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Comparison of uterine and oviductal health status of cows in different metabolic condition

Diploma thesis

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Contents

1.) Introduction

A modern dairy farm is aiming for optimal milk production and reproduction to meet profitability. Diseases negatively affect the economics of dairy farms. A frequent disorder is subclinical ketosis due to negative energy balance after calving. Subclinical ketosis results in direct milk loss, increases the risk for other diseases and consequently leads to reduced reproductive performance (Duffield et al. 2009). Postpartum infections of the uterus and the ovaries as well as hormonal imbalances are well-known impact factors for sub- and infertility in cows (Sheldon and Dobson 2004, Pérez-Marín and España 2007). The oviduct, however, is also essential for good fertility, as it provides the environment for fertilisation and early embryonic development (Hunter 2012).

1.1) Oviduct

The oviduct is the first anatomical structure that affiliates the fertilizable *ovum*, which has only a few hours after ovulation to be fertilized. The oviductal structures are highly influenced by hormones via the systemic blood flow and locally by counter-current transfer (Hunter et al. 1983). The endothelial cells transport and supply the *ovum*. Therefore, the volume of liquid in the oviduct rises and changes in composition during the luteal phase and provides a direction of flow from the *ampulla* into the uterus (Roblero et al. 1976). The physiological function of the oviduct plays an important role in fertilization, and, thus, pathological lesions of the oviduct negatively affect fertility. Azawi (2009) investigated the prevalence of hydrosalpinx, pyosalpinx, salpingitis, adhesions and obstruction of the oviduct in slaughtered buffaloes and found similar bacteria in the oviduct and the uterus. In humans, salpingitis is the primary cause of tubal infertility (Price et al. 2017). Diagnosis of acute salpingitis in humans is based on ultrasonographic examinations, confirmed by diagnostic laparoscopy (Romosan et al. 2013). Because of the lack of symptoms in subclinical diseases, diagnostic options are limited, in particular for subclinical salpingitis, as no on-cow test is available. Approaching the oviduct via laparoscopy is complex; therefore, procedures more relevant for veterinary practice are desirable. In the past, Besenfelder et al. (1998) applied transvaginal endoscopy for embryo transfer in the rabbit and in heifers (Besenfelder et al. 2010). In heifers, Pothmann et al. (2017) tested the transvaginal endoscopic approach to the oviduct and approved this technique to be suitable for cytological examination of the samples specimen.

1.2) Uterine infection

While metritis describes an acute uterine infection with fetid, red-brown discharge and systemic signs of the disease (Sheldon et al. 2006), clinical or chronical endometritis (CE) only shows vaginal discharge with distinct character (Dohmen et al. 1995). Subclinical endometritis (SE) is an inflammatory process in the cow's endometrium *postpartum* (*p.p.*) in the absence of clinical signs (Sheldon et al. 2006). Subclinical endometritis negatively affects fertility, leading to increased days open (Gilbert et al. 2005). The diagnosis of SE is based on endometrial cytology and can be performed with the cytobrush-technique (Kasimanickam et al. 2004, Dubuc et al. 2010). In the course of uterine inflammation, granulocytes are the first line of defence to fight invading agents (Pascottini et al. 2016). The proportion of polymorphonuclear neutrophil granulocytes (PMN) to the total number of endometrial cells in microscopic smears defines SE (Kasimanickam et al. 2004).

1.3) Ketosis

Ketosis is a metabolic disease defined as an excessive amount of ketone bodies in the extracellular fluid, leading to clinical signs of disease or, in cases of subclinical ketosis, to increased concentration in blood, excretion in milk and urine without clinical signs. If the energy balance is negative, often in high performing lactating dairy cows early postpartum, the low blood glucose levels cause a decline of insulin in plasma. In consequence, the mobilisation of body fat rises, leading to elevated concentration of free fatty acids (non-esterified fatty acids, NEFA) in serum. In order to generate energy through the citric acid cycle, NEFA are oxidized to acetyl coenzyme A (Acetyl-coA). Because of the low blood glucose level and eventually the lack of pyruvate, ketone bodies (acetoacetic acid, acetone, and beta-hydroxybutyrate) are produced by splitting AcetylCoA (Guyton et al. 1981). The commonly used threshold of beta-hydroxybutyrate (BHB) for the definition of subclinical ketosis is set at 1.2 mmol/l (Duffield et al. 2009), while concentrations of BHB exceeding 2.9 mmol/l together with clinical signs define clinical ketosis (Dubuc and Buczinski 2018). Clinical signs of ketosis are a loss of condition, reduced milk yield and, more obvious, the refusal of concentrate intake. Disorders of the nerval system like salvation, chewing movements with an empty mouth or licking of parts of the stall are less frequently reported. Furthermore, a sweet smell of the cow´s breath, because of acetone in the exhaled air, can be

detected (Anderson et al. 2004). An increase of NEFA before calving is related to a higher risk of ketosis and endometritis postpartum (Ospina et al. 2010). The cut off for pathological NEFA concentrations *ante partum* is set between ≥ 0.3 mmol/l and 0.6 mmol/l (Ospina et al. 2010).

Although the bovine reproduction tract, including the oviduct, have been in the focus of several research projects, but to our best knowledge no studies have investigated the association between endometritis and salpingitis under conditions of metabolic stress. Therefore, this study focused on the relation of subclinical ketosis, subclinical salpingitis, and subclinical endometritis in cattle, hypothesizing that there is an association between these subclinical conditions. As a part of a larger research study, the suitability of a modified cytobrush-technique via transvaginal endoscopy for sampling the bovine oviduct was tested.

2.) Material and Methods

This study was approved by the institutional ethics committee and the national authority according to §8 of Law for Animal Experiments, Tierversuchsgesetz-TVG (BMWFW-68.205/0162-WF/V/3b/2017).

The study was conducted at the research and teaching farm VetFarm Kremesberg, University of Veterinary Medicine Vienna, Austria. The dairy herd comprised approximately 75 Simmental milking cows, housed in a freestall barn with straw-bedded cubicles. The study took place from June 2018 to December 2019. The annual average milk production per cow was 9,247 and 8,555 kg in 2018 and 2019, respectively. Cows suffering from peripartal diseases, i.e. downer cow syndrome and infections accompanied with systemic signs were excluded from the study. Likewise, cows with *dystocia* and *sectio caesarea* were excluded for the trial. Cows developing signs of a systemic disease with fever during the study were treated according to the diagnosis and were excluded from the study.

2.1) Feeding

Individual feeding was performed in a segregated part of the barn with eleven feeding troughs with individual animal identification and electronic scales (Hokofarm Group B.V, The Netherlands). The forage was delivered by an automating feeding system (Trioliet, Oldenzaal, The Netherlands). Each cow was assigned to a single trough, measuring the daily feed intake, excluding concentrate, which was offered individually in a feeding station. The aim of the feeding concept was to achieve a controlled metabolic stress by offering a ration with different energy content. Prepartum feeding consisted of balanced nutritional supply for Simmental cows (Bayerische Landesanstalt für Landwirtschaft 2020), containing 5.5 MJ NEL/kg DM to meet the requirements (control group, CON). High-energy supply of 6.9 MJ NEL/kg DM was offered to the treatment group (TRE). This study was part of a comprehensive study and aimed to achieve two groups with different metabolic status *postpartum*. Therefore, the *prepartum* grouping of the animals in CON and TRE was not important for further analyses. A scientific assistant controlled the daily feeding and the data collection.

The total mixed ration (TMR) for the cows consisted of hey, straw, grass silage, corn silage, concentrated feed and water. The ingredients were added in order from big to small, scaled

before and after they were put into the distribution trailer of the feeding robot. A deviation of a maximum of 3 % of the amount of each component was accepted in the mixture. The forage was mixed in the feeding mixer for 15 minutes before the single troughs were filled. Once a week, the dry matter in the total mixed ration was calculated. A nutritional analyses of TMR was performed in the laboratory of the agriculture chamber (Futtermittellabor Rosenau, Wieselburg, Austria), when fresh silage (corn, grass) was added to the ration. According to the results, the TMR was adjusted to the nutritional requirements previously mentioned.

2.2) Study groups and sampling

Cows were assigned to groups approximately 42 days before expected calving. Groups were balanced for milk yield in the previous lactation and feed intake in the dry period. Postpartum, cows were categorized into subclinical ketosis (sKET) when BHB-concentrations exceeded ≥ 1.2 mmol/L, and nonKET, if BHB was < 1.2 mmol/L (Duffield et al. 2009).

At the day of enrolment in the study (42 day *a.p.*), animals underwent a clinical examination, including the assessment of general condition, the inner body temperature, auscultation of heart, lungs and rumen. In addition, capillary blood samples were taken from the vulva skin to measure BHB-concentrations with an on-site test (Freestyle Precision, Abbott, Illinois, USA), described in detail by Kanz et al. (2015). Table 1 provides an overview over the detailed sampling plan. The sampling day could deviate for one day because of logistic reasons.

Table 1. Timetable of examinations and sampling

Days	Examination	Samples
42 *a.p.*, 14 *a.p.*, day of calving (d0)	- Clinical examination, - BHB, NEFA	Capillary blood, Serum
1 to 13 *p.p.*	-BHB every second day	Capillary blood
7 *p.p.*	- Clinical examination, transrectal palpation (T), vaginal examination (V) - Cytobrush - BHB	Cytobrush Endometrium Capillary blood
14 *p.p.*	- Clinical examination, T, V, - Cytobrush - BHB, NEFA	Endometrium Capillary blood, Serum
28-34 *p.p.* *	- Clinical examination, T, V - BHB - Cytobrush	Capillary blood endometrium and oviduct (bilateral)
40-46 *p.p.* *	- Clinical examination, T, V, - BHB - Cytobrush	Capillary blood endometrium and oviduct (bilateral)

a.p.: *ante partum*, *p.p.*: *postpartum*, BHB: beta-hydroxybutyrate, NEFA: not esterified fatty acids

* Examinations were only performed once within the indicated timeframe

The cytological sampling of the endometrium was performed as described elsewhere (Pothmann et al. 2018). In brief, a cytobrush (Gynobrush, Heinz Herenz, Hamburg, Germany) screwed on a metal rod and protected by a plastic catheter and a plastic sleeve was inserted into the uterine cavity. In the *corpus uteri*, the plastic sleeve was drawn back and the brush was rolled along the endometrium. After retraction of the instrument, the brush was rolled on a microscope slide, fixed, stained and the proportion of polymorphonuclear neutrophil granulocytes (PMN) was determined by counting of 300 endometrial cells and PMN under a microscope (Melcher et al. 2014).

The diagnosis of CE was performed by evaluation of the vaginal discharge, according to reports of Williams et al. (2005). Subclinical endometritis was defined as condition without vaginal discharge and ≥ 5 % PMN in the cytological smear.

The sampling of the oviductal epithelium was executed with a bi-tubular endoscopic system (50 cm length, Ø11 mm, Karl Storz, Vienna, Austria), as previously reported by Besenfelder et al (2010). Pothmann et al. (2017) described the procedure of CB technique (mini-CB, Ø2 mm, Karl Storz, Vienna, Austria) to obtain oviductal epithelial cells in detail. In brief, the ovary was fixed in place by the examiner via transrectal palpation. The intravaginal endoscope including a trocar was introduced through the *fornix vaginae* and by optic control of the endoscope a cyto-brush was forwarded through the *ostium abdominale* into the oviduct. Through a channel of the endoscope, a mini-cyto-brush was inserted to collect oviductal epithelial cells. There is no gold standard for the definition of subclinical salpingitis, however, the number of counted PMN were expected to be low. Thus, low thresholds of ≥1 % and ≥3 % PMN, resp., in 300 cells (oviductal endothelial cells and PMN) in a cytological smear were assumed for the definition of subclinical salpingitis.

2.3) Statistical analysis

Data were analysed with a statistical software program (SPSS version 20; IBM Corporation, NY, USA) and tested for normal distribution with the Kolmogorov-Smirnov Test. Descriptive statistics compounds median, minimum and maximum BHB- and NEFA-concentration and PMN from oviductal and uterine epithelial samples. Proportion of cows with CE, SE and diagnosis of subclinical salpingitis were compared between cows of nonKET and sKET. Spearman correlation was performed for the relationship of PMN content between right and left oviduct and oviduct und uterus. The linear regression line was calculated by $y=a+b*x$, indicating the intercept (a) and the slope (b) of the regression. The coefficient of determination (r^2) represents the proportion of the variation in the two variables (PMN from oviduct left and right), indicating the strength of the relationship. Level of significance was set at $P \leq 0.05$.

3.) Results

Twenty-six of 54 cows from the entire study underwent vaginal investigation, endoscopy and sampling of the oviduct by cytobrush. From the oviductal specimen, ten and eight were taken on day 28 and 42, respectively. Eight cows were sampled on both time points, i.e. day 28 and 42. The number of utilizable samples referring evaluation of oviductal epithelium and uterine endothelium on destined sampling days are listed in Table 2. Overall, 58 oviductal endothelium samples and 31 endometrium samples were obtained.

Table 2. Samples usable for the evaluation of oviductal epithelium and uterine endothelium.

Cow ID	Oviduct left D28	Oviduct right D28	Oviduct left D42	Oviduct right D42	Uterus D28	Uterus D42
1	x	x			x	
3		x			x	
4	x	x	X	x	x	x
14	x	x		x	x	x
18	x	x			x	
22			X	x		x
29	x				x	
35	x	x	X	x	x	
37	x	x			x	
38			X			x
44	x	x			x	
50	x	x			x	
51			x	x		x
52			x	x		x
54				x		x
55	x	x	x		x	x
56		x		x	x	
58			x	x		x
62	x	x	x	x	x	x
63			x	x		x
73	x	x			x	
80	x	x			x	
82			x	x		
86	x	x				x
91		x		x	x	x

98	x	x	x	x	x	x
Total number	15	17	12	14	17	14
Total number	58				31	

D: sampling day; x: Marks an usable specimen

3.1) Descriptive statistics

Twelve cows were assigned to nonKET, while 14 showed subclinical ketosis (sKET), indicated by a BHB-concentration ≥1.2 mmol/L on a single sampling day. Although five cows of sKET had BHB-concentrations indicating clinical ketosis, as defined by Dubuc and Buczinski (2018), those were not excluded from the study because clinical signs were absent throughout the study period. Twenty-two cows showed NEFA-concentration > 0,3mmol/L on the day of calving, indicating a higher risk of ketosis *postpartum*.

Tab. 3: Median and maximum/minimum (min/max) concentration of not esterified fatty acids (NEFA, mmol/L) *ante partum* and beta-hydroxybutyrate (BHB, mmol/L) *postpartum* on sampling days (D) in cows without subclinical ketosis (nonKET) and with subclinical ketosis (sKET). Day of diagnosis of clinical endometritis (CE) and sampling days (D) of polymorphonuclear neutrophil granulocytes (PMN %) in uterine (UT) and oviductal (OV) microscopic smears.

		NEFA (min/max)	BHB (min/max)	BHB			CE*	UT PMN%	OV left PMN %	OV right PMN%
Group	Cow ID	*a.p.*	D0-7	D14	D28	D42	D	D28/42	D28/42	D28/42
nonKET (n=12)	3	0.15 (0.08/0.26)	0.65 (0.5/0.9)	0.7	0.9	0.7		0.0/0.0	—/—	1.0/—
	4	0.13 (0.1/0.83)	0.6 (0.4/0.6)	1.1	0.9	1.1		**6.0**/4.0	1.0/1.3	0.7/1.0
	14	0.23 (0.07/0.39)	0.55 (0.4/0.6)	0.4	—	—	14	0.0/0.0	0.0/—	0.7/—
	35	0.13 (0.11/0.18)	0.8 (0.7/1.2)	—	0.8	0.9	7	**30.0**/—	0.0/2.7	0.3/**3.3**
	38	0.3	0.7 (0.4/0.8)	0.5	0.7	0.6	7/ 14/ 28	**95.0**/**16.0**	—/0.3	—/—

	44	0.96 (0.23/1.69)	0.8 (0.1/0.9)	0.9	0.8	0.8	7/ 14/ 42	0.0/0.0	0.0/—	1.0/—
	50	0.18 (0.09/0.36)	0.6 (0.5/0.7)	0.5	0.4	0.4	7	0.0/0.0	0.7/—	0.0/—
	55	0.12 (0.1/0.14)	0.7 (0.6/0.8)	0.7	—	—	7/ 14	**69.0**/0.0	1.0/1.3	2.0/—
	62	0.47 (0.16/0.78)	0.8 (0.4/1.1)	0.6	0.6	1.1	28/ 42	0.0/4.0	0.3/0.0	0.0/1.0
	73	0.17 (0.11/0.23)	0.6 (0.5/0.9)	0.5	1	0.6	14	0.0/0.0	0.3/—	0.0/—
	82	0.42 (0.14/0.59)	0.75 (0.6/0.9)	0.8	—	—	7	0.0/—	—/0.0	—/0.7
	98	0.14 (0.08/0.36)	0.6 (0.6/0.7)	0.9	—	—		0.0/0.0	0.0/0.0	0.0/0.7
sKET (n=14)	1	0.44 (0.17/0.68)	0.6 (0.7/1.1)	2.1	3.3	0.8	7/ 14	1.0/0.0	0.0/—	0.0/—
	18	0.33 (0.27/0.39)	0.8 (0.7/1.1)	0.8	1.8	0.6	7/ 14	**10.0**/2.0	0.0/—	0.0/—
	22	0.23 (0.13/0.74)	0.8 (0.7/1.4)	2.1	0.9	2.2	14	0.0/0.0	—/0.0	—/0.0
	29	0.17 (0.17/1.19)	1.45 (1.1/2.9)	0.5	1.9	0.7		2.0/0.0	0.0/—	—/—
	37	0.18 (0.12/0.92)	1.9 (1.4/3.3)	1.8	1.3	0.6	7/ 14	1.0/1.0	0.0/—	0.0/—
	51	0.32 0.19 (0.12/0.64)	1.2 (0.7/2.2)	1.2	1.3	0.9		0.0/2.0	—/0.0	—/0.0
	52	0.29 (0.18/1.06)	0.7 (0.5/1.2)	1.1	1.3	0.3	7	**55.0**/1.0	—/0.0	—/0.0
	54	0.23 (0.06/0.4)	0.7 (0.7/1.1)	2.7	3.2	5.1	7/ 14/ 42	**89.0**/**99.0**	—/—	—/0.3
	56	1.05 (0.21/1.88)	0.6 (0.5/1.7)	1.1	3.8	0.7	14	1.0/—	—/—	1.0/0.0
	58	0.21 (0.13/0.81)	1.0 (0.8/2.1)	1.7	1.9	0.8	7	**10.0**/1.0	0.0/—	0.0/—
	63	0.59 (0.36/1.38)	1.6 (1.2/3.2)	1.4	0.9	0.7		0.0/0.0	—/0.0	—/0.0
	80	0.1 (0,08/0,92)	0.7 (0,6/1,1)	1,4	0,8	0,8	14/ 28	5.0/—	0.0/—	0.0/—
	86	0.2 (0.12/0.81)	1.1 (0.6/2.6)	0.9	0.9	1.8	7/ 14	—/**10.0**	1.3/—	1.0/—
	91	0.21 (0.19/0.47)	1.3 (0.5/2.6)	1.2	1.4	1.8	7/ 14	**14.0**/0.0	—/—	0.7/2.0

—: no value available;

* CE (Clinical endometritis): Day (D) of diagnosis

Bold values: exceeding threshold for diagnosis of subclinical endometritis (≥5 % PMN) and subclinical salpingitis (≥3% PMN), respectively

Overall, the prevalence of CE referred to vaginal evaluation of discharge on day 28 and 42, was 8.3% (3 of 36) and 5.6% (2 of 36), respectively. Subclinical endometritis, defined as ≥ 5% PMN in the cytological evaluation, was diagnosed in 36.0% (9 of 25) and 13.6% (3 of 22) of cows on day 28 and 42, respectively. Research regarding diagnosis of subclinical salpingitis by cytologic evaluation is lacking. Therefore, a threshold of ≥ 3% was determined to define the disease resulting in only one positive sample (Cow ID 35, oviduct right on day 42). Assuming a threshold of ≥ 1% PMN for the diagnosis of subclinical salpingitis resulted in a prevalence of six out of 27 samples of the left oviduct and eight out of 30 samples of the right oviduct. Proportion of detected reproductive diseases (SE, CE, subclinical salpingitis) showed no association with the metabolic status of the cows, i.e. sKET or nonKET (Table 3).

3.2) Correlation

Spearman correlation was calculated to show the association for not normally distributed data of PMN from the oviductal and endometrial smears. A high correlation coefficient of 0.71 was only found between PMN of the left and right oviduct on day 42 (Tab. 4).

Tab. 4: Spearman correlation coefficient (r) of polymorphonuclear neutrophil granulocytes from the left and right oviduct and endometrium on sampling days (D) 28, 42, and both, respectively.

	D 28	P	D 42	P	D 28+42	P
Oviduct right and left (n=14)	0.4	0.16	0.71	0.02*	0.48	0.02*
Oviduct left and uterus (n=14)	0.06	0.84	0.23	0.52	0.08	0.7
Oviduct right and uterus (n=16)	0.22	0.42	0.12	0.72	0.12	0.54

* Indicating significant results (P ≤ 0.05)

Scatterplots of the PMN from the left and right oviduct on days 28, 42, and both are demonstrated in Figure 1 to 3. Number of PMN of the left and right oviduct showed good linear correlation (Fig. 2).

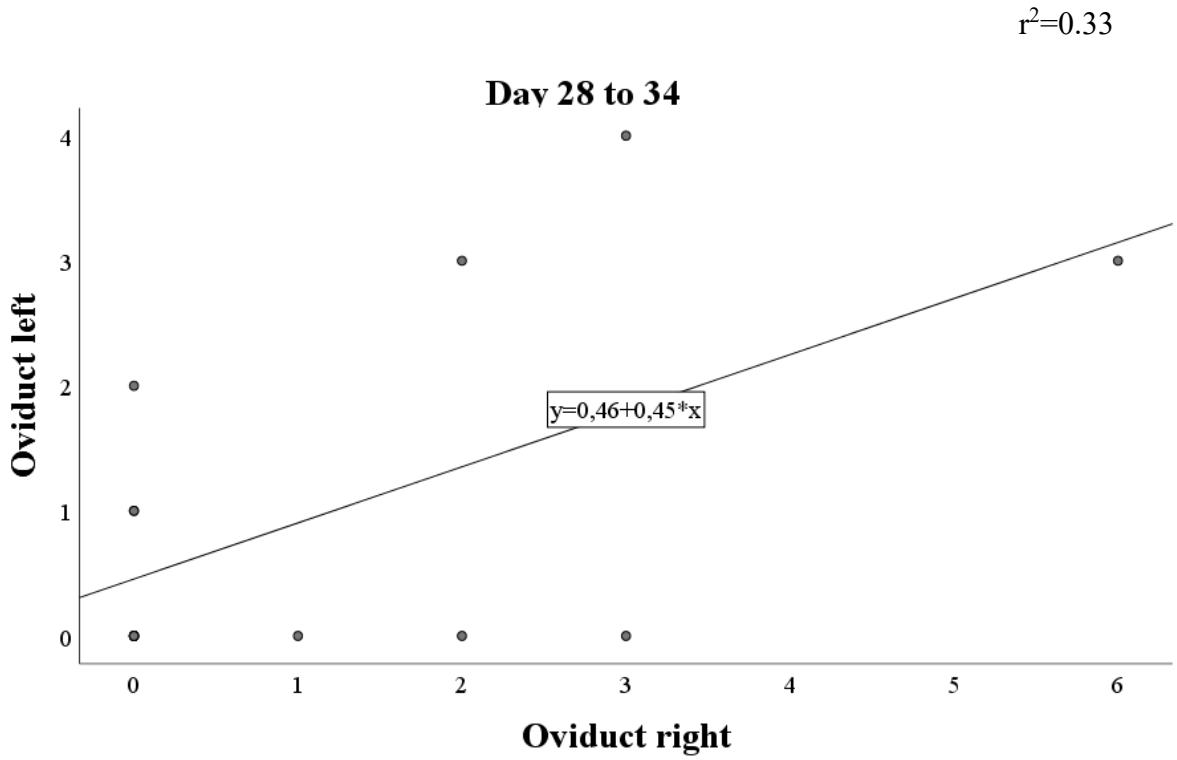

Fig. 1: Diagram of polymorphonuclear neutrophil granulocytes (PMN) from the oviduct left and right on sampling day 28 to 34 showing the coefficient of determination (r^2)

r^2=0.82

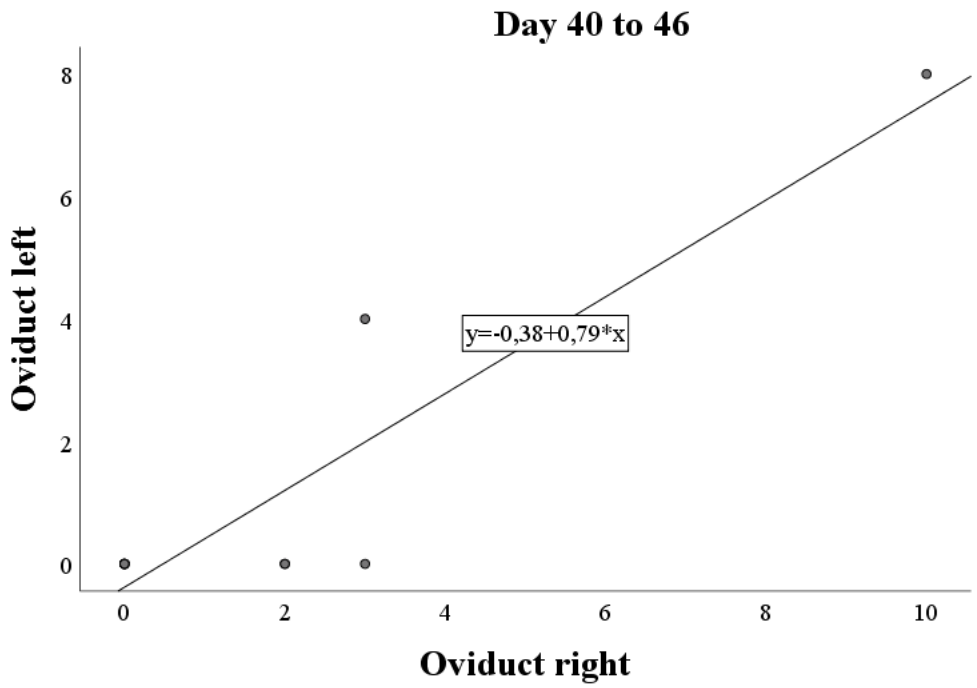

Fig. 2: Diagram of polymorphonuclear neutrophil granulocytes (PMN) from the oviduct left and right on day 40 to 46 showing the coefficient of determination (r^2)

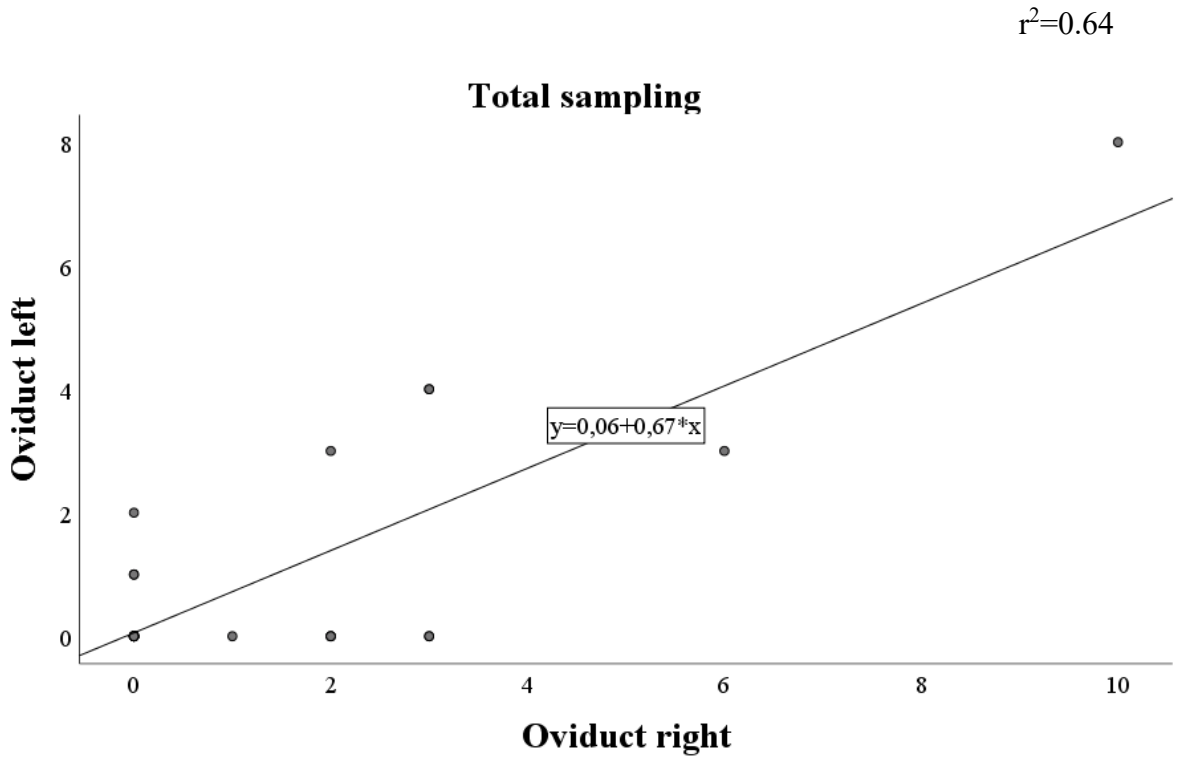

Fig. 3: Diagram of polymorphonuclear neutrophil granulocytes (PMN) from the oviduct left and right on sampling days 28 to 34 and 40 to 46 showing the coefficient of determination (r^2).

4.) Discussion

Approaching the oviduct to obtain samples *in vivo* was labour-intensive until transvaginal endoscopy was successfully applied in heifers (Besenfelder et al. 1998). The oviductal sampling by endoscopy was performed in adult cows in our study for the first time. The procedure revealed a satisfactory proportion of useable oviductal samples, thus cytobrush technique via transvaginal endoscopy was suitable. The results of this study suggest, however, that there was no relationship between endometritis and salpingitis based on cytological evaluation of microscopic smears. Cows showing cytological endometritis revealed no signs of inflammatory processes, i.e. abundance of PMN, in the examined part of the oviduct. In contrast to our results, Owhor et al. (2019) found a strong association between endometritis and salpingitis in samples collected from cows directly after slaughtering. Diagnostic methods, however, were different, as Owhor et al. (2019) determined the inflammatory processes by cytological evaluation of lymphocytes and macroscopic condition of the tissues. Furthermore, it has to be considered that *in vivo* only a small part of the oviduct can be sampled, while *in vitro* methods enable examinations *in toto*. In this study, the applied technique of transvaginal endoscopy enabled the investigators to obtain oviductal samples *in vivo*, followed by a cytological assessment based on the proportion of PMN to a number of epithelial cells. The low proportion of PMN in cows of our study might be because no acute inflammatory processes were present, as neutrophils constitute the first line of defence against invading pathogens *p.p.*, resulting in an increase of PMN within the uterine lumen (Butt et al. 1993). In chronic processes, an abundance of lymphocytes is usually found in the endothelium (Pascottini et al. 2016, Owhor et al. 2019). This aspect of assessment, however, was not the aim of our study. Uterine infection is often a risk factor for transmitting infections to the upper genital tract in humans (Price et al. 2017). Results of the present study revealed a low proportion of clinical endometritis, which might explain the lacking association between endometritis and salpingitis. The abundance of PMN in oviductal samples was very low in our study, indicating no acute inflammation. At the beginning of salpingitis, an increasing ciliary beat frequency of the oviduct was found to eliminate bacteria (Owhor et al. 2019), which could have contributed to the low prevalence of salpingitis in our study. Microbiological analysis of uterine and oviductal fluid revealed the same pathogens, within *Trueperella (T.) pyogenes* was predominant in severe salpingitis (Owhor et al. 2019).

Trueperella pyogenes is a common pathogen in bovine endometritis (Dohmen et al. 1995, Williams et al. 2005), which is causing rapid cell death in the oviductal endothelium leading to impaired immune function (Danesh Mesgaran et al. 2017). Other infectious agents, e.g. *Chlamydia abortus*, altered oviduct cell cultures, thus possibly contributing to bovine infertility. The aim of this thesis, however, was not to investigate the bacteriological load of the oviduct. Therefore, future research should focus on potential causative agents, in particular in dairy cows with metabolic disorders.

There is strong evidence that elevated NEFA concentrations *ante partum* and subclinical ketosis *postpartum* foster inflammatory processes in bovine, e.g. endometritis (Duffield et al. 2009, Ospina et al. 2010). Subclinical ketosis hampers the immune response and favours serious course of peripartal diseases (Esposito et al. 2014). The previously mentioned association between endometritis and salpingitis hypothesize a higher risk for salpingitis in cows with metabolic stress. In our study, subclinical ketosis was related to subclinical and clinical endometritis, but had no effect on the condition of the oviduct. Severe negative energy balance, however, resulted in lower expression of IGFBP-2 and IGFBP-6 in the oviduct, leading to decreased availability of IGF-II, which may hinder embryo development (Fenwick et al. 2008). Further research on the mechanism of metabolic hormones and specific pro-inflammatory factors is desirable to improve the understanding of the oviduct`s contributing role to embryonic mortality in dairy cows.

5.) Conclusion

The oviductal sampling with cytobrush by transvaginal endoscopy in adult cows was applied successfully. Results indicated no association between subclinical endometritis and subclinical salpingitis, not even in cows showing subclinical ketosis. Further research, however, with a higher statistical power and focus on bacterial load as well as inflammatory aspects on molecular level would be of interest. Using the oviductal approach enables researcher to obtain more information of the oviduct´s role relating embryonic losses in cattle.

6.) Summary

In this study, the *in vivo* sampling technique of transvaginal endoscopy was applied in adult cows for the first time to obtain oviductal samples. We successfully collected 58 cytological smears from the oviductal epithelium and 31 cytological smears from the endometrium. Based on the cytological assessment of polymorphonuclear neutrophil granulocytes the endometrial and oviductal health status was compared in healthy cows and in cows with subclinical ketosis. Only one sample of the salpinx indicated subclinical salpingitis, while seven cows showed subclinical endometritis. In conclusion, there was no association between subclinical salpingitis and subclinical endometritis evident, independent of the cows‘ metabolic status. Further research is desirable to investigate the role of the oviduct of cows with metabolic stress potentially contributing to reduced reproductive performance.

Zusammenfassung

In dieser Studie wurden zum ersten Mal bei Kühen in vivo Proben des Eileiters gewonnen. Diese Probenahme erfolgte mittels transvaginaler Endoskopie. Wir entnahmen erfolgreich 58 Schmierproben des Epithels der Eileiter und 31 Proben des *Endometriums*. Die Tiere befanden sich während der Probenahme in unterschiedlichen metabolischen Zuständen. Ausgehend von der Zahl der polymorphkernigen neutrophilen Granulozyten wurde der Gesundheitsstatus des jeweiligen Organs festgestellt und dieser zwischen gesunden Kühen und solchen mit subklinischer Ketose verglichen. Nur eine Probe aus den Eileitern wies auf eine subklinische Salpingitis hin, während sieben Proben auf eine subklinische Endometritis hinwiesen. Zusammengefasst ließ sich kein Zusammenhang zwischen subklinischer Salpingitis und subklinischer Endometritis feststellen unabhängig vom metabolischen Zustand der Kuh. Weitere Studien wären notwendig um zu klären, wieweit der Gesundheitszustand der Eileiter von Kühen unter metabolischem Stress sich auf die verminderte Reproduktionsleistung auswirkt.

7.) Abbreviations:

non-esterified fatty acids	NEFA
total mixed ration	TMR
Acetyl coenzyme A	AcetylCoA
beta-hydroxybutyrate	BHB
subclinical endometritis	SE
polymorphonuclear neutrophil granulocytes	PMN
transrectal palpation	T
vaginal examination	V
ante partum	*a. p.*
postpartum	*p. p.*
insulin-like growth factor	IGF
negative energy balance	NEB

8.) Literature

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9.) Supplementary tables

Tab. 5: Maximal concentration of beta-hydroxybutyrate (BHB) *postpartum* and of not esterified fatty acids (NEFA) *ante partum* on cow level

Cow ID	BHB max (mmol/L)	NEFA (mmol/L)
1	3.3*	0.68*
3	1.1	0.26
4	1.1	0.83*
14	0.6	0.39*
18	1.8*	0.39*
22	2.1*	0.74*
29	2.9*	1.19*
35	1.2	no sample
37	3.8*	0.92*
38	0.8	0.3*
44	0.9	1.69*
50	0.7	0.36*
51	2.2*	0.64*
52	1.3*	1.06*
54	5.1*	0.4*
55	0.8	0.14
56	3.8*	1.88*
58	2.1*	0.81*
62	1.1	0.78*
63	3.2*	1.38*
73	0.8	0.23
80	1.6*	0.92*
82	1	0.42*
86	2.6*	0.81*
91	2.6*	0.47*
98	0.8	0.36*

* Values exceeding threshold of BHB-concentration ≥ 1.2 mmol/L and of NEFA-concentration ≥ 0.3 mmol/L.

Tab. 6: Number of polymorphonuclear neutrophil granulocytes (PMN) in samples of the left oviduct (OV), right OV on sampling days (D) 28 and 42, respectively.

Cow ID	Left OV D 28	Right OV D 28	Left OV D 42	Right OV D 42
1	0	0		

3		3		
4	3	2	4	3
14	0	2		2
18	0	0		
22			0	0
29	0			
35	0	1	8	10*
37	0	0		
38			1	
44	0	3		
50	2	0		
51			0	0
52			0	0
54				1
55	3	6	4	
56		3		0
58			0	0
62	1	0	0	3
63			0	0
73	1	0		
80	0	0		
82			0	2
86	4	3		
91		2		6
98	0	0	0	2

No values, if no sample was available; *exceeding threshold for subclinical salpingitis (i.e. ≥3% PMN out of 300 cells in total)

Tab. 7: Number of polymorphonuclear neutrophil granulocytes (PMN) in endometrial samples at sampling days (D) 28 and 42, respectively. Day of diagnosis of clinical endometritis (CE).

Cow ID	D 28	D 42	CE D28	CE D42
1	3			
3	1			
4	17*	13		
14	0	0		
18	30*			
22		0		
29	6			
35	90*			
37	3			
38		47*		
44	0			
50	0			
51		6		
52		2		
54		296*		x
55	207*	0		
56	4			
58		4		
62	1	13	x	x
63		0		
73	0			
80	16*		x	
86		30*		
91	41*	0		
98	1	1		

No values, if no sample was available; *exceeding threshold for subclinical endometritis (i.e. ≥5% PMN out of 300 cells in total)