mL) and Dextrose (500mL) intravenously and five to seven gallons of Fresh Cow Drench (FCD) orally if ketosis was moderate. If ketosis was mild, 500mL of propylene glycol was administered. Cows with severe or moderate ketosis on the third day were treated as recommended by a veterinarian. Metritis was characterized by red to brown watery fetid uterine discharge with or without the presence of fever. Cows with systemic illness were treated with antibiotic and anti-inflammatory drugs as recommended by a veterinarian and only these cows, with systemic illness, were included in the study. In case of a toxic mastitis (hot, swollen, hard and painful quarter with serous or watery secretion, signs of systemic illness), 2 ml of Oxytocin was administered and quarters were hand stripped at milking. Fluid therapy (1-2 liters of hypertonic saline IV, followed with 5-10 gallons of water mixed with fresh cow drench), antibiotics and non-steroidal treatment were given as indicated by a veterinarian. When the mastitis was non-toxic (clumps in the milk, cow was not sick), cows were treated with intramammary antibiotics. Any displaced abomasum was surgically corrected by a veterinarian. Cows with indigestion (milk deviation, diarrhea, less rumen motility and sometimes bloat) were treated with 2-4 MagOx boluses PO for a total of 2 treatments. Severely lame cows were considered a veterinary emergency. Cows were treated with non-steroidal anti-inflammatory drugs and a vet was scheduled for the next day, unless the foot trimmer was already scheduled for the next day. Moderately lame cows were administered pain management and seen by a vet as soon as possible. Specific treatment was dependent on the condition found (see SOP). The operation procedures described above were developed for Farm 1. Information provided by Farm 2 was not as detailed as for Farm 1, but according to PC Dart©, cows were treated quite similarly if necessary. The calving season was either categorized as spring (March through May), summer (June through August), fall (September through November) or winter (December through February). The body weight was measured by a weight scale in the sorting gate after cows were being milked in the milking parlor in Farm 1.

2.3 Statistical analyses

Additional data (e.g. disease history, milk yield, length of the dry period) were collected using the farm software program. Microsoft Excel (Microsoft Corporation, Richmond, WA) was used to organize all the information. The outcome variable was ovulation within 21 DIM . For the cows that received GnRH this meant that a CL ≥22mm at 21 ± 3 DIM (US2) needed to be found. For the cows that did not receive GnRH a CL ≥22mm within 21 ± 3 DIM or a CL < 22mm in two consecutive US needed to be found. Categorical data were first analyzed by a univariable Chi-Square analysis using the FREQ procedure of SAS (Version 9.3, SAS institute Inc., Cary, NC) (Table 1). 'Dry period length' and 'Calving season' were dichotomized because they had the same trend. That means, for Calving season, 'Summer' was combined with 'Fall' (SumFall) and 'Winter' with 'Spring' (WinSpri). For the length of the dry period, 'Short (<40 days)' and 'Medium (40 -70 days)' were combined into 'Adequate (<70 days)' to compare it with 'Long (>70 days)'. 'Hypocalcaemia' was combined with 'Ketosis' because both variables had the same trend, and the variable was called 'Metabolic problem'. Cows had a metabolic problem if they had hypocalcaemia or ketosis or both. The same applied for 'Displaced abomasum' and 'Indigestion' (these two factors were combined into 'Digestive problem'), for 'Metritis' and 'Mastitis' (these two factors were combined into 'Infection'), and for 'Dystocia', 'Twins', 'DOA', 'Abortion' and 'RFM' (these factors were combined into 'Calving problem'). Moreover, the factors were also combined to see if they would be significant if combined.

For the body weight loss class (BWLC), cows were classified into the variables ‘Lost’ (lost more than 28 kg during the first fourteen DIM) or ‘Kept’ (gained weight or lost 28 kg or less during the first fourteen DIM). This was based on a paper by Feguson, 2006, in which he states that losing 56 kilograms of bodyweight can be compared with losing one unit of BCS. Losing one unit of BCS had a significant effect on ovulation in previous studies (Santos, Rutigliano & Sa Filho 2009) . Average milk yield during the first fourteen days postpartum was classified as ‘Below’ or ‘Above’ average within parity. The average milk yield during the first 14 days postpartum was 31 kg for multiparous cows and 21 kg for primiparous cows.

Multivariable analyses were performed by mixed logistic regression analyses using the GLIMMIX procedure of SAS. Three models were constructed: Model 1 – excluding both DPL (not available for primiparous cows) and BWL (only available for cows from farm 1). Therefore, all cows were included; Model 2 – including DPL and excluding BWL. Hence, only multiparous cows were included in Model 2; Model 3 – including BWL. Since the body weight could only be measured at Farm 1, only animals form this farm were included in the last model. Only variables with *P* ≤0.2 (see Table 1) were included in each model; BCS class (BCSC, Low or Medium), Dystocia (Yes or No), Metabolic problem (Yes or No), Metritis (Yes or No), Digestive problem (Yes or No,), Calving season (SumFall or WinSpri), Dry period length (DPL, Adequate or Long) and Body weight loss class (BWLC, Lost or Kept). Metritis was included separate because only metritis was significant. A manual backward elimination was performed, and variables were removed from the model when the p-value was >0.10. Differences with a P-value ≤ 0.05 were considered statistically significant, and a 0.05 < P ≤ 0.10 was considered a tendency towards statistical difference.

A correlation spearman measure was performed, using the CORR procedure of SAS, to look for correlations between the significant variables from the GLIMMIX models.

3. Results

3.1. Univariable Chi-square analysis

Of all 768 cows, 31.0% (n=238) ovulated within 21 DIM. When a univariable Chi-square analysis is performed, dystocia, ketosis, metritis, displaced abomasum (DA), calving in winter or spring, a long dry period and loss of more than 28 kg of body weight had a significantly negative effect on early ovulation postpartum (Table 1). Indigestion tended to affect ovulation. No significant effect of parity, body condition score, twins, stillbirths, abortions, RFM, hypocalcaemia, mastitis, lameness, or the milk yield was found.

The prevalence of dystocia in this study was 21.6%. Off all cows having dystocia (n=166), 24.1% ovulated within 21 DIM. Cows that did not have difficulties with calving (n=602) were 1.54 times more likely to ovulate within 21 DIM (*P*=0.03, 32.9% ovulated). Cows without ketosis (n=607, 79.0%) were 1.81 times more likely (*P*=0.004) to ovulate (33.4%) within 21 DIM than cows that did have a ketosis (n=161, 21.0%) (21.7% ovulated). 131 cows (17.1%) had a metritis, of which 22.9% ovulated. This was significantly less than the 32.7% of cows that ovulated out of all the cows that did not have a metritis (n=637) (*P*=0.03). Only ten cows in this study had a DA in the first fourteen days postpartum. None of them ovulated within 21 DIM, which was significant (*P*=0.03). 31.4% of the cows that did not have a DA (n=758) ovulated. 21.1% (n=162) of all cows calved in Fall, 9.9% (n=76) in Winter, 12.2% (n=94) in Spring and 56.8% (n=436) in Summer. Cows calving in summer or fall (n=598, 77.9%) were 1.6 times more likely (*P*=0.02) to ovulate (33.1%) than cows calving in winter or spring (n=170, 22.1%)(23.5% ovulated). Furthermore, a long dry period of more than 70 days significantly affected resumption of ovarian cyclicity postpartum (*P*=0.03). Only 23.9% of these cows (n=92) ovulated, compared to 36.2% of the cows that were dried off 70 days or less (n=351) before parturition. Finally, body weight could be measured for 456 cows (Farm 1). 156 of these cows (34.2%) kept their body weight or lost only up to 28kg. They were 1.63 times more likely to ovulate (38.5%, *P*=0.02) than the cows that lost 28 kg or more in the first fourteen days postpartum (n=300, 65.8%), of which 27.7% ovulated. When combined as a variable, metabolic problems, infection and digestive problems impaired cows of ovulating early postpartum, whereas calving problems did not. Having a metabolic problem was more significant (*P*=0.001) to negatively affect ovulation than a digestive problem (*P*=0.03) or an infection (*P*=0.04). The combined factor 'Calving problem' was not significant (*P*=0.11). When variables needed to be included in the multivariate analysis, 'Metabolic problem' and 'Digestive problem' were analyzed as a combined variable because the significance of the variable 'Metabolic problem' was derived from both the variables 'Ketosis' and 'Hypocalcaemia' and so did 'Displaced abomasum' and 'Indigestion' for 'Digestive problem'. Out of all the variables that 'Calving problem consisted of, only 'Dystocia' was significant and so was 'Metritis' for 'Infection'.

Table 1. Descriptive statistics and outcomes of the univariable Chi-square analysis. Odds ratios for the resumption of ovarian cyclicity within 21 DIM for 768 cows. Differences with a P-value ≤ 0.05 were considered statistically significant, and a 0.05 < P ≤ 0.10 was considered a tendency towards statistical difference.

Variable	% Ovulated <21 DIM (Number of cows)	OR	*P*-Value
Parity			
Primiparous	30.4 (85/280)	Referent	0.77
Multiparous	31.4 (153/488)	1.05	
BCSC			
Low	27.2 (50/184)	Referent	0.20
Medium	32.2 (188/584)	1.27	
Dystocia			
Yes	24.1 (40/166)	Referent	0.03
No	32.9(198/602)	1.54	
Twins			
Yes	33.3 (18/54)	Referent	0.70
No	30.8 (220/714)	0.89	
DOA			
Yes	35.4 (23/65)	Referent	0.43
No	30.6 (215/703)	0.80	
Abortion			
Yes	22.2 (2/9)	Referent	0.57
No	31.1 (236/759)	1.58	
RFM			
Yes	23.8 (5/21)	Referent	0.47
No	31.2 (233/747)	1.45	
Calving problem			
Yes	26.8 (60/224)	Referent	0.11
No	32.7 (178/544)	1.33	
Hypocalcaemia			
Yes	12.5 (2/16)	Referent	0.11
No	31.4 (236/752)	3.20	
Ketosis			
Yes	21.7 (35/161)	Referent	0.004
No	33.4 (203/607)	1.81	
Metabolic problem			
Yes	20.7 (35/169)	Referent	0.001
No	33.9 (203/599)	1.96	
Metritis			
Yes	22.9 (30/131)	Referent	0.03
No	32.7 (208/637)	1.63	
Mastitis			
Yes	32.1 (17/53)	Referent	0.86
No	30.9 (221/715)	0.95	
Infection			
Yes	24.6 (42/171)	Referent	0.04
No	32.8 (196/597)	1.50	
Displaced abomasum			
Yes	00.0 (0/10)	-	0.03
No	31.4 (238/758)		
Indigestion			
Yes	21.2 (14/66)	Referent	0.07
No	31.9 (224/702)	1.74	
Digestive problem			
Yes	19.4 (14/72)	Referent	0.03
No	32.2 (224/696)	1.97	
Lameness			
Yes	27.3 (6/22)	Referent	0.70
No	31.1 (232/746)	1.20	

Calving season			
Spring	24.5 (23/94)	1.12	0.75
Summer	31.9 (139/436)	1.62	0.10
Fall	36.4 (59/162)	1.99	0.03
Winter	22.4 (17/76)	Referent	
WinSpri	23.5 (40/170)	Referent	0.02
SumFall	33.1 (198/598)	1.61	
Dry period length			
0-39	42.9 (12/28)	2.39	0.05
40-70	35.6 (115/323)	1.76	0.04
>70	23.9 (22/92)	Referent	
Long (>70d)	23.9 (22/92)	Referent	0.03
Adequate (≤70d)	36.2 (127/351)	1.80	
Body weight loss class			
Lost	27.7 (83/300)	Referent	0.02
Kept	38.5 (60/156)	1.63	
Milk yield			
Below Average	28.7 (98/342)	Referent	0.21
Above Average	32.9 (140/426)	1.22	

3.2. Multivariable GLIMMIX models

3.2.1. First GLIMMIX model (all cows, body weight and length of the dry period excluded)

Table 2a. Outcome of the first logistic regression model. All variables were included except for DPL and BWL. Of all cows in this model (n=768), 31.0% ovulated. Risk factors for anovulation early postpartum were metabolic problems (P=0.003), digestive problems (P=0.05) and calving in winter or spring (P=0.02). Herd was random. OR = odds ratio, CI = confidence interval.

Variable	Level	n	Ovulated within 21 DIM, %	OR	95% CI	*P*-value
Metabolic problem	No	599	33.9	1.9	1.2 – 2.9	0.003
	Yes	169	20.7	-		
Digestive problem	No	696	32.2	1.9	1.0 – 3.5	0.05
	Yes	72	19.4	-		
Calving Season	SumFall	598	33.1	1.6	1.1 – 2.5	0.02
	WinSpri	170	23.5	-		
Dystocia	No	602	32.9	1.5	1.0 – 2.2	0.06
	Yes	166	24.1	-		

When all cows (n=768) were included in the mixed logistic regression analysis, metabolic problems, digestive problems and calving in winter or spring had a significant negative effect on early ovulation postpartum (Table 2a). Cows without a metabolic problem (n=599, 78.0%) were 1.9 times more likely (*P*=0.003, 95%CI: 1.2 – 2.9) to ovulate (33.9%) within 21 DIM than cows that had ketosis, hypocalcaemia or both (n=169, 22.0%) (20.7% ovulated). The incidence of digestive problems was lower than for metabolic problems (n=72, 9.4%). Only 19.4% of these cows ovulated; significantly less

(*P*=0.05, OR: 1.9, 95%CI: 1.0 – 3.5) than the 32.2% of the cows that did not have a DA, indigestion or both. Cows calving in summer or fall (n=598, 77.9%) were 1.6 times more likely (*P*=0.02, 95%CI: 1.1 – 2.5) to ovulate (33.1%) than cows calving in winter or spring (n=170, 22.1%)(23.5% ovulated). Dystocia tended to have a significant negative effect on ovulation within 21 DIM (*P*=0.06).

Variables with a *P* ≤0.2 (see Table 1) were included in the multivariable analyses; BCS class (BCSC, Low or Medium), Dystocia (Yes or No), Metabolic problem (Yes or No), Metritis (Yes or No), Digestive problem (Yes or No,), Calving season (SumFall or WinSpri), Dry period length (DPL, Adequate or Long) and Body weight loss class (BWLC, Lost or Kept). A manual backward elimination was performed, and variables were removed from the model when the *P*-value was >0.10.

3.2.2. Second GLIMMIX model (length of the dry period included, body weight loss excluded)

Table 2b. Outcome of the second logistic regression model. DPL was included as a variable and BWL was excluded. Of all cows in this model (n=443), 33.6% ovulated. Factors that affected the first ovulation postpartum were metabolic problems (P=0.02) and metritis (P=0.05). The length of the dry period tended to have a negative effect on the first ovulation postpartum. Herd was random. OR = odds ratio, CI = confidence interval.

Variable	Level	n	Ovulated within 21 DIM, %	OR	95% CI	*P*-value
Metabolic problem	No	311	38.6	1.8	1.1 – 3.0	0.02
	Yes	132	22.0	-		
Metritis	No	391	35.8	2.2	1.0 – 4.7	0.05
	Yes	52	17.3	-		
DPL	≤70	351	36.2	1.7	1.0 – 2.8	0.07
	>70	92	23.9	-		

488 Multiparous cows were included in this study, but data for the length of the dry period was only available for 443 cows. Therefore, 443 multiparous cows were included in the second GLIMMIX model (Table 2b). 33.6% of them ovulated within 21 DIM. Metabolic problems and metritis significantly affected early ovulation postpartum in this group of cows. The incidence of metabolic problems was higher (n=132, 29.8%) for this group of multiparous cows than for the total group of cows (n=169, 22%). Cows without a metabolic problem (n=311, 70.2%) were 1.8 times more likely (*P*=0.02, 95%CI: 1.1 – 3.0) to ovulate (38.6%) within 21 DIM than cows that had ketosis, hypocalcaemia or both (22.0% ovulated). Metritis occurred in 11.7% of the cows in this group (n=52). Only 17.3% of them ovulated. Cows that did not have a metritis were 2.2 times more likely (*P*=0.05, 95%CI: 1.0 – 4.7) to ovulate: 35.8% out of 391 cows ovulated within 21 DIM. A long dry period (>70 days) tended to have a negative effect (*P*=0.07, OR: 1.7, 95%CI: 1.0 – 2.8).

3.2.3. Third GLIMMIX model (body weight loss included, length of the dry period excluded)

<u>Table 2c.</u> Outcome of the final logistic regression model. BWL was included as a variable and DPL was excluded. Of all cows in this model (n=456), 31.4% ovulated. Factors that affected the first ovulation postpartum were metritis (P=0.03), digestive problems (P=0.05), calving in winter or spring (P=0.01) and the loss of more than 28kg of body weight. Herd was random. OR = odds ratio, CI = confidence interval.

Variable	Level	n	Ovulated within 21 DIM, %	OR	95% CI	*P*-value
Metritis	No	343	34.7	1.8	1.1 – 3.0	0.03
	Yes	113	21.2	-		
Digestive problem	No	386	33.4	1.9	1.0 – 3.5	0.05
	Yes	70	20.0	-		
Calving Season	SumFall	298	35.2	1.8	1.1 – 2.8	0.01
	WinSpri	158	24.1	-		
BWL	Kept	156	38.5	1.6	1.0 – 2.4	0.04
	Lost	300	27.7	-		

In the last model, the length of the dry period was left out and body weight loss was included, leaving only cows from Farm 1 (n=456) (Table 2c). On this farm, 31.4% of the cows ovulated within 21 DIM, which resembles the percentage of the whole study. Metritis, digestive problems, calving in winter or spring and losing more than 28kg in the first fourteen days postpartum significantly affected early ovulation postpartum. The incidence of metritis was 24.8%. 21.1% of 113 cows ovulated, compared to 34.7% of the 343 cows (75.2%) that did not have a metritis. Cows without a metritis were 1.8 times more likely to ovulate (*P*=0.06, 95%CI: 1.1 – 3.0). Cows without a digestive problem (n=386, 84.6%) were 1.9 times more likely (*P*=0.05, 95%CI: 1.0 – 3.5) to ovulate (33.4%) within 21 DIM than cows that had a DA, indigestion or both (n=70, 15.4%) (20.0% ovulated). 35% of the cows on this farm calved in winter or spring. Only 24.1% of these cows ovulated; significantly less (*P*=0.01, OR: 1.8, 95%CI: 1.1 – 2.8) than the 35.2% of the cows that ovulated from the cows that calved in summer or fall (n=158, 65.4%). Finally, cows that kept their body weight or lost less than 28 kg (n=156, 34.2%) were 1.6 times more likely to ovulate (38.5%, *P*=0.04, 95%CI: 1.0 – 2.4) than cows that lost 28 kg or more in the first fourteen days postpartum (n=300, 65.8%), of which 27.7% ovulated.

Overall, cows that had metabolic problems, digestive problems, dystocia, metritis, a long dry period, calved in the winter or spring or lost > 28kg BW in the first 14 DIM experienced a delay in ovarian cyclicity within 21 DIM.

3.3. Spearman correlation

Table 3. Spearman correlation coefficients. H_0: ρ=0, n=768. Values indicate the correlation coefficient (r). Metabolic problems were associated with metritis (P<0.0001) and digestive problems (P<0.0001). Dystocia was associated with metritis (P=0.0002). A negative association was found between dystocia and digestive problems (P=0.01). Metritis was associated with digestive problems (P =0.01).

	Metabolic problem	**Dystocia**	**Metritis**	**Digestive problem**
Metabolic problem	1	0.042 (*P*=0.25)	0.194 (*P*<0.0001)	0.153 (*P*<0.0001)
Dystocia		1	0.132 (*P*=0.0002)	-0.093 (*P*=0.01)
Metritis			1	0.092 (*P*=0.01)

The correlation coefficient of factors that were significant in the multivariable analyses were measured with a spearman correlation measure (Table 3). According to this, metabolic problems were associated with metritis (*r*=0.194, *P*<0.0001) and digestive problems (*r*=0.153, *P*<0.0001). Dystocia was associated with metritis (*r*=0.132, *P*=0.0002), and there was a negative association between dystocia and digestive problems (*r*=-0.093, *P*=0.01). Finally, metritis was associated with digestive problems (*r*=0.092, *P* =0.01).

4. Discussion

In this study, 31% of all cows ovulated within 21 DIM. This shows similarity with the results from previous studies (McCoy 2006, Beam, Butler 1998, Galvao et al. 2010).

Dystocia

Dystocia had a significant effect in the univariable analysis (*P*=0.03) but only tended to have an effect when the first logistic regression analysis was performed (*P*=0.06)(Table 1 and 2a). To our knowledge, no other research has been done on the effect of dystocia on resumption of cyclicity within 21 DIM. Opsomer et al. found that cows that needed assistance (from more than one person) during calving were 3.6 times more likely to develop a delay in ovarian activity postpartum (not cyclic by 50 DIM) (Opsomer et al. 2000). Bicalho et al. measured a hazard ratio of 0.84 (*P*<0.005) for assisted calving against no difficulties with calving on time to conception (Bicalho et al. 2007b). In another study, they reported that assisted calving led to a hazard ratio of 0.72 (*P* <0.001) for being detected as pregnant (Bicalho et al. 2007a). Garbarino et al. could not find any significant difference in their study (Garbarino et al. 2004). Dystocia was significantly correlated with metritis in this study (*r*=0.132, *P* =0.0002) and was listed as a risk factor for metritis by Sheldon et al. (Sheldon, Dobson 2004). Metritis did have a significant effect on early ovulation in this study, which might therefore be an explanation for the negative effect of dystocia on resumption of ovarian cyclicity postpartum.

Metritis

Uterine diseases postpartum such as metritis are highly prevalent in high-producing dairy cows. Metritis affects approximately 20% of lactating dairy cows, with the incidence ranging from around 10% to more than 40% in some farms (Goshen, Shpigel 2006, Dubuc et al. 2010, Benzaquen et al. 2007, Markusfeld 1987, Zwald et al. 2004, Martinez et al. 2012). In cows with risk factors such as dystocia, delivery of twin calves, retained placenta (RP), or stillbirth, an incidence of 50% was found (Goshen, Shpigel 2006). A recent study at the University of Florida observed that the incidence of metritis in this high risk cows was as high as 67% (Martinez et al. 2012). The overall incidence of metritis in our study was 17%. For multiparous cows, the incidence of metritis was slightly lower: 12%. This shows similarity with the results from a study by Goshen et al., in which they reported a higher incidence of metritis for heifers (30%) compared to multiparous cows (18.6%) too (Goshen, Shpigel 2006). Metritis had a significant effect on ovulation within 21 DIM for multiparous cows and for cows on Farm 1 (Table 2b and 2c). 17.3% of the multiparous cows that had a metritis ovulated, compared to 35.8% of the multiparous cows that did not have a metritis (*P*=0.05, OR=2.2). From the cows investigated on Farm 1, a higher percentage suffered from metritis (25%). 21.1% of them ovulated, compared to 34,7% of the cows not having metritis (*P*=0.06, OR=1.8). From our study we can conclude that metritis also significantly influences early ovulation postpartum.

Substantial research has been done on the effect of endometritis on fertility in dairy cows, but not as much as on the effect of metritis (inflammation of the uterus within 21 DIM). Especially about the effect of metritis on early resumption of ovarian cyclicity, limited research is done. Based on progesterone levels, cows that had a metritis were eleven times more likely to have prolonged luteal cycles than cows that did not (*P*<0.001)as observed in a field study in Belgium (Opsomer et al. 2000). Moreover, cows that had abnormal vaginal discharge during their puerperium were 4.5 times more likely to have a delayed ovulation (*P*<0.001) (Opsomer et al. 2000). Delayed cyclicity was formulated in their study as constantly low progesterone levels during the first 50 days postpartum and cows were assigned having a prolonged luteal phase if progesterone levels were elevated for more than 20 days. Garbarino et al. evaluated the effect of metritis on delayed cyclicity (low progesterone concentrations <60 DIM) but it was not significant (*P* =0.73, OR: 1.15) (Garbarino et al. 2004). Another quite new indicator of metritis in cows is the measurement of the concentration of haptoglobin, an acute phase protein, in serum (Huzzey et al. 2009, Dubuc et al. 2010). Increased haptoglobin levels during the first week postpartum (≥0.8g/L) were to be associated with purulent discharge within 20 DIM (OR 2.17, *P*<0.01) (Dubuc2010b). When haptoglobin concentrations were measured 0.3g/L or above in another study of Dubuc et al., the cows were 1.6 times more at risk of a prolonged postpartum anovulation (progesterone levels not exceeding 1ng/L within 63 DIM) (Dubuc et al. 2012). No studies have evaluated the effect of metritis on resumption of ovarian cyclicity within 21 DIM.

Numerous studies have been done on the influence of inflammation on follicular development and function postpartum (e.g. (Sheldon et al. 2002)). Lipopolysaccharides from bacteria such as *E.Coli* suppress the release of LH by the pituitary (Sheldon et al. 2009), which in turn leads to a smaller diameter of postpartum ovarian dominant follicles and as a consequence resulting in lower plasma estradiol levels. These smaller follicles result in smaller corpora lutea that produce less progesterone than normal corpora lutea (Williams et al. 2007, Sheldon et al. 2002). Moreover, luteolysis is impaired because LPS switches the secretion of PGF to PGE by endometrial cells (Sheldon et al. 2009). Peripheral blood neutrophil (PMN) function is also significantly (*P* <0.05) impaired in cows diagnosed with metritis within two weeks postpartum (Hammon 2006). For a detailed description of all the cytokines, chemokines, receptors and cells influenced by uterine infections, see the review of Sheldon et al, 2009 (Sheldon et al. 2009).

Metabolic problems

Ketosis

A metabolic problem, especially ketosis, was the most significant factor in this study to impair early resumption of ovarian cyclicity within 21 DIM. This shows similarity with results from previous studies. In 1998, researchers also concluded that clinical symptoms of ketosis led to cows that were eleven times more likely (P<0.01) to develop a delay in their ovarian function (constantly low progesterone levels (<1ng/mL)) during the first 50 days postpartum (Opsomer et al. 2000). In a study of Garbarino, ketosis led to a delay in ovarian cyclicity as well. Affected cows where 2.6 times more at risk (P =0.03) of having a delayed cyclicity (when progesterone levels were consistently low during the first 60 days postpartum) (Garbarino et al. 2004).

With the rising milk yield over the last decades, fertility has considerably declined (Butler 2003, Pryce 2004, Butler, Smith 1989). Nutritional requirements rise immediately after calving when milk yield increases. Dry matter intake (DMI) is mostly not sufficient enough to keep up with the high demanding milk yield, leading to a negative energy balance (NEB) postpartum in almost every cow (Butler 2003, Butler, Smith 1989). The mobilization of fat as an energy source usually starts a few days before calving and is at its worst around 2 weeks later (nadir) (Butler, Smith 1989, Jorritsma et al. 2003). The more severe the NEB postpartum and the longer the interval to its nadir, the higher the risk of anovulation postpartum (Beam, Butler 1997, Beam, Butler 1998, Opsomer et al. 2000, Garbarino et al. 2004). A NEB has a negative impact on the establishment of the LH-surge and makes the ovaries less responsive to LH (Butler 2003). Furthermore, serum glucose, insulin and IGF-1 concentrations are lower in case of a NEB. Glucose and insulin usually stimulate follicles and upregulate the expression of LH receptors on granulose cells (Butler 2003). IGF-1 stimulates granulosa cells to produce estradiol, increases the number of sites for LH to bind and increases the production of progesterone produced by the CL after ovulation (Beam, Butler 1998). Serum IGF-1 concentrations were higher in cows that ovulated their dominant ovarian follicles from their first follicular wave compared to cows that did not ovulate (Beam, Butler 1998). Cows with ovulatory follicles during the first follicular wave postpartum had significantly less days to the energy balance nadir than cows with nonovulatory follicles (P<0.01) (Beam, Butler 1998).

The effect of elevated β-hydroxybutyrate (BHBA) concentrations (a way of diagnosing ketosis) on other measurements of reproductive performance than ovulation postpartum has been investigated as well (Walsh et al. 2007a). The median time to pregnancy increases when BHBA concentrations are elevated during the first two weeks postpartum: a median days to pregnancy of 124 and 130 days when elevated in one of the weeks or in both the first two weeks postpartum respectively, compared to a 108 days to pregnancy for normal cows. Cows that did not have BHBA levels above the subclinical ketosis threshold were two times more likely to get pregnant from the first service (Walsh et al. 2007b).

Hypocalcemia (milk fever)

Unfortunately, hypocalcemia was not significant as a factor that could affect resumption of early ovarian cyclicity in the univariable analysis (*P*=0.11) (Table 1). As a combined factor, it was found significant in the first two GLIMMIX models, with *P*=0.003 and *P*=0.02 for the first and the second model respectively (Table 2a and 2b). In a study of Risco et al., hypocalcaemic cows also had a longer interval to their first ovulation postpartum than cows without milk fever (30.8±3.1 days vs. 20.4±3.3 days, respectively (*P*<0.05)) (Risco et al. 1994). To our knowledge, no other studies have evaluated the effect of hypocalcaemia on early ovulation postpartum.

In this research we found a correlation between metabolic problems (either hypocalcemia, ketosis or both) and metritis. Although the correlation was not strong (r=0.194, r^2=0.038); it was highly significant (*P*<0.0001) (Table 3). A few other studies have also found a correlation between these two factors (Markusfeld 1987, Risco et al. 1994, Benzaquen et al. 2007, Duffield et al. 2009, Martinez et al. 2012). Martinez et al. defined a serum concentration of ≤8.59 mg/dL in at least 1 sample in the first 3 DIM (when collected every day) as subclinical hypocalcaemia (SCH) using a receiver operator characteristic curve. They found that the concentration of neutrophils was lower and their function was impaired in cows with SCH. This could be an explanation for the correlation between having hypocalcaemia and the risk of developing metritis, since hypocalcaemic cows were 3.24 times more at risk (*P*<0.01) of developing metritis than normal cows. They also determined that with every 1 mg/dL increase in serum calcium, the relative risk of developing metritis was reduced by 22%. Moreover, cows with serum calcium below ≤8.59 mg/dL had higher serum levels of nonesterified fatty acids (NEFA) and BHBA compared to normocalcaemic cows. In contrast to our study, they did not find any effect of SCH on ovulation (by 38 DIM), only a longer interval to pregnancy (Martinez et al. 2012). In Risco's study mentioned before, the uterine horns of cows having milk fever had a greater diameter during days 15 to 32 postpartum than control cows (*P*<0.05)(Risco et al. 1994), suggesting a correlation between hypocalcaemia and metritis.

Another correlation we found in this study was the one between metabolic problems and digestive problems (DA, indigestion or both) (r=0.153, r^2=0.023, *P*<0.0001). This finding is in agreement with previous studies (Chapinal et al. 2011, Massey et al. 1993). In a study by Chapinal et al., serum calcium levels of ≤2.2 mmol/L were associated with a DA (Chapinal et al. 2011). Hypocalcaemic cows were 4.9 times more at risk of developing a DA in Massey's research (Massey et al. 1993). One explanation is a reduced number of contractions of the abomasum due to lower serum calcium levels (Daniel 1983). Chapinal and her colleagues could not find any correlation between hypocalcaemia and metritis.

Extensive research has been done about the association between ketosis and metritis and the association of ketosis and digestive problems (Duffield et al. 2009, Chapinal et al. 2011, Dubuc et al. 2010). Elevated BHBA levels in the first week postpartum were correlated with a higher risk of metritis (OR = 3.35, 95%CI: 1.55 - 7.24) and a DA abomasum (OR = 2.60, 95% CI: 1.25 - 5.21) (Duffield et al. 2009). Cows with a metritis had less DMI ($P<0.001$) and higher serum NEFA ($P<0.01$) and BHBA ($P<0.05$) levels postpartum in a study by Hammon et al (Hammon 2006). Like with hypocalcaemia, Chapinal et al. could not find any correlation between ketosis and metritis, but they did find that elevated pre- and postcalving serum NEFA levels were associated with having a DA (Chapinal et al. 2011). Dubuc et al. could neither find any significant association between elevated non esterified fatty acids (NEFA, an indicator for a NEB) and BHBA levels postpartum and metritis. Only cows with higher NEFA concentrations prepartum (≥0.6 mmol/L) were 1.6 times more likely to develop metritis (Dubuc et al. 2010). An impairment of neutrophil function ($P<0.01$) in cows with a NEB could be an explanation of the correlation between ketosis (subclinical or clinical) and a metritis (Hammon 2006).

Digestive problems

The overall incidence of digestive problems (DA, indigestion or both) in this study was 9.4%: 1.3% of all cows had a DA, 8.6% a digestive problem and 0.5% had both. Digestive problems significantly affected the time of first ovulation postpartum in the first (P=0.05, OR: 1.9, 95%CI: 1.0 – 3.5) and the third (P=0.05, OR: 1.9, 95%CI: 1.0 – 3.5) GLIMMIX model (Table 2a and 2c). Walsh found an incidence of a DA of 3.1% in his study with 1341 cows. Cows that had a DA were 2.3 times more likely to be anovulatory by 46 and 60±7 DIM ($P<0.01$), based on two milk progesterone samples (Walsh et al. 2007a). To our knowledge, no more research has been done on the effect of digestive problems on (early) ovulation postpartum. Cows that had a surgical intervention to correct the left DA had more days from calving to insemination and from calving to conception ($P<0.01$) as shown in a study with 100 cows in the UK (Pedersen 2006). Further research needs to be done to explain the effect of digestive problem on ovulation postpartum. From our study, we can only conclude digestive problems are correlated to metabolic problems postpartum (r=0.153, r^2=0.023, $P<0.0001$) (Table 3).

Calving season

Previous studies showed that cows that calved in summer or fall were more likely to ovulate later postpartum than cows that calved in the cold season (Hansen, P J Hauser,E R. 1983, Opsomer et al. 2000, Walsh et al. 2007a, Santos, Rutigliano & Sa Filho 2009, Kim, Ill Jeong,Jae Kang, Hyun 2012, Dubuc et al. 2012). In 1984, Peters at al. reviewed that cows calving in winter or spring had a longer interval to the first estrus postpartum (Peters 1984). Dubuc et al. determined that from cows calving in summer, the highest percentage was ovulating early postpartum and the least percentage was anovulatory by 63 DIM (OR=0.42, $P<0.01$) (Dubuc et al. 2012). Santos et al. also found that cows calving in summer had more chance ($P<0.0001$) to have ovulated by 65 days postpartum than cows that calved in winter or spring (Santos, Rutigliano & Sa Filho 2009). Opsomer et al. stated that cows calving in winter were at risk for a delay in ovarian cyclicity ($P<0.0001$) (Opsomer et al. 2000). Their results were in agreement with the results from Hansen et al.: in that study they also determined that the interval from calving to first estrus was prolonged ($P<0.01$) for cows calving in winter (Hansen, P J Hauser,E R. 1983). Moreover, in a study by Walsh et al., cows calving in fall were most likely ($P<0.05$) to ovulate at 46 and 60±7 DIM (Walsh et al. 2007a); according to Kim et al., cows calving in fall were 2.32 times more likely ($P<0.003$) to ovulate within three to five weeks postpartum (Kim, Ill Jeong,Jae Kang, Hyun 2012). Finally, cows calving in summer (HR = 1.03, $P=0.018$) and fall (HR = 1.04, $P=0.001$) were more likely to be pregnant within 100 DIM than cows calving in spring according to Pinedo et al. (Pinedo, Risco & Melendez 2011). With our study, we confirmed that the first ovulation postpartum is also positively affected by calving in summer or fall. Cows that calved in summer or fall were 1.6 times more likely ($P=0.02$) to ovulate within 21 DIM (33.1% ovulated) than cows calving in winter or spring (23.5% ovulated) (Table 2a and 2c).

It is remarkable that summer also seems to have a positive effect on fertility in Florida, since the weather in Florida can be extreme, reaching over a 30 degrees Celsius (with an annual average of 20 – 24 degrees) and a humidity around 90% in summer (annual average of >78%) (Thatcher 1974). 'Heat stress' namely, has been known to affect production and reproduction in dairy cattle in the Southern parts of the U.S. as well. Indeed, the South (e.g. Florida) and West part (e.g. California) of the U.S. have the longest calving intervals. Remarkably, the Southern region has the highest conception rates after the first service. Apparently this is because they have the highest percentage of cows being bred by natural service (Jordan 2003). In various studies, conception rate (CR) and estrus expression were lower when the temperature or the temperature humidity index (THI) increased (Chebel et al. 2004, Badinga et al. 1985, Ingraham, Stanley & Wagner 1976, Cartmill et al. 2001). Furthermore, when follicles were aspirated and the collected follicles were matured in vitro, more good quality oocytes were obtained and the development into morula stages was greater in the cool season (February to March) than in the hot season (May to June) ($P=0.02$ and $P=0.06$ respectively) (Rocha et al. 1998). Moreover, in a study of Wilson and colleagues, cows were randomly assigned to either the thermo neutral treatment (19^{o}C, TN) or the heat stress treatment (29^{o}C, HS). Cows receiving the TN were more likely to ovulate the dominant follicle of the second follicular wave ($P<0.001$) than HS cows. The TN cows had higher serum estradiol concentrations ($P < 0.001$) and their follicles underwent a structural luteolysis ($P < 0.001$)(Wilson et al. 1998). Overall, CR can be 20 – 30% lower in summer than in winter (Cavestany, El-Wishy & Foote 1985, Badinga et al. 1985, De Rensis, Scaramuzzi 2003). Cooling cows using fans or showers has positive effects on estrus behavior and CR (Ealy et al. 1994, Her et al. 1988). Collier et al. found no significant difference in days to first progesterone rise, days to observed estrus, services per conception or days open when comparing cows provided shade or no shade during summer before calving. However, roughage intake was dramatically reduced (Collier et al. 1982). Since this study was done over 30 years ago, results are outdated. To our knowledge, no positive effect has been found from calving in the cool season on early ovulation within 21 DIM in previous studies. Garbino et al. determined that cows calving in summer had a delayed cyclicity during 60 days postpartum. However, in that particular study no significance was found (*P*=0.4) (Garbarino et al. 2004).

Since cows are not seasonal breeders, several researchers have hypothesized to explain the significant effect of season on ovulation postpartum. According to Santos et al., increased ovulation rate during summer or fall can be explained by different photoperiod stimulation, based on a study by Dahl et al. (Dahl, Buchanan & Tucker 2000, Rhodes et al. 2003). Melatonin, which is decreased when day length increases, has a negative effect on the secretion of IGF-1 by cows (Dahl, Buchanan & Tucker 2000). As mentioned before, IGF-1 has positive effects on ovulation (Beam, Butler 1998). Hansen et al. performed research on photoperiod length and ovulation in cows. When they ovariectomized heifers after exposing them to either 18 hours of light and 6 hours of darkness or 6 hours of light and 18 hours of darkness, a stronger estrogen-induced LH release was observed in the group that received a longer photoperiod (Hansen, P J Kamwanja, L A Hauser, E R. 1982). In another study by Hansen et al., cows exposed to the 18 hours of light ovulated earlier postpartum (*P*<0.025) (Hansen 1984).

More research is required regarding the effect of calving season on ovulation within 21 DIM, since 78% of our cows calved in summer or fall. Also, we did not take humidity or temperature into account, but only the calving season. Further and more precise research like Chebel et al. in 2004 may be done to gain new information on the relationship between calving season and the resumption of ovarian cyclicity postpartum. To our knowledge, no research has been done on the effect of length of daylight on ovulation within 21 DIM in dairy cows.

The length of the dry period

According to Opsomer et al., the longer the dry period lasts, the higher the odds ratio for a delayed ovulation postpartum is. Cows were 2.9 times more likely to develop a delay in ovarian cyclicity when having a dry period of 77 days, compared to cows that were dried off 60 days before calving (*P*<0.05). In our study, a longer dry period tended to have a negative effect on early ovulation postpartum (*P*=0.07, OR: 1.7, 95% CI: 1.0 – 2.8) in the second GLIMMIX model (Table 2b). According to the outcomes of the univariable analysis, cows that had been dried off 70 days or earlier before parturition were 1.8 times more likely to have an early resumption of ovarian cyclicity than cows with a longer dry period (P=0.03) (Table 1). In a study from Watters et al., cows were divided into two groups, undergoing two different dry periods: a traditional dry period of 55±11 days (T) or a short dry period (S) of 34±14 days. At 40 DIM, 62.7% of the S-cows were cycling, compared to 45.8% of the T-cows (P=0.001). Moreover, only 8% of the S-cows remained anovulatory by 70 DIM, whereas 18% of the T-cows did. The findings of this research also demonstrate an improved reproductive performance when cows had a shorter dry period (Watters et al. 2009). Walsh et al. were not able to find any significance concerning dry period length and the moment of ovulation postpartum (Walsh et al. 2007a). The calving-to-first-service interval, calving to conception interval and number of services per conception increased significantly with the number of dry days in the study by Pinedo et al. Furthermore, cows with a shorter dry period had more chance to become pregnant from the first service (Pinedo, Risco & Melendez 2011). All these studies implicate lesser reproductive performance of cows with a longer dry period, but to our knowledge, no one has investigated the effect of the length of the dry period on ovulation within 21 DIM. Opsomer and his colleagues had two explanations for the negative effect of a longer dry period on ovulation postpartum: a previous lactation with a longer dry period can indicate a longer calving interval and therefore impaired reproductive performance overall. Additionally, cows are in an anabolic condition for a longer time and hence are at more risk of calving with a higher BCS (Opsomer et al. 2000). In a study by Rastani et al. in 2005, prepartum dry matter intake was increased for cows that had a shorter dry period (28 days) compared to cows that had a traditional dry period (56 days) (P<0.001), which resulted in a more positive energy balance (EB) postpartum for the cows that had a shorter dry period (P<0.05) (Rastani et al. 2005). Additionally, in a study by Watters et al. in 2008, cows that had a shorter dry period, had lower serum concentrations of NEFA (P=0.02) postpartum, displaying a more positive energy balance. This finding could be an explanation for the positive effect of a shorter dry period on early ovulation postpartum, which is in agreementwith Beam and Butler who also observed that a less negative energy balance accounted for earlier ovulation postpartum (Beam, Butler 1998).

BCS at enrollment and body weight loss

BCS at enrollment (17±3 DIM) showed not to be significant in our study (P=0.20). Santos et al. discovered that a lower (<3.00) or higher (≥3.75) BCS one week after calving (BCSc) was associated with decreased resumption of cyclicity by 65 days postpartum (P<0.0001). 74.2% of the cows with a BCSc of <3.00 were cyclic, compared to 76.7% of the cows with a BCSc of 3.00 – 3.50, and 75.1% having a BCSc of ≥3.75. Cows with a BCSc of <3.00 had lower conception rates at 30 days after AI than cows with a BCSc of 3.00 – 3.50 and a BCSc of ≥3.75 (34.9% vs. 38.7% and 34.9% vs. 40.1% (P=0.004), respectively). Also, conception rates at 58 days were lower (30.0% vs. 33.1% and 30.0% vs.

36.2% (*P*=0.001), respectively) (Santos, Rutigliano & Sa Filho 2009). In a study by Suriyasathaporn in 1998, cows had a lower chance of first insemination during 45 and 135 DIM when their BCSc (five days after calving) was ≤2.75, compared to cows with a BCSc of 3 and more (*P*<0.05) (Suriyasathaporn et al. 1998). Information on the effect of the BCS at a certain time after calving on resumption of ovarian cyclicity is limited.

BCS-loss turns out to be of a lot more value when it comes to predicting the influence on the first ovulation postpartum (Opsomer et al. 2000, Santos, Rutigliano & Sa Filho 2009, Shrestha et al. 2005, Suriyasathaporn et al. 1998). Cows that lost ≥1 unit BCS from two weeks before to seven weeks after calving experienced a delay in the first ovulation (*P*=0.014) as shown in a study by Shrestha et al. (Shrestha et al. 2005). Santos et al. found that cows that had no change in BCS from calving to AI (around 70 days postpartum) were 2.39 times more likely to ovulate than cows that lost one unit or more (*P*<0.0001); cows that lost less than one unit were 1.96 times more likely to be cyclic by 65 days postpartum than cows that lost one unit or more (*P*<0.0001) (Santos, Rutigliano & Sa Filho 2009). According to researchers in the Netherlands, loss of BCS within 45 DIM was associated with more days open (Suriyasathaporn et al. 1998). Opsomer also stated that loss of BCS within the first month postpartum was an important risk factor for a delay in ovarian function (Opsomer et al. 2000). Garbarino et al. and Beam and Butler found no significant difference in cows losing BCS or body weight after calving compared to cows that did not on ovulation postpartum (Garbarino et al. 2004, Beam, Butler 1998, Beam, Butler 1997).

In our study, we were not able to evaluate the change in body condition score from calving to the time of enrollment, as the BCS at calving was recorded for only a small percentage of all the cows. The information we could obtain was the BCS at the time of enrollment at 17±3 DIM (BCSe) and hence the body weight loss during the first fourteen days postpartum at one of the farms. According to Ferguson, 2006, the loss of 56 kg of body weight can be compared with the loss of one unit of BCS. Losing one unit of BCS on a five-point scale had a significant effect on ovulation by 65 DIM in the study by Santos et al. (Santos, Rutigliano & Sa Filho 2009). Therefore, we analysed if losing half a unit of body condition score (28kg) or more would significantly affect the resumption of ovarian cyclicity postpartum. Body weight loss had a significant effect on early ovulation postpartum when a univariable analysis was performed (*P*=0.02) and in the third GLIMMIX model (*P*=0.04) (Table 2c). Cows that kept their body weight or lost less than 28 kg were 1.6 times more likely to ovulate (*P*=0.04, 95%CI: 1.0 – 2.4) than cows that lost 28 kg or more in the first fourteen days postpartum. In a study by Beam and Butler in 1998, body weight change and BCS change was similar for cows that had a nonovulatory or an ovulatory dominant follicle in their first follicular wave postpartum (Beam, Butler 1998). El-Din. Zain et al. found that significant weight loss within 30 days postpartum was highly correlated with the length of the postpartum interval to first ovulation (*r*=0.736, *P*<0.001) and that the amount of food intake was significant negatively associated with the postpartum interval to first ovulation (*r*=-0.837, *P*<0.001) (El-Din. Zain et al. 1995). Furthermore, limited research is done on the change in body weight shortly after parturition and its effect on early ovulation postpartum. Current literature does not show any significant correlation between body weight loss and a metabolic problem, we suggest that cows that lose more weight postpartum have a more severe NEB compared to cows that keep their body weight or lose less weight and therefore are at higher risk of being anovulatory postpartum.

Data validity

The incidence of postpartum diseases was higher on Farm 1 than on Farm 2. This, because on Farm 2 sick cows were excluded from the study. This was done since data on Farm 2 were collected for the GnRH study from June 2012 – August 2012 and they excluded cows in the hospital barn. In future studies, more sick cows can be included. One should take into account that both the farms were located in Florida, with the weather conditions mentioned above when extrapolating results to other areas in the world. Also, data was only gathered from 2 farms. For future research, more and different types of farms can be used. To gain a better overview of follicle development in sick cows postpartum, starting the examinations at calving or earlier postpartum might have given better insight. The measurement of serum progesterone next to performing US could have improved the sensitivity and specificity of this study (Bicalho et al. 2008). Another point of improvement for this research was that the assessment of the general health of fresh cows after calving was performed by farm personnel and not by veterinarians. Data were collected from the farm software, so we assumed data were filled out correctly. BCS recordings were done by three to four persons. Together with the fact that scoring of a body condition is subjective, that could have led to biased data. Neither did we take into account the severity of any disease: The levels for the variables were either ‘Yes’ or ‘No’. Furthermore, we categorized cows being either primiparous or multiparous. For future research, we recommend to separate the cows in primiparous, biparous (second lactation) and multiparous (third lactation or more) cows, like Santos et al. and Dubuc et al. did (Dubuc et al. 2012, Santos, Rutigliano & Sa Filho 2009) did. Finally, we stopped following most of the cows after they ovulated and did not determine the effect on other reproductive performances.

5. Conclusion

The aim of this study was to evaluate risk factors affecting ovulation within 21 DIM in dairy cows. It is known from previous studies that cows that ovulated within 21 DIM had a better uterine health and reproductive performance when compared with cows that ovulated for the first time around 50 DIM or had not ovulated at all by the end of the VWP (Galvao et al. 2010). Dystocia, ketosis and metritis were the factors with the highest prevalence among cows in our study. Our study confirmed that the transition period around calving is of major importance for the reproductive performance early, and hence later in lactation. All cows experience a negative energy balance postpartum and confining the severity of the negative energy balance can prevent metabolic problems like ketosis. Metabolic problems that result from a bad transition period and thus a negative energy balance, not only have a negative influence on postpartum ovarian function themselves, they were also associated with uterine inflammation and digestive problems (both with a $P<0.0001$), which in turn can negatively affect early ovulation postpartum. Impaired fertility is multifactorial and a lot of factors are associated with each other, like dystocia was associated with metritis ($P=0.0002$). In conclusion, cows that had metabolic problems, digestive problems, dystocia, metritis, were dried off 70 days or more before calving, calved in the winter or spring or lost > 28kg of body weight in the first 14 DIM had more risk of being anovulatory within 21 DIM. This study provides preliminary data for future research regarding ovulation postpartum within 21 DIM.

6. Appendix

Table 4. Incidence rates of the different factors overall, on the different farms, in multiparous vs. primiparous cows and in the warm vs. the cold season.

Variable	Overall (%)	Farm 1 (%)	Farm 2 (%)	Multiparous Cows (%)	Primiparous Cows (%)	Warm Season (SumFall) (%)	Cold Season (WinSpri) (%)
Parity							
Multiparous	63.5	53.6	78.5	-	-	-	-
Primiparous	36.5	46.4	21.5	-	-	-	-
BCSC							
Low	24.0	25.2	22.1	25.8	20.7	24.1	23.5
Medium	76.0	74.8	77.9	74.2	79.3	75.9	76.5
Dystocia	21.6	16.3	29.6	17.6	28.6	20.7	24.7
Twins	7.0	1.7	15.0	7.0	7.1	7.9	4.1
DOA	8.5	5.2	13.4	6.4	12.1	9.2	5.9
Abortion	1.2	1.1	1.3	1.0	1.4	1.0	1.8
RFM	2.7	1.7	4.2	3.9	0.7	3.2	1.2
Calving problem	29.2	22.1	39.7	40.8	37.5	28.8	30.6
Hypocalcaemia	2.1	2.8	1.0	3.3	0.0	2.2	1.8
Ketosis	21.0	28.2	10.1	26.4	11.4	19.9	24.7
Metabolic problem	22.0	29.3	11.1	28.1	11.4	20.9	25.9
Metritis	17.1	24.7	5.5	11.5	26.8	14.7	25.3
Mastitis	6.9	9.8	2.6	7.0	6.8	7.4	5.3
Infection	22.3	31.7	8.1	17.0	31.4	19.9	30.6
DA	1.3	2.2	0.0	1.8	0.4	1.3	1.2
Indigestion	8.6	14.1	0.3	8.6	8.6	6.9	14.7
Digestive problem	9.4	15.4	0.3	9.6	8.9	7.7	15.3
Lameness	2.9	4.1	1.0	3.1	2.5	2.5	4.1
Calving season							
Spring	12.2	18.4	2.9	11.1	14.3	-	-
Summer	56.8	29.9	97.1	64.5	43.2	-	-
Fall	21.1	35.1	0.0	20.9	21.4	-	-
Winter	9.9	16.5	0.0	3.5	21.1	-	-
DPL *							
Adequate	79.2	82.4	75.3	79.2	-	79.0	80.6
Long	20.8	17.6	24.7	20.8	-	21.0	19.4
BWLC **							
Lost	65.8	65.8	-	76.6	53.3	69.1	59.5
Kept	34.2	34.2	-	23.4	46.7	30.9	40.5
Milk yield							
Below	44.5	52.5	32.6	42.6	47.9	44.8	43.5
Above	55.5	47.5	67.4	57.4	52.1	55.2	56.5

*DPL could be determined for only 443 cows
** BWL could be determined for only 456 cows

7. References

SOP: http://extension.vetmed.ufl.edu/files/2012/01/WEB_VERSSION_-2011-Dairy-Unit-Complete-SOPs-vers-11-07-01-with-Title-page.pdf

Ferguson 2006:

http://research.vet.upenn.edu/DairyPoultrySwine/DairyCattle/PennConf1996/ImplementationofaBCSPrograminDairyHerds/tabid/1730/Default.aspx

Badinga, L., Collier, R.J., Thatcher, W.W. & Wilcox, C.J. 1985, "Effects of climatic and management factors on conception rate of dairy cattle in subtropical environment", *Journal of dairy science,* vol. 68, no. 1, pp. 78-85.

Beam, S.W. & Butler, W.R. 1998, "Energy balance, metabolic hormones, and early postpartum follicular development in dairy cows fed prilled lipid", *Journal of dairy science,* vol. 81, no. 1, pp. 121-131.

Beam, S.W. & Butler, W.R. 1997, "Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat", *Biology of reproduction,* vol. 56, no. 1, pp. 133-142.

Benzaquen, M.E., Risco, C.A., Archbald, L.F., Melendez, P., Thatcher, M.J. & Thatcher, W.W. 2007, "Rectal temperature, calving-related factors, and the incidence of puerperal metritis in postpartum dairy cows", *Journal of dairy science,* vol. 90, no. 6, pp. 2804-2814.

Bicalho, R.C., Cheong, S.H., Galvao, K.N., Warnick, L.D. & Guard, C.L. 2007a, "Effect of twin birth calvings on milk production, reproductive performance, and survival of lactating cows", *Journal of the American Veterinary Medical Association,* vol. 231, no. 9, pp. 1390-1397.

Bicalho, R.C., Galvao, K.N., Cheong, S.H., Gilbert, R.O., Warnick, L.D. & Guard, C.L. 2007b, "Effect of stillbirths on dam survival and reproduction performance in Holstein dairy cows", *Journal of dairy science,* vol. 90, no. 6, pp. 2797-2803.

Bicalho, R.C., Galvao, K.N., Guard, C.L. & Santos, J.E. 2008, "Optimizing the accuracy of detecting a functional corpus luteum in dairy cows", *Theriogenology,* vol. 70, no. 2, pp. 199-207.

Butler, W.R. 2003, "Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows", *Livestock Production Science,* vol. 83, no. 2, pp. 211.

Butler, W.R. & Smith, R.D. 1989, "Interrelationships between energy balance and postpartum reproductive function in dairy cattle", *Journal of dairy science,* vol. 72, no. 3, pp. 767-783.

Cartmill, J.A., El-Zarkouny, S.Z., Hensley, B.A., Rozell, T.G., Smith, J.F. & Stevenson, J.S. 2001, "An alternative AI breeding protocol for dairy cows exposed to elevated ambient temperatures before or after calving or both", *Journal of dairy science,* vol. 84, no. 4, pp. 799-806.

Cavestany, D., El-Wishy, A.B. & Foote, R.H. 1985, "Effect of Season and High Environmental Temperature on Fertility of Holstein Cattle", *Journal of dairy science,* vol. 68, no. 6, pp. 1471-1478.

Chapinal, N., Carson, M., Duffield, T.F., Capel, M., Godden, S., Overton, M., Santos, J.E. & LeBlanc, S.J. 2011, "The association of serum metabolites with clinical disease during the transition period", *Journal of dairy science,* vol. 94, no. 10, pp. 4897-4903.

Chebel, R.C., Santos, J.E., Reynolds, J.P., Cerri, R.L., Juchem, S.O. & Overton, M. 2004, "Factors affecting conception rate after artificial insemination and pregnancy loss in lactating dairy cows", *Animal Reproduction Science,* vol. 84, no. 3-4, pp. 239-255.

Collier, R.J., Beede, D.K., Thatcher, W.W., Israel, L.A. & Wilcox, C.J. 1982, "Influences of environment and its modification on dairy animal health and production", *Journal of dairy science,* vol. 65, no. 11, pp. 2213-2227.

Dahl, G.E., Buchanan, B.A. & Tucker, H.A. 2000, "Photoperiodic effects on dairy cattle: a review.", *Journal of dairy science,* vol. 83, no. 4, pp. 885-893.

Daniel, R.C. 1983, "Motility of the rumen and abomasum during hypocalcaemia", *Canadian journal of comparative medicine.Revue canadienne de medecine comparee,* vol. 47, no. 3, pp. 276-280.

De Rensis, F. & Scaramuzzi, R.J. 2003, "Heat stress and seasonal effects on reproduction in the dairy cow--a review", *Theriogenology,* vol. 60, no. 6, pp. 1139-1151.

De Vries, A. 2006, "Economic value of pregnancy in dairy cattle", *Journal of dairy science,* vol. 89, no. 10, pp. 3876-3885.

Dubuc, J., Duffield, T.F., Leslie, K.E., Walton, J.S. & LeBlanc, S.J. 2012, "Risk factors and effects of postpartum anovulation in dairy cows", *Journal of dairy science,* vol. 95, no. 4, pp. 1845-1854.

Dubuc, J., Duffield, T.F., Leslie, K.E., Walton, J.S. & LeBlanc, S.J. 2010, "Risk factors for postpartum uterine diseases in dairy cows", *Journal of dairy science,* vol. 93, no. 12, pp. 5764-5771.

Duffield, T.F., Lissemore, K.D., McBride, B.W. & Leslie, K.E. 2009, "Impact of hyperketonemia in early lactation dairy cows on health and production", *Journal of dairy science,* vol. 92, no. 2, pp. 571-580.

Ealy, A.D., Arechiga, C.F., Bray, D.R., Risco, C.A. & Hansen, P.J. 1994, "Effectiveness of short-term cooling and vitamin E for alleviation of infertility induced by heat stress in dairy cows", *Journal of dairy science,* vol. 77, no. 12, pp. 3601-3607.

El-Din. Zain, A., Nakao, T., Abdel Raouf, M., Moriyoshi, M., Kawata, K. & Moritsu, Y. 1995, "Factors in the resumption of ovarian activity and uterine involution in postpartum dairy cows", *Animal Reproduction Science,* vol. 38, no. 3, pp. 203-214.

Ferguson, J.D., Galligan, D.T. & Thomsen, N. 1994, "Principal descriptors of body condition score in Holstein cows", *Journal of dairy science,* vol. 77, no. 9, pp. 2695-2703.

Galvao, K.N., Frajblat, M., Butler, W.R., Brittin, S.B., Guard, C.L. & Gilbert, R.O. 2010, "Effect of early postpartum ovulation on fertility in dairy cows", *Reproduction in domestic animals = Zuchthygiene,* vol. 45, no. 5, pp. e207-11.

Garbarino, E.J., Hernandez, J.A., Shearer, J.K., Risco, C.A. & Thatcher, W.W. 2004, "Effect of lameness on ovarian activity in postpartum holstein cows", *Journal of dairy science,* vol. 87, no. 12, pp. 4123-4131.

Gautam, G., Nakao, T., Yamada, K. & Yoshida, C. 2010, "Defining delayed resumption of ovarian activity postpartum and its impact on subsequent reproductive performance in Holstein cows", *Theriogenology,* vol. 73, no. 2, pp. 180-189.

Goshen, T. & Shpigel, N.Y. 2006, "Evaluation of intrauterine antibiotic treatment of clinical metritis and retained fetal membranes in dairy cows", *Theriogenology,* vol. 66, no. 9, pp. 2210-2218.

Gumen, A., Guenther, J.N. & Wiltbank, M.C. 2003, "Follicular size and response to Ovsynch versus detection of estrus in anovular and ovular lactating dairy cows", *Journal of dairy science,* vol. 86, no. 10, pp. 3184-3194.

Hammon, D.S. 2006, "Neutrophil function and energy status in Holstein cows with uterine health disorders", *Veterinary immunology and immunopathology,* vol. 113, no. 1, pp. 21.

Hansen, P J Hauser,E R. 1983, "Genotype x environmental interactions on reproductive traits of bovine females. III. Seasonal variation in postpartum reproduction as influenced by genotype, suckling and dietary regimen.", *Journal of animal science,* vol. 56, no. 6, pp. 1362-1369.

Hansen, P J Kamwanja, L A Hauser, E R. 1982, "The effect of photoperiod on serum concentrations of luteinizing and follicle stimulating hormones in prepubertal heifers following ovariectomy and estradiol injection.", *Theriogenology,* vol. 18, no. 5, pp. 551-559.

Hansen, P.J. 1984, "Photoperiodic alteration of postpartum reproductive function in suckled cows", *Theriogenology,* vol. 22, no. 1, pp. 1.

Her, E., Wolfenson, D., Flamenbaum, I., Folman, Y., Kaim, M. & Berman, A. 1988, "Thermal, productive, and reproductive responses of high yielding cows exposed to short-term cooling in summer", *Journal of dairy science,* vol. 71, no. 4, pp. 1085-1092.

Huzzey, J.M., Duffield, T.F., LeBlanc, S.J., Veira, D.M., Weary, D.M. & von Keyserlingk, M.A. 2009, "Short communication: Haptoglobin as an early indicator of metritis", *Journal of dairy science,* vol. 92, no. 2, pp. 621-625.

Ingraham, R.H., Stanley, R.W. & Wagner, W.C. 1976, "Relationship of temperature and humidity to conception rate of Holstein cows in Hawaii", *Journal of dairy science,* vol. 59, no. 12, pp. 2086-2090.

Jordan, E.R. 2003, "Effects of heat stress on reproduction", *Journal of dairy science,* vol. 86, pp. E104.

Jorritsma, R., Wensing, T., Kruip, T.A., Vos, P.L. & Noordhuizen, J.P. 2003, "Metabolic changes in early lactation and impaired reproductive performance in dairy cows", *Veterinary research,* vol. 34, no. 1, pp. 11-26.

Kim, Ill Jeong,Jae Kang, Hyun 2012, "Field investigation of whether corpus luteum formation during weeks 3-5 postpartum is related to subsequent reproductive performance of dairy cows.", *Journal of Reproduction and Development,* vol. 58, no. 5, pp. 552-556.

Markusfeld, O. 1987, "Periparturient traits in seven high dairy herds. Incidence rates, association with parity, and interrelationships among traits", *Journal of dairy science,* vol. 70, no. 1, pp. 158-166.

Martinez, N., Risco, C.A., Lima, F.S., Bisinotto, R.S., Greco, L.F., Ribeiro, E.S., Maunsell, F., Galvao, K. & Santos, J.E. 2012, "Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease", *Journal of dairy science,* vol. 95, no. 12, pp. 7158-7172.

Massey, C.D., Wang, C., Donovan, G.A. & Beede, D.K. 1993, "Hypocalcemia at parturition as a risk factor for left displacement of the abomasum in dairy cows", *Journal of the American Veterinary Medical Association,* vol. 203, no. 6, pp. 852-853.

McCoy, M.A. 2006, "Milk progesterone profiles and their relationship with fertility, production and disease in dairy cows in Northern Ireland", *Animal Science,* vol. 82, no. 2, pp. 213.

Opsomer, G., Grohn, Y.T., Hertl, J., Coryn, M., Deluyker, H. & de Kruif, A. 2000, "Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: a field study", *Theriogenology,* vol. 53, no. 4, pp. 841-857.

Pedersen, S.L. 2006, "Analysis of reproductive performance, milk production and survival following surgery for a left displaced abomasum in dairy cattle", *Cattle practice,* vol. 14, no. 3, pp. 221-226.

Peters, A.R. 1984, "Reproductive activity of the cow in the post-partum period. I. Factors affecting the length of the post-partum acyclic period", *British Veterinary Journal,* vol. 140, no. 1, pp. 76.

Pinedo, P., Risco, C. & Melendez, P. 2011, "A retrospective study on the association between different lengths of the dry period and subclinical mastitis, milk yield, reproductive performance, and culling in Chilean dairy cows", *Journal of dairy science,* vol. 94, no. 1, pp. 106-115.

Pryce, J.E. 2004, "Fertility in the high-producing dairy cow", *Livestock Production Science,* vol. 86, no. 1, pp. 125.

Rastani, R.R., Grummer, R.R., Bertics, S.J., Gumen, A., Wiltbank, M.C., Mashek, D.G. & Schwab, M.C. 2005, "Reducing dry period length to simplify feeding transition cows: milk production, energy balance, and metabolic profiles", *Journal of dairy science,* vol. 88, no. 3, pp. 1004-1014.

Rhodes, F.M., McDougall, S., Burke, C.R., Verkerk, G.A. & Macmillan, K.L. 2003, "Invited review: Treatment of cows with an extended postpartum anestrous interval", *Journal of dairy science,* vol. 86, no. 6, pp. 1876-1894.

Risco, C.A., Drost, M., Thatcher, W.W., Savio, J. & Thatcher, M.J. 1994, "Effects of calving-related disorders on prostaglandin, calcium, ovarian activity and uterine involution in postrartum dairy cows", *Theriogenology,* vol. 42, no. 1, pp. 183-203.

Rocha, A., Randel, R.D., Broussard, J.R., Lim, J.M., Blair, R.M., Roussel, J.D., Godke, R.A. & Hansel, W. 1998, "High environmental temperature and humidity decrease oocyte quality in Bos taurus but not in Bos indicus cows", *Theriogenology,* vol. 49, no. 3, pp. 657-665.

Santos, J.E., Rutigliano, H.M. & Sa Filho, M.F. 2009, "Risk factors for resumption of postpartum estrous cycles and embryonic survival in lactating dairy cows", *Animal Reproduction Science,* vol. 110, no. 3-4, pp. 207-221.

Sheldon, I.M., Cronin, J., Goetze, L., Donofrio, G. & Schuberth, H.J. 2009, "Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle", *Biology of reproduction,* vol. 81, no. 6, pp. 1025-1032.

Sheldon, I.M. & Dobson, H. 2004, "Postpartum uterine health in cattle", *Animal Reproduction Science,* vol. 82-83, pp. 295-306.

Sheldon, I.M., Noakes, D.E., Rycroft, A.N., Pfeiffer, D.U. & Dobson, H. 2002, "Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle", *Reproduction (Cambridge, England),* vol. 123, no. 6, pp. 837-845.

Shrestha, H.K., Nakao, T., Suzuki, T., Akita, M. & Higaki, T. 2005, "Relationships between body condition score, body weight, and some nutritional parameters in plasma and resumption of ovarian cyclicity postpartum during pre-service period in high-producing dairy cows in a subtropical region in Japan", *Theriogenology,* vol. 64, no. 4, pp. 855-866.

Suriyasathaporn, W., Nielen, M., Dieleman, S.J., Brand, A., Noordhuizen-Stassen, E.N. & Schukken, Y.H. 1998, "A Cox proportional-hazards model with time-dependent covariates to evaluate the relationship between body-condition score and the risks of first insemination and pregnancy in a high-producing dairy herd", *Preventive veterinary medicine,* vol. 37, no. 1-4, pp. 159-172.

Thatcher, W.W. 1974, *Effects of Season, Climate, and Temperature on Reproduction and Lactation1*, American Dairy Science Association.

Walsh, R.B., Kelton, D.F., Duffield, T.F., Leslie, K.E., Walton, J.S. & LeBlanc, S.J. 2007a, "Prevalence and risk factors for postpartum anovulatory condition in dairy cows", *Journal of dairy science,* vol. 90, no. 1, pp. 315-324.

Walsh, R.B., Walton, J.S., Kelton, D.F., LeBlanc, S.J., Leslie, K.E. & Duffield, T.F. 2007b, "The effect of subclinical ketosis in early lactation on reproductive performance of postpartum dairy cows", *Journal of dairy science,* vol. 90, no. 6, pp. 2788-2796.

Watters, R.D., Wiltbank, M.C., Guenther, J.N., Brickner, A.E., Rastani, R.R., Fricke, P.M. & Grummer, R.R. 2009, "Effect of dry period length on reproduction during the subsequent lactation", *Journal of dairy science,* vol. 92, no. 7, pp. 3081-3090.

Williams, E.J., Fischer, D.P., Noakes, D.E., England, G.C., Rycroft, A., Dobson, H. & Sheldon, I.M. 2007, "The relationship between uterine pathogen growth density and ovarian function in the postpartum dairy cow", *Theriogenology,* vol. 68, no. 4, pp. 549-559.

Wilson, S.J., Marion, R.S., Spain, J.N., Spiers, D.E., Keisler, D.H. & Lucy, M.C. 1998, "Effects of controlled heat stress on ovarian function of dairy cattle. 1. Lactating cows", *Journal of dairy science,* vol. 81, no. 8, pp. 2124-2131.

Zwald, N.R., Weigel, K.A., Chang, Y.M., Welper, R.D. & Clay, J.S. 2004, "Genetic selection for health traits using producer-recorded data. I. Incidence rates, heritability estimates, and sire breeding values", *Journal of dairy science,* vol. 87, no. 12, pp. 4287-4294.