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2020-08-20

Grahofer , A , Björkman , S & Peltoniemi , O 2020 , ' Diagnosis of endometritis and cystitis in sows : use of biomarkers ' , Journal of Animal Science , vol. 98 , no. Suppl 1 , pp. S107-S116 . <https://doi.org/10.1093/jas/skaa144>

<http://hdl.handle.net/10138/333340>

<https://doi.org/10.1093/jas/skaa144>

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Alexander Grahofer, Stefan Björkman, Olli Peltoniemi, Diagnosis of endometritis and cystitis in sows: use of biomarkers, Journal of Animal Science, Volume 98, Issue Supplement_1, August 2020, Pages S107–S116

is available online at:

https://academic.oup.com/jas/article/98/Supplement_1/S107/5894009

<https://doi.org/10.1093/jas/skaa144>

1 **Diagnosis of endometritis and cystitis in sows: use of biomarkers**

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18 Review-ICPD 2019

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20 **Abstract**

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

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43 **1. Introduction: biomarkers of the urogenital tract in sows**

44

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

63

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

69 2017). A continuous evaluation of the uterine diameter after the birth process can be used as a
70 monitoring biomarker, which is characterized by serial measurements to detect changes in the tissue
71 (Myers et al., 2017, Grahofer et al., 2019, Meile et al., 2019). A predictive biomarker evaluates the
72 effect from a specific intervention or exposure (Myers et al., 2017). An example would be the
73 intramuscular use of oxytocin in a sow with dystocia to provoke uterine contractions (Almond et al.,
74 2006). Prognostic biomarkers are used to identify the likelihood of a clinical event, disease,
75 recurrence or progression of the disease (Myers et al., 2017). An example would be the vaginal cell
76 lipidome of weaned female piglets, which essentially defines the reproductive potential of a gilt
77 (Casey et al., 2018). An increase in antibodies after vaccination of a sow can be used as a response
78 biomarker, which evaluates the reaction to a treatment (Myers et al., 2017; Arsenakis et al., 2019).
79 Safety biomarkers were defined to indicate the reaction of the intervention (Myers et al., 2017). An
80 example from swine research would be the recent study from Bill et al. (2017) that conducted a
81 dose-finding study on Prostaglandin E2 in sows during the birth process to evaluate the effect of the
82 drugs.

83

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

86

87 **2. Diagnosis of endometritis**

88 Currently, the markedly extended farrowing in hyper-prolific sows (Oliviero et al., 2019) increases
89 the incidence of postpartal disorders, especially endometritis, and thereby negatively affects the
90 subsequent reproductive cycle and performance of the sow (Oliviero et al., 2013; Björkman et al.,
91 2018, Grahofer et al., 2019). Therefore, a rapid and accurate diagnostic approach for sows is needed
92 by pig farmers.

93 **2.1. Definition of endometritis**

94 Endometritis is defined as an inflammation of the endometrium or uterine lining and occurs due to
95 an imbalance between external factors and the sow`s immune defence of the uterus. The majority of
96 sows with uterine abnormalities show endometritis instead of metritis (Dial and MacLachlan, 1988).
97 The uterine discharge of affected sow vary extensively, depending on the pathogenic
98 microorganism, duration of infection, and the stage of the estrous cycle (Dial and MacLachlan,
99 1988). Endometritis is causes through several factors and therefore an accurate diagnostic work up
100 is necessary to avoid fertility disorders in sow herds.

101 To date, there is still no consistent clinical or histopathological nomenclature for endometritis in
102 sows. The endometritis can be distinguished as non-puerperal and puerperal, depending on the time
103 point of occurrence in the reproductive cycle (Kauffold, 2008). In addition, it can be categorized
104 into sub-clinical (without clinical symptoms), acute and sub-acute endometritis, which are clinically
105 apparent (Muirhead, 1986; De Winter et al.,1994; Dalin et al., 2004; Heinritzi et al., 2006;
106 Kauffold, 2008; Tummaruk et al., 2010). The severity of endometritis can be classified according
107 to the percentage of tissue containing inflammatory cell infiltrate, ranging from mild to severe
108 (Novakovic et al., 2018). Furthermore, the number of immune cells and damage to the endometrial
109 tissue can differentiate the time course of an infection of the endometrium in sows (de Winter et al.,
110 1992). Nevertheless, the interpretation of endometritis based on histological examination varies
111 depending on the stage of the oesturs cycle (Kaeoket et al., 2001; Dalin et al., 2004) and therefore
112 might lead to misinterpretation of the results.

113 **2.2. Vaginal discharge**

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

119 However, this finding may lead to an incorrect diagnosis because discharge can originate from the
120 urinary bladder or the vagina. The colour, consistency, and quantity of vaginal discharge vary
121 regardless of whether the vaginal discharge is of physiological or pathological origin (Noakes et al.,
122 1992). The colour can vary from clear, whitish, yellowish to reddish (Fig. 2).
123
124 The consistency varies from watery to creamy with lumps, and the volume can reach 500 ml
125 (Muirhead, 1986; Naokes et al., 1992). Increased volumes of vaginal discharge are associated with
126 endometritis, but there is no significance between the occurrence of endometritis and the colour of
127 the vaginal discharge (Muirhead, 1986). Mucopurulent to purulent and greyish-yellowish vaginal
128 discharge is often associated with predominant infection by *Streptococcus* several species
129 (spp.)and/or *Staphylococcus* spp (Heinritzi et al., 2006). Less frequently, vaginal discharge is
130 observed in endometritis caused by *Escherichia coli*, when the vaginal discharge is ofgreyish-white
131 colour (Heinritzi et al., 2006). Other bacteria, such as *Chlamydia* spp. (Kauffold et al., 2006;
132 Kauffold, 2008), and anaerobic microbes (i.e. *Fusobacterium necrophorum*, *Prevotella* spp.) are
133 also kown to cause vaginal discharge (Oravainen et al., 2006). Several studies have shown that
134 vaginal discharge occurs frequently postpartum in healthy and diseased animals (Nachreiner and
135 Ginther, 1972; Hermansson et al., 1978; Morkoc et al., 1983), with the highest incidence between
136 day 2 and 4 postpartum (Madec and Leon, 1992; Grahofer et al., 2019). Obstetrical intervention and
137 prolonged farrowing increase the risk of vaginal discharge in the puerperium (Bará and Cameron,
138 1996, Grahofer et al., 2019) and lead to higher incidence of endometritis in sows (Björkman et al.,
139 2018). Vaginal discharge has also been associated with the production environment, such as
140 overcrowding, restriction of movement by crating, poor hygiene and lack of enrichment
141 materials.(Oravainen et al., 2006; 2007). Besides puerperal discharge, non-puerperal discharge can
142 occur in breeding farms. The ethology as well as pathogenesis is more challenging and an
143 investigation is warranted, when herd prevalence is more than 3 percentage (Kauffold et al., 2008).

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

146

147 **2.3. Body temperature**

148 Fever is a cardinal symptom of inflammation and the most frequently used variable to evaluate the
149 health status of a sow in the puerperal period. Importantly, several parameters effect the body
150 temperature of sows such as the circadian rhythm (Stiehler et al., 2015), parity (Stiehler et al.,
151 2015), variations if compared between sequential measurements (Mead and Bonmarito, 1949), and
152 positioning of the thermometer in the rectum (Rotello et al., 1996). There is a large discrepancy in
153 the reference values for fever in sows with puerperal disorders, ranging from 39°C (Tummaruk and
154 Sang-Gassanee, 2013) to 40°C (Papadopoulos et al., 2010).

155

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

160

161 **2.4. Vaginal cytology and histology of the uterus**

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

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

194

195 **2.5. Microbiology**

196 Endometritis in gilts and sows is often caused by several species of bacteria (Dial and MacLachlan,
197 1988), but also fungi and rarely viral pathogens can cause uterine inflammation (Kauffold, 2008).
198 Especially in sows with acute or subacute endometritis, half of the animals showed positive
199 bacteriological results while only 17% of the uteri with chronic endometritis and 13% of the
200 histologically normal uteri were positive (de Winter et al., 1995). The most common pathogens that
201 are found in sows with puerperal and non-puerperal endometritis are Gram-positive pyogenic
202 bacteria such as *Staphylococcus* spp. and *Streptococcus* spp. and Gram-negative bacteria such as
203 *Escherichia coli* (D'Allaire et al., 1987; Muirhead, 1986; de Winter et al., 1995; Glock and Bilkei,
204 2005; Oravainen et al., 2007; Tummaruk et al., 2010). Results suggest that an endometritis
205 associated with vaginal discharge is most likely an ascending infection of pathogens from the vulva
206 and the urinary bladder (de Winter et al., 1995). Furthermore, sows with chronic cystitis are 3.5
207 times more likely to develop endometritis (Biksi et al., 2002). These findings were also confirmed
208 in a study from Austria, where a bacteriological and pathological investigations of culled sows with
209 reproductive disorders revealed that 84,6 % of the animals (n=39) had an endometritis and cystitis
210 (Sipos et al., 2014). Therefore, an investigation of a uterine swab and a urine sample may be useful
211 in sow herds with endometritis. A speculum (Fig. 3) with a double-guarded swab should be used to
212 obtain a representative sample from the uterus and to avoid contamination of the bacterial flora
213 from the vagina (Oravainen et al., 2007, Grahofer et al., 2017).

214

215 **2.6. Acute phase proteins**

216 Acute-phase proteins are plasma proteins that increase, when an infection, inflammation or trauma
217 occurs in the host. It would be logical to assume that in cases where a systemic inflammation
218 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., 2019; 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).

244

245 **3. Cystitis**

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

263

264

265 **3.1. Definition and aetiology of cystitis**

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

269 cystitis (Bellino et al., 2013). In addition, the uterus can be a reservoir for a possible infection of the
270 urinary tract and vice versa (Gmeiner, 2007). Bacteria can also arise from the intestinal tract of the
271 sows or from a housing system with suboptimal hygiene. *Escherichia coli* is the predominant
272 bacterial species associated with about 70% of cystitis cases (Biksi et al., 2002, Grahofer et al.,
273 2014). *Escherichia coli* occurs mainly in monoculture, but also as mixed culture with
274 *Staphylococcus* spp., *Streptococcus* spp., *Proteus* spp. and others (Biksi et al., 2002). Normally, the
275 immune system of the sow is able to eliminate infections from the urinary bladder unless it is
276 impaired. Parturition itself decreases immunity and causes constipation, which increases the risk of
277 bacteria and toxins entering the blood system (Oliviero et al., 2010; Kaiser et al., 2018). Therefore,
278 Berner (1987), Wendt et al. (1990) and Biksi et al. (2002) established a connection between cystitis
279 and postpartum dysgalactia syndrome (PDS). Wendt et al. (1990) found that 77% of pigs with PDS
280 had the same bacteria in the urinary bladder. Furthermore, sows with chronic cystitis were six times
281 more likely to have PDS (Wendt et al., 1990). Biksi et al. (2002) found that sows with chronic
282 cystitis had 3.5 times higher odds of developing endometritis. Berner (1987) considered cystitis to
283 be both a cause and a result of PDS. Therefore, we recommend that sows suffering from PDS are
284 examined for whether the aetiology of the syndrome is caused by cystitis. For optimal treatment, the
285 exact cause of PDS needs to be determined. If not diagnosed and treated, chronic cystitis can
286 increase piglet mortality before weaning and reduce pregnancy rate and litter size at next breeding
287 (Thorup, 1994; Tolstrup, 2017). Further, cystitis has also been linked with increased number of
288 stillborn piglets (Tolstrup, 2017). This shows the importance of diagnosing cystitis even before
289 parturition in order to prevent birth complications. Several parameters can be evaluated to diagnose
290 cystitis in sows.

291

292 **3.2. Urinalysis**

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

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

318

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

330

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

334

335

336

337 **3.3. Bacteriological investigation**

338 Bacteriological investigation of the urine is regarded as a generally reliable method for diagnosing
339 cystitis in live animals. Sensitivities and specificities are similar to those for urine turbidity
340 evaluation and measurement of blood and protein using the urine stick testing (Table 1). Dipslides
341 can be used for bacteriological evaluation. They are placed into urine for about 10 seconds and the
342 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 10^4 and 10^5 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 10^3 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).

368 Microscopically, acute cystitis is characterized by epithelial loss and bacterial colonies found on the
369 surface of the urinary bladder. The lamina propria mucosae is oedematous and has a diffuse
370 infiltration with neutrophilic granulocytes. In addition, superficial hyperaemia and bleeding occur in
371 the tissue (Weiss, 1999; Liebhold et al., 2005; Newman et al., 2007). Chronic cystitis is associated
372 with diffuse thickening of the mucosa and a hypertrophic muscle layer. Depending on the
373 inflammatory reaction, diffuse, follicular or polypoid changes appear in the urinary bladder (Weiss
374 1999; Newman et al., 2007; Bellino et al., 2013). The diffuse forms may result in detachment of the
375 epithelium and excessive infiltration of the submucosa with mononuclear inflammatory cells and
376 few neutrophilic granulocytes, whereas, the follicular forms exhibit disseminated, nodular,
377 submucosal proliferations of lymphoid nodules (Weiss 1999; Newman et al., 2007). These
378 lymphoid follicles are often surrounded by a hyperaemic zone. In addition, there is usually a
379 diffusely thickened, hyperplastic lymphoid follicle and a chronic lymphoplasmacellular infiltrate
380 and fibrosis in the lamina propria mucosae. In several cases, the tunica muscularis is hypertrophic
381 (Weiss 1999; Newman et al., 2007). The chronic polypoid cystitis is characterized by single or
382 multiple nodular mucosal proliferation consisting of fibrous connective tissue and infiltration of
383 neutrophilic granulocytes and mononuclear leukocytes. The proliferative tissue is ulcerated or
384 covered with a hyperplastic epithelium with goblet cell metaplasia (Liebhold et al., 1995). Hence,
385 animals affected with the polypoid form show haematuria (Weiss 1999; Newman et al., 2007).
386 In conclusion, a pathological investigation of the urinary bladder appears to be a useful method to
387 estimate urinary tract infection in a sow herd (Bellino et al., 2013; Sipos et al., 2014, Grahofer et al.,
388 2014, Sipos et al., 2017)

389

390 **3.5. Ultrasonography**

391 Kauffold et al. (2010) studied ultrasonographic characteristics of the urinary bladder with defined
392 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 smoothing 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.

452

453

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659

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662

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

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

667

668 **Figure 2:** Puerperal vaginal discharge of different colours. 0= clear, 1= reddish, 2=yellowish and
669 3= whitish (Grafofer et al., 2019)

670

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

674

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

678

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

686

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

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

Figure 1

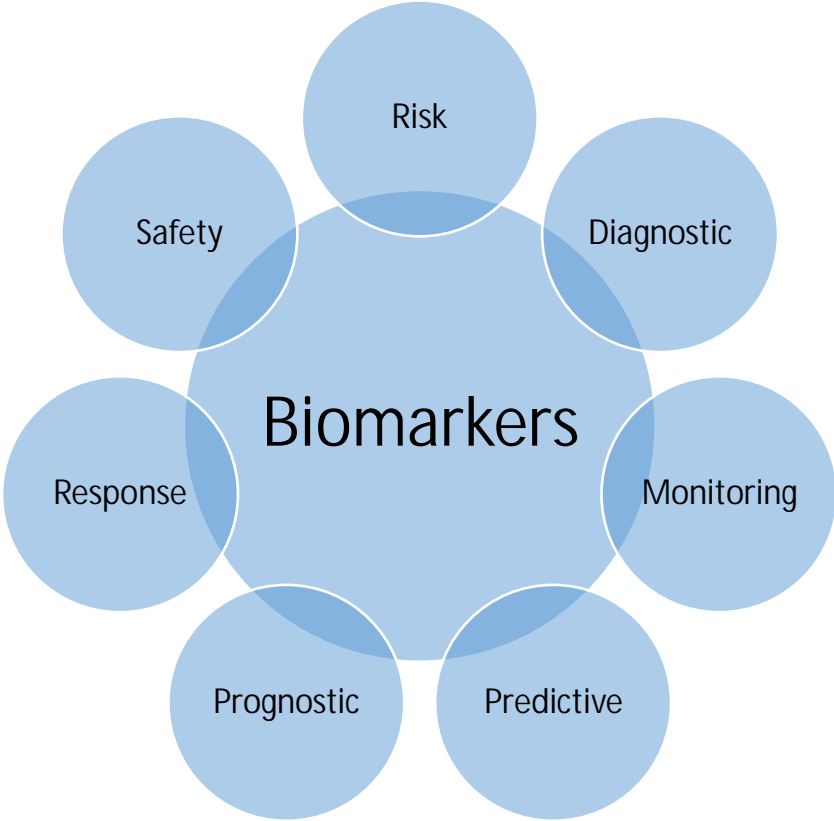


Figure 2



Figure 3

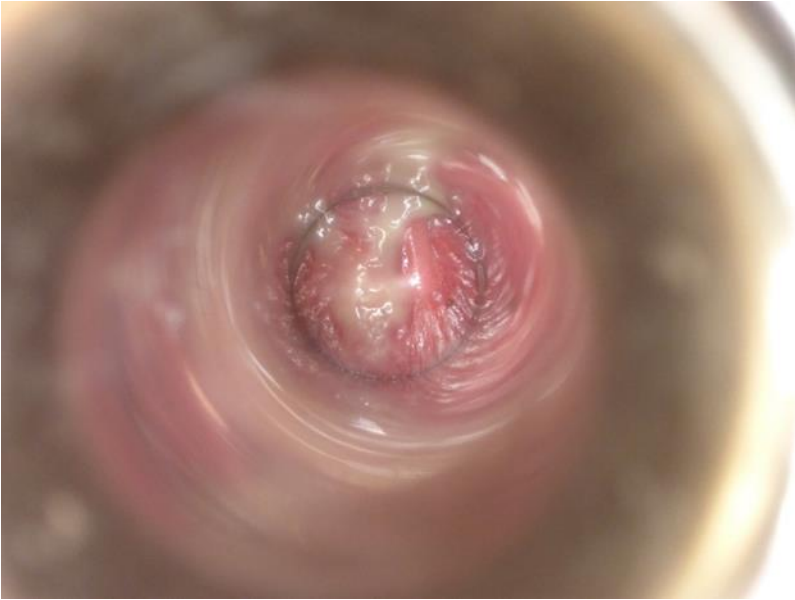


Figure 4

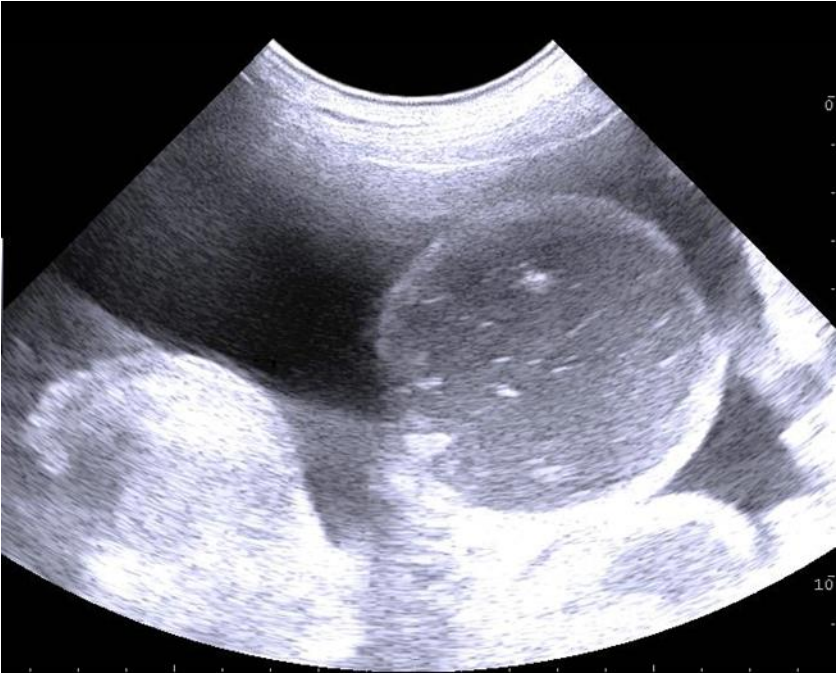


Figure 5

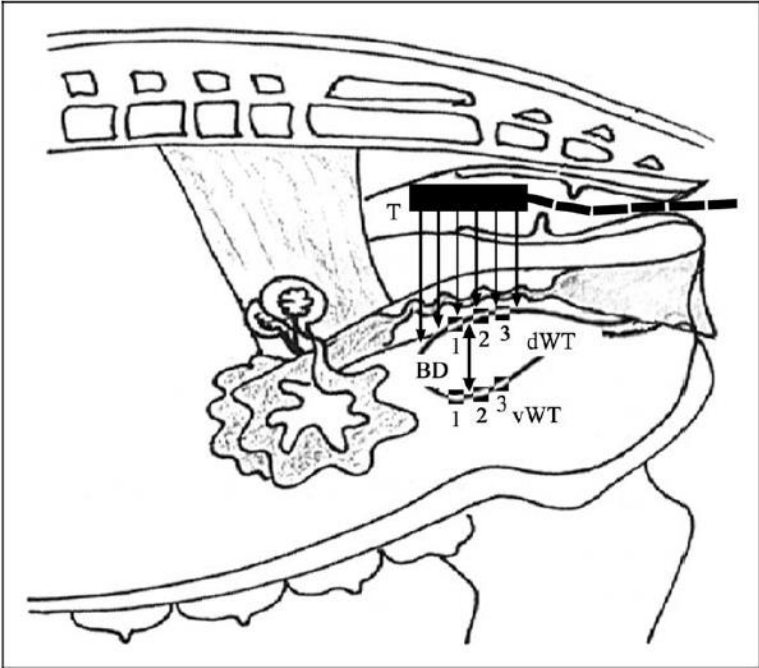
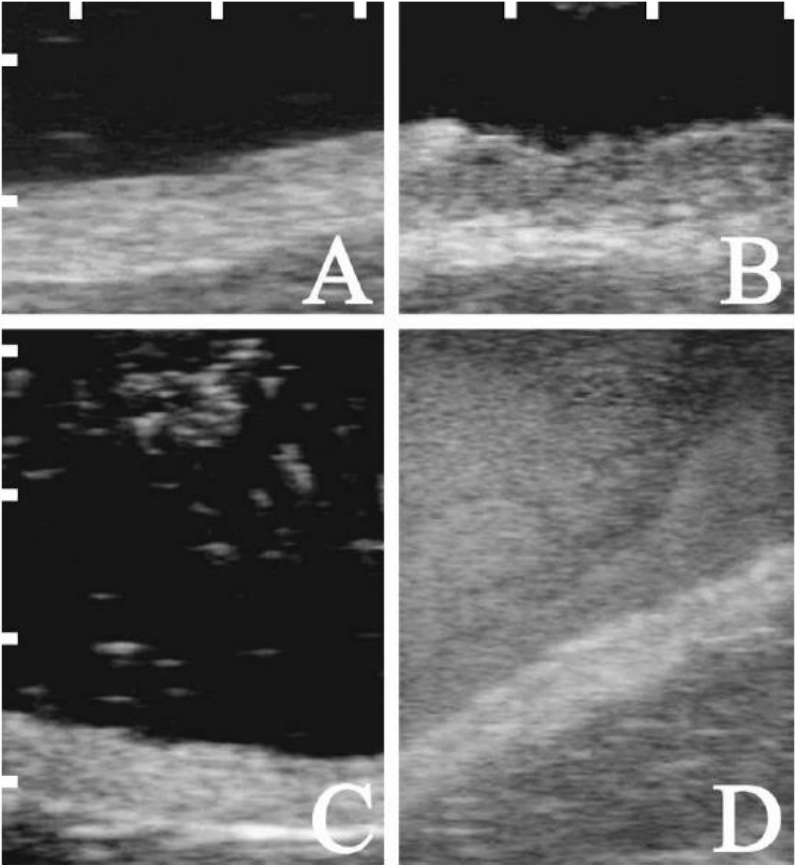


Figure 6



696