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A literature review about risk factors for fertility disorders in dairy cattle

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1 Introduction

Early detection of reproductive diseases is crucial to improve health and welfare of dairy cattle. Due to demanding challenges to metabolism and the functions of the immune system, the most critical phases for dairy cows to develop fertility disorders are the late pregnancy- and the post-partum-period. Fertility disorders play an important role because their prevalence contributes to poorer fertility and higher culling rates (Gilbert 2016).

The background of this thesis originated in the framework of the collaboration between D4Dairy (https://d4dairy.com/en/#start) and the University of Veterinary Medicine, Vienna. Based on latest technologies, D4Dairy network establishes effective management strategies by generating data platforms, data analyses and a precise decision support for dairy farmers.

The D4Dairy project is subdivided into two main areas. The focus in "area 1" is on the utilization of data that has been collected on farms in recent years. Data derives from for example feed automations or health-related sensors. D4Dairy aims to connect them and use them as base for the development of a precise decision support for farmers and veterinarians. Strategies which help to minimize the use of antibiotics on dairy farms to prevent resistances are also in process and main target of the company. The focus in "area 2" is on early detection of risk factors and diseases based on data collection to maintain health and welfare of dairy cattle. Furthermore, the registration and validation of novel traits are of main interest to establish ideal breeding programs.

The main aim of the thesis was to summarize relevant risk factors that affect pathogenesis of selected fertility disorders. The thesis generate knowledge about risk factors for several fertility disorders compared to previous publications that dealt only with single diseases. The work covers briefly and concisely important risk factors that have a major influence on the occurrence of fertility diseases, overall health and well-being of dairy cows. Results provide the essential basis for further development of mathematical models that will enable early prediction of diseases and will therefore provide a great support for dairy farmers.

The scope of the thesis neither allows the analysis of all described risk factors, nor of all possible fertility disorders. Therefore, the focus of the thesis was to do a literature research on scientific papers and describing which of the observed risk factors are the most relevant in dairy cattle. Further, significant associations between various risk factors have been explored. The following literature reviews journal articles mainly published since the year 2000.

2 Methods and Materials

The content of the thesis is based on a literature research that has been carried out from March until November 2019. Data were collected from scientific articles reported on Pubmed (https://www.ncbi.nlm.nih.gov/pubmed/), Science direct (https://www.sciencedirect.com/) and Google scholar (https://scholar.google.com/). The thesis summarizes mainly knowledge of literature that has been published in the year 2000 or more recent. The research has been conducted in areas with highly developed dairy industry (Europe, North America and New Zealand). This thesis contains exclusively knowledge from literature in English or German language.

To get the appropriate information online, the following keywords were used: “post-partum disease”, “reproductive disorders”, “fertility disorder”, “fertility performance”, “retained foetal membranes”, “retained placenta”, “metritis”, “uterine infectious diseases”, “endometritis”, “ovarian cysts”, “cystic condition”, “anovulation”, ”anoestrus”, “dystocia”, “dairy cattle”, “risk factor”, “risk”, and “fertility”.

The aim of the study was to find studies that report risk factors mainly as odds ratios with the corresponding confidence intervals (CI 95 %), which indeed was in fact not possible for all described risk factors. Studies that observed a big amount of dairy cattle were considered as more important and were therefore discussed more intensively. To illustrate the most relevant data from the reviewed papers, adapted tables were added. Furthermore, limitations in the comparability of the different studies existed due to the various thresholds of single disorders and the various techniques that have been used for data collection.

This literature review contains knowledge of all together 82 scientific papers. These papers were selected based on their large numbers of cows or datasets that were enrolled in the studies. Further, studies that worked with odds ratios were preferred to others. Only literature in English or German language were included. The research focused on studies from Europe, North America and New Zealand.

Thus, 66 papers were excluded. However, this large number results from an inaccurate research method used at the beginning. The literature research stretched over an extended period. However, over this period the latest articles were always read and either included or excluded.

3 Definitions of reproductive diseases

According to current state of research, the most frequent reproductive diseases are metritis, endometritis, retained foetal membranes and ovarian disorders. To give an overview, the next chapter describes these conditions briefly and underline that they all kind of interrelate with each other.

Retained placenta (RP)

After calving the placenta is expelled physiologically within six hours. Cattle are diagnosed with retained placenta via vaginal examination if this event does not occur within 24 hours after parturition. Disease incidences among dairy cattle are between five and fifteen percentage. The exact pathogenesis of retained placenta is not completely clear yet but it seems to have various triggering factors (Gilbert 2016, Sheldon et al. 2008).

Kimura et al. (2002) findings underline that RP affected cows have an impaired neutrophil function and a lower concentration of interleukin-8, which is a crucial chemoattractant. In fact, the maternal immune system is not able to recognize the placenta as a “foreign tissue” and does not give the signal to expel it.

However, RP is one of the most frequent and important post-partum disorder of multifactorial nature. This thesis deal with risk factors causing RP. Additionally, several authors (Bruun et al. 2002, Dubuc et al. 2010b, Gilbert 2016, Sheldon et al. 2008) describe RP as a risk factor for further uterine infectious diseases like metritis and endometritis.

Puerperal metritis and endometritis

The expulsion of foetal membranes after parturition, the involution of the uterus and endometrial regeneration are the main physiological events that are necessary in post-partum cows to remain healthy and fertile after their calving (Sheldon et al. 2008).

They are important factors for prevention of bacterial contamination. Post-partum uterine diseases have been summarized and categorized by Sheldon et al. (2006).

Individuals are diagnosed with puerperal metritis (PM) if they have enlarged uterine horns and abnormal red-brown vaginal discharge. Furthermore, clinical noticeable are signs of systemic illness like elevated body temperature that occur during the time between parturition and day 21 pp. Classification for clinical metritis is not totally clear, but it can be defined including all signs of PM except systemically sickness.

Cows presenting purulent vaginal discharge including more than 50 % pus, or mucopurulent discharge containing approximately 50 % pus and 50 % mucus 21 days pp or later suffer from clinical endometritis. Endometritis is more superficial and is located only in the uterine epithelium. Metritis though affects also deeper layers and is more severe (LeBlanc et al. 2002, Sheldon et al. 2006, Sheldon et al. 2009).

After calving it is normal that the uterus is contaminated with several aerobic and anaerobic bacteria. However, *Trueperella pyogenes (T. pyogenes)*, *Fusobacterium necrophorum* and *Prevotella* species are associated with the appearance of uterine infectious disorders. *T. pyogenes* and *Escherichia coli (E. coli)* are the most commonly detected pathogens (Potter et al. 2010).

Most of the cattle (24 % - 40 %) suffer from clinical metritis in the first two weeks pp. Twenty percent of the affected animals further develop clinical endometritis. If no signs for clinical endometritis are detected, cows can either be healthy or they can have subclinical endometritis. If uterine samples are collected within day 21 and 33 pp for cytology and if they contain more than 18 % neutrophils, animals are diagnosed with subclinical endometritis. If samples are taken between day 34 and 47 pp, there should be less than ten percent neutrophils to diagnose cattle as healthy. It is crucial to detect subclinical sick cows without obvious signs of illness as well, because it can have a negative impact on future fertility (Sheldon et al. 2006, Sheldon et al. 2008).

Ovarian cysts

If follicles do not ovulate during oestrus, they often persist as ovarian cysts. They are defined as cysts if follicles with a diameter of 25 mm or bigger and no corpus luteum are found at the same time. Due to this event cows are further often unable to ovulate again and get infertile. The real cause for the development of ovarian cysts is not completely investigated yet. However, some publications suggest that the lower released amount of gonadotropin-releasing hormone (GnRH) plays an important role in the pathogenesis. During oestrus GnRH physiological triggers, the luteinizing hormone LH until it reaches its peak and initiate ovulation. A defect in the hypothalamus, which makes it insensitive for the oestradiol surge, may is an important cause of follicular cysts (Crane et al. 2006, Gümen and Wiltbank 2005).

4 Diagnostic methods regarding fertility disorders

Clinical endometritis is known as one of the most common diseases, which negatively affects dairy fertility resulting in tremendous economic losses. To minimize endometritis cases in a dairy herd, it is important to take supporting measures for prevention by using proper diagnostic methods. Thereby affected individuals can be treated on time and with the right medication (Barlund et al. 2008, Lee and Kim 2007, Machado et al. 2012).

Several researchers discuss different diagnostic techniques, which evaluate the uterine health status. To examine ovarian structures, the uterus and their functionality, although it has a low sensitivity and specificity, transrectal palpation is most widely used in veterinary cattle practice to detect endometritis cases (Hanzen et al. 2000, LeBlanc et al. 2002, Mee et al. 2009). Recent findings indicate that rectal palpation alone is not reliable enough to detect clinical endometritis (Wagener et al. 2017).

Another diagnostic method to check either a cow has endometritis or not, is the ultrasonography which is meanwhile widely used among veterinarians. It is an inexpensive equipment, which can be used to check pregnancy and the reproductive health status through the assessment of uterine fluid volume and endometrial thickness (Barlund et al. 2008, Machado et al. 2012, Runciman et al. 2008).

Furthermore a "vaginal mucus scoring system" enables the detection of uterine clinical endometritis. Šavc et al. (2016) found out, that the combination of both methods ("vaginal mucus scoring system" and ultrasonography) offers the better diagnostic value for well-timed intervention to increase reproductive performance.

Other diagnostic methods to evaluate vaginal mucus and uterine inflammation are vaginoscopy, hysteroscopy and the use of an intravaginal device, the so-called Metricheck (Barlund et al. 2008, Machado et al. 2012, McDougall et al. 2007, Pleticha et al. 2009).

To practice only visual inspection with the help of a speculum, when cows with obvious purulent or mucopurulent vaginal discharge are diagnosed as endometritic, can lead to many false positive results (Dubuc et al. 2010a).

A German study of Pleticha et al. (2009) described the comparison between vaginal examination with gloved hands and the usage of the Metricheck device as diagnostic tools. Vaginoscopy was the reference testing technique. They observed 1,002 Holstein-Friesian cows and results underline that the Metricheck device diagnosed more cows as endometritic than the other methods. Results are demonstrated in table 4-1.

Table 4-1 (Pleticha et al. 2009); Spread of vaginal discharge scores "VDS (0-3)" using different diagnostic tools:

Diagnostic method	n	Score 0 % (CI 95 %)	Score 1 % (CI 95 %)	Score 2 % (CI 95 %)	Score 3 % (CI 95 %)
Speculum	328	63.1 (57.7-68.2)	15.5 (11.5-19.3)	9.8 (6.4-12.8)	11.6 (8.0-14.9)
Metricheck Device	337	52.5 (47.0-57.7)	16.0 (12.0-19.8)	16.9 (12.8-20.8)	14.5 (10.6-18.2)
Gloved hand	337	63.2 (57.9-68.2)	10.4 (7.0-13.5)	11.6 (8.0-14.8)	14.8 (10.9-18.5)

"Vaginal discharge score: Score 0 = clear or translucent mucus; Score 1 = mucus containing flecks of white or off-white pus; Score 2 = discharge containing less than 50 % white or off-white mucopurulent material; Score 3 = discharge composed of more than 50 % white or yellow pus or is sanguineous; CI = confidence interval" (Pleticha et al. 2009).

Interestingly there were more positive results using the Metricheck technique if the same cow underwent vaginoscopy first. Authors believe that the first method they had applied could have stimulated uterine contractions and therefore afterwards a higher rate of mucus score can be detected. Further research must be done to prove the hypothesis. However, the appearance of the higher prevalence by using the Metrichek tool cannot be explained in detail yet. Further all examination tools are indirect tests, where the presence of purulent vaginal mucus suggest an inflammation of the endometrium, but some positive tested cows may just have vaginitis and not endometritis. Which leads to false positive results, also proven by (Dubuc et al. 2010a).

As maintained by Machado et al. (2012), the “uterine lavage sample optical density” (ULSOD) method eliminates false-positive diagnoses better than other techniques, because it detects and evaluates only the mucopurulent secretion located inside the uterus.

The researchers included 1,742 cows from three dairy farms located around Ithaca (New York, USA) in their study and used low-volume uterine lavage to collect samples from the cows that were 35 d ± 3 days in milk. After cleaning and disinfection of the perineum, a plastic infusion pipette was pushed carefully through vagina and cervix into the uterus. With the help of sterile saline solution, the sample of uterine fluid was aspirated and then analysed always by the same investigator. (Score 0: without purulent or mucopurulent exudate; Score 1: bloody sample without pus; score 2: purulent sample). Cows belonging to score 2 were diagnosed with clinical endometritis. Among all observed cows the incidence for clinical endometritis was only 10 %, which is relatively low. Several wavelengths were used to measure endometritis affected cows through absorption of the exudate. Six-hundred-twenty nanometres turned out to be the best one, because it presented the greatest area under the curve (AUC) in receiver operating characteristics (ROC). As shown in Table 4-2 they did a second ROC analyse, in which the neutrophils of the uterine lavage samples were used as the gold standard. Cows with more than 18 % neutrophils were diagnosed as endometritis positive, cows with 18 or less percent neutrophils were diagnosed as endometritis negative (Machado et al. 2012).

Table 4-2 (Machado et al. 2012); Sensitivity and specificity for “uterine lavage sample optical density at 620 nanometres” (ULSOD 620 nm) for the detection of endometritis:

	AUC	**ROC threshold**	**Sensitivity %**	**Specificity %**
ULSOD 620	0.85	0.058	76.3	78.3
ULSOD 620*	0.862	0.059	100	82.2

“ULSOD 620 nm = uterine lavage sample optical density at 620 nanometres; n = 554; ULSOD 620 > 18 % neutrophils endometritis positive; ≤ 18 % endometritis negative; AUC = area under the curve; ROC = receiver operating characteristics” (Machado et al. 2012).*

Machado et al. (2012) used a multivariable logistic regression model which displays, that the variables APYO (Presence of *Trueperella pyogenes* in the lavage sample), MET (presence of metritis) and RP (presence of retained placenta) were significantly associated with the dichotomized ULSOD 620. Results are demonstrated in Table 4-3. Through the multivariable cox proportional hazard model the researchers found out that cows having a ULSOD 620 of 0.058 or less were 1.21 times more likely to get pregnant, correlated to cows with ULSOD 620 higher than 0.058 (P < 0.01) (Machado et al. 2012).

Table 4-3 (Machado et al. 2012); Associations between the dichotomized uterine lavage optical density at 620 nm and the presence of T. pyogenes, metritis and retained placenta:

variable	n	Odds for ULSOD620 > 0.058 (%)	P
TPYO positive	151	59.6	< 0.001
TPYO negative	1.577	24	< 0.001
MET positive	265	39.6	= 0.035
MET negative	1.477	24.9	= 0.035
RP positive	98	44.9	= 0.04
RP negative	1.644	26.1	= 0.04

n = number of cows;"ULSOD 620 = uterine lavage optical density at 620 nanometres; MET = presence of metritis; TPYO = presence of Trueperella pyogenes; RP = presence of retained placenta" (Machado et al. 2012).

The appearance of *Trueperella pyogenes* in the uterine lavage samples resulted in a higher mean ULSOD 620. *T. pyogenes* causes fatal endometrial lesions, which can result in an anti-inflammatory response. Therefore, a high ULSOD 620 can also be used as an indicator of the outcome of the endothelial inflammatory process caused by this pathogen.

Machado et al. (2012) recommended ULSOD 620 detection for endometritis, because it takes less laboratory time, the operation is simple and demands no special training.

In Ireland Šavc et al. (2016) tried to figure out which method can be used as a predictor of the future reproductive performance in 493 Holstein-Friesian dairy cows. They compared the mucus scoring- with the ultrasonographic- method between day 21 and 38 after parturition.

To collect the vaginal mucus, experienced operators used the practical cup-shaped Metricheck device. Then the mucus was scored as MS 0 (no or clear mucus was found), MS 1 (mucus with specks of purulent material), MS 2 (< 50 % of pus) and MS 3 (> 50 % of pus). The vaginal mucus enables to identify cervical and vaginal inflammation caused by several pathogens like *T. pyogenes*, *Fusobacterium necrophorum* or *Bacteroides spp.* (Runciman et al. 2008, Šavc et al. 2016).

The uterine score (UTS) was measured via ultrasound and ranked as shown in Table 4-4. Cows with "Uterine Score 2" and above were positive and suffered, quite certainly, from uterine inflammation.

Table 4-4 (Šavc et al. 2016); Definitions of uterine score (UTS 0-4) measured via ultrasound:

Uterine score (UTS)	Uterine characteristics
0	No fluid or small volume ≤0.2 cm in the lumen, completely involuted with infolding of the endometrium.
1	No fluid or small volume ≤0.2 cm in the lumen, with a spoke wheel-shaped hyperechoic (white) lumen with infolding of the endometrium.
2	Moderate volume ≥0.2 cm ≤0.5 cm of anechoic (black) fluid in the lumen with infolding endometrium and enlarged center.
3	Moderate volume ≥0.2 cm ≤0.5 cm of mixed echogenicity (gray or white) fluid in a spoke wheel-shaped lumen with enlarged/stellate center.
4	large volume ≥0.5 cm mixed echogenicity (gray or white) fluid with hyperechoic particles in a circular lumen without infolding of the endometrium

As demonstrated in the Table (Table 4-5), the uterine health, weather measured through the Metricheck device (MS) or ultrasound (UTS), is an important indicator which affects the positive pregnancy status at the end of the season.

Šavc et al. (2016) developed a univariate cox proportional hazard, in which they used the PS to describe the reproductive performance of the Holstein-Friesian dairy cows, which seasonally calved.

Table 4-5 (Šavc et al. 2016); Effects of the uterine health status measured through ultrasound uterine scores (UTS 1-4) or through the Metricheck device (MS) on the hazard ratio (HR) of the positive pregnancy status (PS):

Uterine health status	HR of PS	P
UTS 1	1	< 0.01
UTS 2	0.84	< 0.01
UTS 3	0.53	< 0.01
UTS 4	0.78	< 0.01
MS 0	1	< 0.01
MS 1	1.02	< 0.01
MS 2	0.51	< 0.01
MS 3	0.75	< 0.01

"HR = hazard ratio; PS = positive pregnancy status; UTS = Ultrasound; MS = Metricheck device; MS 0 = no or clear vaginal mucus; MS 1 = mucus with specks of purulent material; MS 2 = less than 50% of pus; MS 3 = more than 50% pus in the sample

UTS 1 = No fluid or small volume ≤ 0.2 cm in the lumen, with a spoke wheel-shaped hyperechoic lumen with infolding of the endometrium; UTS 2 = Moderate volume ≥ 0.2 cm ≤ 0.5 cm of anechoic fluid in the lumen with infolding endometrium and enlarged centre; UTS 3 = moderate volume ≥ 0.2 cm ≤ 0.5 cm of mixed echogenicity fluid in a spoke wheel-shaped lumen with enlarged/stellate centre; UTS 4 = large volume ≥ 0.5 cm mixed echogenicity, fluid with hyperechoic particles in a circular lumen without infolding of the endometrium" (Šavc et al. 2016)

Including the age as a variable, younger cows (lactation < 5) with UTS and MS scores of 0 and 1 indicated a significantly better reproductive performance. Results of the receiver-operating characteristic analysis let Šavc et al. (2016) conclude, that UTS and MS results can be different. However, the researcher figured out, that cows with a positive UTS (UTS 2 and above) were 7.75 times more likely to be MS positive scored as well ($P < 0.01$). A high MS score has a lower negative impact on PS than a high UTS score ($P < 0.05$). Mismatches between the two techniques exist, because the vaginal content is not of the same quality and quantity as the uterine content.

Šavc et al. (2016) show that there was a 64 % sensitivity for cows without purulent uterine fluid (UTS 0) to be ranked MS 0 as well. The specificity amounted 25.7 %. The sensitivity decreased with increasing UTS scores and was for MS 1 22.0 %, MS 2 33.3 % and MS 3 26.3 %.

The combination of MS 2 and MS 3 cows were diagnosed as positive and when using them as the threshold, the sensitivity was 39.1 % and the specificity 91.7 %.

The study of Šavc et al. (2016) underlines that well-timed intervention and successful treatments help to minimize fertility disorders in dairy herds. Cows scored with UTS 3 during pre-breeding season conceived earlier, because they were identified from the farmer and were well treated before their earliest service date (Šavc et al. 2016).

Mee et al. (2009) included 5,751 cows that calved seasonally in their study and examined them via ultrasound prior to breeding to assess their URTS, ultrasound reproductive tract , score. Five-point five percent of the cows had a mild endometritis or incomplete uterine involution (URTS 3 and 4). Only 2.2 % suffered from pyometra. The study underlines the association between URTS score and uterine involution. An incomplete uterine involution at day 53 post-partum is in accordance with recent findings on subclinical endometritis. Furthermore, pyometra turned out to be a significant risk factor for late embryonic mortality, hence the affected cows had a lower pregnancy rate.

Like Šavc et al. (2016), also Runciman et al. (2008) maintain, that scoring the vaginal mucus via vaginoscopy is a practical tool to detect cows with a lower reproductive performance. He also used a scale from zero to three to describe the mucus inside the vaginal discharge but defined it differently (0 = no discharge or clear mucus; 1 = mucus with ≤ 50 % pus; 2 = mucus and > 50 % pus; 3 = entirely pus).

Runciman et al. (2008) observed 1325 cows, which were in higher risk of getting endometritis. He ranked them as "higher risk cows to get endometritis" because of the following risk factors, which turned out to be significant among this study.

1. Retained foetal membrane (RFM > 24 h after calving)
2. Dystocia
3. Dead calf (after or within 24 hours of calving)
4. Hypocalcaemia (detected at or within 24 hours of calving)
5. Twin birth
6. Calving induction (with dexamethasone trimethylacetate)
7. Observed vaginal discharge (any vaginal discharge found ≥ 7 days after calving)

The odds ratios displayed in Table 4-6, show that a positive visual vaginal (VV) score (1-3) directly correlated with low body condition scores, primiparity, intrapelvic or large uterus, poor uterine tone, retained foetal membrane (RFM), vaginal discharge, dystocia, dead calf and twins. All in all, cows that were diagnosed as positive had a lower chance to conceive to first service than negative cows (Runciman et al. 2008).

Table 4-6 (Runciman et al. 2008);

Table 5. Adjusted odds ratios for factors associated with a positive visual vaginal (VV) status within a subset of dairy cows selected on the basis of being at high risk of reproductive tract disease (n = 1265)

Risk factor	Proportion VV positive	n	Coeff	SE	P-value	Odds ratio	95% CI	
							Lower	Upper
Herd[a]					< 0.001			
Dystocia								
No	0.25	792				1.00		
Yes	0.24	473	0.491	0.186	< 0.01	1.63	1.14	2.35
Dead calf								
No	0.25	1028				1.00		
Yes	0.24	237	0.455	0.208	0.03	1.58	1.05	2.37
Twins								
No	0.24	1204				1.00		
Yes	0.34	61	0.782	0.335	0.02	2.19	1.13	4.21
RFM								
No	0.22	923				1.00		
Yes	0.30	342	0.867	0.187	< 0.001	2.38	1.65	3.43
Vulval discharge								
No	0.24	1135				1.00		
Yes	0.31	130	0.793	0.263	< 0.01	2.21	1.32	3.70
Uterus position								
Abdominal	0.25	868				1.00		
Pelvic	0.24	397	0.511	0.201	0.01	1.67	1.12	2.47
Uterus size (cm)			0.253	0.077	< 0.001	1.29	1.11	1.50
Uterine tone								
Flaccid	0.30	701	0.408	0.200	0.04	1.50	1.01	2.23
Normal/turgid	0.18	564				1.00		
CEI (weeks)			−0.266	0.047	< 0.001	0.77	0.70	0.84
Age (years)					0.016			
2	0.30	173				1.00		
3	0.19	207	−0.711	0.282	0.01	0.49	0.28	0.85
4–8	0.25	658	−0.767	0.243	0.002	0.46	0.29	0.75
> 8	0.25	227	−0.673	0.292	0.02	0.51	0.29	0.90
BCS					0.022			
< 4.5	0.29	400	0.177	0.177	0.3	1.19	0.84	1.69
4.5	0.27	494				1.00		
5	0.17	286	−0.490	0.214	0.02	0.61	0.40	0.93
> 5.0	0.13	85	−0.503	0.403	0.2	0.60	0.27	1.33
Constant			−0.835	0.682	0.22			

[a]Individual herd differences not tabulated (17 herds). Hosmer-Lemeshow (HL) P-value = 0.69, ROC = 0.79.

Barlund et al. (2008) observed 221 cows and they tried to identify which method is the best to detect endometritis affected cows. They compared five different diagnostic methods: vaginoscopy, ultrasonographic assessment of uterine fluid volume, ultrasonographic assessment of endometrial thickness, endometrial cytology collected by cytobrush and endometrial cytology collected by uterine lavage.

Cytobrush cytology had the best repeatability among all the techniques and therefore it was considered as reference test. It turned out to be the most reliable technique to detect endometritis affected individuals with a specificity of 89.9 %. The sensitivity was 12.9 %. The big difference between the percentage of sensitivity and specificity indicates that there are other risk factors than endometritis for cows to not get pregnant (Barlund et al. 2008).

Westermann et al. (2010) analysed the associations between positive vaginal discharge findings, bacteriological and cytological results associated with clinical endometritis. Aim of the study was to define the proportion of samples that were diagnosed false positive for endometritis by vaginoscopy. Overall, 17.3 % of the cows that were controlled via intrauterine bacteriology as referent test were diagnosed false positive by vaginoscopy. Vaginoscopy resulted in 28.5 % false positive results when they used cytology as the referent test. The most frequent found bacteria was *T. pyogenes* and they determined a significant correlation between a positive VDS and the appearance of *T. pyogenes*. Additionally, the paper proofs a significant correlation between the PMN fraction size and the occurrence of *T. pyogenes.* Interestingly *E. coli* or other pathogens, which play a key role in endometritis cases did not show the same significant effect. Results indicate that T. pyogenes causes more severe endometritis than other pathogens and is more demanding for the cellular immune response (Westermann et al. 2010).

The routinely cytological diagnostic of post-partum dairy cows is additionally important to recognize subclinical endometritis affected individuals. Cytological material can be collected via cytobrush technique or low-volume flushing of the uterus. Both methods do not influence the endometrial health and further conception rates. Thresholds for subclinical endometritis are described differently in various studies and range from the presence of 5 to 18 % PMN, depended on the time pp.

Although the uterine cytology is a relatively time consuming examination, Wagener et al. (2017) suggest it as a helpful monitoring tool for veterinarians, if a herd shows poorer fertility, to detect causal problems.

Chapter summary:

Finding the appropriate diagnostic tool to assess uterine health was subjected in a lot of latest researches and there is general agreement that a well-timed and well-performed examination is crucial. If sick individuals do not get diagnosed as sick, they are in higher risk to get infertile later. The previous discussed papers underline the importance of diagnostic tools, which cannot just be practiced by veterinarians, but some also by farmers, to minimize the risk for uterine infectious diseases. To detect uterine infectious diseases various contents, like the uterine or vaginal ones were used. Because of different qualities and quantities of the collected mucus, not all the study results are comparable.

While some authors suggest that the assessment of uterine fluid, collected directly from the inside of the uterus is the most reliable sample. Other papers instead point out that the vaginal mucus scoring is the most practicable and solid technique.

The biggest advantage of the ULSOD diagnostic technique is the objective assessment, because of the fixed numerical value. On the other hand, also the cytology technique can yield reliable results with a high specificity. A big disadvantage is that the cytology method requires more specific knowledge and labour time (Westermann et al. 2010).

Apart from the applied technique, many papers indicate the importance of taking the same investigator for each examination, in order to not get too many variations. Except the cytology and the ULSOD tool, all of them are measurements underlying subjective judgement. Additionally, it is essential to find the best time post-partum and/or pre breeding and to do examinations exactly on the same day.

Authors point out that the risk factor interrelates with other important ones, like lactation number or BCS. These factors will be discussed later in the course of the thesis.

In general, it can be assumed that diagnostic check-ups should be part of farm management, to collect important parameters, like contents of fluids in combination with clinical signs, to reduce herd prevalence of endometritis and maintain also health of single cows. Although there does not exist a gold standard test yet, ULSOD measurements and cytology techniques seem to be the most objective ones and come along with a high sensitivity and specificity. Vaginoscopy in turn is more practicable but end up with more cows diagnosed as false positive.

The following table (table 4-6) reviews the findings of the previous described literature. It must be kept in mind, that investigators worked with different starting points and thresholds.

Table 4-7; overview of the chapter 4 "Diagnostic methods regarding fertility disorders":

Author	**Number of cows/Breed**	**Breed**	**Risk factor/ diagnostic tool**	**Sensitivity/Specificity (%)**
Barlund et al. (2008)	221	Holstein-Friesian	Vaginoscopy; ultrasonography of uterine volume; endometrial thickness; cytology via cytobrush and uterine lavage	12.9/89.9
Machado et al. (2012)	1,742	Holstein-Friesian	ULSOD 620 ULSOD 620*	76.3/78.3 100/82.2
Runciman et al. (2008)	1,325		Visual vaginal scoring via vaginoscopy (VV 0-3)	

Šavc et al. (2016)	493	Holstein-Friesian	Vaginal mucus scoring via Metricheck (MS 0-3); Uterine score (UTS 0-4)	39.1/91.7
Westermann et al. (2010)	230	Holstein-Friesian	Vaginal discharge scoring (VDS 0-3)	53.9/95.4
Mee et al. (2009)	5, 751	Holstein-Friesian	URTS scoring pre breeding	

"ULSOD 6020 = ULSOD 620 nm = uterine lavage sample optical density at 620 nanometres;
ULSOD 620 > 18 % neutrophils endometritis positive; ≤ 18 % endometritis negative" (Machado et al. 2012).*
"VV Score = visual vaginal score (0 = no discharge or clear mucus; 1 = mucus with ≤ 50 % pus; 2 = mucus and > 50 % pus; 3 = entirely pus)" (Runciman et al. 2008).
"Metricheck MS Score =; MS = Metricheck device; MS 0 = no or clear vaginal mucus; MS 1 = mucus with specks of purulent material; MS 2 = less than 50% of pus; MS 3 = more than 50% pus in the sample;
UTS Score = ultrasound uterine score; UTS 1 = No fluid or small volume ≤ 0.2 cm in the lumen, with a spoke wheel-shaped hyperechoic lumen with infolding of the endometrium; UTS 2 = Moderate volume ≥ 0.2 cm ≤ 0.5 cm of anechoic fluid in the lumen with infolding endometrium and enlarged centre; UTS 3 = moderate volume ≥ 0.2 cm ≤ 0.5 cm of mixed echogenicity fluid in a spoke wheel-shaped lumen with enlarged/stellate centre;
UTS 4 = large volume ≥ 0.5 cm mixed echogenicity, fluid with hyperechoic particles in a circular lumen without infolding of the endometrium" (Šavc et al. 2016).
"VDS Score = Vaginal discharge score: Score 0 = clear or translucent mucus; Score 1 = mucus containing flecks of white or off-white pus; Score 2 = discharge containing less than 50 % white or off white mucopurulent material; Score 3 = discharge composed of more than 50 % white or yellow pus or is sanguineous" (Pleticha et al. 2009).
"URTS = Ultrasound reproductive tract scoring" (Mee et al. 2009).

5 Associations between nutrition/metabolism and fertility disorders

5.1 Risk factor - Body condition score (BCS)

To assess the nutritional status and energy reserves of dairy cows, the body condition scoring system (BCS) was invented. The BCS is an assessment of the proportion of body fat and plays a crucial role in dairy cattle management. Normally the BCS is visually scored by using a five-point scale. Based on this scale, one means thin and five means over conditioned (Roche et al. 2009).

BCS status during pregnancy, parturition and post-partum is an important risk factor of endometritis and retained foetal membranes. In addition, a constant BCS over three is important for many physiological functions, which are necessary for successful reproduction, for example the ovulation of a high-quality oocyte (Hoedemaker et al. 2009).

In the study of Hoedemaker et al. (2009) 234 German Holstein-Friesian cows in North Germany were observed. The researchers concluded that cows with a lower BCS (< 3.0) were, at any time point, at calving or early lactation, more likely to suffer from endometritis or retained placenta. Furthermore, they needed more time to return to cyclicity. The Table 5-1 demonstrates the odds ratios for body condition scores < 3.0. During the study, none of the cows were extremely over- or underconditioned. Throughout the antepartum period, it is normal that cows lose weight. However, the degree of loss was minor than in other studies and did not affect the incidence of metritis (Hoedemaker et al. 2009).

Table 5-1 (Hoedemaker et al. 2009); Odds ratios for fertility disorders for a body condition score (BCS) < 3.0:

Disease	**OR**
Not cyclic at 3-5 weeks pp	2.56-2.79
Endometritis	2.95
Risk of dystocia 10 weeks pp	4.10
Retained placenta	2.78

BCS = Body condition score; pp = Post-partum; OR = Odds ratios

Controversially Kim and Suh (2003) came to the following conclusion: cows which suffer a massive BCS loss during dry period have a bigger risk of getting metritis, than cows with moderate condition loss (62 % vs. 23 %). They divided 67 Holstein dairy cows into two different groups. “The moderate condition loss group”, involved cows that lost 0-0.75 points in BCS (0-0.75 points, n = 41) and “the marked condition loss group” (1.0-1.5 points, n = 26). Interestingly there was no higher risk for the marked condition loss group to get retained placenta (Kim and Suh 2003).

Runciman et al. (2008) observed cows with a higher risk of getting endometritis and demonstrated (Table 4-6), that there is a difference in odds for a positive vaginal discharge between cows with a BCS of 4.5 and BCS of 5.0 (OR = 1 vs 0.61).

Roche et al. (2009) also stated that a low BCS, or a loss in the BCS pre-partum or early post-partum, are risk factors for uterine infectious diseases. Furthermore, the study shows that associations between BCS loss, calf size and sex of the following calf exist. Bigger calf size and male sex in turn are risk factors for dystocia (Roche et al. 2009).

The effect of BCS change on dystocia could not be shown in the study of Berry et al. (2007), that analysed data sets of 15 years were retrospectively. BCS changes did not influence the likelihood of dystocia, hence difficult calving or stillbirth. However, cows that suffered from the previous mentioned fertility disorders, had a lower BCS, reduced milk yield and poorer fertility later on (Berry et al. 2007).

BCS changes have a negative impact on a regular cyclicity. Ovarian cyclicity of cows that undergo BCS changes during early lactation, or during the period until their first oestrus, need more time to get restarted (Chagas et al. 2007).

While comparing the CIDR treated with the Ovsynch treated group, Crane et al. (2006) found out that cows with a BCS lower than three (BCS < 3) had a 0.49 times lower chance to have a CL after timed insemination, than cows with a higher body condition score, which means, that they needed more time to return to normal cyclicity. The average probability to get pregnant was also lower among cows with a BCS lower than three (Crane et al. 2006).

Variable body condition scores are associated with infrequent luteinizing hormone pulses, poor follicular response to gonadotropin and reduced functional competence of the follicle (Chagas et al. 2007).

According to Diskin et al. (2003), anoestric conditions start, if dairy cattle lose 22-24 % of their earliest body weight.

A negative energy balance can either influence directly the hypothalamic GnRH secretion or manipulate through the insulin-like growth factor- (IGF) hormone insulin axis. However, there are many other risk factors like season and environment, which have an effect on the cooperative work between brain and ovarian function (Diskin et al. 2003).

López-Gatius et al. (2002b) monitored during the pre- and post-partum periods risk factors of 650 Friesian dairy cows, which may have an impact on ovarian cysts development. Low BCS or BCS changes to a lower BCS unit (pre- or post-partum) did not negatively influence the arise of cysts during the early post-partum period. Controversially, cows with a BCS gain of one-unit (for example three to four) pre-partum (60 days until parturition) were 8.4 times more likely to develop ovarian cysts. The BCS had no effect on the spontaneously regression of cystic structures. It is not well-known why some regress without treatment, and some do not. In addition, the pathogenesis of ovarian cysts is not clear, although they may occur because of a neuro-endocrine imbalance. The disproportion leads to one preovulatory follicle, that is unable to ovulate and persist as a cystic structure (López-Gatius et al. 2002b).

5.2 Risk factor - Negative Energy Balance (NEB)

After parturition many cows experience a negative energy balance (NEB), which can be a critical point for their health status and fertility. The metabolic stress caused by the NEB does not only affect uterine health, but also the follicular and luteal development. Under physiological conditions, a cow ovulates again 20-30 days after parturition. If cows have a NEB, follicle waves occur at the right time, but the follicles are not capable to undergo full development. When deviation and maturation take part during the NEB nadir, the likelihood of ovulation can be lower, because the follicle cannot grow to ovulatory size (Wathes et al. 2007).

Furthermore Wathes et al. (2007) describe, that cows having a NEB status, experience more difficulties regarding the post-partum uterine involution. After parturition, the endometrial tissue must get repaired and the uterus must get free of bacterial components. NEB interrupt uterine involution, which is proved by the study of Wathes et al. (2007). By histological examination they found more segmented inflammatory cells, but fewer mononuclear cells in cows that faced a severe negative energy balance, compared to cows with a mild negative energy balance. In conclusion, a NEB after parturition is a risk factor for remaining uterine inflammation pp, that can end up in endometritis (Wathes et al. 2007).

LeBlanc et al. (2011) conclude that the number of circulating non esterified fatty acids (NEFAs) rises, if the feed intake is low. Meanwhile the risk for a fatty liver increases and therefore the functionality of the neutrophils shrinks. Cows often get into a pro-inflammatory stage post-partum (LeBlanc et al. 2011).

In the study of Colazo et al. (2009) 27 pregnant Holstein cows were involved and fed with different sources of fatty acids, *ad libitum* (AL) or via 24 % feed restriction (FR) during the dry period. Eight percent dry matter was the main feed component and canola (oleic), linola (linolenic) or flax (linoleic oil) were supplemented. The aim of the study was, to find out which effect restrict feeding and supplementation of fatty acids during transition period have on pp diseases.

While FR cows felt into a negative energy balance pre-partum, the dietary source had no influence on the energy status. Cows that were fed *ad libitum,* were more likely to suffer from uterine infection, but less of them had ovarian cysts. The likelihood of ovarian cysts did not differ among the three fat dietary groups. Further, there was no higher or lower incidence for retained foetal membranes in the various dietary groups (Colazo et al. 2009).

Table 5-2 (Colazo et al. 2009); Incidences for fertility disorders in the "feed restriction" (FR) and "*ad libitum*" (AL) fed groups:

	FR	**AL**
Energy balance	Pre-partum negative EB; pp less negative EB	
Uterine infections	10 out of 37 (P = 0.01)	2 out of 35 (P = 0.01)
Mean ± SE interval from calving to uterine involution	26. 8 ± 1.8 d	26. 8 ± 1.8 d
Ovarian cysts	7 out of 35 (P < 0.09)	2 out of 35 (P < 0.09)
Proportion of short estrous cycle < 17 d	↑ number (P = 0.12)	↓ number (P = 0.12)

FR = feed restriction; AL = ad libitum; EB = energy balance

Table 5-3 (Colazo et al. 2009); Effects of oil supplementation on the interval from calving to first ovulation post-partum (pp):

	Canola oil	**Linola oil**	**Flax oil**
Interval from calving to first ovulation pp	34.7 d ± 3.1 d (P = 0.02)	23.7 d ± 3.2 d (P = 0.02)	21.0 d ± 3.1 d (P = 0.02)

Only 7.7 % of the cows in the AL group fed with canola oil (C), compared to the FR cows (also fed with C 45.4 %, developed cysts. The supplementation of either linoleic (L) or flax (F) acids helped to shorten the time between calving and first ovulation pp.

In contrast to other studies like Huzzey et al. (2007), that demonstrate that fewer dry matter intake (DMI) during the pre-partum period is a risk factor for uterine infections, Colazo et al. (2009) suggest that it is not. The likelihood for uterine infections among the AL group was significantly higher than in the FR group.

During transition period an adequate DMI is important to reduce the NEB post-partum. The feeding ration 21 days pre-partum should contain enough protein-, energy and a sufficient mineral supply so that metabolic mechanisms can work physiological also during the challenging lactation period. Throughout the study cows with a mild metritis did not eat enough dry components. The lack of DMI further resulted in a severe metritis after parturition. Ten minutes less feeding time during seven days before parturition let the risk for acute metritis raise (OD = 1.72). One kilogram less intake of dry matter had the consequence that cows were three times more likely to get a severe metritis (Huzzey et al. 2007).

Table 5-4 (Huzzey et al. 2007); Decreased daily dry matter intake (DMI) rate between day 7 and day 2 pre-partum in healthy cows compared to mildly and severely metritic cows:

Healthy cows	Mildly metritic cows	Severely metritic cows
- 0.15 kg/d	- 0.21 kg/d	- 0.33 kg/d

Also Hammon et al. (2006) found that already metritic cows have a lower daily DMI intake than healthy ones. Cows with a minimum intake of dry matter until two weeks pre-partum had significantly ($P < 0.01$) lower peripheral blood neutrophil myeloperoxidase (PMN) activity, that play a necessary role in immunity (Hammon et al. 2006).

Feeding fat in the dry period and in early lactation seems a promising factor to improve fertility by increasing diameter of the ovulatory follicle, increasing progesterone concentrations, better oocyte quality and later embryo quality. In addition, it can help to maintain pregnancy.

Santos et al. (2008) tried to find out which sort of fatty acid is the best one to improve fertility health and performance. Fatty acids of the n-6 family had better outcomes than n-3 acids. It is now known that C18:2 n-6 and C18:3 n-3 fatty acids cannot be synthesized by the cattle itself, although they are essential for a good reproductive process.

Like mentioned before, cattle are fed with fat in their diet to get the negative energy status balanced, but they seldom consume more, and the overall energy intake stays the same. Arachidonic acid (AA; C20:4 n-6) plays an important role in PGF2α synthesis, which in turn is important for uterine health. It is responsible for an adequate emission of the placenta and uterine contents. Too little PGFα secretion is a risk factor for retention of foetal membranes and uterine health afterwards (Santos et al. 2008).

Santos et al. (2008) tried to conclude several studies and results show that some health problems can be reduced by feeding a certain type of fat. Especially the unsaturated n-3 and n-6 fatty acid groups had helpful effects on prevention of fertility disorders.

5.3 Risk factor - Hypocalcaemia

Hypocalcaemia, defined by a calcium concentration of 2.14 mmol/L or less, has a negative impact on the resumption of cyclicity of dairy cows during the early pp period. Hypocalcaemic cows need more time until they reach their first ovulation after calving. In the study of Ribeiro et al. (2013) 957 multiparous cows were observed and 43.3 % of them were diagnosed with subclinical hypocalcaemia. It had the highest prevalence among the subclinical diseases noticed. Cows diagnosed with a subclinical hypocalcaemia had a higher likelihood to have metritis (4.0 % vs. 7.9 %; $P < 0.03$) and subclinical endometritis (8.9 % vs. 24.1 %; $P < 0.03$). The difference between healthy and subclinical hypocalcaemic cows regarding the incidence for evolved clinical endometritis was not that high. (13.3 % vs. 18.1 %; $P < 0.09$) (Ribeiro et al. 2013).

To prevent hypocalcaemia, it is important to feed a mixture of anionic salts with an adequate bulk of calcium and magnesium supplemented. Minerals are also important for the acid base status and positively affect the dry matter intake and therefore the NEB status post-calving. Hypocalcaemia and a low selenium concentration are risk factors for dystocia and retained placenta. Calcium is needed for functional muscle contractility. If the contractility does not work, the ruminal function gets impaired and cows end up with a NEB status.

Furthermore, the muscles of the uterus cannot work appropriate, hypocalcaemic cows are not able to expulse foetal membranes and hence they are in higher risk of showing RP.

The calcium demand during lactation is extremely high, because cows lose 2.3 grams of it for each litre of milk they produce. If the cow does not get enough Ca through the feed intake, the parathormone (PTH) starts to work by stimulating Ca resorption out of bones. Through the formation of 1,25 dihydroxy-vitamin D (1,25 $(OH)_2$Vitamin D) in the kidneys, the blood calcium can rise because of the resorption out of the gut. These two mechanisms are self-help for cows to get their blood calcium balanced. But if the feed intake pre calving consists of high calcium concentrations, these two mechanisms do not start in time. Then cows are high-risked getting hypocalcaemia, which is also known as milk fever (MF). The other way around, a diet with low calcium concentrations pre-calving can boost the mobilization of Ca from the bones and gut. Therefore, it is preventive for milk fever (Wilde 2006).

Lean et al. (2006) did a meta-analysis involving 2,545 datasets from calving cattle. Particularly they analysed the ideal dietary cation-anion difference (DCAD) pre-partum, which can minimize the occurrence of clinical hypocalcaemia. The Figure 5-1 demonstrates that there is a linear relationship between DCAD and milk fever risk.

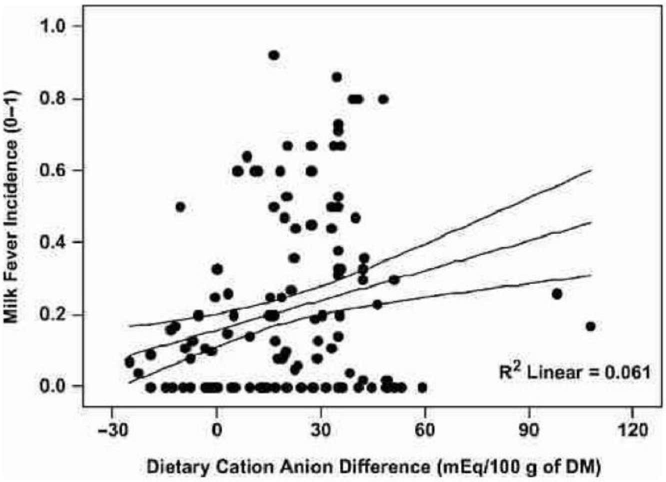

Figure 5-1 (Lean et al. 2006); "unweighted linear regression for incidences of milk fever in trials against dietary cation anion difference"

If more magnesium was fed before calving (from 0.3 % to 0.4 % of the DMI), cows had a 62 % lower risk for developing MF. If the calcium amount of the diet was about one percent of the DMI, the risk for hypocalcaemia and following problems decreased. However, diets with 1.1 % to 1.5 % calcium content led to a climax of milk fever risk. Lean et al. (2006) cannot recommend yet the ideal Ca concentration a pre-partum diet should contain.

A rise in the phosphoric components of the diet from 0.3 % to 0.4 % of the DMI led to an 18 % higher risk for MF. Phosphoric components negatively influenced the work of PTH and therefore, the essential active calcium resorption. A high potassium content may negatively affected the magnesium absorption (Wilde 2006).

Several authors like Pontes et al. (2015) or Qu et al. (2014) reported the effect of vitamin E as a risk factor for RP. Qu et al. (2014) observed 32 healthy cows (H), 32 cows diagnosed with RP and 32, that developed other diseases (OD). Results show that RP cows had lower concentrations of α-tocopherol in their serum than healthy cows three weeks before calving until the day after calving, because of their overall lower feed intake. Therefore, low α-tocopherol increases the risk for cows to develop RP (Qu et al. 2014).

Table 5-5 (Qu et al. 2014); Serum α-tocopherol concentrations in the healthy (H) compared to the group that developed retained placenta (RP) within the first 28 days post-partum:

Indicator	**H group (n = 32)**	**RP group (n = 32)**			**P-value**
α-tocopherol µM (3- and 2-weeks pre-partum)	12.4 ± 0.6	8.5 ± 0.6			< 0.001
α-tocopherol µM (last week pre-partum)	12.6 ± 0.6	8.6 ± 0.6			< 0.001
α-tocopherol µM (morning after calving)	10.3 ± 0.5	7.1 ± 0.5			< 0.001

H group = Healthy group; RP = rained placenta; n = number of cows

The aim of Pontes et al. (2015) study was, to find out, if the tendency for retained foetal membranes (RFM) was lower in cows treated with a Vitamin E injection three weeks pre calving. All together 890 cows were enrolled. Cows were fed with Vitamin E and in addition they were treated with three intramuscular injections of 1.000 IU dL α- tocopherol (= Vitamin E).

Table 5-6 (Pontes et al. 2015); Incidences of retained foetal membranes (RFM) in the control group and Vitamin E treated group:

Item	Control group	Vitamin E	AOR^2 (95% CI)	P-value
Number of cows	441	449		
RFM; %	20.1	13.5	1.68 (1.15– 2.44)	0.007

AOR = adjusted odds ratios; 95 % CI = 95 % confidence interval RFM = retained foetal membranes

As the Table 5-6 demonstrates, cows treated with Vitamin E pre-partum show a decreased incidence for RFM compared to the control-, untreated group (13.5 % vs. 20.1 %) (Pontes et al. 2015).

Chapter summary:

Numerous of investigations have been done to check the nutritional status of cows and its impact on fertility disorders. Authors are in general agreement that a BCS loss pre-partum constitutes an increased risk for the aetiology of several disease complexes.

Regarding the BCS various studies dealt with different BCS changes before calving. While some of the authors describe the effect of a mild BCS change, others discuss severe BCS losses and their effects. However, there is a tendency among the selected literature, that a BCS under 3 let the risk for the occurrence of endometritis, RP and delayed return to ovarian cyclicity raise. On the opposite, a gain in BCS values increases the risk for the development of ovarian cysts.
Beside the BCS, also the NEB status pre- and post-partum can be analysed and should be kept, especially pp, to a minimum. The NEB pp was subjected in many recent studies and can be reduced by a sufficient dry matter intake or by supplementation of the missing nutrients. A NEB pp negatively affects the uterine involution and further represents higher risk for endometritis. Results of various studies mentioned above, point out that fatty acids, the unsaturated n-3 and n-6, should be supplemented to prevent fertility disorders.
This chapter also underline the importance that all described risk factors cannot be seen separately and interrelate with each other. For example, already metritic cows are at higher risk

to eat less dry matter pre- partum and consequently suffer from reduced immunological functions.

Furthermore, the risk factor, hypocalcaemia was taken up in this chapter of the thesis, because it is relevant, especially in the context of milk fever. The discussed meta-analysis cannot give yet recommendations about the ideal Ca concentration a cow should be fed. Further investigations need to be done.

Comparing the studies, which dealt with the importance of Vitamin E, although they have observed a different number of cows, authors conclude the same, namely, that a lower Vitamin E concentration constitutes a higher risk for RP.

The Table 5-7 briefly gives an overview of the described risk factors, odds ratios or the occurrences of disorders. It must be noted that data are adapted and not all of them are comparable, because, among other things results originated through different methods. Additionally, the listed OR are varying, because of the deviating thresholds used by the investigators.

Table 5-7; Overview of the chapter 5 "Associations between nutrition/metabolism and fertility disorders":

Data adapted from	**Number/ breed**	**Risk factor**	**Fertility disorder**	**OR**	**CI (95 %)**	**Disorder occurrence**
Runciman et al. (2008)	1, 325 cows	BCS 4.5 vs. BCS 5.0	Positive VV	1 vs. 0.61	0.40-0.93	
López-Gatius et al. (2002b)	650 cows	BCS change/loss	Ovarian cysts	4.3	1.19-15.45	

Kim and Suh (2003)	67 cows	Massive- vs. moderate BCS loss	Metritis; MF			62 % vs. 32 %
Crane et al. (2006)	401 cows	BCS < 3	CL after timed insemination	0.49	0.26-0.92	
Hoedemaker et al. (2009)	234 cows	BCS < 3	Endometritis RP Not cyclic at 3-5 weeks pp Risk of dystocia 10 weeks pp	2.95 2.78 2.24-3.99 4.10	1.22-7.12 1.18 1.12–4.46 1.66–9.60 1.39-12.05	
Huzzey et al. (2007)	101 cows	DMI ↓; NEB ↑ 10 mins less feeding time 1 kg less DMI intake	Metritis	 1.72 2.87	 1.08-2.75 1.16-7.09	
Colazo et al. (2009)	29 cows	Feeding FR vs. AL	uterine infections ovarian cysts mean ± interval calving to			27.02 % vs. 5.71 % 20 % vs. 5.71 % 26.8 ±1.8d Vs. 26.8 ± 1.8d

			uterine involution			
Ribeiro et al. (2013)	957 cows	Hypocalcaemia	Metritis vs. healthy subclinical endometritis vs. healthy			7.9 % vs. 4.0 % 24.1 % vs. 8.9 %
Pontes et al. (2015)	890 cows	Vitamin E ↓	RFM	1.68	1.15-2.44	

OR = Odds ratios; CI 95 % = Confidence interval; BCS = Body condition score; MF = Milk fever; RP = Retained placenta

"Positive VV (1-3); VV 1 = mucus with ≤ 50 % pus; VV 2 = mucus and > 50 % pus; VV 3 = entirely pus)" (Runciman et al. 2008).

CL = Corpus luteum; DMI = Dry matter intake; NEB = negative energy balance; Feeding FR = feed restriction; Feeding AL = Ad libitum; RFM = retained foetal membranes

6 Risk factor - Immune dysfunction

6.1 Immune dysfunction during transition period

Immune dysfunctions, local and systemic, play an important role as a risk factor for infectious diseases during transition period. Compared to healthy cows, animals suffering from metritis show fewer number of circulating neutrophils, which undergo chemotaxis and produce reactive oxygen species (ROS) (Cai et al., 1994).

The immune responses of the adaptive and innate immune system are negatively influenced by the plasma concentration of several hormones including prolactin, growth hormone, IGF and insulin. However, not the altered concentrations of steroid hormones are the major risk for immune dysfunction pre calving. Instead, the DMI and the higher nutrition requirements are responsible for a misbalanced immune system and hence risk factor for infectious diseases in dairy cattle (Sordillo 2016).

6.2 Immune dysfunction after parturition

Normally the uterus is sterile, but during and after the parturition, the uterus is dilated, relaxed and more susceptible for bacterial pathogens. Pathogens like *Escherichia coli (E. coli), Trueperella pyogenes* and *Herpesvirus 4* can easily enter the uterine lumen and can damage the endometrial tissue. Two weeks after calving it is normal in half of the dairy cows, that the uterus is contaminated with bacteria. Therefore, the immune status of the cow is an important risk factor for remaining uterine infection, which can lead to acute and severe endometritis. Bacterial DNAs in healthy cows get detected from toll-like receptors, which are located on the surface of endometrial granulosa cells. Once the DNA is recognized, the lipopolysaccharides (LPS) get active and the secretion of proinflammatory cytokines, chemokines and antimicrobial peptides (AMPs) is induced.

Neutrophils and macrophages come over because of the chemokines to fight against the bacteria. If neutrophils persist, without present bacteria, it is a sign for subclinical endometritis.

A dysfunction in the endometrial immunity is an important risk factor for uterine diseases after parturition. Uterine diseases further negatively influence the cyclicity. Affected cows are less likely to ovulate. Also, the prostaglandin synthesis switch trough a phospholipase A2-mediated mechanism from Prostaglandin F to Prostaglandin E series, that stop luteolysis. Luteal phases in cattle with uterine diseases are extended because of a low progesterone profile. The IGF-1 influences the somatotropic axis, which affects the puerperal phase immunomodulatory (Sheldon et al. 2009).

Maintained also by Hammon et al. (2006) the uterus is more susceptible for foreign bacteria during the pp period due to immunosuppression. They examined 83 cows, 18 (21.7 %) had puerperal metritis, 43 (51.8 %) had subclinical endometritis and 22 (26.5 %) had physiological uterine conditions.

Puerperal metritis diagnosed cattle showed a significantly less working peripheral blood neutrophil (PMN) functions. PMNs could not work accurate because of the minor cytochrome-c activity and determining myeloperoxidase activity. Neutrophils generally are important to reduce bacterial colonization of the uterus during the pp phase (Hammon et al. 2006).

Kimura et al. (2002) observed 142 periparturient dairy cattle to find out if an impaired immunity can be a risk factor, respectively a cause for RP. Cows were diagnosed with RP if they did not expulse the placenta within 24 hours pp. To check the immune function, they took a closer look at neutrophils, their myeloperoxidase activity and the Interleukin-8. The myeloperoxidase activity of neutrophils is an indicator for their killing ability. Interleukin-8 is a chemoattractant for the neutrophils. Cows with a RP had a significantly lower myeloperoxidase activity shortly before calving and the nadir on the calving day. To measure myeloperoxidase activity, it was tested if cells were able to incorporate radioactive iodine (125I) into trichloroacetic acid and precipitable parts. Results are shown in Table 6-1. Unlike the “non-retained placenta” (NRP) cows, the “retained placenta” (RP) cows had a decrease of the myeloperoxidase activity until it reached a nadir on calving day (Kimura et al. 2002).

Table 6-1 (Kimura et al. 2002); Myeloperoxidase activity and Interleukin-8 in "non-retained placenta" (NRP) and "retained placenta" (RP) cows:

Parameters on calving day	NRP cows	RP cows
Myeloperoxidase activity	103 ± 4.2 %	64.7 ± 5.4 %
Interleukin-8	134 ± 11 pg/ml	51 ± 12 pg/ml

NRP cows = non-retained placenta cows; RP cows = retained placenta

The immune system is responsible for recognizing the placenta as foreign so it can get expelled after parturition. If the immune system is not able to recognize it, because of less active neutrophils, the risk for RP is getting higher. In addition, neutrophils must be attracted to the "foreign" tissue with the help of the chemoattractant Interleukin-8 (IL-8). As demonstrated in Table 6-1 the plasma of RP individuals had reduced concentrations of IL-8 on the day of parturition. According to Kimura et al. (2002) study, IL-8, as a chemoattractant for neutrophils, and their myeloperoxidase activity are determining factors for RP as a fertility disorder (Kimura et al. 2002).

Chapter summary:

This chapter gives a brief insight into challenges the immune system of dairy cattle must handle around the calving. It is known that the transition period and the time after parturition are very demanding phases. The immune system must work at high-performance to provide the cows health. Due to the higher nutritional requirements it is important to ensure a reliable and sufficient energy supply.

Variable studies underline that the uterus is more susceptible for bacteria after parturition, which can be for example, noted as a risk factor for uterine infectious diseases. However, the risk cannot be easily reduced. Also, measurements of diverse markers for the immune system functionality, like IL-8 or myeloperoxidase activity, are described in the literature, but can be barely practicable used.

Although the risk factor “immune dysfunction” seems to be less relevant, I have taken it up in the thesis, because, like other mentioned factors, it also points out the importance of an adequate energy supply pre- and post-partum to prevent the occurrence of fertility disorders.

7 Risk factor - Milk yield

Milk production has become the highest value in genetic selection of dairy cattle. In contrast to high milk yield, fertility and health traits are still not that important for breeding plans. However, more recently these parameters are considered as more important in breeding selection (Miglior et al. 2005).

Koeck et al. (2014) studied genetic parameters for reproductive disorders trough data from the Austrian cattle data base (Rinderdatenverbund) of 33,362 Austrian Fleckvieh cows. From 1,038 cows with RP, 94.2 % developed RP during the first seven days post calving.
Eighty-three-point-eight percent (83.8 %) of the puerperal diseases (n = 549) occurred in the first seven days after parturition.
The incidence for metritis (n = 1024) was highest between day 15 and 60, for anoestrus or silent heat (n = 1.889) and cysts (n = 2455) between day 30 and 60, after calving. The cows in the study had an average milk yield of 28.5 kg on the first two days after calving.
Fertility disorder traits were divided into three different groups. The early- (EREPRO), the late- (LREPRO) and the general- reproductive disorders (REPRO) group. Disorders like RP, PURERP and puerperal metritis (MET) belonged mainly to the EREPRO group, whereas LREPRO group consisted primarily of cysts (CYST), anoestrus or silent heat and metritis. Correlations between Milk yield and these trait groups are shown in Table 7-1.

Table 7-1 (Koeck et al. 2014); Genetic correlations between milk yield and fertility traits:

Trait	**MY**
EREPRO	0.309 (0.108)
LREPRO	0.209 (0.078)
REPRO	0.267 (0.115)

EREPRO = early reproductive disorders; all reproductive disorders that occurred and were treated within 30 d after calving; LREPRO = late reproductive disorders; all reproductive diseases occurring between 31 and 150 d after calving; REPRO = all reproductive disorders that occurred within 150 d after calving;MY = Milk yield; Standard errors for estimates are shown in the parentheses

Results from the unfavourable associations between MY, EREPRO LREPRO and REPRO ranged from 0.21 to 0.31, and highlight, that selection for high milk yield ends up with more cows contracted with fertility disorders. Further, their fertility will be negatively affected as well with a higher non-return rate to normal cyclicity at 56 days, with a longer interval from calving to first insemination and interval from first to last insemination.

Not only Koeck et al. (2014) proved the unfavourable genetic associations between milk yield and fertility disorders. But also López-Gatius et al. (2002b) proved that the incidence for cysts increased for 1.05 times with each kilogram milk yield increase. In addition, there was a negative association between high milk production and spontaneous early cyst recovery. The likelihood for spontaneous cyst recoveries increased 1.06 times with each kilogram decrease in milk yield.

Cycle disbalances increased in the last decades of years, which is associated with the demand on high milk production. Wathes et al. (2007) point out that most studies deal with the total milk yield and not the shape of the lactation curve. Results indicate that individuals who have reached their lactation early after calving were more susceptible for fertility disorders (Wathes et al. 2007).

As maintained by Fleischer et al. (2001) there is a significant correlation between the current high milk yield and fertility disorders, more precisely for ovarian cysts and MF. There was a probability for the associations between the milk yield of the last lactation, RP and MF. No correlation between metritis and high milk production was found. The study included 2197 lactations of 1,074 Holstein-Friesian cows from ten different farms in Lower Saxony (Fleischer et al. 2001).

Contrary to Fleischer et al. (2001) findings, Gröhn and Rajala-Schultz (2000) reviewed that cows with a higher milk yield had an increased likelihood to develop early metritis as well compared to lower yielding dairy cattle.

Table 7-2 and Figure 7-1 demonstrate "estimate probability of appearance" (EPA) curves. Obviously, the curve for ovarian cysts has one of the steepest rises correlated to the milk yield in the second lactation. The inverse coefficient of the regression describes the one percent increase in EPA which goes along with the amount of milk yield in kilogram.

Table 7-2 (Fleischer et al. 2001); Associations between incidences for fertility disorders and milk yield:

Disorder complex	**Model**	**EPA (%) (12.000 kg; 3. LN)**	**EPA (%) (12.000 kg in 3. LN)**	**Kg of milk per 1 % increase in EPA (kg)**
RP	P	6.4	17.0	570
Metritis	P	16.9 (NS)	25.6 (NS)	695 (NS)
OC	C	9.0	27.5	323
Milk fever	P	4.0	13.2	650

RP = retained placenta; OC = ovarian cysts; P = milk yield from the previous lactation; C = milk yield from the current lactation; kg of milk per 1% increase in EPA = Average yield increase in the 6000 to 12,000 kg of milk yield range, associated with an EPA increase of 1%; NS = not significant

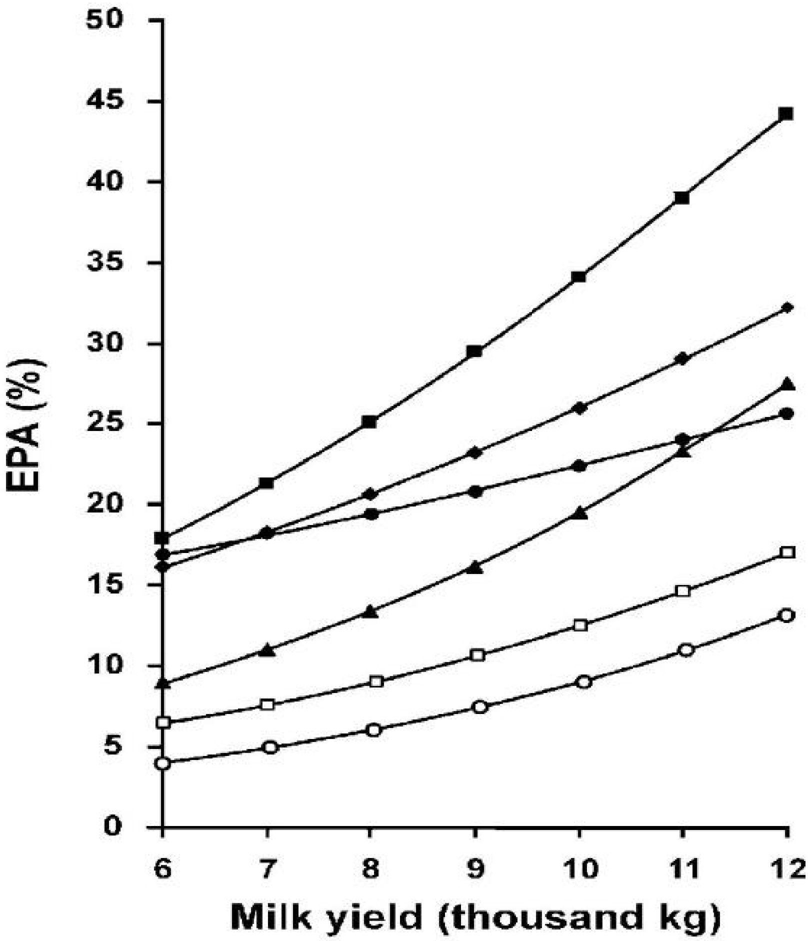

Figure 1. Estimated probability of appearance (EPA) of mastitis (■)[1], claw diseases (◆)[2], metritis (●)[1,3], ovarian cysts (▲)[2], retained placenta (□)[1], and milk fever (○)[1] in the third lactation dependent on 305-d milk yield ([1]milk yield from the previous lactation, [2]milk yield from the current lactation, [3]taken from insignificant correlation).

Figure 7-1: "The Relationship Between higher milk yields and the estimated probability of appearance (EPA) of metritis, ovarian cysts, retained placenta and milk fever" (Fleischer et al. 2001).

Although the results seem to be clear and totally significant, interpretations are limited. Only data from dairy herds in Lower Saxony were observed. There are several other risk factors causing fertility disorders, which were not included in the statistic models, for example management factors or animal genetics. All of these are typical for herds in Lower Saxony area but not somewhere else (Fleischer et al. 2001).

Table 7-3 (Yániz et al. 2008); Associations between milk yield rise and different ovarian disorder rates of the last decades:

	1991-1995	1996-2000	2003-2007
Number of cows	6306	6405	10 493
Ovarian hypofunction (%)	3.8	10.2	12.0
Ovarian cysts (%)	7.2	7.3	6.3
Annual milk production/cow (kg)	8 300	9 660	11 221

A synonym for ovarian hypofunction is follicular anovulation. A follicular structure of 8-15 mm, but no CL or ovarian cyst is found. In addition, the cow does not show oestrus signs and does not undergo ovulation. The biggest surge regarding ovarian hypofunction took place between the year periods of 1991-1995 and 1996-2000. The incidences ranged from 3.8 % to 10.2 % until they were 12.0 % in the time period of 2003-2007. In the past decades there were invented different treatment- and prevention- programs, like the Ovsynch protocol or the progesterone-releasing intravaginal device. They are declaratory for the only minimal rise in the incidence, although the milk yield was increasing rapidly from year 1996-2007. The variation of the percentage of ovarian cysts was not high, and decreased just a little bit in the last five years (7.3 %-6.3 %) (Yániz et al. 2008).

Costa et al. (2019) studied the genetic correlations of milk lactose percentage (LP), lactose yield (LY) and other milk solids between fertility disorders. They included datasets of 142,285 lactations from 84,289 Austrian Fleckvieh cows in uni- and bivariate statistics. Lactose originates from blood sugar and is known as the main solid of bovine milk. It gets synthesized in the mammary glands.
High milk producing dairy cows produce more lactose than low milk producing cows. (maximum of 4.4 kg/d). Lactose is important for the water gain through osmosis from the cytosol and hence a crucial factor for milk volume (Aschenbach et al. 2010).

For a high amount of LY and LP basics, like high concentrations of glucose in the blood, a positive energy status and an adequate expression of glucose transporters are essential (Ouweltjes et al. 2007).

The Austrian Fleckvieh is the most common breed in Austria with an average lactation milk yield of 7.345 kg, containing 4.16 % fat and 3.42 % protein (Zucht Data 2017).

Results of the study highlight that cows with a low LP percentage (≤ 4.553 %) were more likely to suffer health disorders compared to cows that yielded a higher LP amount (≥ 5.045 %).

Both, milk fever (MF) and ovarian cysts (CYS) presented a genetic correlation of approximately 0.20 to LY.

LY decreased with parity and multiparous cows had a higher incidence for MFV (3.76 %) than primiparous individuals with high LY (0.29 %). Ovarian cysts incidences did not differ a lot between primiparous individuals with high LY (4.69 %) and multiparous cows with low LY (5.87 %). Also, the incidences for retained placenta did not really altered a lot (primiparous high LY: 1.23 % and multiparous with low LY: 2.56 %). Interestingly, the likelihood for cysts shrank with higher LY and increased with lower LY values (Primiparous high LY incidence for CYS: 4.69 % vs. low LY CYS: 3.46 %). Multiparous cattle data bases had an equal tendency (Costa et al. 2019).

Chapter summary:

Researchers are in general agreement that each kilogram uptake in milk yield constitutes a higher risk for the occurrence of reproductive disorders. There exist proofed unfavourable genetic correlations between high MY and reproductive diseases. (genetic correlation: 0.209-0.309; standard error: 0.108 and 0.078).

Metritis incidences are subject of controversial debate. Some describe a higher risk for the development of metritis by increased MY, and some do not.

Interpretation of variable study results is limited, because most of them worked with the total milk yield, instead of lactation curves.

However, one previously quoted study implicates that cows who have reached the peak of the lactation curve fast after calving have been more susceptible for fertility disorders.

Researchers concluded that cows with a low LP percentage were by trend more likely to suffer MF but no other reproductive diseases. High milk producing dairy cows produce more lactose than low producing ones. Which means, high producing dairy cattle have been in advantage. However, only a single Austrian study, including datasets of 142,285 lactations, has been mentioned in the thesis above, and more research should be made to get a convincing conclusion. However, the LP is linked to another risk factor of high importance, namely the parity number. Cows with a higher parity number had a lower lactose yield and were more likely to suffer MF. The risk factor parity number can be found in chapter 9.

8 Environmental conditions as risk factors for fertility disorders

8.1 Risk factor - climate

Heat stress is an important factor in dairy industries worldwide. It negatively affects health and biological functions of dairy cattle. Consequently milk yields decreases and the reproductive performance is getting poorer (Polsky and Keyserlingk 2017).

Cows are homeotherm animals with regulation mechanism to keep their body temperature the same, independent of environmental temperature conditions. Still dairy cows have their climatic comfort zone, which ranges from five degree Celsius (°C) to 25 °C. Ideal ambient temperatures allow the body to work with maximum power, while keeping the body temperature balanced. In fact, then physiological mechanisms need minimal energy to function appropriate and productivity reaches its peak. Temperatures above 25 °C lead to heat stressed cows. Because of the long-lasting negative effects of heat stress, it is of main interest to minimize its intensity by cooling stables through evaporation and feeding ideal rations. The application of hormones is used to maintain a firm fertility. As claimed by Rensis et al. (2015) cows with a rectal temperature of 39 °C and above are hyper thermic and under heat stress. Environmental temperatures of 29.7 °C cause a raise in rectal temperature and lead to 39 °C.

Solar radiation, relative humidity (RH), wind speed and rainfall are additional factors that influence the intensity of heat stress in dairy cows. The temperature-humidity index (THI) represents the combination of environmental temperature and RH. The most presentable index for heat stress is the maximum THI, which generates from the maximum temperature and the minimum RH value. Performance problems can accrue if the THI value is 75 and above. Climate raises affect fertility parameters like the fertility-, and conception rate or average pregnancy rates. Heat stress is a risk factor for dysbalanced cycles, anovulatory follicles and ovarian cysts. As demonstrated in Figure 8-1 high ambient temperature negatively affects animal welfare on different levels. Clinical noticeable are, for example, lethargic behaviour, increased thirst, hunger and aggression. Heat stressed individuals do not show oestrus behaviour, therefore 80 % of oestric cows are not registered.

Theca and granulosa cells are negatively influenced by heat stress, so they are not able to produce the physiological amount of oestradiol. Oestradiol raises under normal cyclic conditions and triggers oestrus behaviour (Polsky and Keyserlingk 2017, Rensis et al. 2015). Gene expression profiles monitored by Vanselow et al. (2016) underline decreases in pre-ovulatory follicle growth rate and oestradiol production in acute heat exposed cows. Stress hormone genes of granulosa cells are not expressed, but follistatin gene expression get boosted. Follistatin suppresses the activing function and the release of FSH from the pituitary gland (Esch et al. 1987).

Comparing past decades 1991-1995, 1996-2000 and 2003-2007, ovarian hypofunction always appeared more often during warm seasons. The occurrence for ovarian cysts during hot periods changed from 13.2 % in 1991-1995 to 6.5 % in 2003-2007. The decline can be explained by the usage of modern cooling systems like fans (Yániz et al. 2008).

Schüller et al. (2017) results prove, that the intensity of oestrus signs depends on the amount of oestrus discharge, contractility of the uterus and the pink vaginal mucosa examined. The THI value and oestric follicle sizes are inversely proportioned factors. With a THI rise, at the start of 68, follicle sizes begin to shrink.

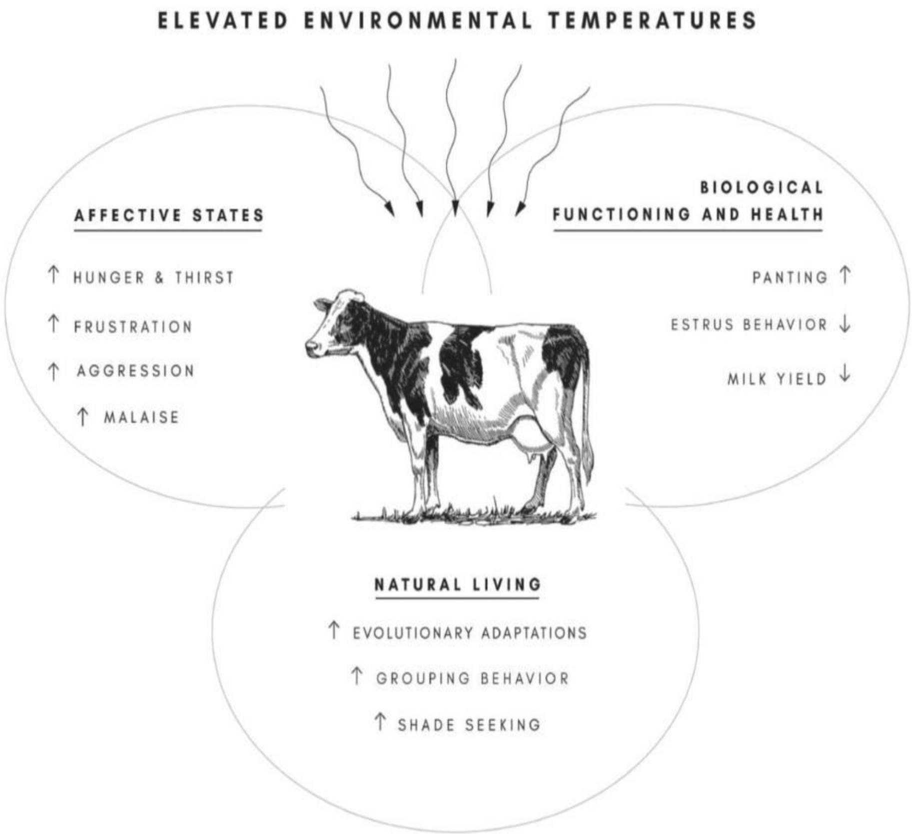

Figure 8-1: "The influences of elevated environmental temperatures on the 3 key constructs of animal welfare (biological functioning and health; affective states; natural living)" (Polsky and Keyserlingk 2017)

Several researchers prove heat stress as a relevant risk factor for ovarian cysts (López-Gatius et al. 2005, Roelofs et al. 2010). As maintained by López-Gatius et al. (2005), cows that were artificial inseminated during warm periods had a 3.9 times higher likelihood for ovulation failure. During hot season they performed 663 AIs and 12.4 % had ovulation failure. In contrast, during the cool period, only 3.4 % of 1254 AIs failed because of missed ovulation, although all the cows showed oestrous signs that were properly detected. Luteal structures could not be found in cows that missed ovulation and in 23 individuals the follicle remained. One-hundred-two cows developed ovarian cysts, sized 18-32 mm in diameter (López-Gatius et al. 2005).

The Table 8-1 shows that warm season is a risk factor for the development of early ovarian cysts. The risk for getting early cystic structures was 2.6 times higher in individuals that calved in summer than those calved in winter. If cystic conditions occur during the first 43 to 49 days post-partum, they are defined as “early ovarian cystic conditions post-partum”. Odds ratios for “late ovarian cystic conditions” detected on days between 57 and 63 pp were 2.33 but results were not significant (López-Gatius et al. 2002b).

Table 8-1 (López-Gatius et al. 2002b); Odds ratios for “early cystic conditions post-partum” for calving warm periods:

Factor	**n**	**OR**	**CI 95 %**	**P-value**
Calving in the warm period	350	2.6	1.71-3.95	< 0.0001

n = number of cows; OR = Odds ratios; CI 95 %= 95 % Confidence interval;

Due to lack of investigations, the consequences of heat stress on the post-partum health of dairy cows is not clear yet. However, it can be assumed that heat stress suppresses the eventual maturation and steroidogenesis of the follicle. To draw conclusions from current stage of research, it can be assumed, that under heat stress the cows feed intake reduces and therefore the NEB pp gets more intense. Subsequently anovulatory periods get extended (López-Gatius et al. 2002b).

It is also maintained by Rensis and Scaramuzzi (2003) that heat stress indirectly affects cows health pp, because of the worse negative energy status. A prolonged NEB status leads to anovulation. Additionally Pascottini et al. (2017) findings demonstrate the indirect correlation between the heat stress induced NEB and cytological endometritis. Cytological endometritis (CYTO) diagnosed with the help of the “cytotape technique” during the most critical point, the time of AI, in Holstein dairy cows, appeared more often in warm (July, August, September) than in cold months.

There were no significant correlations between winter months (October, November, December, February, March) and the occurrence of CYTO. Odds ratios of the multivariable mixed effects analyses are demonstrated in Table 8-2 and point out the difference between summer and winter months (Pascottini et al. 2017).

Table 8-2 (Pascottini et al. 2017); Odds ratios for cytological endometritis (CYTO) during artificial insemination (AI) comparing summer and winter months:

Month of the AI	**OR**	**95 % CI**	**P-value**
July	2.9	1.65-5.27	0.0002
August	2.3	1.39-3.87	0.001
September	1.4	1.22-1.69	0.001
October	0.7	0.43-1.07	0.09
November	1.1	0.77-1.79	0.46
December	1.1	0.77-1.79	0.46
February	1.4	0.91-2.17	0.13
March	0.7	0.44-1.15	0.16

OR = Odds ratios; 95 % CI = 95 % Confidence interval

The average THI value influences the occurrence of pp diseases. Cows exposed to a higher THI in average are more likely to develop pp diseases during the period of day zero until day ten after calving. Due to global warming, there are more heat stress days dairy cattle must cope with now, than in the past decades (Gernand et al. 2019, Solymosi et al. 2010).

Gernand et al. (2019) did not only consider the THI but also repeated trait evaluations from individual test-days and health documentations of the previous lactation. Health traits incidences in the period of zero until ten days pp were 10.8 % for RP and 13.9 % for puerperal disorders (PD). Figure 8-2 and Figure 8-3 illustrate clearly correlations between the THI and RP, or rather PD incidences. For each THI increase the likelihood of RP and PD gained 0.01 % during zero- and five-days pp.

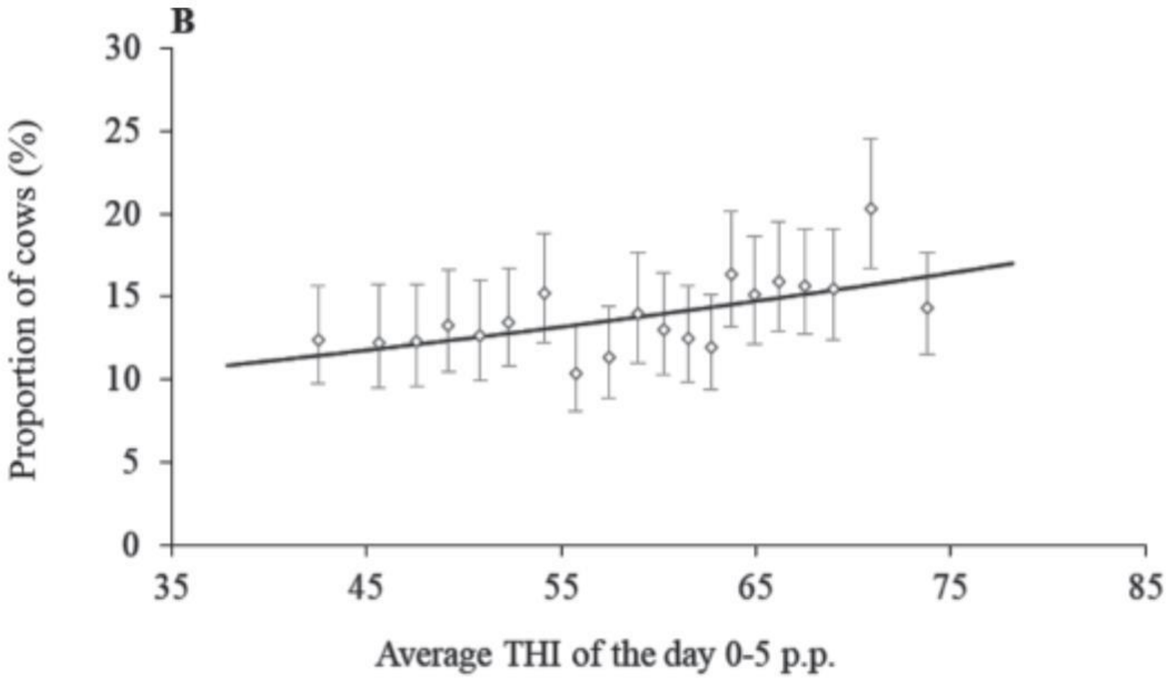

Figure 8-2: "Least squares means for incidences of disease trait (puerperal disorders 0 to 10 days post-partum) (±95% CI represented via vertical stripes) by temperature-humidity index (THI) class effects (5% percentiles for THI classes) and estimates from the linear regression analyses (solid line)." (Gernand et al. 2019)

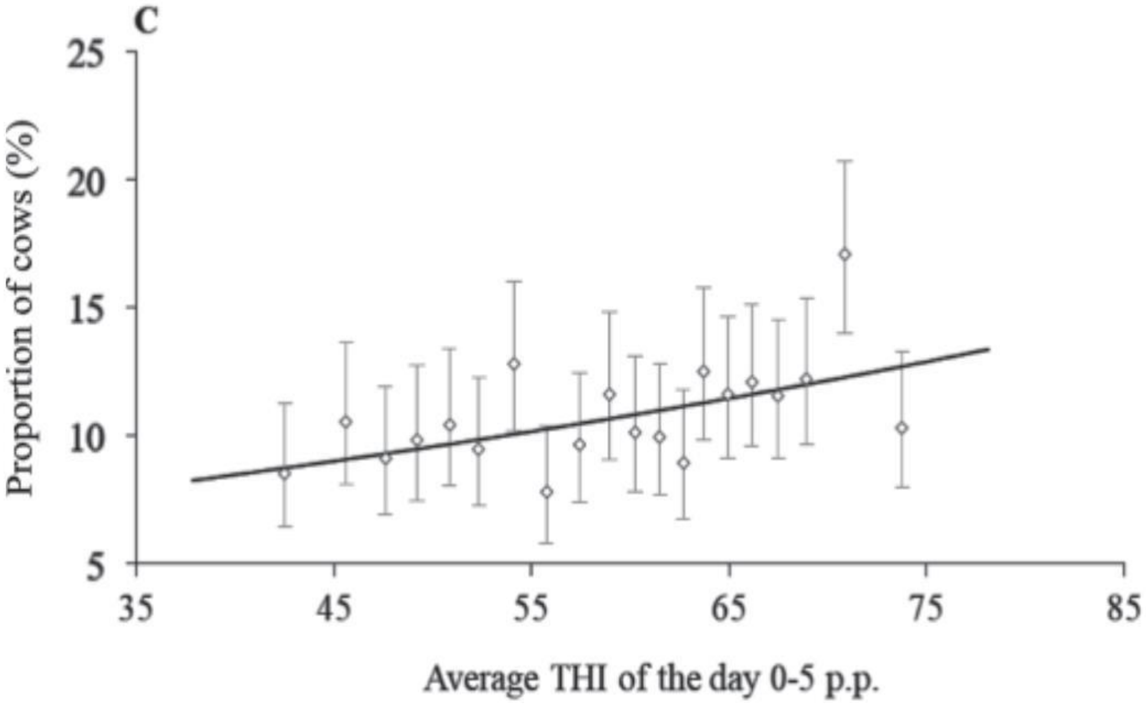

Figure 8-3: ""Least squares means for incidences of disease trait (retained placenta 0 to 10 days post-partum) (±95% CI represented via vertical stripes) by temperature-humidity index (THI) class effects (5% percentiles for THI classes) and estimates from the linear regression analyses (solid line)." (Gernand et al. 2019)

Controversially Sanker et al. (2013) did not find significant correlations between the THI during summer periods and incidences for fertility treatments. The study included records of all veterinary treated cases of ovarian cysts, endometritis and anovulatory conditions over two years from eight dairy herds in Lower Saxony. Results may appear not significant, because the THI is a limited factor, that does not exactly include all climatic factors, like wind speed and thermal radiation. In addition, there are cow-side risk factors, such as high milk yield or high body weight, that influence heat resistance (Sanker et al. 2013). Different study outcomes exist, because fertility disorders pp are multifactorial disorders and environmental status cannot be summed up in one single parameter. Husbandry conditions, gestation lengths, feeding components and feeding technique for example are risk factors as well and cannot all get included into statistical models (Gernand et al. 2019).

Maintained by Benzaquen et al. (2007) correlations between risk factor parity and risk factor calving season exist. The odds for puerperal metritis (PM) for primiparous cows that gave birth during warm season (THI ≥ 76.2) were lower than during cold season (THI < 76.2). However, this tendency did not occur among multiparous cows (Benzaquen et al. 2007).

Table 8-3 (Benzaquen et al. 2007); Logistic regression analyses risk factor (parity x season) for "puerperal metritis" (PM):

Parity	**Season**	**Incidence of PM**	**n**	**AOR**	**95% CI**	**P - value**
Primiparous	**Warm season**	12.7	6/47	0.24	0.09-0.62	0.001
Primiparous	**Cold season**	39.4	54/137	Referent	Referent	
Multiparous	**Warm season**	18.1	12/66	1.43	0.65-3.18	0.001
Multiparous	**Cold season**	11.0	22/200	Referent	Referent	

PM = puerperal metritis; n = number of cows; AOR = adjusted odds ratios; 95% CI = 95 % confidence interval

The retrospective study of 61,124 Finnish Ayrshire cows demonstrates the correlation between calving season and appearances of different fertility disorders. In accordance with the research outcomes of Benzaquen et al. (2007), winter calvers were more likely to suffer from metritis or retained placenta than individuals, that calved during summer periods. Unlike other research results mentioned above (López-Gatius et al. 2005; Roelofs et al. 2010; Rensis and Scaramuzzi 2003), cold weather conditions implicated higher risk for ovarian cysts and silent heat. Detailed values are shown in Table 8-4 (Gröhn and Rajala-Schultz 2000).

Table 8-4 (Gröhn and Rajala-Schultz 2000); "Odds ratios" (OR) for fertility disorders (ovarian cysts; early metritis; late metritis; silent heat) with calving season as risk factor:

Calving season	OR Ovarian cysts	OR Early metritis	OR Late metritis	OR Silent heat
Jan-Apr	1.0	1.6	1.0	1.9
May-Aug	1.0	1.0	1.6	1.9
Sept-Dec	1.6	1.7	1.9	1.0

OR = Odds ratios

Chapter summary:

The risk factor heat stress overall is a complex matter, which is not easy to interpret because it is influenced by many variable parameters.

Due to global warming it currently has become subject of many researches and discussions. Investigators invented the maximum THI value to make assessments as objective as possible. Many authors share the same view that heat stress does not only influence the occurrence of fertility disorders in a negative way, but also the animal welfare. However, it is not scope of this thesis to deal with the welfare in detail, but it in fact, heat stress affects the productivity. If dairy cattle do not feel comfortable, they are less likely to show oestrus behaviour, their follicles do not grow to ovulatory size and they suffer more likely from ovarian cysts.

Some of the OR mentioned in this chapter illustrate clearly that cytological endometritis occurs more often during summer than during winter months. Same tendency was determined for RP and other puerperal diseases pp.

However, some authors could not find significant correlations or, they even conclude the completely opposite.

The previous quoted study, which took place in Lower Saxony does not show significant correlations between the THI in summer periods and fertility treatments (Sanker et al. 2013).

Another up taken, relevant topic is the crossed factor parity number and season. Interestingly the exact opposite trend was observed. In comparison to summer calvers, winter calvers were more susceptible for metritis or retained placenta (Benzaquen et al. 2007).

Summed up heat stress is a highly interesting risk factor for fertility disorder which depends on many other factors, like for example the breed. Breeds bear heat differently and some get more used to it than others. The studies took place in various degrees of latitude and thus, different climatic zones. Furthermore, housing conditions and the used cooling techniques are not the same. Comparability therefore is limited.

Quoted studies and their main adapted results are demonstrated in Table 8-5.

Table 8-5; Overview of the chapter 8.1 "Risk factor climate":

Data adapted from	**Number of cows/ breeds**	**Risk factor**	**Fertility disorder**	**OR**	**CI (95 %)**	**Disorder occurrence**
López-Gatius et al. (2005)	1,792 cows	Warm season	Ovulation failure; Early cystic condition pp	3.85 2.6	2.63-5.56 1.75-3.95	
Pascottini et al. (2017)	837 Holstein-Friesian	Warm season	CYTO	2.9	1.65-5.27	
Gernand et al. (2019)	22,212 Holstein-Friesian	Average THI ↑	RP; PD			Each increase in THI -> 0.01 % higher likelihood

Benzaquen et al. (2007)	450 calvings	THI > 76.2	PM	0.24	0.09-0.62	
Gröhn and Rajala-Schultz (2000);	61,124 Finnish Ayrshires; 13,307 New York Holsteins	Calving season	Ovarian cysts; metritis	Sept-Dec 1.6 cysts 1.7 metritis		

OR = odds ratios; CI = Confidence interval; CYTO = cytological endometritis; THI = Temperature-humidity index; RP = Retained placenta; PD = puerperal disorders; PM = puerperal metritis

8.2 Risk factor - Herd size, group stress

The time pre-partum is a highly sensitive phase for the health of dairy cattle. Competition among herd mates minimizes feed intake and affects standing behaviour. As claimed by Proudfoot et al. (2009) displacements away from the feed bunk happen more frequently if one bin is used to feed two animals. Cows then are under competitive pressure. One week before parturition, cows fed under competitive environment, stay longer at the feed bunk to eat their ration, have fewer meals on a day, but spend more time having their ration (Hosseinkhani et al. 2008, Proudfoot et al. 2009).

However, this tendency was not the same among multiparous cows one-week pre- and post-partum. They used 28 % less time having their meal. Consequently, their daily DMI decreased. Primiparous cows observed in the study, that were competitively fed, got 25 times displaced on a day. During the same period non-competitive ones, faced only seven displacements per day (25 vs. 7/d ± SE = 2.2; $P < 0.02$). The multiparous competitive fed group was involved in 24, meanwhile the non-competitive fed group was involved in ten movements at the bin per day (24 vs. 10/d, ± 3.8, $P = 0.02$). Competitive pressure did only influence the standing behaviour of primiparous, not of multiparous cows. Longer standing times without eating, for multiparous competitive fed cows were monitored in the period of one-week before calving. Lying time was 494 ± 41 minutes per day among competitive fed multiparous cows and 641 ± 41 minutes per day for the non-competitive fed ones. Two weeks after calving no differences among the two feeding groups were detected (Proudfoot et al. 2009).

The stocking density (SD) is associated with behavioural changes. (Hosseinkhani et al. 2008, Proudfoot et al. 2009) The study of Silva et al. (2014) underlines, that there is no meaningful higher risk for post-partum diseases if comparing a 100 % SD with a 80 % SD. Cows, that did not calved yet, were separated from the rest. Within the 80 % SD group 48 headlocks were available for 38 animals. Within the 100 % SD group there was one headlock per animal. Results for pp disorders are demonstrated in Table 8-6. To stock an already existing pre-partum herd with new animals every week or every two weeks does not implicate a higher risk for fertility disorders pp (Silva et al. 2014).

Table 8-6 (Silva et al. 2014); Incidences for post-partum (pp) disorders in a group with 100 % stocking density (SD) compared to a group with 80 % SD:

Disorder	Incidence (%) for 80 SD group	Incidence (%) for 100 SD group	AOR (95 % CI)	P-value
RFM	5.1	7.8	1.55 (0.78, 3.07)	0.19
Metritis	21.2	16.7	0.71 (0.46, 1.09)	0.11
Acute metritis	9.9	9.4	0.87 (0.45, 1.66)	0.64
Vaginal purulent discharge at 35 ± 3 DIM	5.8	7.9	1.41 (0.65, 3.05)	0.35
Cyclic by 35 DIM	45.5	48.2	1.19 (0.72, 1.95)	0.47
Cyclic by 45 DIM	60.0	66.3	1.30 (0.67, 2.55)	0.40

DIM = days in milk; RFM = retained foetal membrane; AOR = Adjusted odds ratios; 95 % C = confidence interval; SD= stocking density

Bigger herd sizes tend to increase the occurrence of vaginal discharge and dystocia. The study included 80 conventional dairy cattle farms. The farms were grouped into four different herd size classes. Class one (C1) contained of herds with less than 100, C2 100-299, C3 300-499, and C4 more than 500 animals. There was a significant interplay between season and group size as well. Vulvar discharge was less common examined within the C1 group during summer periods (1.0 ± 0.6 %) compared to the C4 group during winter (2.9 ± 0.5 %) and summer (3.5 ± 0.5 %). For the sake of convenience, only results of summer periods are illustrated in Table 8-7 (Gieseke et al. 2018). Bruun et al. (2002) did not determine associations between bigger herd size and higher incidence risks for metritis.

Table 8-7 (Gieseke et al. 2018); Incidences for dystocia and vulvar discharge within different herd group sizes C1 to C4:

Indicator	C1	C2	C3	C4	P-value
Cows with vulvar discharge (%)	1.0	1.8	1.7	3.5	0.41
Dystocia within 12 months (%)	6.1	6.6	5.3	7.8	< 0.05

C1 = Class 1, containing herds with less than 100 animals; C2 = Class 2, herds with 100-299 animals; C3 = Class 3 containing herds with 300-499 animals; C4 = Class 4 containing herds with more than 500 animals

Chapter summary:

Some researchers share the same opinion that a bigger herd size implicates less space per each individual and further can lead to stress in dairy cows.

The term "stocking density" was invented to describe the number of cows living on one defined section of the pasture for a certain time period. Study results do not show differences if comparing a SD of 80 % with a SD of 100 %. The table 8-6 clearly demonstrates the outcome. On the other hand, other authors prove that indoor housed cows that have less space on the feeding bin, due to a bigger herd size, are more likely to get displaced and consequently their daily feed intake decreases. It is proved that these cows, fed under pressure consume less dry matter. A low DMI itself constitutes a higher risk for pp diseases. For more detailed information I also refer to chapter 5.2, "Risk factor - Negative Energy Balance (NEB)". Another paper thematises the higher risk for the development of dystocia and vaginal discharge, which appears if cows live in bigger sized herds.

It should be kept in mind that stress can be caused by an enormous number of factors and it is not easy to measure it through a single risk factor. However, the risk factor "bigger herd size" is often debated in the literature and have a significant impact on the occurrence of fertility disorders. Further this chapter underlines that risk factors interrelate with each other. It is assumed that a bigger herd size causes stress in dairy cows, which in turn reduces the DMI.

A lower DMI itself constitutes an important hazard that can end up in a negative energy balance and unhealthy individuals.

8.3 Risk factor - housing system

Housing systems are risk factors for subclinical endometritis (SE) in the pp phase. Post-partum cows kept in free stalls did not develop SE as often as cows kept on bedded packs. The herd-level prevalence was, SCE = 19.4 % for free stall housing and SCE = 36.1 % for bedded packs. Calving pens with straw as bedding substance minimized the prevalence for SE during the post-partum period by 10.7 % (Cheong et al. 2011).

Prunner et al. (2014) investigated risk factors for clinical- (CE) and SE in cows living on small- or medium sized commercial dairy farms in Austria. SE was diagnosed via cytobrush technique. Cows were detected SE positive if the PMN value reached 5 % or more. The prevalence was 27.3 % for CE and 21.0 % for SE among 400 individuals. Eight out of ten farms had free-stall furniture with cubicles and slatted groundings. The other two farms were equipped with concrete floors. Before calving, pregnant cows were separated and kept in calving pens (eight farms) or in tie stalls (two farms). Risk factors were not the same for CE and SE. Relevant ones are demonstrated in Table 8-8 (Prunner et al. 2014).

Table 8-8 (Prunner et al. 2014); Odds ratios for the risk factors "calving assistance" and "calving pen" for "clinical endometritis" (CE) and "subclinical endometritis" (SE) in cows examined 20-30 days post-partum:

	OR	**CI 95 %**	**P - value**
Risk factor of CE			
Calving assistance (0 = no intervention; 1 = assisted calving)	1.79	1.12 - 2.86	0.02
Risk factor of SE			
Calving pen (0 = calving box; 1 = tie stall)	0.47	0.24 – 0.94	0.03
Farm (1 - 10)	1.11	1.02 – 1.20	0.02

OR = Odds ratios; CI = 95 % confidence interval; CE = cows with endometritis Score 1-3; SE = cows with endometritis score 0, but more than 5 % of PMN in cytology

In contrast Bruun et al. (2002) did not assess an association between odds for metritis and flooring systems. Barns with concrete or slats and bedding on it seem to be clearer than without bedding. However, this variable depends a lot on management and therefore it is not a reliable one to measure environmental cleanliness. Prunner et al. (2014) also maintain the severe effect of management actions. Herd sizes were smaller than in other studies mentioned (Cheong et al. 211, Silva et al. 2014) and reached from ≤ 40 to ≥ 81 animals per herd (Bruun et al. 2002).

Not many investigations were made in past few years, to find out, if a dirty calving environment is an important risk factor for uterine disorders pp or not. Kaneene and Miller (1995) have already reported that pasture groundings are less contaminated with bacteria than barn floors. Calving in pasture based environment therefore is a decreasing factor for odds of metritis (Kaneene and Miller 1995).

Potter et al. (2010) analysed associations between "assisted calving", "faecal consistency score" "cow cleanliness score" and clinical endometritis. These factors were used as measurement of the hygienic status. *Escherichia coli* and *Trueperella pyogenes* are the most often detected bacteria in cows having uterine diseases. They are able to live in the environment and are likely to transfer from there into the cattle's organism (Potter et al. 2010, Sheldon et al. 2009).

Controversially Prunner et al. (2014) did not report *E. coli* as the most frequent pathogen. Instead *Trueperella pyogenes* was the most crucial microorganism for the incidence of clinical endometritis (OR = 5.72; 95% CI = 3.07-10.83). No correlations between *E. coli* and signs of CE were found (Prunner et al. 2014).

Although "faecal consistency" and "cow cleanliness" seem to be relevant risk factors for endometritis, the study did not result in significant associations. Odds ratios for endometritis correlated to calving assistance were significant. Contaminations may evolve through human skin that is covered with pathogens from faeces and environment. Probability values and odds ratios are listed in Table 8-9.

Table 8-9 (Potter et al. 2010); Odds ratios and statistical probability (P-values) for the association between risk factors and clinical endometritis:

Variable	OR	P-values
Assisted calving	2.83	0.002
Faecal consistency score	1.27	0.349
Cow cleanliness score	0.83	0.294

OR = Odds ratios

Based on 57 surveys veterinarians confirm 21, noticed during practice, risk factors for endometritis. "Induced calving" is on the sixth place, "dirty calving equipment" on the tenth. "Calving indoors" is ranked higher, on place thirteen, compared to "calving outside", ranked as the last of all 21. "Faecal consistency" score has its role not only in theory, but also in practice and can be registered on place 16.

Interestingly, consulted veterinarians also establish "movement between management groups at the time of calving" as a risk factor for endometritis (Potter et al. 2010).

Chapter summary:

The briefly subjected risk factor “housing system” supplies controversial findings. There exists the general tendency that a dirty environment constitutes a higher risk for the development of metritis and endometritis. Also, some authors share the opinion that pasture based floors are cleaner and freer from pathogens than barn floors. However, according to several studies “housing systems” is a risk factor that is highly depended on management. Consequently, it is difficult to draw an objectified conclusion. Because it is a multifactorial factor depending a lot on human actions, I added survey outcomes. In the survey veterinarians ranked “induced calving” and “dirty calving equipment” under the top ten of risk factors for endometritis. Followed by “calving indoors” and “faecal consistency”. It can be assumed that a hygienic status is relevant for the maintenance of health in dairy cows.

9 Risk factor - parity and lactation number

Publications have conflicting outcomes when discussing effects of parity on disorders pp in dairy cattle (Costa et al. 2019, Gilbert et al. 2005, Gilbert 2016, Pascottini et al. 2017).

Gröhn and Rajala-Schultz (2000) used logistic regression and analysed the association between parity and incidences for fertility disorders in 61,124 Finnish Ayrshires. High parity turned out as a significant risk factor for retained placenta and ovarian cysts. Incidences for retained placenta in cows that calved twice were 1.0. In animals that calved more than three times before, incidences were 1.5. Odds ratios for ovarian cysts were 1.0 in primiparous and 1.4 in multiparous (parity > 3) cattle (Gröhn and Rajala-Schultz 2000).

Costa et al. (2019) determined an overall lower frequency of health disorders pp in primiparous than in multiparous cows. This may can be explained by the higher metabolic stress caused by dry period and former lactations multiparous ones must cope with. High parity number in combination with yielded milk containing a low lactose percentage (LP) constitutes the highest risk for diseases pp. The Table 9-1 shows the mean standard deviation (SD) of subclinical endometritis and frequencies of ovarian cysts and retained placenta. Cows were divided into three different LP (LP "low", "medium" and "high") classes. SCE appearance can be seen most frequent in the "low-LP" class in multiparous and most rarely in the "high-LP" class in primiparous cows. The higher the assessed LP value, the higher the incidence for ovarian cysts gets in both parity categories (Costa et al. 2019).

Table 9-1 (Costa et al. 2019); The "mean standard deviation" (SD) of "subclinical endometritis" (SCE) and frequency of health disorders among different "lactose percentage" (LP) classes (Low; medium; high):

Class	Lactose percentage range	n	SCE (Mean SD)	Ovarian cysts (%)	Retained placenta (%)
Primiparous					
Low	4.200-4.664	2,402	2.454 (1.564)	3.46	1.25
Medium	4.665-5.116	83,459	1.512 (1.261)	4.40	1.08
High	5.117-5.30	1.705	1.014 (1.130)	4.69	1.23
Multiparous					
Low	4.180-4.553	5,400	3.538 (1.764)	5.78	2.56
Medium	4.554-5.004	187,366	1.848 (1.510)	7.08	2.01
High	5.045-5.345	3,861	1.084 (1.269)	10.52	2.18

SD = mean standard deviation; SCE = subclinical endometritis; LP = lactose percentage; n = number of cows

Researches about associations between age and ovarian cysts supply different outcomes. Some support the assumption that parity or lactation number are risk factors for cyst occurrence (Costa et al. 2019, Lee and Kim 2006, Nelson et al. 2010), others do not (López-Gatius et al. 2002b).

Early cystic conditions diagnosed within day 43 and 49 pp did not appear more frequent in cows with a higher lactation number. But lactation number did affect spontaneous early cyst recovery. Overall, 313 dairy cows during the first, 244 during the second and 316 during the third lactation were observed. Each unit decline in lactation number was accompanied with a 1.4-fold higher risk of cyst regression.

Table 9-2 (López-Gatius et al. 2002b); Rates for spontaneous cyst recovery for different lactation numbers (before 60 days post-partum):

	First lactation, n* (%)	**Second lactation, n** (%)**	**Third lactation or more, n*** (%)**
Cyst recovery	16 (80)	10 (30.3)	18 (29.5)
Cyst persistence	4 (20)	23 (69.7)	43 (70.5)
Total cysts	20 (17.5)	33 (28.9)	61 (53.5)

n = 313 cows; n** = 244; n*** = 316*

Lee and Kim (2006) determined a significant lower risk for cows in their first parity to develop ovarian cysts than in cows with a higher parity number. Figure 9-1 demonstrates ovarian cyst incidences for parity one, two, three, four and higher than five ($p < 0.05$).

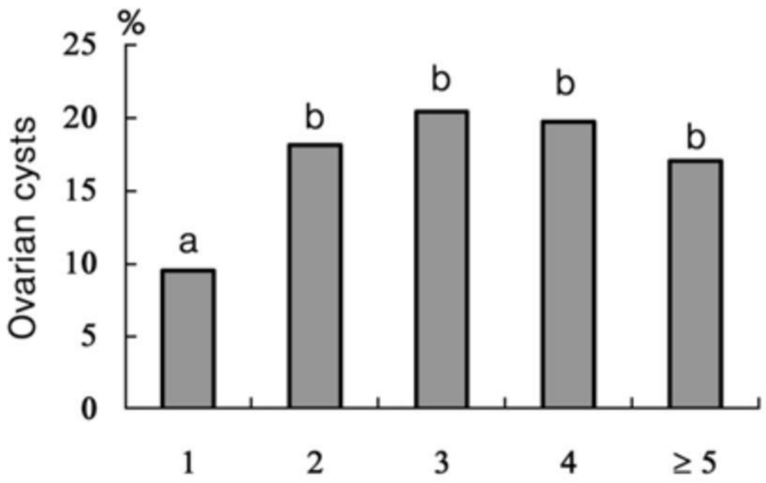

Figure 9-1: "Occurrence of ovarian cysts (%) in cows with parity 1, 2, 3, 4 and 5 or higher; letters a to c denote significant differences between parities ($p < 0.05$)" (Lee and Kim 2006)

As maintained by Nelson et al. (2010) increasing parity can be marked as a relevant risk factor for dairy cattle to suffer from "cystic ovarian disease" (COD). The study included datasets of

15,0000 herds of Norwegian dairy Red cattle. Disorders were diagnosed in the period from 40 until 165 DIM. Detailed COD incidences are shown in Table 9-3. Additionally, a multivariable cox survival model proves associations between COD incidences and COD in earlier lactation. Risk factor parity, calving season and geographical seasons were significantly interrelated. Highest risk for COD appearance existed among cattle that calved during autumn and had a greater parity (Nelson et al. 2010).

Table 9-3 (Nelson et al. 2010); "Cystic ovarian disease" (COD) incidences among different parities:

Parity number	**n**	**COD %**
1	845	0.40
2	1251	0.81
3	1211	1.21
4	780	1.36
> 4	671	1.25

n = number of cows; COD = cystic ovarian disease

Investigations of Hooijer et al. (2001) dealt with genetic associations between lactation number and COD incidences. The risk for COD was slightest in cattle undergoing their first lactation and highest for cattle in lactation number three or higher (3+). Results are demonstrated in Table 9-4. The p-value was less than 0.001.

Table 9-4 (Hooijer et al. 2001); Association between "cystic ovarian disease" (COD) incidences and lactation number:

Lactation number	**N Calvings**	**COD incidence (%)**
1	4379	5.89
2	3500	8.34
3+	7683	8.51

N calvings = number of calvings; COD = cystic ovarian disease

As claimed by Opsomer et al. (2000), based on progesterone measurements, cows in parity one had a lower probability for prolonged luteal periods than cows in parity four and above. Fifteen percent out of 103 primiparous cows and 32 % out of 82 multiparous animals had an extended luteal phase. The main issue about a prolonged luteal phase is that the uterus involution needs more time to get completed (Opsomer et al. 2000).

Researches indicate a connection between parity and RFM. Incidences of RFM raise with increasing parity. Andela et al. (2019) observed 83 primiparous and 213 multiparous Holstein dairy cattle. Thirty-three percent of all were diagnosed with RFM. The disease complex occurred in 29.3 % of cows in parity number two and in 46.3 % in cows in parity number three. Prevalence for parities four and higher was 26.5 % to suffer from RFM (Andela et al. 2019). Through explanatory and predictive models, Vergara et al. (2014) tried to find out relevant risk factors for disorders within 30 DIM. They concluded that high parity can trigger the occurrence of retained placenta. The likelihood for pp diseases increased a lot, if two risk factors, like parity and calving abnormality were combined (Vergara et al. 2014).

As maintained by Potter et al. (2010) primiparity is a significant risk factor for clinical endometritis. All together 293 animals from four different Holstein dairy herds were examined and 80 were diagnosed with endometritis. Overall disease prevalence was relatively high. To achieve more reliable and meaningful results they consulted the "proportion of cows exposed to the risk factor" (Pe) and "the population attributable fraction" (PAF). Pe amounted 0.259 and the PAF was 0.384. Odds ratios amounted 2.3 with a 95 % confidence interval of 1.2-4.4. Derived risk ratios (RR) with a 95 % confidence interval of 1.17-2.46 were 1.78. Additional they conducted interviews with 57 veterinarians who shared their practical experiences. Out of 21 risk factors that often initiated endometritis in practice, parity ranked place fifteen (Potter et al. 2010).

Another publication of Giuliodori et al. (2013) marks primiparity as a risk factor for metritis occurrence in dairy cattle. Metritis was diagnosed if cows had a "Vaginal Discharge Score" three between five and eight DIM based on Sheldon et al. (2006)s scoring system. Incidences for primiparous and multiparous amounted 48.9 % and 35.2 % respectively. Odds ratios for multiparous cows were 0.646 with a 95 % confident interval of 0.371-1.125 and a P-value of 0.08.

Pascottini et al. (2017) observed 873 Holstein dairy cows from 18 farms and determined an averaged prevalence of 28.1 % for cytological endometritis recognized at the time of AI.

In contrast to Potter et al. (2010)s conclusion, this study points out that a parity number of two or higher ($P \geq 2$) is a significant risk factor for cows to get diagnosed with cytological endometritis. Odds ratios for $P \geq 2$ were 1.8 with a P-value of 0.008.

The results are in accordance with LeBlanc et al. (2002) findings. Compared to Pascottini et al. (2017) there was a bigger dataset available. LeBlanc et al. (2002) study dealt with datasets of 1865 individuals. They were examined within day 20 and 33 in milk. The prevalence for clinical endometritis valued 16.9 % and was lower than in other publications (Gilbert et al. 2005, Pascottini et al. 2017, Potter et al. 2010). Percentage for endometritis diagnosed cattle increased with increasing mature. Incidence for endometritis was twelve percent in primiparous cows and 21 % in cows that were in third lactation or elder.

In the study of Benzaquen et al. (2007), 26.0 % of 266 registered cows, that calved previously, developed clinical endometritis within day 20 and 30 pp. Disease incidence was 23.3 %, hence a little less, among primiparous individuals (n = 184). A significant correlation between the two risk factors season and parity were disclosed regarding puerperal metritis. P-value amounted less than 0.001. Cows that had their first calving during the warm period, had a lower chance to get puerperal metritis, than during the cold period (AOR = 1.43; 95 % CI = 0.65-3.18).

Gilbert et al. (2005) contrary, revealed parity not as a risk factor for endometritis diagnosed via cytology (P = 0.53). Among the 141 observed Holstein cattle there was a 53 % occurrence of cytological endometritis within day 40 and 60 pp.

Bicalho et al. (2017) evaluated bacterial flora components and the “total bacterial load” in Holstein dairy cows. Results demonstrated in Figure 9-2 show that the “total bacterial load” collected was significantly higher in primiparous- than in multiparous cattle (P < 0.001). Differences were determined at day three and day seven pp. Samples derived from vaginal swabs from 111 dry cows. The “total bacterial load” was measured by quantitative PCR. The “least squares means” for the “total bacterial load” is listed as “log10 16S rDNA gene copies” in Figure 9-2 and explains a higher number of microbes in primiparous- than multiparous individuals (Bicalho et al. 2017).

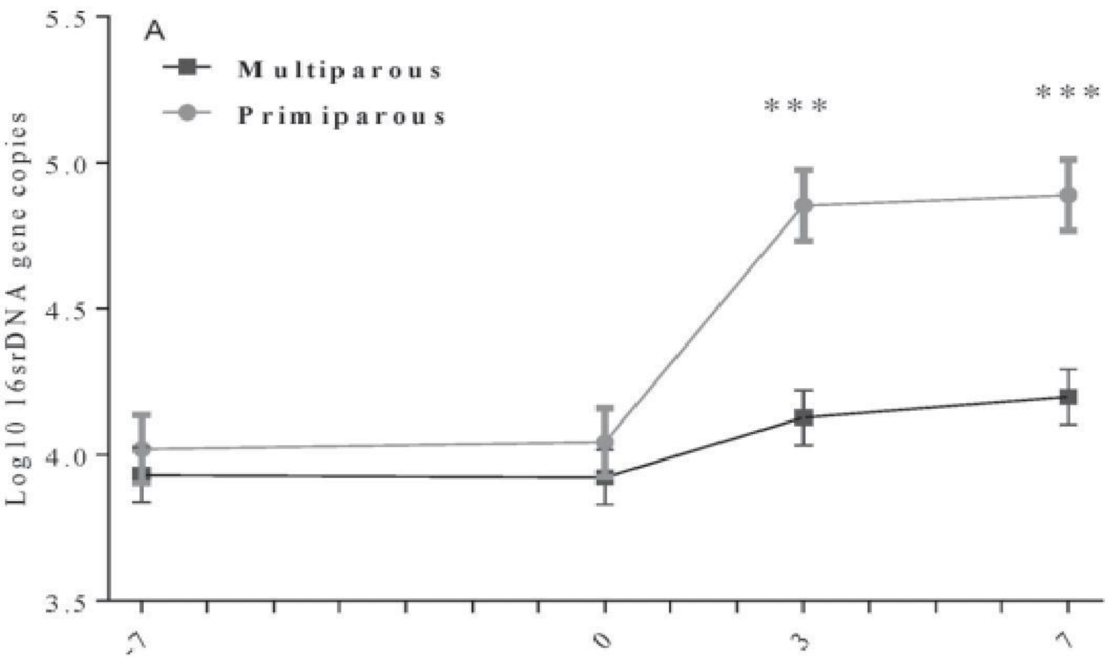

Figure 9-2: “Least squares means ± SEM for total bacterial load (log10 16S rDNA gene copies) detected in vaginal samples from dairy cows at d −7, 0, 3, and 7 relative to parturition. The Graph compares multiparous and primiparous cows. P < 0.001” (Bicalho et al. 2017).

Some authors describe an increased risk for subclinical endometritis for primiparous cows that produce more milk while multiparous cows show an opposite tendency.

Their risk to develop subclinical endometritis decreases with increasing milk (Cheong et al. 2011, Galvão et al. 2010). Incidence of subclinical endometritis was among multiparous and primiparous observed cows nearly the same (26.4 % vs 20.1 %) (Cheong et al. 2011). Occurrences were also the same among other studies (Galvão et al. 2009, Gilbert et al. 2005).

Chapter summary:

Odds ratios for ovarian cysts and RP are among the quoted studies by trend increasing with lactation number three or higher. Multiparous cows may be more susceptible for diseases than their younger herd mates because they are under demanding metabolic stress. Additionally, primiparous individuals are more likely to undergo spontaneous cyst recovery. Regarding metritis, endometritis and cytological endometritis authors conclude controversial results. The study conducted in Norway is highly interesting because the research group included a big dataset and did investments on correlations between several risk factors, like parity, calving season and geographical season.

It is proven that prolonged luteal phase and hence prolonged uterine involution are more frequently diagnosed in multiparous, than primiparous cows.

Adapted odds ratios are assembled in table 9-3 If comparing the study results by ignoring the differences in breeds, dataset number and statistical approach, they all show the same tendency regarding the likelihood for RP and ovarian cysts.

Due to the enormous amount of literature, which deal with parity- or lactation number as a risk factor for fertility disorders, it is for certain, one of the most relevant risk factors among the ones debated in this thesis. Lactation number and calving abnormalities together may constitute the highest hazard for the appearance of fertility disorders. More details about abnormal calving are given in the following chapter, chapter 10.

Figure 9-3; Overview of the chapter 9 “Parity and lactation number”:

Data adapted from	**Number/ breed**	**Risk factor**	**Fertility disorder**	**OR**	**CI (95 %)**	**Disorder occurrence**
Gröhn and Rajala-Schultz (2000)	61,124 Finnish Ayrshires	Parity > 3	Ovarian cysts	1.4		
López-Gatius et al. (2002a)	873 cows	Lactation number	Late ovarian cystic condition	1.36	1.17-1.58	
Nelson et al. (2010)	4,758 cows; 597,722 lactations	Higher parity number	COD			P 4* 1.36 % P 1 0.40 %
López-Gatius et al. (2002a)	873 cows	Higher lactation number	Spontaneous cyst recovery			L** 1 80 % L ≥ 3 29.5%
Hooijer et al. (2001)	15,562 lactations of Dutch Black and White	Higher lactation number	COD			L1 5.89 % L ≥ 3 8.51 %

	dairy cattle					
Opsomer et al. (2000)	326 cows					
Andela et al. (2019)	83 primiparous; 213 multiparous	Higher parity number	RFM			P2 29.3 % P3 46.3 %
Vergara et al. (2014)	1,309 Holstein		RP			
Potter et al. (2010)	293 cows	Primiparity	Clinical endometritis	2.3	1.2-4.4	
Giuliodori et al. (2013)	303 cows	Primiparity	Metritis	0.646, 95% CI: 0.371–1.125 OR for multip.		
Pascottini et al. (2017)	873 cows	P ≥ 2	Cytological endometritis	1.8	0.95-2.48	

LeBlanc et al. (2002)	1,865 cows	Higher lactation number	Clinical endometritis			L1: 12 % L ≥ 3: 21 %
Bicalho et al. (2017)	111 cows	Primiparity	Total bacterial load			

OR = Odds ratios; CI = confidence interval; COD = cystic ovarian disease; RFM = retained foetal membranes; RP = retained placenta
** Parity number*
*** Lactation number*

10 Risk factor – Dystocia

Benzaquen et al. (2007) categorized cows as experiencing either abnormal calving, or normal calving. If dystocia, twins, retained foetal membranes, or a mix of the mentioned conditions were noticed, animals were considered as having an abnormal calving status. Probabilities for puerperal metritis were higher among cows having an abnormal calving status. Abnormal calving turned out as a significant risk factor for metritis in the study of Giuliodori et al. (2013) as well. Detailed results are demonstrated in Table 10-1 (Benzaquen et al. 2007, Giuliodori et al. 2013). The cumulative negative effect of retained placenta and dystocia on the incidence for metritis was also reported by Bruun et al. (2002). Authors did not find differences among various breeds regarding risk factor RP. They assumed that the foetal membranes are ideal breeding ground for bacterial infection.

Table 10-1 (Benzaquen et al. 2007, Giuliodori et al. 2013); Incidences of puerperal metritis in cows with abnormal and normal calving status:

Author	**Calving status**	**Incidence of puerperal metritis (%)**	**Incidence of puerperal metritis (n)**	**Adjusted odds ratios (AOR)**	**95 % CI**	**P-value**
(Benzaquen et al. 2007)	Normal	13.1	43/327	Reference	Reference	0.001
	Abnormal	41.4	51/123	4.8	2.9-8.0	0.001
(Giuliodori et al. 2013)	Normal	34.6	90/260	Reference	Reference	0.008
	Abnormal	65.1	28/43	2.576	1.189-5.559	

AOR = Adjusted odds ratios; 95 % = confidence interval; Abnormal calving status = appearance of dystocia, twins, retained foetal membranes, or a mix of these conditions; normal calving status = cows without calving-related problems

Examinations have been done from day 20-30 pp to check if cows have signs for clinical endometritis or not. They were diagnosed positive, if one or more of the following points were noticed: a bigger diameter than six cm of the cervix; one bigger uterine horn with fluid inside; vaginal discharge with or without pus. Twenty-four percent of the observed animals developed clinical endometritis. Abnormal calving turned out to be a risk factor for odds of clinical endometritis. Out of 123 cows that had birth complications, 40.6 % further generated clinical endometritis. Compared with this, only 17.7 % of 327 cows with a normal calving process were later diagnosed with clinical endometritis. Incidences are shown in Table 10-2.

Table 10-2 (Benzaquen et al. 2007); Incidences of clinical endometritis in cows at 20 to 30 days post-partum with abnormal or normal calving status:

Calving status	**Incidence of clinical endometritis (%)**	**Incidence of clinical endometritis (n)**	**Adjusted odds ratios (AOR)**	**95 % CI**	**P-value**
Normal	17.7	58/327	Reference	Reference	0.001
Abnormal	40.6	50/123	2.8	1.7-4.9	0.001

"AOR = Adjusted odds ratios; 95 % = confidence interval; Abnormal calving status = appearance of dystocia, twins, retained foetal membranes, or a mix of these conditions; normal calving status = cows without calving-related problems

Metritic individuals were subdivided into three classes that contained of different rectal temperature ranges. "Mittemp (MT)-1" cows had puerperal metritis, but no higher body temperature than 39.4°C. Metritic cows with a rectal temperature of 39.4°C and above were classified as MT-2 cows. MT-3 individuals did not have signs of puerperal metritis at all. Puerperal metritis did affect odds for clinical endometritis in a negative way. The adjusted odds for clinical endometritis amounted 2.1 in category MT-1 and 2.2 in category MT-2.

No differences between the "Mittemp groups" can be registered. Table 10-3 illustrates detailed outcomes" (Benzaquen et al. 2007).

Table 10-3 (Benzaquen et al. 2007); Odds for clinical endometritis for cows with different "metritis status":

Puerperal Metritis status	**Incidence of clinical endometritis (%)**	**Incidence of clinical endometritis (n)**	**Adjusted odds ratios (AOR)**	**95 % CI**	**P-value**
No metritis	20.2	72/356	Reference	Reference	
Puerperal metritis	38.2	36/94	2.2	1.1-3.9	0.005
MT-1	38.4	21/55	2.1	1.09-4.1	0.02
MT-2	38.1	15/39	2.2	1.07-4.6	0.02

"AOR = Adjusted odds ratios; 95 % = confidence interval; Abnormal calving status = appearance of dystocia, twins, retained foetal membranes, or a mix of these conditions; normal calving status = cows without calving-related problems; Puerperal metritis = presence of a watery, brown-coloured, fetid discharge from the vulva with or without a rectal temperature ≥ 39.4°C within 13 d post-partum. MT-1 = cows with puerperal metritis without fever; MT-2 = cows with puerperal metritis and fever" (Benzaquen et al. 2007).

Dubuc et al. (2010b) point out the importance of seeing endometritis and purulent vaginal discharge as separated disorders. A positive vaginal discharge status does not give the evidence for endometrial inflammation. In fact, risk factors are not the same for both health disorders. Cytological endometritis was diagnosed 35 plus or minus three days in milk if more than six percent of the collected mucus sample were polymorphonuclear cells.

Calving processes that needed external help, like pulling of two or more people, usage of calf-jack for a longer period than fifteen minutes, were defined as "dystocia".

The occurrence for purulent vaginal discharge was greater in cows having dystocia, twin births or metritis. Odds ratios amounted 2.13, 2.16 and 2.33. If cows had retained placenta, they were more likely to develop metritis. But metritis also occurred without previous retained placenta. Risk factors for cytological endometritis were primarily associated with metabolic disbalances throughout the peripartum phase (Dubuc et al. 2010b).

Table 10-4 (Dubuc et al. 2010b); "Odds ratios" (OR) for risk factors causing purulent vaginal discharge:

Risk factor	Class	n	OR	95% CI	P-value
Twins	No	1,216	Reference		
	Yes	79	2.16	1.09-4.27	0.03
Dystocia	No	971	Reference		
	Yes	324	2.13	1.43-3.98	<0.01
Metritis	No	1,107	Reference		
	Yes	188	2.33	1.41-3.77	<0.01

OR = odds ratios; 95 % CI = confidence interval

Bicalho et al. (2017)s study dealt with the "total bacterial load" (TBL) and demonstrates that primiparous cows had a higher TBL pp than their multiparous counterparts. The findings suggest that cows experiencing their first calving often suffer from small tissue trauma and necrosis initiated by calving assistance. Calving assistance were more frequent performed in primiparous than in mature individuals. Also twinning increased the risk for a higher TBL in the uterus due to the need of calving assistance. The Figure 10-1 shows the TBL seven days pre- and post-partum in cows that faced external support during their calving compared to cows that had a complication-free parturition (Bicalho et al. 2017).

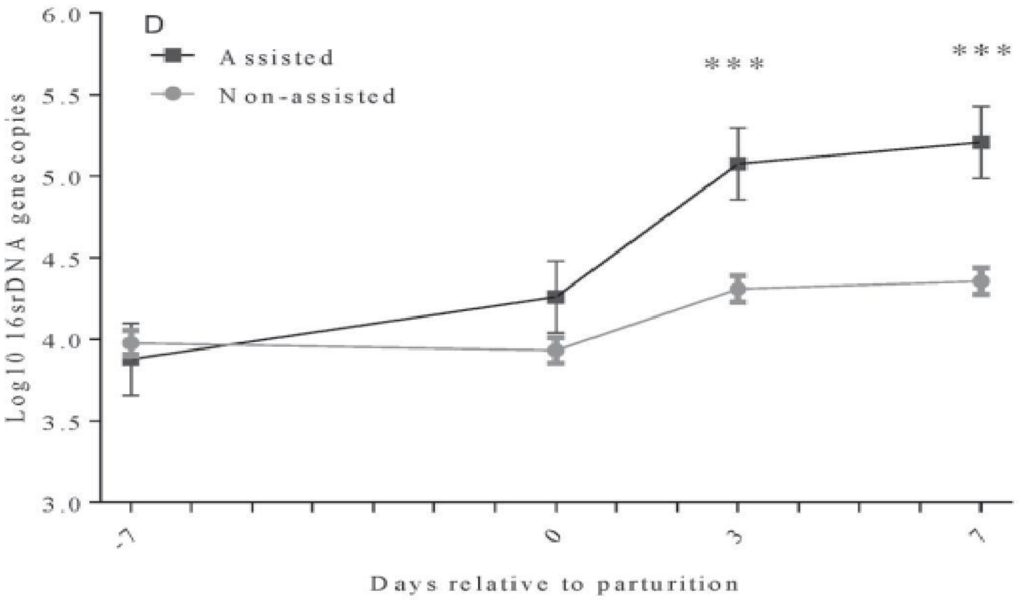

Figure 10-1: “Least squares means ± SEM for total bacterial load (log10 16S rDNA gene copies) detected in vaginal samples from dairy cows at d −7, 0, 3, and 7 relative to parturition. The Graph compares assisted with non-assisted calvings. P < 0.001” (Bicalho et al. 2017).

The Figure 10-2 illustrates the TBL in cows that gave birth to twins, males, females or stillbirths from day seven pre-partum until day seven pp. Controversially to previous findings (Linden et al. 2009) only twins had a negative effect on the TBL pp. Gilbert (2016) conclude that twin births increase the risk of metritis. Additionally, they name RP and obstetric complications as risk factors for PM. The occurrence of RP gets triggered by abortion, dystocia, birth of more than one calf, abortion and stillbirth (Gilbert 2016).

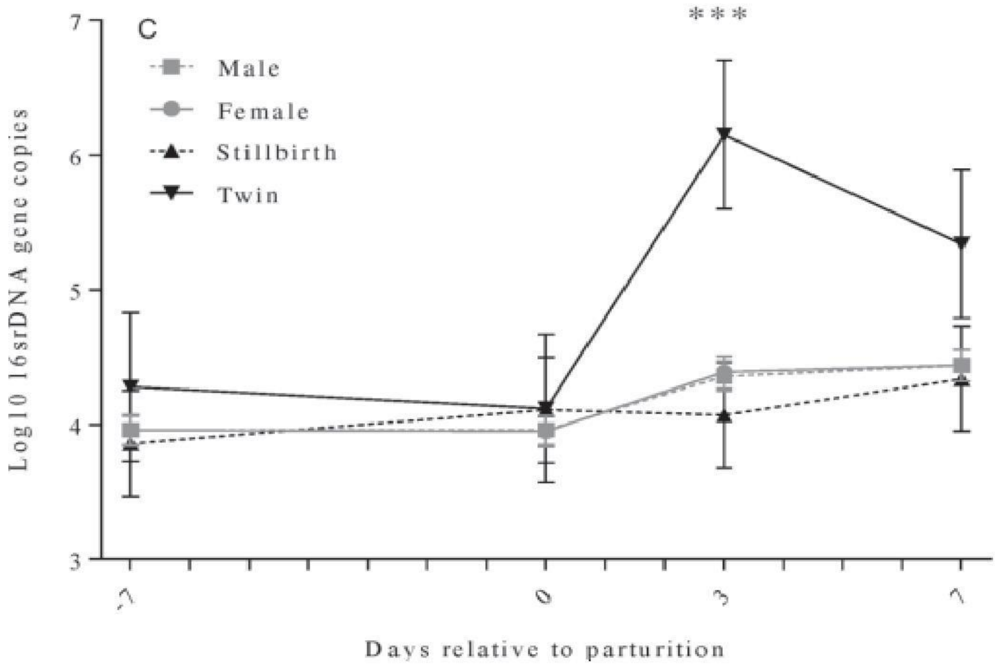

Figure 10-2: "Least squares means ± SEM for total bacterial load (log10 16S rDNA gene copies) detected in vaginal samples from dairy cows at d −7, 0, 3, and 7 relative to parturition. The Graph shows the different calf categories. P < 0.001" (Bicalho et al. 2017).

The research of Potter et al. (2010) included 293 animals from four different dairy farms. Twenty-seven of the observed animals were diagnosed with clinical endometritis. Univariate logistic regression models demonstrate the highest OR for RFM (OR = 34.29; P < 0.001). Odds ratios for the risk factors stillbirth, twins, assisted calving and the birth of male calf amounted 7.94, 4.98, 2.83, and 1.40 respectively. Significant risk factors throughout multivariable analyses are shown in Table 10-5 and agree with other research outcomes (Dubuc et al. 2010b, Potter et al. 2010). RFM turned out to be most relevant. The necrotic material seems to be ideal breeding ground for pathogens and further due to RFM the uterine involution and the endometrial reconstruction get interrupted. The births of male calves are often demanding because they are usually bigger sized than female individuals. Calving assistance and male calves are therefore directly connected parameters (Potter et al. 2010).

An expert panel containing of 57 veterinarians was surveyed and they ranked RFM as the most important risk factor for endometritis as well. Dystocia, twins and stillbirth ranked second, third and fourth. Results of the multivariable and surveyed ones are matching, expect twinning (Potter et al. 2010).

Table 10-5 (Potter et al. 2010); Risk factors for the incidence of endometritis as a result of two conditional logistic regression models:

Variable	OR	SE	P-value	95 % CI (OR)	RR	95 % CI (RR)	Pe	PAF
RFM	40.3	42.1	0.001	5.0-326.4	3.96	2.58-4.24	0.042	0.148
Stillbirth	7.5	3.5	0.001	3.0-18.5	3.09	2.09-3.83	0.091	0.236
RFM	43.5	46.6	0.001	5.3-355.8	3.97	2.65-4.23	0.042	0.148
Assisted calving	2.1	0.8	0.064	1.0-4.4	1.67	0.97-2.51	0.206	0.302
Male calf	1.8	0.6	0.044	1.0-3.3	1.54	1.01-2.15	0.528	0.632

OR = Odds ratios; RR = derived risk ratios; 95 % CI = Confidence intervals; Pe = Proportion of cows exposed to each risk factor; PAF = population attributable fractions

Ribeiro et al. (2013) findings underline that calving problems are a major risk for the evolution of metritis which in turn constitute a risk for clinical endometritis. Incidences of metritis increased with higher prevalence of calving complications from 3.0 %-30.9 %. The likelihood of clinical endometritis had the same tendency and increased from 13.4 %-34.7 %. Metritic cows were then more likely to suffer from clinical endometritis (12.9-55.1 %) and clinical endometritic cows in turn, were at higher risk to get a subclinical endometritic status. (10.1 % vs. 29.6 %) (Ribeiro et al. 2013).

Through predictive and explanatory models Vergara et al. (2014) analysed the risk factors for the combined factor TXR30 for multi- and primiparous cows separated. TXR30 included treatment and removal from the herd within 30 DIM. The treatment category contained of cattle that were treated because of MF, RP, metritis and other non-fertility disorders.

Dystocia, twins and stillbirths were defined together as one factor, “calving abnormality” and were noticed in 14.1 % of the multiparous animals. Likelihood for dystocia amounted 7.2 %, for twinning 6.8 % and for stillbirths 3.3 %. The odds ratios for cattle in the calving abnormality group were 3.3 times higher regarding TXR30 (95 % CI: 2.0-5.4). Interestingly a longer previous lactation period (longer than 349 days) in combination with abnormal calving, strongly influenced the TXR30. They had 8.3 times increased odds ratios (95 % CI: 3.7-18.7). However, further investigations are needed to find out if there exist explainable biological associations between lactation length and abnormal calving. Among the primiparous observed cattle, calving abnormality also triggered odds of TXR30 (OR = 3.9; 95 % CI: 2.3-6.8; $P < 0.01$). Primiparous cows with a shorter gestation length and abnormal calving status were additionally more likely to get treated or removed from the herd (OR = 0.6; 95 % CI: 0.4-0.9 for each six days increase in gestation length ($P < 0.01$) (Vergara et al. 2014).

López-Gatius et al. (2002b) combined twin births, RP, ketonuria and metritis cases in one factor and named it “abnormal puerperium”. The study points out that an abnormal puerperium is a significant risk factor for the development of ovarian cysts. Odds ratios for the “early ovarian cystic condition”, within day 43 and 49 pp, amounted 1.93 for cows with an abnormal puerperium ($P < 0.005$). Interestingly this tendency was not reported for late ovarian cystic condition.

Pesántez et al. (2016) also suggest that a pathological puerperium increases the risk for the development of cysts. While 20 % of the cattle with a normal puerperium were diagnosed with ovarian follicular cysts, 26 % in the abnormal puerperium group showed cystic structures.

Chapter summary:

Abnormal calving is a wide ranging-topic including dystocia, twins, retained foetal membranes or even a mix of two or more of these factors. Based on existing knowledge retained foetal membranes are ideal breeding ground for pathogens.

All of them constitute a significant higher risk for the development of metritis and endometritis. Although the investigators used different statistical methods, for example predictive and explanatory models or univariate cox model, they share the same overall outcome. The table 10-6 demonstrates the adapted results clearly. It is one of the strongest risk factors with the most clearly, comparable results this thesis deals with. It is also proven that cows giving their first birth need more often calving assistance then their multiparous counter partners. At this point I want to refer to the previous chapter "parity and lactation number".

Table 10-6: overview of the chapter 10 "Abnormal calving":

Data adapted from	**Number/ breed**	**Risk factor**	**Fertility disorder**	**OR**	**CI (95 %)**	**Disorder occurrence**
Benzaquen et al. (2007)	450 calvings	Abnormal calving	Metritis; Clinical endometritis	4.8 2.8	2.9-8.0 1.7-4.9	41.4 % 40.6 %
Giuliodori et al. (2013)	303 cows	Abnormal calving	Metritis	2.567	1.189-5.559	65.1 %
Dubuc et al. (2010b)	1,663 Holstein-Friesian	Dystocia; Twin births; Metritis	Metritis; cytological endometritis	2.13 2.16 2.33	1.43–3.98 1.09–4.27 1.41–3.77	
Linden et al. (2009)	1,245 calvings					
Bicalho et al. (2017)	111 cows					

Potter et al. (2010)	293 cows	Stillbirth; Assisted calving; Male calf; RFM	Clinical endometritis	7.5 2.1 1.8 43.5	3.0-18.5 1.0-4.4 1.0-3.3 5.0-326.4 5.3-355.8	
Ribeiro et al. (2013)	957 cows	Abnormal calving	Metritis: Clinical endometritis			Normal 3.0 % 13.4 % Abnormal. 30.9 % 34.7 %
Vergara et al. (2014)	1,309 Holstein	Abnormal calving multiparous; And primiparous	TXR30	3.3 8.3	2.0-5.4 3.7-18.7	
López-Gatius et al. (2002a)	873	Abnormal puerperium	Early ovarian cystic condition	1.93	1.21-3.08	
Pesántez et al. (2016)	1,249 reproductive records	Normal vs. abnormal puerperium				20 % vs. 26 %

OR = odds ratios; CI = confidence interval; RFM = retained foetal membranes; TXR 30 = factor, which included treatment and removal from the herd within 30 days in milk

11 Discussion

Due to the fact, that fertility is one of the most present and discussed issue in dairy cattle farming, a vast amount of scientific papers is available. Therefore, the literature research about risk factors for fertility disorders in dairy cattle turned out more challenging than expected. The first task set was to define the most common and most intensively analysed fertility disorders. In order to facilitate further research, the focus was set on the following diseases: retained foetal membranes, puerperal metritis and endometritis, ovarian cysts, cystic disbalances. As stated in the introduction chapter, it was not scope of the thesis to include all fertility disorders described in dairy cows.

On the one hand the thesis stands out from other published papers, because it includes more than a single disease and gives a short and good overview of the risk factors for metritis, endometritis, ovarian cysts and cystic disbalances. The generated knowledge about the risks that influence the occurrence of fertility diseases is crucial for the maintenance of overall health and well-being of dairy cows. The results provide the essential basis for further development of mathematical models that will enable early prediction of diseases and will therefore provide a great support for dairy farmers.

On the other hand, it was not the ideal approach to deal with several diseases, retrospectively. The scope of the thesis in general is too broad. For more precise outcomes it would have been preferable to write the paper about one specific disorder, like for example endometritis. It would have ended up in more detailed results, including more intense debates about the large variability in material, methods and thresholds practiced in the different studies. Additionally, more risk factors could have been considered.

If consulting the analyses of the first chapter “diagnostic methods regarding fertility disorders” deviating approaches can be seen clearly. Different methods with variating sensitivity and specificity to detect disease complexes were established. It is of high importance to choose suitable tools for the detection of diseases to start interventions timely. Acting quickly is crucial to minimize disorder prevalence and further resulting financial costs.

These inequalities do not only exist within diagnostic techniques, but they already start within disease definitions.

When should individuals be diagnosed as sick? How is the threshold set for subclinical patients? Accordingly, there are limitations regarding the comparability of the studies.

The purpose was to originate a useable summary of the most relevant risk factors, outlined by meaningful odds ratios, for the "D4Dairy" project. Therefore, the thesis includes many tables and figures. Limitations for the interpretations are among others present because of the different diagnostic approaches, thresholds, various housing and managing conditions.

Additionally, there is a huge range regarding disease incidences and prevalence if comparing quoted studies. This problem also arises because of an unsimilar starting point, used methods, frequency in testing, person, who does the testing and deviating interpretations.

Another critical point to name is the variability in investigation places. It was the aim to only include researches that has been conducted in Europe or near it. This was not possible for all risk factors and is accompanied with limitations in interpretations. Different countries keep different breeds, have different housing systems, climatic conditions, ethnical foundations et cetera.

Furthermore, each risk factor is discussed separately although, they all kind of interrelate with each other. Attempts have been made, especially throughout the short chapter summaries to mention correlating risk factors as well. During the literature research it also turned out that retained foetal membranes, itself, as a fertility disease constitutes a high risk for endometritis.

The thesis doubtlessness does not include all the risk factors and their odds ratios. However, the mentioned ones do have a decisive impact on the likelihood for cows to develop fertility disorders**.** BCS, NEB, parity number and calving abnormalities are hotly debated among authors and the most influential risk factors based on my research. Additionally, higher milk yields evolved over the last years into a frequently discussed risk. Judgements must be made carefully, because there is not always the same tendency among all fertility diseases: A higher BCS (BCS > 3) for instant implicates higher incidences for ovarian cysts. Meanwhile a BCS < 3 constitutes a higher risk for uterine infectious diseases, RFM and cyclic disbalances. BCS and NEB are two of the most manageable factors, so great attention should be made to get them controlled. Higher parity number in turn is beyond the farmers´ or veterinarian doctors' control.

The assessment of environmental risk factors is not easy and due to this fact, they are more demanding in interpretation as well. Papers tried to include many circumstances, but it is not achievable to give attention to every single influential factor. The THI value was invented to measure temperature conditions more precise and uniform throughout the studies, which is a good point to get the heat stress monitored and minimized.

Further detailed investigations focused on one disorder are may reasonable. Additionally, a meta-analyses would be advisable, particularly for the “D4Dairy” project, to get meaningful mathematical and statistical results.

12 Conclusion

Fertility disorders are of multifactorial origin and therefore there exist various risk factors. It is not easy to rank them by importance. However, BCS, NEB, parity number and calving abnormalities turned out through this literature research as the most debated and most influential risk factors. Additionally, higher milk yields evolved over the last few years into a meaningful risk for the development of fertility disorders. Some of the discussed factors can be easily minimized, others cannot. Because of the varying aetiology of the disease complexes, risk factors can hardly be generalized. Furthermore, it is not the right approach to deal with each factor separately, because they all kind of interrelate with each other.

13 Summary

Reproductive diseases are hotly debated in dairy cattle farming. They contribute a large part to general health and welfare of dairy cows. It is of high importance to detect disorders timely so interventions can be made, which not only improve the well-being of individuals and whole herds, but also minimize consequential cost factors for farmers. Disorders most frequently occur during the post-partum period, which is a critical phase due to appearing higher metabolic needs. A cow's body must work like a machinery to yield as much milk as possible without getting sick itself.

Regular puerperal checks should be made in dairy cattle practice to reduce cases of disease in herds. Abnormal calving turned out through the research as the main risk factor for the occurrence of metritis and endometritis. It is recommended to highly put attention into hygienic conditions if calving assistance is needed.

During pregnancy, calving and lactation the body of dairy cattle must operate at full stretch. To prevent cows from getting sick itself, it is important to provide ideal feeding rations during these periods. A body condition score lower than 3 or changes in BCS pre-partum constitute highly relevant risk factors for the occurrence of uterine infectious diseases, RFM and cyclic disbalances. Meanwhile a gain in BCS comes along with more ovarian cyst cases. The BCS can be assessed easily by veterinarians or trained farmers. It should be measured regularly to reduce fertility disorders within herds and to control the feed ration. It is not easy to meet the nutritional requirements of in calf and lactating dairy cows, but a lot of attention should be paid to it, so cows do not face a negative energy balance status.

Beside the NEB and the BCS, hypocalcaemia is defined as a risk factor for distractions in fertility health, regarding particularly milk fever. However, the question about the ideal preventive Ca concentration the feeding rations must contain, remains unclear. A Vitamin E deficiency can be found in the literature as a risk factor for RFM, but it has for sure less impact than factors mentioned above.

The older the cattle gets the more fertility disorders appear. A lactation number of 3 or higher is described by several authors as one of the most relevant risk factors for ovarian cysts and

RFM. The effect of a higher parity on the likelihood of metritis and endometritis is controversial discussed.

Based on genetic research the unfavourable correlations between high milk yield and reproductive diseases is proven. High milk yield plays a crucial role these days because of the constantly rising demand in milk production.

Adequate environmental circumstances are influential to the overall cow comfort. If the comfort is thrown off track cows are more susceptible for diseases. Heat stress evolved into a meaningful factor, also in our latitudes due to the climatic change. The climatic comfort zone for dairy cattle are defined between 5 and 20°C. Authors rank a THI value above 75 as critical and influential to fertility disorder incidences.

Furthermore, winter calvers are more likely to suffer from reproductive diseases. However, it is a factor highly depending on the husbandry- and management- system. Also, the climatic conditions can be managed differently by using fans as cooling systems, stables with access to open air et cetera. Among the reviewed studies controversial findings exist regarding associations between odds for metritis and flooring systems. An important risk factor constitutes contaminations through the environment and through human skin contaminated with pathogens from faeces. The cow cleanliness score and the faecal consistency score can help to assess possible contaminations which can lead further to endometritis. Bigger herd size and a higher stocking density must be considered as well, because cows can get stressed and evolve easily any disorder. Environmental factors in general are very specific to each farm, because of differences in housing, flooring, cooling, feeding and milking systems, hence, an overall conclusion is impossible to make.

All together it can be said that fertility disorders are very common, of multifactorial origin and should be well monitored in dairy cattle farms. Risk factors are in some cases the same for all describes disorders, but sometimes not. Lots of them interrelate with each other and therefore all of them should be included into the statistical models of the "D4Dairy" project.

14 Zusammenfassung

Reproduktionskrankheiten sind ein intensiv diskutiertes Thema in der Milchviehhaltung. Sie tragen einen großen Teil zur generellen Gesundheit und dem Wohlbefinden von Milchkühen bei. Die rechtzeitige Erkennung von Fertilitätsstörungen ist äußerst wichtig, damit frühzeitig Interventionen getätigt werden können, welche nicht nur das Wohlbefinden auf Einzeltier- und Herdenbasis verbessern, sondern auch die Folgekosten für Landwirte und Landwirtinnen minimieren. Fertilitätsstörungen treten am häufigsten in der Zeit nach der Geburt auf, einer kritischen Phase, die durch einen höheren Stoffwechselbedarf gekennzeichnet ist. Der Körper einer Kuh muss wie eine Maschine arbeiten, um konstant so viel Milch wie möglich zu produzieren, ohne dabei selbst krank zu werden. Um die Anzahl der Krankheitsfälle in den Herden zu reduzieren, sollten regelmäßige Puerperalkontrollen durchgeführt werden.

Abnormales Abkalben erwies sich im Zuge der Literaturrecherche als der Hauptrisikofaktor für das Auftreten von Metritis und Endometritis. Es wird empfohlen, in hohem Maße auf hygienische Bedingungen zu achten, wenn bei der Kalbung externe Hilfe benötigt wird. Während der Trächtigkeit, des Kalbens und der Laktation muss der Körper von Milchkühen auf Höchstleistung arbeiten.

Um zu verhindern, dass die Kühe selbst krank werden, ist es wichtig, ihnen speziell in diesen kritischen Zeiten ideale Futterrationen bereitzustellen. Ein Body Condition Score von weniger als drei oder Veränderungen des BCS prä partum stellen hochrelevante Risikofaktoren für das Auftreten von uterinen Infektionskrankheiten, RFM und zyklischen Ungleichgewichten dar, während umgekehrt ein Anstieg des BCS mit mehr Fällen von Eierstockzysten einher geht. Der BCS kann von Tierärzt*innen oder geschulten Landwirten*innen leicht erhoben und beurteilt werden. Er sollte regelmäßig gemessen werden, um Fruchtbarkeitsstörungen innerhalb der Herde zu reduzieren und die Futterration unter Kontrolle zu halten. Es ist nicht einfach, den Nährstoffbedarf von tragenden und laktierenden Milchkühen zu decken. Dennoch sollte viel Aufmerksamkeit darauf gerichtet werden, damit die Kühe in keinen negativen Energiebilanzstatus gleiten.

Neben dem NEB und dem BCS wird Hypokalzämie als Risikofaktor für Störungen der Fruchtbarkeitsgesundheit definiert, insbesondere im Hinblick auf Milchfieber.

Die Frage nach der idealen präventiven Ca-Konzentration, die die Futterrationen enthalten sollte, bleibt jedoch bisher unklar. Ein Vitamin-E-Mangel ist in der Literatur als Risikofaktor für RFM zu finden, hat aber mit Sicherheit weniger Auswirkungen als die oben erwähnten Faktoren.

Weiters spielt das Alter der Kühe eine zentrale Rolle. Je älter das Tier wird, desto eher treten Fruchtbarkeitsstörungen auf. Eine Laktationszahl von drei oder höher wird von mehreren Autoren*innen als einer der relevantesten Risikofaktoren für Ovarialzysten und RFM beschrieben. Die Auswirkung einer höheren Parität auf die Wahrscheinlichkeit von Metritis und Endometritis wird kontrovers diskutiert.

Auf der Grundlage genetischer Forschung sind die ungünstigen Korrelationen zwischen hoher Milchleistung und Reproduktionskrankheiten erwiesen. Eine hohe Milchleistung spielt heutzutage aufgrund der ständig steigenden Nachfrage in der Milchproduktion eine entscheidende Rolle.

Umweltbedingungen haben allgemein einen großen Einfluss auf den Gesamtkuhkomfort. Wenn der Komfort aus der Bahn geworfen wird, sind die Kühe anfälliger für diverse Krankheiten. Hitzestress hat sich, aufgrund des Klimawandels auch in unseren Breitengraden zu einem bedeutsamen Faktor entwickelt. Die klimatische Komfortzone für Milchkühe liegt zwischen 5 und 20°C. Wissenschaftler*innen stufen einen THI-Wert über 75 als kritisch und einflussreich für das Auftreten von Fruchtbarkeitsstörungen ein.

Darüber hinaus leiden im Winter kalbende Kühe mit größerer Wahrscheinlichkeit häufiger an Reproduktionskrankheiten als im Sommer kalbende. Dies ist jedoch ein Faktor, der stark vom Haltungs- und Managementsystem abhängt. Auch die klimatischen Bedingungen können unterschiedlich beeinflusst werden, indem Ventilatoren als Kühlsysteme, Ställe mit Zugang zum Freien usw. eingesetzt werden. Unter den zitierten Studien gibt es kontroverse Befunde bezüglich der Wechselwirkungen zwischen dem Vorkommen von Metritis und den unterschiedlichen Bodensystemen. Ein wichtiger Risikofaktor ist die Kontamination durch die Umwelt und durch die menschliche Haut, wenn diese mit Pathogenen aus Fäkalien in Kontakt gekommen ist. Die Bewertung der Kuhsauberkeit und die Bewertung der Kotkonsistenz können helfen, mögliche Kontaminationen zu erkennen, die dann folglich zu Endometritis führen können. Erhöhte Herdengrößen und Besatzdichten müssen ebenfalls in Betracht gezogen werden, da die Kühe unter Stress gesetzt werden und dadurch leichter Störungen entwickeln können. Umweltfaktoren sind im Allgemeinen sehr spezifisch für jeden Betrieb, da die Stallungssysteme, der Bodenbelag, das Kühlungs-, das Fütterungs- und das Melksystem unterschiedlich sind, sodass eine allgemeine Schlussfolgerung nicht möglich ist.

Alles in allem kann gesagt werden, dass Fruchtbarkeitsstörungen sehr häufig auftreten, multifaktoriellen Ursprungs sind und in Milchviehbetrieben gut überwacht werden sollten. Die Risikofaktoren sind in einigen Fällen für alle beschriebenen Störungen gleich, in manchen unterscheiden sie sich aber. Viele von ihnen stehen in Wechselbeziehung zueinander, weshalb alle in die statistischen Modelle des "D4Dairy"-Projekts miteinbezogen werden sollten.

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16 List of Abbreviations

Abbreviation	Explanation
T. Pyogenes	Trueperella pyogenes
AI	Artificial insemination
AL	Ad libitum
AMP	Antimicrobial peptides
AOR	Adjusted odds ratios
AUC	Area under the curve
Ca	Calcium
CIDR	Controlled Internal Drug Release
CIDR	Controlled Internal Drug Release
CL	Corpus luteum
COD	Cystic ovarian disease
COD	Cystic ovarian disease
CYTO	Cytological endometritis
DCAC	Dietary cation anion difference
DF	Dominant follicle
DIM	Days in milk
E. coli	Escherichia coli
EB	Estradiol benzoate
eCG	Equine chorionic gonadotropin
EPA	Estimate probability of appearance
FA	Fatty acid
FR	Feed restriction
FSH	Follicle-stimulating hormone
FTAI	Fixed time artificial insemination
GnRH	Gonadotropin releasing hormone
IGF	Insuline-like growth factor
IL-8	Interleukin 8
IU	International units

LH	Luteinizing hormone
LP	Lactose percentage
LP	Lactose percentage
LPS	Lipopolysaccharides
LY	Lactose yield
MET	Metritis
MF	Milk fever
MS	Metricheck device
NEFA	non esterified fatty acids
PAF	Population attributable fraction
PAI	Pregnancy per artificial insemination rate
Pe	Proportion of cows exposed to the risk factor
PG	Progesteron
PGF2α	Prostaglandin-2α
PM	Puerperal metritis
PMN	Polymorphonuclear leukocytes
Pp	Post-partum
PS	Pregnancy status
PSM	Planned start of mating
PTH	Parathormone
RFM	Retained foetal membrane
RH	Relative humidity
ROC	Receiver operating characteristics
ROS	Reactive oxygen species
RP	Retained placenta
RR	Risk ratios
SD	Stocking Density
SE	Subclinical endometritis
SE or SCE	Subclinical endometritis
THI	Temperature humidity index
ULSOD	Uterine lavage sample optical density

UTS	Uterine score
UTS	Ultrasound
VDS	Vaginal discharge score
VV	Visual vaginal

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