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Diagnosis of endometritis and cystitis in sows: use of biomarkers

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Abstract

The health status of breeding sows is critical for physiological reproductive performance in the herd and has a major impact on animal welfare, as well as on the economic output of a farm. Diseases of the urogenital tract in particular, such as endometritis and cystitis, occur on farms characterized by low reproductive performance. It is very important to recognize and treat the causes of these as soon as possible, and consequently a range of biomarkers have been used and described. This article summarizes those most relevant to endometritis and cystitis in sows. Particular biomarkers can be used for both cystitis and endometritis, such as vaginal discharge and body temperature, whereas others are more specific, for instance, ultrasound and cytology of the uterus for endometritis and analysis and bacteriology of urine for cystitis. Nevertheless, due to the low sensitivity of individual markers, a combination of clinical parameters and several biomarkers are needed. Nonetheless, evaluation of biomarkers can be unrewarding in the diagnosis of cystitis and endometritis in live animals, usually because the infections are subclinical. Therefore, pathological examination of the urogenital tract of slaughtered sows also needs to be performed in herds of a low reproductive performance. Overall, it is important that the clinician be aware of the limitations of each biomarker for diagnosing urogenital infections in sows so as to not over- or underestimate the prevalence of disease at herd level.

1. Introduction: biomarkers of the urogenital tract in sows

The health status of breeding sows is critical for physiological reproductive performance in the herd and has a major impact on animal welfare, as well as on the economic output of a farm (Koketsu et al., 2017). One of the most frequent reasons for culling a sow from a breeding farm is a reproductive disorder, during farrowing, the suckling period or at the insemination. Diseases of the urogenital tract in particular, such as endometritis and cystitis, frequently occur on farms with differing within herd prevalence (Chagnon et al., 1991; Christensen et al., 1995; Dalin et al., 1997; Heinonen et al., 1998, Biksi et al., 2002; Schnurrbusch et al., 2009; Bellino et al., 2013). It is very important to recognize and treat such reproductive disorders as soon as possible to avoid negative effects on the subsequent reproductive cycle and performance of the sow. Therefore, a diagnostic approach is necessary that recognizes pathological disorders at an early stage of the disease. During recent years, biomarkers have been extensively used in veterinary and human medicine to evaluate the health status and diagnose or predict disease, but also to monitor responses of the animal /human patient to therapy (Myers et al., 2017). Therefore, the number and type of biomarkers in veterinary medicine has increased over recent times (Myers et al., 2017). Ideally, biomarkers should be easy to perform, cheap, non-invasive and allow for detection of affected animals before the onset of clinical disease. (Koene et al., 2012; Myers et al., 2017; Casey et al., 2018). Hence, a great diversity of biomarkers is available and, depending on usage, can be classified into seven categories (Figure 1) (FDA-NIH Biomark. Work. Group. 2016).

A risk biomarker indicates the likelihood of an animal developing a disease (Myers et al., 2017). For instance, prolonged farrowing (more than 300 min) would be a risk biomarker for postpartum disorders in sows (Oliviero et al., 2008; Björkman et al., 2017; Björkman et al., 2018). A diagnostic biomarker identifies animals with a specific disease or condition, such as a positive bacteriological result in cases of urinary tract infection (Grahofer et al., 2014; Sipos et al., 2014; Myers et al.,

2017). A continuous evaluation of the uterine diameter after the birth process can be used as a monitoring biomarker, which is characterized by serial measurements to detect changes in the tissue (Myers et al., 2017, Grahofer et al., 2019, Meile et al., 2019). A predictive biomarker evaluates the effect from a specific intervention or exposure (Myers et al., 2017). An example would be the intramuscular use of oxytocin in a sow with dystocia to provoke uterine contractions (Almond et al., 2006). Prognostic biomarkers are used to identify the likelihood of a clinical event, disease, recurrence or progression of the disease (Myers et al., 2017). An example would be the vaginal cell lipidome of weaned female piglets, which essentially defines the reproductive potential of a gilt (Casey et al., 2018). An increase in antibodies after vaccination of a sow can be used as a response biomarker, which evaluates the reaction to a treatment (Myers et al., 2017; Arsenakis et al., 2019). Safety biomarkers were defined to indicate the reaction of the intervention (Myers et al., 2017). An example from swine research would be the recent study from Bill et al. (2017) that conducted a dose-finding study on Prostaglandin E2 in sows during the birth process to evaluate the effect of the drugs.

This article aims to summarize the relevant biomarkers for endometritis and cystitis in sows that can be implemented as a rapid diagnostic approach on farms exhibiting reproductive problems.

2. Diagnosis of endometritis

Currently, the markedly extended farrowing in hyper-prolific sows (Oliviero et al., 2019) increases the incidence of postpartal disorders, especially endometritis, and thereby negatively affects the subsequent reproductive cycle and performance of the sow (Oliviero et al., 2013; Björkman et al., 2018, Grahofer et al., 2019). Therefore, a rapid and accurate diagnostic approach for sows is needed by pig farmers.

2.1. Definition of endometritis

Endometritis is defined as an inflammation of the endometrium or uterine lining and occurs due to an imbalance between external factors and the sow`s immune defence of the uterus. The majority of sows with uterine abnormalities show endometritis instead of metritis (Dial and MacLachlan, 1988). The uterine discharge of affected sow vary extensively, depending on the pathogenic microorganism, duration of infection, and the stage of the estrous cycle (Dial and MacLachlan, 1988). Endometritis is causes through several factors and therefore an accurate diagnostic work up is necessary to avoid fertility disorders in sow herds.

To date, there is still no consistent clinical or histopathological nomenclature for endometritis in sows. The endometritis can be distinguished as non-puerperal and puerperal, depending on the time point of occurrence in the reproductive cycle (Kauffold, 2008). In addition, it can be categorized into sub-clinical (without clinical symptoms), acute and sub-acute endometritis, which are clinically apparent (Muirhead, 1986; De Winter et al.,1994; Dalin et al., 2004; Heinritzi et al., 2006; Kauffold, 2008; Tummaruk et al., 2010). The severity of endometritis can be classified according to the percentage of tissue containing inflammatory cell infiltrate, ranging from mild to severe (Novakovic et al., 2018). Furthermore, the number of immune cells and damage to the endometrial tissue can differentiate the time course of an infection of the endometrium in sows (de Winter et al., 1992). Nevertheless, the interpretation of endometritis based on histological examination varies depending on the stage of the oesturs cycle (Kaeoket et al., 2001¦ Dalin et al., 2004) and therefore might lead to misinterpretation of the results.

2.2. Vaginal discharge

Physiological vaginal discharge, which is watery or slightly cloudy, can be observed immediately after parturition, insemination and shortly before oestrus (Muirhead, 1986; Meredith, 1991; de Winter et al., 1992; Almond et al., 2006). Expelled seminal fluids may lead to a physiological vaginal discharge after insemination (Meredith, 1991). Vaginal discharge 14 – 20 days after oestrus is a clinical sign of endometritis in sows (Almond et al., 2006) and can be used as a biomarker.

However, this finding may lead to an incorrect diagnosis because discharge can originate from the urinary bladder or the vagina. The colour, consistency, and quantity of vaginal discharge vary regardless of whether the vaginal discharge is of physiological or pathological origin (Noakes et al., 1992). The colour can vary from clear, whitish, yellowish to reddish (Fig. 2).

The consistency varies from watery to creamy with lumps, and the volume can reach 500 ml (Muirhead, 1986; Naokes et al., 1992). Increased volumes of vaginal discharge are associated with endometritis, but there is no significance between the occurrence of endometritis and the colour of the vaginal discharge (Muirhead, 1986). Mucopurulent to purulent and greyish-yellowish vaginal discharge is often associated with predominant infection by *Streptococcus* several species (spp.)and/or *Staphylococcus* spp (Heinritzi et al., 2006). Less frequently, vaginal discharge is observed in endometritis caused by *Escherichia coli*, when the vaginal discharge is ofgreyish-white colour (Heinritzi et al., 2006). Other bacteria, such as *Chlamydia* spp. (Kauffold et al., 2006; Kauffold, 2008), and anaerobic microbes (i.e. *Fusobacterium necrophorum*, *Prevotella* spp.) are also kown to cause vaginal discharge (Oravainen et al., 2006). Several studies have shown that vaginal discharge occurs frequently postpartum in healthy and diseased animals (Nachreiner and Ginther, 1972; Hermansson et al., 1978; Morkoc et al., 1983), with the highest incidence between day 2 and 4 postpartum (Madec and Leon, 1992; Grahofer et al., 2019). Obstetrical intervention and prolonged farrowing increase the risk of vaginal discharge in the puerperium (Bará and Cameron, 1996, Grahofer et al., 2019) and lead to higher incidence of endometritis in sows (Björkman et al., 2018). Vaginal discharge has also been associated with the production environment, such as overcrowding, restriction of movement by crating, poor hygiene and lack of enrichment materials.(Oravainen et al., 2006; 2007). Besides puerperal discharge, non-puerperal discharge can occur in breeding farms. The ethology as well as pathogenesis is more challenging and an investigation is warranted, when herd prevalence is more than 3 percentage (Kauffold et al., 2008).

After ruling out the aforementioned physiological vaginal discharge reasons, all other vulva discharges are classified abnormal (Kauffold et al. 2008; Almond et al., 2009).

2.3. Body temperature

Fever is a cardinal symptom of inflammation and the most frequently used variable to evaluate the health status of a sow in the puerperal period. Importantly, several parameters effect the body temperature of sows such as the circadian rhythm (Stiehler et al., 2015), parity (Stiehler et al., 2015), variations if compared between sequential measurements (Mead and Bonmarito, 1949), and positioning of the thermometer in the rectum (Rotello et al., 1996). There is a large discrepancy in the reference values for fever in sows with puerperal disorders, ranging from 39°C (Tummaruk and Sang-Gassanee, 2013) to 40°C (Papadopoulos et al., 2010).

In conclusion, body temperature above 40.0°C cannot be used as the sole criterion for detecting endometritis in sows. However, a body temperature of more than 39.5°C, together with clinical signs such as abnormal general behaviour (i.e. lethargy, apathy), reduced feed intake and abnormal vaginal discharge, are associated with endometritis (Stiehler et al., 2015, Grahofer et al., 2019).

2.4. Vaginal cytology and histology of the uterus

Vaginal cytology is a non-invasive and often used method in other animals, such as cows, mares and dogs, to evaluate the health status of the uterus. Compared to other domestic animals, less is kwon about the vaginal cytology in pigs. The histological changes of the uterus have been the main focus of attention in recent years (Kaeoket et al., 2005; Busch et al., 2007; Oravainen et al., 2007; Tummaruk et al., 2010; Entenfellner, 2016). An older study distinguished between acute, subacute and chronic endometritis, according to the immigration of inflammatory cells in the endometrium and lumen of the uters (de Winter et al., 1992). Essentially, the oestrus cycle must be considered for the

histological interpretation (de Winter et al., 1992; Busch et al., 2007) because the number and type of immune cells on one hand depend on the oestrus cycle of sows and on the other hand on the stage of endometritis (Tummaruk et al., 2010). During the normal reproductive cycle, more neutrophilic granulocytes and lymphocytes are present in the follicular phase compared with the luteal phase (de Winter et al., 1992; Tummaruk et al., 2010). In addition, the endometrium of heathy sows always contain inflammatory cells. The number and type of inflammatory cells in the endometrium per visual field in the x400 magnification of the light microscopy gets used to classify an classification into acute and chronic endometritis. In sows with acute endometritis more than 20 neutrophilic granulocytes can be detected in a field (de Winter et al., 1995). In comparison, chronic endometritis is defined as the presence of more than 20 lymphocytes, plasma cells or histiocytes in a field (de Winter et al., 1995). Until today, the understanding of where and what type of cells are mainly found in the endometrium is still lacking. One study indicates that leukocytes are mainly located in the glandular layer of the endometrium (Tummaruk et al., 2010). This finding is not consistent with those of other studies (Kaeoket et al., 2005; Entenfellner, 2016), where leukocytes were mainly found in the sub-epithelial layer or migrated diffusely into the endometrium. It is known that numerous leukocytes are found in the endometrium of sows with vaginal discharge. In another study in Finland, the numbers of leukocytes found in the cervix area of sows with vaginal discharge were related to the amount of discharge and also associated with vaginoscopic findings in sows with symptoms (Oravainen et al., 2007). In sows without vaginal discharge, the endometrium contains a low number of neutrophilic and eosinophilic granulocytes as well as plasma cells (Kaeoket et al., 2005, Oravainen et al., 2007). Neutrophilic granulocytes are found in both epithelial and sub-epithelial connective tissue of the endometrium (Kaeoket et al., 2005; Tummaruk et al., 2010). An increase in leukocytes is found in sows with puerperal diseases on the second, fourth and sixth day postpartum in the cytological examination of cervical smears and therefore can be used as a diagnostic biomarker (Winkler, 1987).

2.5. Microbiology

Endometritis in gilts and sows is often caused by several species of bacteria (Dial and MacLachlan, 1988), but also fungi and rarely viral pathogens can cause uterine inflammation (Kauffold, 2008). Especially in sows with acute or subacute endometritis, half of the animals showed positive bacteriological results while only 17% of the uteri with chronic endometritis and 13% of the histologically normal uteri were positive (de Winter et al., 1995). The most common pathogens that are found in sows with puerperal and non-puerperal endometritis are Gram-positive pyogenic bacteria such as *Staphylococcus* spp. and *Streptococcus* spp. and Gram-negative bacteria such as *Escherichia coli* (D'Allaire et al., 1987; Muirhead, 1986; de Winter et al., 1995; Glock and Bilkei, 2005; Oravainen et al., 2007; Tummaruk et al., 2010). Results suggest that an endometritis associated with vaginal discharge is most likely an ascending infection of pathogens from the vulva and the urinary bladder (de Winter et al., 1995). Furthermore, sows with chronic cystitis are 3.5 times more likely to develop endometritis (Biksi et al., 2002). These findings were also confirmed in a study from Austria, where a bacteriological and pathological investigations of culled sows with reproductive disorders revealed that 84,6 % of the animals (n=39) had an endometritis and cystitis (Sipos et al., 2014). Therefore, an investigation of a uterine swab and a urine sample may be useful in sow herds with endometritis. A speculum (Fig. 3) with a double-guarded swab should be used to obtain a representative sample from the uterus and to avoid contamination of the bacterial flora from the vagina (Oravainen et al., 2007, Grahofer et al., 2017).

2.6. Acute phase proteins

Acute-phase proteins are plasma proteins that increase, when an infection, inflammation or trauma occurs in the host. It would be logical to assume that in cases where a systemic inflammation response to the infectious cause of endometritis or cystitis is found, a systemic response in terms of

219 acute phase proteins would be detectable. There are only a few studies available on acute phase
220 proteins and cystitis / endometritis. Oravainen et al. (2006) explored the acute phase response of
221 sows suffering from vaginal discharge syndrome in 19 / 824 animals (2.3%) on 26 farms. They
222 reported no obvious rise in C-reactive protein or haptoglobin. They concluded that endometritis
223 might usually be a limited infection without a systemic response. However, involvement of more
224 pathogenic bacteria could potentially trigger a systemic response, which may be detectable by a rise
225 in acute phase proteins (Oravainen et al., 2006).
226
227 **2.7.Ultrasonography**
228 Ultrasonography has gained recent attention in the characterization of the reproductive tract in
229 sows, diagnosing uterine changes during the postpartal and non-puerperal period. Ultrasound is
230 beneficial in examination of the uterine health status and allows a rapid diagnosis of uterine
231 disorders such as endometritis or a retained piglet or placenta (Kauffold and Wehrend, 2014,
232 Björkman et al., 2018, Grahofer et al., 2019; Kauffold et al., 2019). In evaluation of the structure of
233 the uterus, the parameters of fluid echogenicity, echotexture, and size are measured in order to
234 provide a comprehensive diagnosis (Figure 4; Kauffold and Althouse, 2007; Peltoniemi et al.,
235 2016; Björkman et al., 2018; Grahofer et al., 219; Meile et al., 2019). In a sow with an acute
236 endometritis, the uterus size as well as the echotexture, are increased (Kauffold and Althouse,
237 2007). However, the days postpartum and the parity should be taken into account when evaluating
238 the uterine parameters (Kauffold and Althouse, 2007; Björkman et al., 2018). A recent study
239 showed no statistically significant difference in uterus size between the different parities (Meile et
240 al., 2019). In addition, fluid echogenicity in the uterus can be used as an indicator for an exudative
241 inflammation of the uterus (Kauffold and Althouse, 2007) and is positively correlated with the
242 number of total and stillborn piglets, the application of obstetrical intervention and prolonged
243 farrowing (Björkman et al., 2018, Grahofer et al., 2019).

3. Cystitis

In swine cystitis has been reported throughout the world. Its incidence is increasing and seems to be linked with changes in the management of modern pig production, particularly with confinement housing causing a decrease in hygiene and physical activity and an increase in stress (Drolet, 2019). Cystitis is usually subclinical and systemic reactions are rare, making diagnosis of cystitis challenging. Possible clinical signs include frequent urination, vulval discharge and fever, yet these are often related to endometritis or vaginitis rather than cystitis alone (Tolstrup, 2017). In both human and small animal medicine, standardized diagnostic guidelines are available, including stick testing, microscopic urine evaluation and urine culture in combination with symptoms and clinic signs (Tolstrup, 2017). There are no general guidelines for diagnosing cystitis in sows. In pigs, urinalysis and urine culture are mostly used (Gmeiner, 2007). Nevertheless, these tests often give false positive results because of effects by the sampling procedure (Gmeiner, 2007). Correct diagnosis is crucial for appropriate treatment, which in turn is very important for minimizing antibiotic use and increasing reproductive performance of sows and health and survival of piglets. Different diagnostic procedures have been investigated, including macroscopic pathological urinary bladder examination, macroscopic and microscopic urine evaluation, urine stick testing, urine culture, ultrasonography and cystoscopy. The following section will summarize these biomarkers and their usefulness in the diagnostic approach to cystitis.

3.1. Definition and aetiology of cystitis

The current incidence rate for cystitis is high and varies between 15.3 and 62.5, mainly depending on management and housing system (Tolstrup, 2017). Non-specific and opportunistic organisms inhibiting the vagina and urethra usually ascend into the urinary bladder and may eventually cause

cystitis (Bellino et al., 2013). In addition, the uterus can be a reservoir for a possible infection of the urinary tract and vice versa (Gmeiner, 2007). Bacteria can also arise from the intestinal tract of the sows or from a housing system with suboptimal hygiene. *Escherichia coli* is the predominant bacterial species associated with about 70% of cystitis cases (Biksi et al., 2002, Grahofer et al., 2014). *Escherichia coli* occurs mainly in monoculture, but also as mixed culture with *Staphylococcus* spp., *Streptococcus* spp., *Proteus* spp. and others (Biksi et al., 2002). Normally, the immune system of the sow is able to eliminate infections from the urinary bladder unless it is impaired. Parturition itself decreases immunity and causes constipation, which increases the risk of bacteria and toxins entering the blood system (Oliviero et al., 2010; Kaiser et al., 2018). Therefore, Berner (1987), Wendt et al. (1990) and Biksi et al. (2002) established a connection between cystitis and postpartum dysgalactia syndrome (PDS). Wendt et al. (1990) found that 77% of pigs with PDS had the same bacteria in the urinary bladder. Furthermore, sows with chronic cystitis were six times more likely to have PDS (Wendt et al.,1990). Biksi et al. (2002) found that sows with chronic cystitis had 3.5 times higher odds of developing endometritis. Berner (1987) considered cystitis to be both a cause and a result of PDS. Therefore, we recommend that sows suffering from PDS are examined for whether the aetiology of the syndrome is caused by cystitis. For optimal treatment, the exact cause of PDS needs to be determined. If not diagnosed and treated, chronic cystitis can increase piglet mortality before weaning and reduce pregnancy rate and litter size at next breeding (Thorup, 1994; Tolstrup, 2017). Further, cystitis has also been linked with increased number of stillborn piglets (Tolstrup, 2017). This shows the importance of diagnosing cystitis even before parturition in order to prevent birth complications. Several parameters can be evaluated to diagnose cystitis in sows.

3.2. Urinalysis

Urinalysis is a valuable tool in the diagnosis of cystitis. It is preferred to collect spontaneous midstream urine in a transparent tube. The best time to collect urine is in the morning before feeding because results can be effected it (Kraft et al., 2005). Urinalysis includes macroscopic and microscopic urine evaluation, and urine stick testing. For macroscopic urine evaluation, the colour, smell, and turbidity have to be evaluated (Gmeiner, 2007). The colour can vary between light yellow and dark yellow, depending on urinary concentration. The colour should not be red or brown, which would indicate haematuria or myoglobinuria. The turbidity of the urine should be clear. Cloudy or turbid appearance can indicate the presence of bacteria. Presence of bacteria can also increase ammonia in the urine and cause a putrid odour. Nevertheless, macroscopic urine evaluation is very subjective. Christensen et al. (1995) and Bellino et al. (2013) reported a sensitivity for diagnosis of cystitis of 0.74 and 0.80 and a specificity of 0.92 and 0.50 for the urine turbidity evaluation, respectively (Table 1). Nevertheless, if urine is yellow and clear the probability that the sow is suffering from no cystitis is 0.85 (Becker et al., 1985). A cloudy or flocculent appearance, or a strong ammoniac or putrid odour, could indicate the presence of bacteria in the urine (Tolstrup, 2017).

After macroscopic evaluation, a microscopic evaluation of the urine has to be performed. For the microscopic evaluation, a urine sample has to be centrifuged at 2000 x g, the supernatant discarded (Kraft et al., 2005) and the sediment then evaluated using light microscopy at x400 magnification. Erythrocytes, leukocytes and epithelial cells are counted**.** Urine of healthy sows should not contain erythrocytes and only small numbers (1 – 4 per visual field) of leukocytes (Bellino etal., 2013). A sample is considered positive when there are more than five white blood cells per visual field (Bellino et al., 2013). Bellino et al. (2013) reported a sensitivity of 0.34 and specificity of 0.90 for this biomarker (Table 1). Furthermore, the presence of transitional epithelial cells and bacteria, and a specific gravity of the urine higher than 1.020, can be indicative for cystitis (Gmeiner, 2007; Tolstrup, 2017).

Another method to evaluate blood and leukocytes is urine stick testing. Tolstrup (2017) summarized the diagnostic performance of different diagnostic tests, with histopathological cystitis lesions as the gold standard (Table 1). The following parameters can be evaluated: protein, pH, nitrite, blood and leukocytes. If nitrite is detected, urine contains Gram-negative bacteria. On the other hand, if no nitrite is detected, the presence of Gram-negative bacteria cannot be excluded; which can be the case in the absence of nitrate. The sensitivity of this test is low (0.19; Table 1) but can be increased from 0.88 to 0.93 if potassium nitrate is added to the urine (Gmeiner, 2007). Other parameters with low sensitivity are leukocytes and pH. The normal pH is between 5.5 and 8 and an increase above 8 is indicative of the presence of bacteria. On the other hand, many other factors can increase the pH such as feeding, other diseases and medication. Thus, these factors need to be considered when interpreting the pH. Parameters with good sensitivity are blood and protein (Table 1).

In conclusion, a macroscopic evaluation and urine stick testing are cheap and easy methods to perform on farm. All mentioned biomarkers need to be interpreted together and there is no single biomarker with very good sensitivity and specificity for cystitis.

3.3. Bacteriological investigation

Bacteriological investigation of the urine is regarded as a generally reliable method for diagnosing cystitis in live animals. Sensitivities and specificities are similar to those for urine turbidity evaluation and measurement of blood and protein using the urine stick testing (Table 1). Dipslides can be used for bacteriological evaluation. They are placed into urine for about 10 seconds and the bacterial growth is evaluated approximately 18-24 h later. In human medicine, 10x5 colony forming

343 units (cfu)/mL urine are used as a threshold for a urinary tract infection. This threshold has been
344 adopted also in veterinary medicine (Kraft et al., 2005). Results between 10x4 and 10x5 cfu/mL
345 need to be considered as borderline and be interpreted carefully. Including other biomarkers such as
346 urine turbidity evaluation and urine stick testing into the diagnosis can assist in this. Results below
347 10x3 cfu/mL are usually due to bacterial contamination in the urine from the urethra and vagina
348 (Gmeiner, 2007). Dipslides can also be submitted to the laboratory for specification of the bacteria
349 and antibiogram.
350
351 In conclusion, bacterial growth evaluation can be a reliable biomarker if used in combination with
352 other biomarkers. Furthermore, it allows determination of the exact bacteria and antibiotic
353 sensitivities. In order to minimize antibiotic resistance, this biomarker needs to be included in the
354 diagnostic workup of cystitis.
355
356 **3.4. Pathological investigation**
357 Pathological examination of the urinary bladder can provide useful information about causal
358 diagnostic findings (Wendt et al., 1990; Liebhold et al., 1995; Bellino et al., 2013). Importantly, the
359 urinary bladder should be removed quickly post mortem to gain the best diagnostic results. Hence, a
360 rapid autolytic process of the tissue may cause misleading findings. Acute cystitis caused by non-
361 specific pathogens may be catarrhal, haemorrhagic, fibrinous, ulcerative, phlegmonous or
362 diphtheroid necrotic (Weiss, 1999; Bellino et al., 2013). Depending on the inflammatory character,
363 the urinary bladder contains urine with blood coagula, fibrin, pus and necrotic tissue in varying
364 amounts (Bellino et al., 2013). Oedematous mucous membranes appear mostly cloudy and without
365 shine, and have a diffuse reddening (Weiss, 1999). In addition, petechiae or areal haemorrhages, as
366 well as thickening of the urinary bladder wall, can be detected in infected animals (Berner et al.,
367 1968; Berner 1981; Weiss, 1999; Biksi et al., 2002).

Microscopically, acute cystitis is characterized by epithelial loss and bacterial colonies found on the surface of the urinary bladder. The lamina propria mucosae is oedematous and has a diffuse infiltration with neutrophilic granulocytes. In addition, superficial hyperaemia and bleeding occur in the tissue (Weiss, 1999; Liebhold et al., 2005; Newman et al., 2007). Chronic cystitis is associated with diffuse thickening of the mucosa and a hypertrophic muscle layer. Depending on the inflammatory reaction, diffuse, follicular or polypoid changes appear in the urinary bladder (Weiss 1999; Newman et al., 2007; Bellino et al., 2013). The diffuse forms may result in detachment of the epithelium and excessive infiltration of the submucosa with mononuclear inflammatory cells and few neutrophilic granulocytes, whereas, the follicular forms exhibit disseminated, nodular, submucosal proliferations of lymphoid nodules (Weiss 1999; Newman et al., 2007). These lymphoid follicles are often surrounded by a hyperaemic zone. In addition, there is usually a diffusely thickened, hyperplastic lymphoid follicle and a chronic lymphoplasmacellular infiltrate and fibrosis in the lamina propria mucosae. In several cases, the tunica muscularis is hypertrophic (Weiss 1999; Newman et al., 2007). The chronic polypoid cystitis is characterized by single or multiple nodular mucosal proliferation consisting of fibrous connective tissue and infiltration of neutrophilic granulocytes and mononuclear leukocytes. The proliferative tissue is ulcerated or covered with a hyperplastic epithelium with goblet cell metaplasia (Liebhold et al., 1995). Hence, animals affected with the polypoid form show haematuria (Weiss 1999; Newman et al., 2007).
In conclusion, a pathological investigation of the urinary bladder appears to be a useful method to estimate urinary tract infection in a sow herd (Bellino et al., 2013; Sipos et al., 2014, Grahofer et al., 2014, Sipos et al., 2017)

3.5. Ultrasonography

Kauffold et al. (2010) studied ultrasonographic characteristics of the urinary bladder with defined volumes in healthy sows and compared the findings with those for sows with cystitis.

393 Ultrasonographic examination was performed transrectally using a 5 MHz-linear probe (Kauffold et
394 al. 2010). The urinary bladder was longitudinally imaged and the following parameters were
395 assessed: urinary bladder depth (Figure 5), dorsal and ventral wall thickness (Figure 5), wall
396 regularity (Figure 6), mucosal wall surface (Figure 6) and sediment (Figure 6) (Kauffold et al.
397 2010). Kauffold et al. (2010) demonstrated clear volume dependent changes in both the dorsal and
398 ventral wall thickness, as well as in the wall regularity and mucosal wall. Increased volume of the
399 urinary bladder was associated with decreased wall thickness, increased wall regularity and
400 smoothening of the mucosal surface. Kauffold et al. (2010) interpreted these changes to be a result
401 of wall stretching and decrease of epithelial height and flattening of epithelial folds. Thus, it is
402 necessary to know the volume of the urinary bladder in order to interpret these parameters. Kauffold
403 et al. (2010) suggest using the urinary bladder depth as a volume equivalent because the parameters
404 were strongly associated. Overall, dorsal and ventral wall measurement, as well as wall regularity
405 and mucosal wall surface obtained with ultrasonography, seem to be unreliable for diagnosis of
406 cystitis (Kauffold et al. 2010). Interestingly, animals with cystitis more often had high and moderate
407 amounts of sediment compared with animals without cystitis (Kauffold et al. 2010). Furthermore,
408 Gmeiner (2007) reported that all sows with cystitis had moderate to high amounts of sediment. In
409 contrast, half of the sows without cystitis had none to small amounts of sediments and the other half
410 of the sows had moderate to large amounts of sediment (Kauffold et al. 2010).
411
412 In conclusion, ultrasonographic examination of the urinary bladder may not reliably diagnose
413 cystitis, but evaluation of sediment can detect those sows that suffer from cystitis.
414
415 **3.6. Endoscopy**
416 Cystoscopy has been advocated for urinary bladder assessment and has been helpful in the
417 diagnosis of chronic cystitis (Wendt and Ängenheister, 1989). Wendt and Ängenheister (1989)

418 described the examination of the urinary bladder with a flexible scope in a standing sow without
419 anaesthesia. After the scope is inserted, the urinary bladder must be emptied and filled with air for
420 its systematic inspection. The state of the urinary bladder can be estimated by the colour and state of
421 the mucosa as well as blood, fibrin and pus depositions. Wendt and Ängenheister (1989) found
422 good correlations between endoscopic findings and parameters of urinalysis, especially for sensory
423 parameters, proteinuria, leukocyturia and significant bacteriuria. Though cystoscopy is a good tool
424 to survey the initial or chronic symptoms of cystitis, especially when urine is nearly unchanged, it
425 requires skill and involves the risk of iatrogenic infection (Wendt and Ängenheister, 1989). In
426 addition, this method is conducted in sows without anaesthesia, for that reason it is not
427 contemporary anymore for a diagnostic approach, due to animal welfare reasons. Therefore,
428 cystoscopy is rarely used in practice.
429
430 **Conclusions**
431 In this review, we summarized the relevant biomarkers for endometritis and cystitis in sows.
432 Urogenital diseases are common reproductive disorders on sow farms and lead to substantial losses
433 due to reduced reproductive performance. Hence, practical and accurate diagnostic work to early
434 detect urinary tract infections is important. Ultrasonography is a practical tool for evaluating the
435 urinary tract system and confirming endometritis in a live animal. A limitation of ultrasonographic
436 examination can be found in evaluating the urinary bladder because the volume of the bladder can
437 lead to misinterpretation of the wall structure. Therefore, only bladder sediment is indicative for
438 cystitis. Pathological investigation is a useful and feasible tool to detect even subclinical infections
439 of the urogenital tract in sows. A substantial limitation of this diagnostic approach is that only
440 culled and euthanized animals can be evaluated, although this approach is often used to evaluate the
441 herd prevalence of endometritis and cystitis. Furthermore, bacteriological investigation using
442 selective enrichment is useful to detect the causative agent of the urogenital tract infection, which is

443 usually non-specific bacteria. In the sampling process, it is crucial to avoid contamination with the
444 environmental flora when detecting the causative agent. Therefore, midstream urine and uterine
445 swabs taken with a speculum represent the best testing material for bacteriological investigations. In
446 addition, clinical parameters such as characteristics of the vaginal discharge and body temperature
447 can be easily evaluated in the herd, but the sensitivity is lower compared with the other test
448 methods. Thus, a combination of various parameters increases specificity and sensitivity of
449 detection of urogenital tract infections. Overall, the described biomarkers can be used in diagnosis
450 of reproductive disorders in sows. Importantly, clinicians should be aware of the limitations for
451 each biomarker so as to not over- or underestimate the disease prevalence at herd level.
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663 **Table 1.** Overview of sensitivity (Se) and specificity (Sp) adapted from Tolstrup (2017) for

664 different diagnostic procedures in different studies using histopathology as the gold standard.

Study	Procedure		Se	Sp
Christensen et al. (1995)	Urine turbidity evaluation		0.74	0.92
	Urine stix testing:	- protein	0.81	0.60
		- pH	0.39	0.95
		- blood	0.77	0.55
		- nitrite	0.19	1.00
		- leukocytes	0.16	1.00
	Urine culture		0.83	0.95
Biksi et al. (2002)	Macroscopic bladder examination		0.48	0.88
	Urine culture		0.63	0.71
Bellino et al. (2013)	Urine turbidity evaluation		0.80	0.50
	Urine microscopy:	- more than 5 WBC/HPF*	0.34	0.90
		- presence of bacteria	0.43	0.90
	Urine culture		0.49	0.97

* WBC = white blood cells, HPF = high power field

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Figure 1: Overview of the classification system of biomarkers in veterinary and human medicine

Figure 2: Puerperal vaginal discharge of different colours. 0= clear, 1= reddish, 2=yellowish and 3= whitish (Grahofer et al., 2019)

Figure 3: Collecting process of a uterus swab. The speculum is inserted into the vagina and put forward to the closed cervix. Reddening of the cervical area and excessive grey vaginal content were detected. (Grahofer et al., 2017)

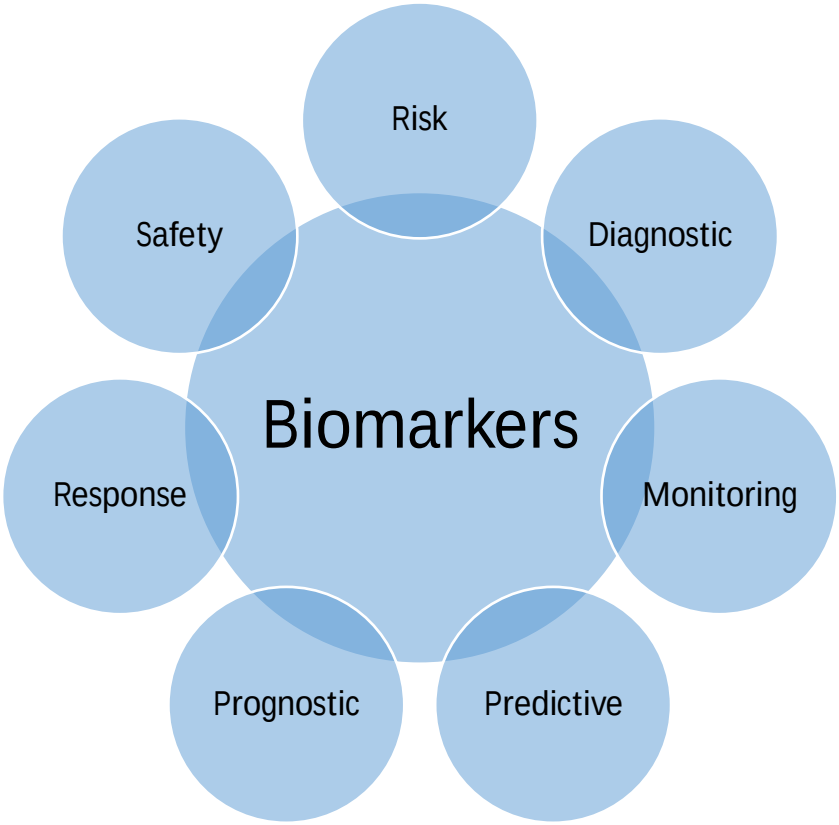
Figure 4: Transabdominal ultrasonograhic picture of endometritis in a sow 3 days postpartum. The uterus diameter is enlarged (70mm) and hyperechogenic content is visible in the uterus tissue. (Grahofer et al., 2019)

Figure 5. Schematic illustration of the procedure of transrectal ultrasonographic examination of the urinary bladder in sows adapted from Kauffold et al. (2010) with the permission of Prof. Kauffold, https://www.vetmed.uni-leipzig.de. Rectal position of the transducer (T), with arrows indicating ultrasound waves. The urinary bladder was imaged longitudinally. The dorsal (dWT) and the ventral (vWT) wall thickness were measured at three places (1 – 3). dWT and vWT were calculated as the average of the three measurements. The arrow within the urinary bladder indicates where the bladder depth (BD; distance between dWT and vWT) was measured.

Figure 6. Ultrasonographic images of parts of the longitudinal imaged urinary bladder of sows adapted from Kauffold et al. (2010) with the permission of Prof. Kauffold, https://www.vetmed.uni-leipzig.de/. Grading of wall regularity (0 – 3 for smooth and slightly irregular, moderately irregular

and strongly irregular, respectively), mucosal wall surface (regularity of the ventral wall; 0 – 3 as described for wall regularity) and sediment (1 – 4 for non, low, moderate and high, respectively). (A) Slightly irregular wall (score 1) with smooth mucosal wall surface (score 0). (B) Moderately irregular wall (score 3) with moderately irregular mucosal surface (score 2). (C) Small amounts of sediment (score 2) and both bladder wall regulatory and mucosal wall surface slightly irregular (score 1). (D) Large amounts of sediment (score 4).

Figure 1


Risk
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Diagnostic
Biomarkers
Response
Monitoring
Prognostic
Predictive

Figure 2

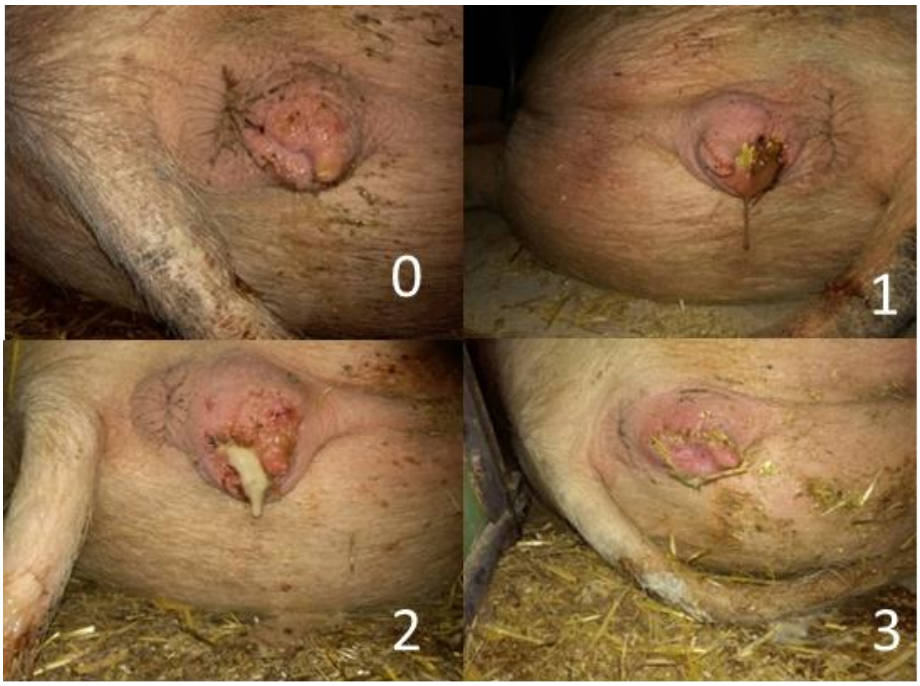


Figure 3

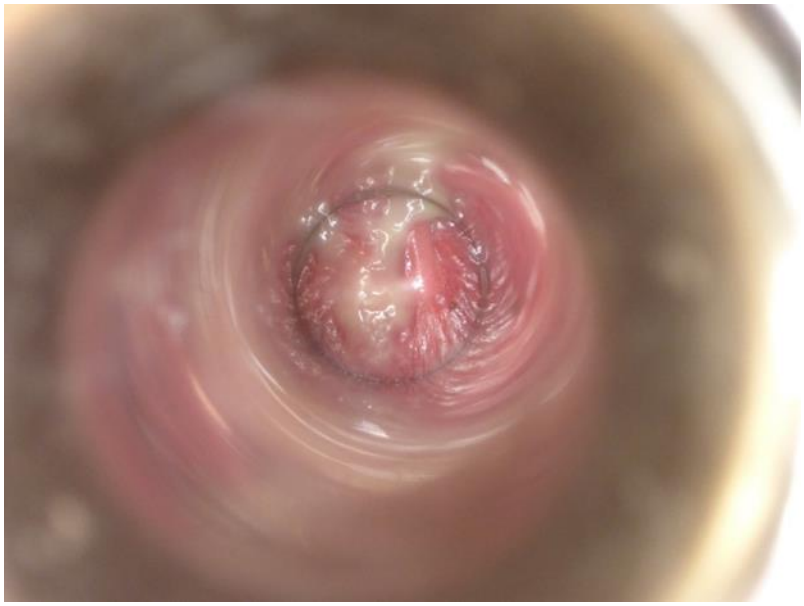


Figure 4

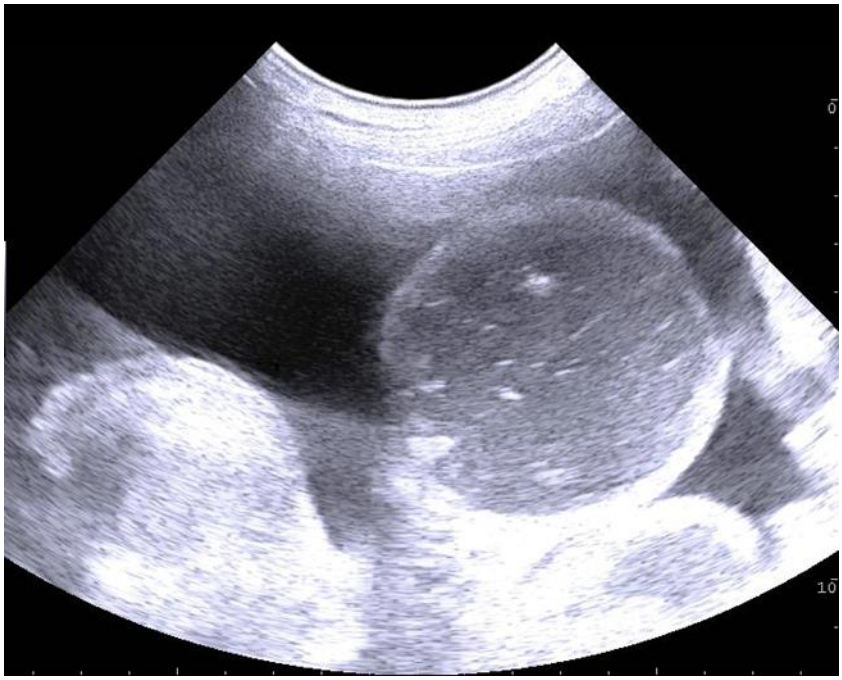


Figure 5

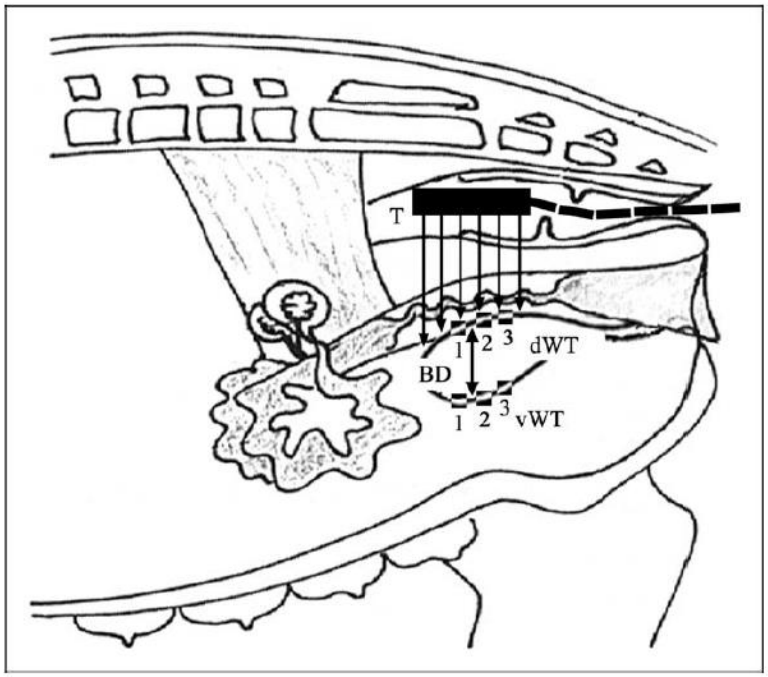
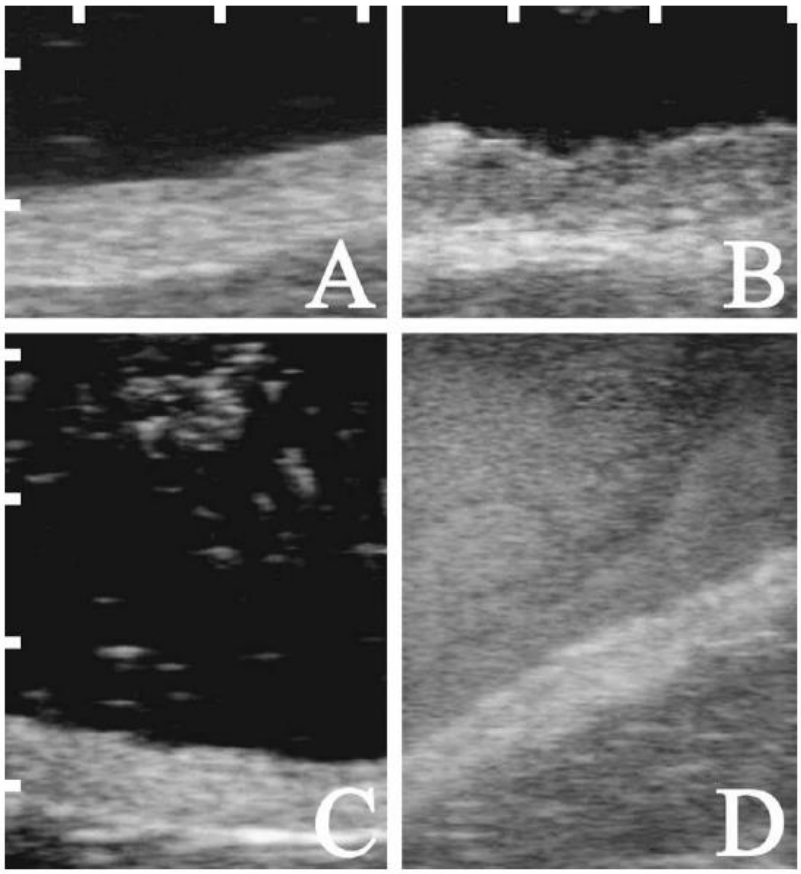


Figure 6



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