The influence of calcium status and ketone body status on the development of metritis and endometritis after parturition in dairy cows.

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Abstract

The aims of the study were to investigate if the calcium concentration after parturition and the ketone body concentration influenced the development of metritis and endometritis after parturition and to investigate if the calcium concentration was correlated to the ketone body concentration after parturition. The present study was conducted on eight dairy farms located across the Netherlands. In total, 632 cows were studied. Within 48h after parturition and at week 1&2 after parturition blood samples were taken for calcium and BHB status, respectively. Furthermore, at week 1, 2, 4 and 8 cows were examined, rectally palpated and scanned to check for metritis and endometritis. The calcium status and ketone body status were not correlated after parturition. Furthermore, calcium status and ketone body status did not influence the development of endometritis. Although not significant, an effect of the BHBA concentration was detected on the development of metritis after parturition. Calcium status and ketone body status did not affect the development of endometritis after parturition. Differences between farms and parity exist. A primiparous cow has the largest odds for developing metritis within two weeks after parturition. Furthermore, the higher a cow is in parity, the larger the odds are for developing endometritis at week 4 after parturition. More research is needed to find which risk factors are of influence on primiparous cows and the rising parity compared to larger odds in developing metritis and endometritis after parturition.

Introduction

Fertility problems are, besides a decreased milk production and claw health, important reasons for culling on modern dairy farms (Milian-Suazo *et al.,* 1989). After parturition, a dairy cow shifts from non-lactating to lactation and is challenged to return to a fertile oestrus cycle within a considerable amount of time. Major events during this period are reduction of the uterine size by myometrial contractions, elimination of bacterial contamination, expulsion of lochia, endometrial repair, resumption of ovarian function and of the reproductive tract (Senger, 2005). Metritis has been associated with a negative effect on reproductive rate and overall pregnancy risk (Bromfield *et al*., 2015, Gilbert *et al*., 2005, Bicalho *et al.,* 2017). Contamination of the uterus after parturition is nearly inevitable, however, only a fraction of the animals will develop metritis (Sicsic *et al.,* 2018). If an infection arises, toll-like receptors on endometrial cells will detect parts of the bacteria, resulting in the secretion of cytokines, chemokines and anti-microbial peptides that will attract phagocytes and start elimination (Sheldon *et al.,* 2009). Nevertheless, recent parturition, the effects of producing colostrum and a high milk production, contribute to a decreased periparturient immunity and, therefore, the possibility of not eliminating the bacteria, having metritis as a result (Aleri *et al*., 2016, Smith Bradford, 2015).

Ketosis has been associated with the development of metritis and subclinical and clinical endometritis (Duffield *et al.,* 2009, Cheong *et al*., 2011, Shin *et al.,* 2015). After parturition, input of energy must equal or exceed output needed for milk production and maintenance to prevent a negative energy balance (NEB). However, the capacity of the animal to consume sufficient feed is not enough to meet the requirements for the energy needed for milk production (Smith Bradford, 2015). Despite good quality feed, during the rising curve of lactation every healthy high producing dairy cow will have some level of physiological NEB, and it will last until energy intake meets the energy requirement for milk production (Smith Bradford, 2015). When energy intake meets the energy requirement for milk production varies among cows, but approximately takes three weeks (Smith Bradford, 2015). During NEB fat is mobilised, and Non Esterified Fatty Acids (NEFA) are presented to the liver. Using the β-oxidation Acetyl-CoA is formed which is used for the citric acid cycle. When this route is satisfied Acetyl-CoA is used for metabolising ketone bodies as an alternative energy source (Adewuyi *et al*., 2005). The ketone body concentration keeps increasing until clinical signs of ketosis appear. However, there exists a varying degree of signs between individuals, a graduate loss of appetite and a slow decrease in milk production for several days may account as the start for some animals, others will show an immediate drop in milk production. However, the NEFA concentration is elevated in every postpartum animal and weight is lost (Smith Bradford, 2015, Caixeta *et al.,* 2015). The presence of clinical signs of ketosis and subclinical ketosis cause economic losses through deceased milk yield, and increased risk for postpartum diseases such as metritis, and increased culling (Shin *et al.,* 2015). Furthermore, ketosis has also been linked to immunosuppression (Hammon *et al.,* 2006, Kimura *et al*., 1999). This may also contribute to the development of metritis.

Calcium status has been related to a higher chance of developing ketosis and metritis (Rodríguez *et al*., 2017, Martinez *et al*., 2012). Cows diagnosed with subclinical hypocalcaemia were 5.5 times more likely to develop ketosis, and 4.3 times more likely to develop metritis after parturition (Rodríguez *et al.,* 2017). Due to the sudden use of calcium by the mammary gland for synthesizing and secreting colostrum and a challenging milk production, serum calcium concentration decreases (Goff, 2008). Smooth muscle contraction is negatively affected by a low serum calcium concentration, which reduces uterus contractility and could therefore be related to developing metritis after parturition (Martinez *et al*., 2012, Smith Bradford, 2015).

The hypothesis of the current study was that calcium- and ketone body status of a dairy cow are influencing the development of metritis and endometritis after parturition. Even though some studies agree on the statement, there are also reports claiming the opposite and no correlation is found between calcium and/ or ketone body status and de development of metritis and endometritis (Mahen *et al*., 2018, Chamberlin *et al.,* 2013, Bicalho *et al.,* 2017). Because of missing unambiguous evidence, the aims of the present study were to investigate if the calcium concentration within 48h after parturition and the ketone body concentration within 1&2 weeks after parturition were correlated to the development of metritis and endometritis at week 1, 2, 4 and 8 after parturition and to investigate if the calcium concentration within 48h after parturition was correlated to the highest ketone body concentration at week 1&2 after parturition.

Materials & Methods

Clinical data collection

The present study was conducted on eight commercial dairy farms, located across the Netherlands and was part of the Sense of Sensors in Transition Management project in collaboration with Nedap, Vetvice, Utrecht University, Wageningen University and Boehringer Ingelheim. A research group visited the farms once a week at the same day. This weekly visiting enrolled all cows which were within 48 hours after parturition for blood sampling of calcium. At week 1 and 2 a blood sample was taken for BHBA concentration and at week 1, 2, 4 and 8 the cows were clinically examined and rectally palpated and scanned to check for metritis and endometritis, as can be seen in table 1.

Table 1: Timeline of actions performed at the cows during the study

Days	Performed action
0	Parturition
0-2	Blood sample for calcium concentration
0-7	Blood sample for BHBA concentration Examining + rectally palpating and scanning for metritis
7-14	Blood sample for BHBA concentration Examining + rectally palpating and scanning for metritis
21-28	Examining + rectally palpating and scanning for endometritis
50-56	Examining + rectally palpating and scanning for endometritis

Enrolled farms & cows

In total, 632 cows among eight dairy farms were enrolled In the study. The mean number of cows on the farms was 139, with a range of 101 to 194 cows. The mean milk production per year per cow was 9659 kilogram, with a range of 8951 kilogram to 11.152 kilogram milk. Of 632 cows, 147 were primiparous (23.3%) and 485 (76.7%) were of second parity and higher (multiparous). Parities varied from 1 to 13. Figure 1 shows the distribution of primiparous and multiparous cows among the eight farms. Multiparous cows were differentiated in groups of second (179 cows), third (133 cows), fourth (88 cows) and fifth and higher (85 cows) parity. Parity distribution within the farms can be seen in figure 2. Second calving was the group which contains the most cows. Second largest group are the primiparous cows. After second calving, the higher the parity of the cows, the less cows the group contained.

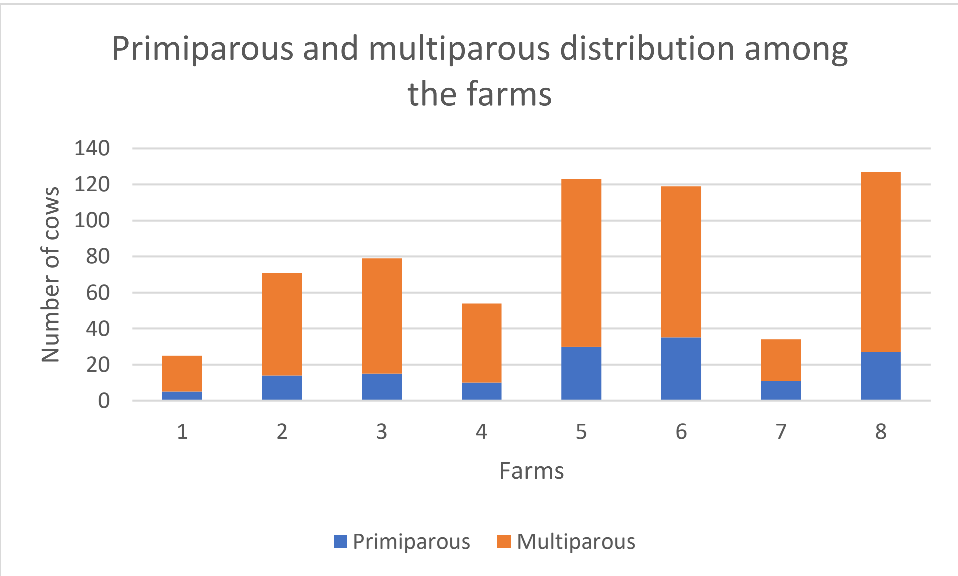

Figure 1: Distribution of 147 primiparous and 485 multiparous cows among the eight farms.

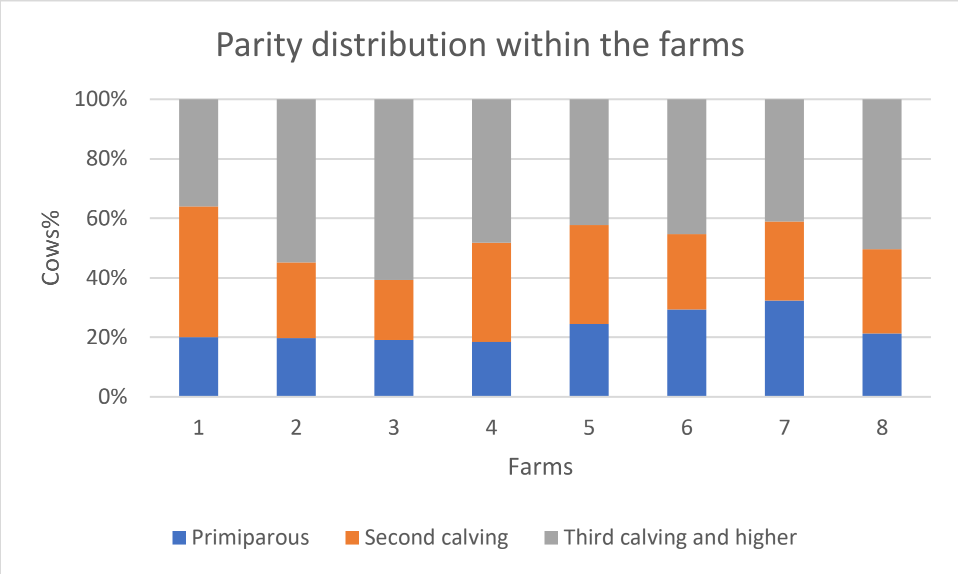

Figure 2: The parity distribution of 632 cows within the eight farms.

Calcium concentration

Blood samples were collected from the coccygeal vein within 48 hours after parturition via evacuated blood collection tubes containing heparin as anticoagulant. Blood samples were stored in a cool container, taken to the lab and were analysed the same day. The blood sample was centrifuged for 10 minutes at 4500 rpm. Serum was pipetted in Eppendorf cups by hand-held pipette and stored at -20 Celsius until further analysis. Quantitative determination of calcium concentration of the serum was performed by an Olympus AU640 (Calcium Arsenazo III, Colorimetric, Dialab) (Korean, 1994).

Ketone body concentration

Blood samples were collected from the coccygeal vein in week 1 and week 2 after parturition using a syringe and a using a Precision Xceed device (Abbot Diabetes Care) on site. A drop of blood was applied on the test strip and after 10 seconds the value appeared and was then recorded. The Precision Xceed device measures the concentration of BHBA in mmol/L. The highest value of both BHBA tests in week 1&2 was used in this study.

Metritis and endometritis scores

Cows were rectally palpated, using a portable ultrasound (Tringa Linear, Esaote-Pie Medical), performed at week 1, 2, 4 and 8 after parturition. The performer was a veterinarian or a well-trained veterinary student in his Masters. Metritis and endometritis scores are based on the morphology of Sheldon *et al.,* 2006 and the findings using the ultrasound. If during rectally palpating and scanning the uterus was enlarged and filled with flaky content, combined with the morphology of Sheldon *et al.,* 2006, the cow was diagnosed with a metritis or endometritis depending on the time after parturition. Metritis was scored until 21 days after parturition and was defined as an animal with an abnormally enlarged uterus, fetid watery red-brown uterine discharge, associated with signs of systemic illness, and fever >39.5°C. Endometritis was scored in week 4 and week 8, from day 21-28 and day 50-56, and is characterised by the presence of purulent of mucopurulent uterine discharge in the vagina, not accompanied by systemic signs (Sheldon *et al.,* 2006).

There were three moments of scoring metritis and endometritis in this study. Considering the fact that the risk of diagnosing metritis at day 3 after parturition is very small, the data of week 1 and 2 were combined to one scoring value. If a cow was diagnosed with metritis in week 1 or week 2 or in both weeks, it was implied in the data as metritis. If a cow was not diagnosed with metritis in week 1 or week 2, it was implied in the data as no metritis. So finally, the dataset contained one combined moment of scoring metritis (week 1&2) and two moments of scoring endometritis (week 4 and 8).

Data

R studio 1.1456 for Windows 10 with Generalised Linear Models was used to analyse the data. Following on Generalised Linear Models was the Logistic Regression. Factors included were 'Farm' and 'Parity' (Par). Calcium concentration (Calcium) and BHBA concentration (BHBA) were implemented in de model. Every scoring moment of metritis (Met) and endometritis (Endomet1 and Endomet2) was separately implemented in the formula and correlated to calcium and BHBA and the factors 'Farm' and 'Parity'. When running the Logistic Regression a model reduction based on Akaike Information Criterion (AIC value) was implemented.

Excel 2016, CORRELATION(CALCIUM;BHBA) was used to calculate a correlation and p value between the calcium concentration and BHBA concentration.

Table 2: Scoring moments with appurtenant statistic model

Scoring moment	Statistical model
Metritis	fit <- glm (Met~factor (Farm) + factor(Par) + Calcium + BHBA, family = binomial)
Endometritis (week 4)	fit2 <- glm (Endomet1~factor (Farm) + factor(Par) + Calcium + BHBA, family = binomial)
Endometritis (week 8)	fit3 <- glm (Endomet2~factor (Farm) + factor(Par) + Calcium + BHBA, family = binomial)

Table 3: Final models used for the three scoring moments with appurtenant final statistic model

Scoring moment	Model in R studio	Model reduction
Metritis	fit <- glm (Met~factor (Farm) + factor(Par) + BHBA, family = binomial)	Based on the AIC value. Lowest AIC value was removed by the 'step(fit)' function. Factors were left in.
Endometritis (week 4)	fit2 <- glm (Endomet1~factor (Farm) + factor(Par), family = binomial)	Based on the AIC value, variables were removed from the model by the 'step(fit2)' function. Factors were left in.
Endometritis (week 8)	fit3 <- glm (Endomet2~, family = binomial)	Model reduction on all data. No variables or factors had an AIC value large enough to be implied in the model.

'Summary(fit)' calculated the log odds used for the results.

Results

The incidences of metritis (week 1 and 2 combined) and endometritis at week 4 and 8 in the study population were 32.3% (204 of 632), 31.0% (176 of 567), 14.8% (78 of 528), respectively. Differences were measured between farms, as can be seen in figure 4.

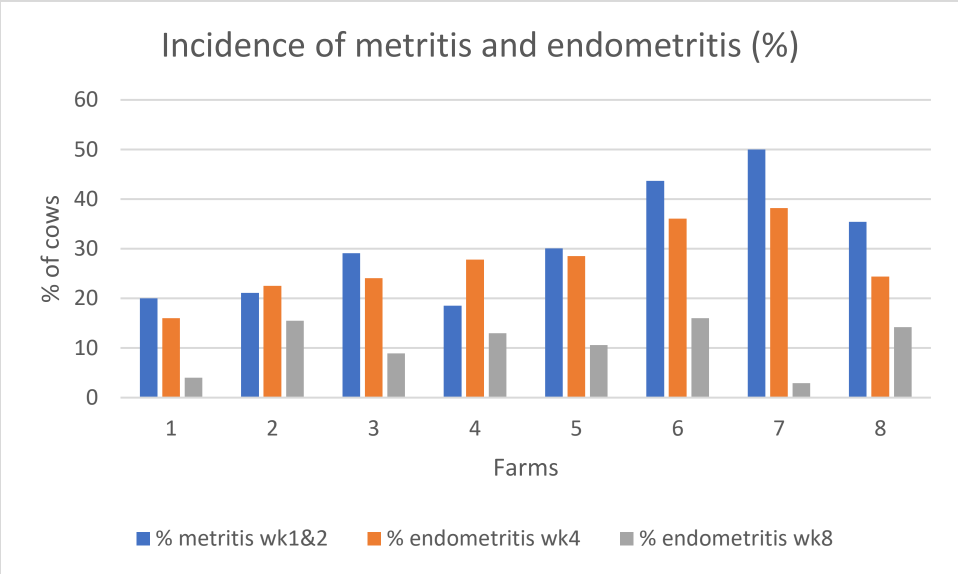

Figure 4: Incidence of metritis and endometritis as a percentage of the cows per farm for the different scoring moments in week 1&2, 4 and 8.

Aims of the study

Are the calcium concentration within 48h after parturition and the highest ketone body concentration correlated to the development of metritis after parturition?

The model with the best fit according to the AIC value excluded the calcium concentration, which indicates that there is no correlation between calcium and the development of metritis after parturition. As can be seen in table 4, log odds of the BHBA concentration was 0.21 ($P<0.07$). A cow with a BHBA concentration of 1,4 mmol/L compared to a cow with a BHBA concentration of 0,4 mmol/L, has an 1,24 larger odds for developing metritis in week 1&2 after parturition.

Differences between farms exist. Compared to farm 1, farm 6 had a larger odds for developing metritis. Compared to parity 1, parity 2, 3, 4 and 5 had smaller odds for developing metritis. A cow in fourth parity has the smallest odds for developing metritis after parturition. A primiparous cow has the largest odds for developing metritis.

Table 4: Log odds of factors and variables. Bold digits in column 'P value' indicate that the number is significant (P<0.05)

	Log OR	SE	P value	OR	2.5%	97.5%
Intercept	-1.1095	0.5348	0.0380	0.3297	0.1039	0.8811
Factor farm 2	0.0798	0.5846	0.8914	1.0830	0.3603	3.7102
Factor farm 3	0.5376	0.5655	0.3417	1.7119	0.5972	5.7002
Factor farm 4	-0.1463	0.6166	0.8124	0.8638	0.2654	3.1048
Factor farm 5	0.5397	0.5440	0.3212	1.7154	0.6286	5.5216
Factor farm 6	1.1386	0.5394	**0.0347**	3.1223	1.1576	9.9828
Factor farm 7	1.1134	0.6272	0.0758	3.0447	0.9322	11.261
Factor farm 8	0.8084	0.5394	0.1339	2.2443	0.8316	7.1745
Factor parity 2	-0.6010	0.2408	**0.0125**	0.5482	0.3410	0.8775
Factor parity 3	-0.5375	0.2638	**0.0415**	0.5842	0.3468	0.9770
Factor parity 4	-1.1413	0.3213	**0.0003**	0.3193	0.1668	0.5908
Factor parity 5	-0.8540	0.3134	**0.0064**	0.4256	0.2273	0.7792
BHBA	0.2103	0.1157	0.0691	1.2339	0.9820	1.5497

Are the calcium concentration within 48h after parturition and the highest ketone body concentration correlated to the development of endometritis at week 4 after parturition?

The model with the best fit according to the AIC value excluded the calcium concentration and ketone body concentration. This indicates that the concentrations of calcium and ketone bodies have no effect on the development of endometritis at week 4 after parturition.

Differences between farms exist as illustrated by an odds ratio (OR) of 3.8 between farm 6 and farm 1 (P = 0.0228). Although, only significant in parity 5, parity shows an increase in OR. The higher a cow is in parity, excluding second parity, the larger the odds are for developing endometritis at week 4 after parturition.

Table 5: Log odds of the factors and variables. Bold digits in column 'P value' indicate that the number is significant (P<0.05)

	Log OR	SE	P value	OR	2.5%	97.5%
Intercept	-1.7885	0.5830	0.0021	0.1672	0.0460	0.4774
Factor farm 2	0.3514	0.6247	0.5737	1.4210	0.4477	5.4785
Factor farm 3	0.4331	0.6175	0.4830	1.5420	0.4945	5.8808
Factor farm 4	1.0112	0.6421	0.1152	2.7489	0.8350	10.9061
Factor farm 5	0.9031	0.5924	0.1273	2.4672	0.8422	9.0731
Factor farm 6	1.3431	0.5901	**0.0228**	3.8308	1.3157	14.0416
Factor farm 7	1.2758	0.6636	0.0545	3.5814	1.0383	14.7188
Factor farm 8	0.5210	0.5933	0.3798	1.6836	0.5723	6.1934
Factor parity 2	-0.2509	0.2754	0.3622	0.7780	0.4529	1.3367
Factor parity 3	0.2536	0.2851	0.3737	1.2886	0.7370	2.2588
Factor parity 4	0.5256	0.3107	0.0907	1.6914	0.9189	3.1157
Factor parity 5	0.8890	0.3158	**0.0048**	2.4326	1.3134	4.5420

Are the calcium concentration within 48h after parturition and the highest ketone body concentration correlated to the development of endometritis at week 8?

The model with the best fit according to the AIC value excluded the calcium concentration, ketone body concentration and the factors. This indicates that there is no correlation between the calcium concentration within 48h after parturition, the ketone body concentration and the development of endometritis at week 8.

To investigate if the calcium concentration within 48h after parturition was correlated to the highest ketone body concentration at week 1&2 after parturition.

A correlation of relevance between the concentration of calcium and ketone bodies was not found. The result came out as -0.15 (P<0.0001). This is only a small correlation of minor relevance.

Discussion

The aims of this study were to investigate if the calcium concentration within 48h after parturition and the highest ketone body concentration were of influence to the development of metritis and endometritis at week 1, 2, 4 and 8 after parturition and to investigate if the calcium concentration within 48h after parturition was correlated to the highest ketone body concentration at week 1&2 after parturition. The incidences of metritis and endometritis (week 4 and 8) in the study population were 32.3% (204 of 632), 31.0% (176 of 567), 14.8% (78 of 528), respectively.

No association was found between calcium concentration and the incidence of metritis and endometritis after parturition. This was similarly as found by Chamberlin *et al.,* 2013, Mahen *et al.*, 2018 and Caixeta *et al*., 2015, but in contrast to Martinez *et al*., 2012, Rodríguez *et al.,* 2017 and Whiteford and Sheldon, 2005. This may characterise a lack of association or it may be that the incidence of metritis and endometritis in de study population was too low to detect an association. Chamberlin *et al*. 2013, Mahen *et al*., 2018 and Caixeta *et al*., 2015 state the incidence of metritis in their study populations 8.0%, 25% and 10%, respectively. Which is considerably smaller than in the present study (32.3%, 31.0% and 14.8%). Studies that found a correlation were Martinez *et al*., 2012, Rodríguez *et al., 2017* and Whiteford and Sheldon, 2005, with an incidence of metritis of 47.3%, 37.4% and no incidence stated, respectively. Furthermore, study population varied among these six studies, from 38 to 764. This makes it unclear if the incidence of metritis and endometritis in the study population is of influence on the results. An interesting note is that the current study had no divergent values from other study prevalence's and had a relatively large study population.

In the present study, scoring metritis and endometritis was performed by a veterinarian or a well-trained veterinary student in his Masters. Considering a veterinary student to have little experience in performing ultrasounds, this may have influenced the results.
The definition of metritis and endometritis used in the present study was defined by Sheldon *et al.,* 2006. Metritis was scored until 21 days after parturition and was defined as an animal with an abnormally enlarged uterus, fetid watery red-brown uterine discharge, associated with signs of systemic illness, and fever >39.5°C. Endometritis was scored in week 4 and week 8, from day 21-28 and day 50-56, and is characterised by the presence of purulent of mucopurulent uterine discharge in the vagina, not accompanied by systemic signs (Sheldon *et al.,* 2006). A different definition of metritis and endometritis may enrol different cows for the diagnose of metritis or endometritis. Therefore, the definition for metritis and endometritis might have influenced our results.

In the present study, although not significant an association was found between the ketone body concentration and developing metritis after parturition. This was similarly reported by Shin *et al*., 2015, Duffield *et al.,* 2009 and Cheong *et al.,* 2011, who found an association between the ketone body concentration and the development of metritis and endometritis. On the contrary Hammon *et al*., 2006, found that elevated levels of BHBA had no association with any form of uterine disease. Hammon *et* al., 2006 had a small study population (83 cows). As in the present study, Shin *et al*., 2015, Duffield *et al.,* 2009 and Cheong *et al.,* 2011 used a considerably large study population. A larger study population gives a better validity and therefore makes results more reliable. The size of the study population might influence the outcome of a possible association between the ketone boy concentration and the development of metritis.

A difference between the data of Shin *et al*., 2015, Duffield *et al*., 2009 and Cheong *et al*., 2011, is that the present study used no cut-off value of 1.2 mmol/L for the determination of ketosis in their statistical model. According to unpublished literature from Hut *et al.* cows differ in the amount of ketone bodies they suffer from. Primiparous cows may already suffer from a BHBA concentration below 1.2 mmol/L, while multiparous cows do not. That's why the present study did not include a cut-off value. This may influence the results of the present study different than the results of Shin *et al*., 2015, Duffield *et al*., 2009 and Cheong *et al*., 2011.
A possible point of improvement could have been the measurement of glucose. Bicalho *et al*. 2017 measured glucose after parturition and found an association of glucose with the development of metritis after parturition. However, ruminants are dependent on the gluconeogenesis and measuring glucose as a metabolite may not be reliable (Koster and Opsomer, 2016).

Conclusion

In this study calcium status and ketone body status were not correlated after parturition. Although not significant, an effect of the BHBA concentration was detected on the development of metritis after parturition. Calcium status and ketone body status did not affect the development of endometritis after parturition. Differences between farms and parity exist. A primiparous cow has the largest odds in developing metritis within two weeks after parturition. Furthermore, the higher a cow is in parity, the larger the odds are for developing endometritis at week 4 after parturition. More research is needed to find which risk factors are of influence on primiparous cows and the rising parity compared to larger odds in developing metritis and endometritis after parturition.

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