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DIAGNOSING AND TREATMENT OF EQUINE ENDOMETRITIS - ESTONIAN PRACTICE
HOBUSTE ENDOMETRIIIDI DIAGNOOSIMINE JA RAVI - EESTI PRAKTIKA

Graduation Thesis
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Equine endometritis, inflammation of uterine inner layer, is described as one of the major causes of infertility in horses. The aim was to observe used diagnosing and treatment protocols in one Estonian practice, follow the treatment outcome and study the complex of this disease as whole. Study was based on one Estonian equine practitioner’s breeding datasheet gathered in season 2017. Data was collected from EquiBreedVet reproduction management database and processed with Microsoft Excel. Also, laboratory results were included in the study. Observation of clinical practice was performed in spring 2018. In breeding season 2017 in total 76 mares were intended to be bred in this veterinarian practice. Infectious endometritis was found from nine mares, with the frequency of 11,8% in studied population. The main microbes isolated were genital commensals E.coli and Streptococcus equi ssp. zooepidemicus. Two mares developed a fungal infection which was caused by Candida ssp. Pregnancy rates in infected mares was considerably lower compared to healthy mares (44,4 vs. 84,4%). Infected mares were also older (8,9 vs. 11,7 years). The pathological findings and frequency were similar as presented in the literature. Methods in researched practice were evidence based. Used diagnostic and treatment protocols were similar as the methods in literature. Antimicrobial treatment was based on pathogens sensitivity. To improve diagnostics biopsy from endometrium could be added in diagnostic protocol. Also, uterine lavage as treatment option might be demonstrated more often and additionally used in combination together with antibiotics.

Keywords: mare, infertility, gynaecology, cytology, bacteriology
# TABLE OF CONTENTS

ABSTRACT ............................................................................................................. 2
LÜHIKOKKUVÕTE ................................................................................................. 3
TABLE OF CONTENTS ......................................................................................... 4
LIST OF ABBREVIATIONS ................................................................................... 6
INTRODUCTION ..................................................................................................... 7
1. LITERATURE REVIEW ....................................................................................... 9
   1.1 Equine endometritis .................................................................................... 9
      1.1.1 Classification of uterine pathologies in mares ..................................... 9
      1.1.2 Reproductive tract barriers and defence mechanisms ...................... 10
      1.1.3 Normal physiological reactions and uterine clearance ...................... 11
      1.1.4 Pathogens and their features ............................................................... 12
   1.2 Diagnosing .................................................................................................. 12
      1.2.1 Reproductive examination of mare .................................................... 13
      1.2.2 Ultrasound .......................................................................................... 13
      1.2.3 Culture and cytology ......................................................................... 14
      1.2.4 Biopsies ............................................................................................... 15
      1.2.5 Endoscopy ........................................................................................... 16
   1.3 Treatment .................................................................................................... 16
      1.3.1 Correction of anatomical defects ....................................................... 17
      1.3.2 Cervical failure ................................................................................... 17
      1.3.3 Uterine lavage ..................................................................................... 17
      1.3.4 Ecbolics ............................................................................................... 18
      1.3.5 Immunomodulators .......................................................................... 18
      1.3.6 Antimicrobials .................................................................................... 19
         1.3.6.1 Intrauterine antimicrobials ............................................................ 19
         1.3.6.2 Systemic antimicrobials ................................................................. 20
         1.3.6.3 Evaluation of antimicrobial treatment ......................................... 21
      1.3.7 Other intrauterine treatments ............................................................. 21
      1.3.8 Management and feeding ................................................................. 22
   2. AIM OF THE STUDY ....................................................................................... 23
3. MATERIALS AND METHODS ............................................................................. 24
3.1 Materials ................................................................................................. 24
  3.1.1 Practice ............................................................................................... 24
  3.1.2 Animals ............................................................................................... 24
  3.1.3 Diagnostic procedures ......................................................................... 25
3.2 Methods .................................................................................................... 26
4. RESULTS ..................................................................................................... 27
  4.1 Mares ....................................................................................................... 27
  4.2 Pathogens and treatment of IE ................................................................. 28
5. DISCUSSION .............................................................................................. 32
6. CONCLUSIONS .......................................................................................... 36
SUMMARY ..................................................................................................... 37
LIST OF REFERENCES ...................................................................................... 39
HOBUSTE ENDOMETRIIDI DIAGNOOSIMINE JA RAVI – EESTI PRAKTIKA........ 43
APPENDIXES .................................................................................................. 46
  Appendix 1. Non-exclusive licence ............................................................... 46
LIST OF ABBREVIATIONS

AI=Artificial insemination

B(M)IE=Breeding (mating) induced endometritis

CEM=Contagious equine metritis

cs=Cooled semen

df=Deep frozen semen

fs=Fresh semen

IE=Infectious endometritis

IU=Intra uterine

nc=Natural covering

NSAID=Non-steroidal anti-inflammatory drug

PB(M)IE=Persistent breeding (mating) induced endometritis

PGF2a=Prostaglandin F2-a

PMNs=Polymorphonuclear neutrophils

PRP=Platelet-rich plasma
INTRODUCTION

The thesis focuses on equine infectious endometritis, an inflammation of uterine endometrium, which is one of the major causes of infertility in mares (Leblanc, Causey 2009). Diagnosis of equine infectious endometritis is a complex procedure and can be challenging in some cases (Ferris 2016: 481), but without proper diagnosis and/or treatment it can lead to infertility (Leblanc, Causey 2009), pregnancy loss (Pasolini et al. 2016: 285) or birth of the septicaemic foal (Causey 2006).

According to literature 10 to 20% of mares either have endometritis or are prone to it (Scoggin 2016: 500). According to Equine database of Estonian Agricultural Registers and Information Board approximately 700 foals are registered in Estonia every year. Based on this, it can be suggested that endometritis potentially affects more than 70 Estonian mares each year and may also have negative effect on birth rates as some mares fail to conceive. As mares are seasonally polyestrous, in Estonia breeding season starts in the beginning of April and continues until the end of August. Different breeding methods are used - natural breeding, artificial insemination with fresh, cooled and frozen semen and fresh embryo transfer. Unfortunately, accurate data of breeding methods are not available in Estonia.

In this thesis the complex of equine infectious endometritis was observed through a case series in one Estonian equine practice. The aim was to observe used diagnosing and treatment protocols in this Estonian practice, report possible limitations or deficiencies in procedures and follow the treatment outcome and compare the statistics and findings to literature. Study was based on one Estonian equine practitioner’s breeding datasheet gathered in season 2017. Data was collected from EquiBreedVet reproduction management database and processed with Microsoft Excel. Accompanying laboratory results were included in the study. Breeding season 2017 included the data from 76 mares. From these 74 mares were bred - 20 covered naturally and others artificially inseminated (11 fresh semen, 3 cooled semen, 40 frozen semen). Two mares were not bred as planned due to severe uterine infection. From all mares included in the study, nine were later diagnosed with infectious endometritis. Frequency of infectious endometritis in study population was 11,8 %. The observation of clinical practice was done in spring 2018 and additional treatment outcomes were included to database.
During this study process I have received guidance and help from many people. So, I want to thank Ants Kavak and Ulrika Tuppits for all the help and support. Special thanks to Ulrika Tuppits for introducing me with this extremely fascinating field of veterinary medicine and sharing all the information during this process. Thanks also to Estonian horses for participating my study and enabling the learning experience during my inspiring practice in Estonia. Now, at the time of my graduation, I want also express my sincere gratitude to all my loved ones for supporting me in these past years during my studies – I really appreciate your patience.
1. LITERATURE REVIEW

1.1 Equine endometritis

The important component of mare’s fertility is normal uterus, especially healthy endometrium (Kenney 1978). There are several factors potentially disturbing uterine environment such as failure to remove spermatozoa, inflammatory exudate and bacteria post-mating (LeBlanc, Causey 2009). Healthy uterus can resist pathogens and clear infections rapidly, but if defence mechanisms are altered the risk of mare to develop uterine infection increases (Ferris 2016: 482).

Mares have been classified as susceptible and resistant according to their ability to resist bacterial uterine infections (Causey 2006; LeBlanc, Causey 2009). Susceptible mares are more prone to endometritis as they fail to eradicate debris and inflammatory products (LeBlanc, Causey 2009). Predisposal factors include for instance poor perineal conformation, decreased muscular tone of vulva, cervical defects, weakened uterine contractility, pendulous uterus and poor body condition (Ferris 2016: 482). Also old mares are in higher risk in developing endometritis (Woodward, Troedsson 2014).

Endometritis is not clearly defined in equine species and is also often undiagnosed and underdiagnosed in clinical practice (personal communication U. Tuppits). Some mares may hide endometritis and the lack or absence of clinical signs may lead to diagnostic failure (LeBlanc, Causey 2009). Both clinical and subclinical endometritis have equally negative effect on fertility (LeBlanc, Causey 2009).

1.1.1 Classification of uterine pathologies in mares

Persistent endometritis can be divided in four groups; venereal/sexually transmitted, chronic infectious endometritis, persistent breeding-induced endometritis (PBIE) and chronic degenerative endometritis (Troedsson 1999). Uterine infections arise from different origins; some of the infections are caused by strict reproductive pathogens and some are clearly caused by mare’s susceptibility (Causey 2006).

It is important to distinguish the origin of endometritis as it needs to be noticed when making decision concerning the treatment- in venereal and chronic infections the treatment should be directed to known pathogen and in PBIE aim is to support the uterine clearance.
In clinical practice it may be challenging or even impossible to apart bacterial uterine infection and breeding induced endometritis (BIE) even though they are usually studied separately (Woodward, Troedsson 2013).

Venereal infections are spread by stallion and produce infection regardless of mare’s susceptibility (Causey 2006). *Taylorella equigenitalis* is highly pathogenic and causes Contagious Equine Metritis (CEM) (Troedsson 2011: 2609). Infection spreads via the infected stallion and mares become infected after mating (LeBlanc 2009). Other potentially venereal pathogens are e.g. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Troedsson 2011: 2609).

Infectious endometritis results commonly from uterine contamination and is most typical in old, pluriparous mares (LeBlanc 2009). Also fungal infection is more commonly seen in older mares with anatomical defects and repeated foalings (LeBlanc 2008).

Post-breeding infections result after natural breeding or artificial insemination and are mostly caused by genital commensals (Causey 2006). It should be noted that breeding-induced endometritis (MIE) occurs in all mares, but only 10-15% of these mares develop persistent endometritis (PMIE) (Troedsson et al. 2008). Post-breeding infections usually last less than 48h, but in susceptible mares can prolonged (Causey 2006).

### 1.1.2 Reproductive tract barriers and defence mechanisms

The natural uterine defence is based on physical barriers including the vulva, the vestibule, the vagina and the cervix (Troedsson 2011: 2608). Also vestibule-vagina sphincter has important role on sealing the mares reproductive tract (Canisso et al. 2016: 466). During the oestrus vestibule-vagina sphincter is the only barrier that remains functional and prevents aspiration of air and debris in to the cranial vagina (Canisso et al. 2016: 466). Physical barriers protect the uterus from external contamination (Troedsson 2011: 2608).

Vulvar conformation has a significant role in resisting the uterine infections (Hemberg et al. 2005). Ideally vulva should be oriented vertically and 2/3 of the vulvar length should be located below the pelvic brim (Canisso et al. 2016: 466). Vulvar lips should be perfectly apposed and mare should have enough fat pad surrounding the vulva (Canisso et al. 2016: 467). If vulvar lips are incompletely closed, vulvoplasty might be beneficial in order to resist uterine infection and improve fertility (Hemberg et al. 2005).
The cervix is an important natural sphincter which takes care of the uterine health. During the estrus it is extremely important that cervix opens adequately and is soften. Open cervix allows the sperm to enter in to the uterus and dilatation is also needed for a uterine drainage. Inadequate opening of the cervix after insemination complicates elimination of sperm and inflammatory products and uterine drainage becomes insufficient. Cervix also has an important role in maintaining pregnancy. For successful pregnancy it is vital that cervix is closed tightly and it seals the uterus and entrance of contaminants is blocked. (Katila 2012)

1.1.3 Normal physiological reactions and uterine clearance

There are many humoral, cellular and mechanical defence mechanisms which aid to resistance of persistent uterine inflammation (Troedsson 1999). Activated polymorphonuclear neutrophils (PMNs) eradicate bacteria, debris and sperm cells from uterus in presence of opsonin trough a phagocytosis (Canisso et al. 2016; Troedsson 1999).

Transient inflammatory reaction including influx of polymorphonuclear neutrophils (PMN) is normal physiological process post-breeding in response to spermatozoa and is demonstrated in both artificial insemination and natural breeding. Kotilainen 1994 et al. reported highest neutrophil counts in mares inseminated with concentrated fresh semen or frozen semen. Influx of neutrophils seems to be associated to introduction of spermatozoa rather than bacteria or seminal plasma. (Kotilainen et al. 1994)

One preventive key factor in avoiding persistent endometritis and endometrial damage is PGF2-a induced myometrial contractions. These contractions aim in removal of prejudicial inflammatory products rising from the PMN-phagocytosis and programmed cell death of PMNs. PGF2-a is released from activated PMNs and possibly from the endometrium. If the infection persists and is still present 5 days after ovulation when embryo enters in to the uterus it will result to embryonic loss as release of PGF2-a interferes corpus luteum. It has also considered that detrimental inflammatory uterine environment may cause embryonic loss. (Troedsson et al. 2008)

Lymphatic vessels and veins also play a part in uterine drainage (Katila 2011: 2601). Endometrial oedema is physiological reaction occurring during oestrus and is resolved naturally after ovulation (LeBlanc, Causey 2009). Also good blood supply is needed as it takes part in several functions such as hormonal signalling, uterine contractility, placentation and faeto-endometrial interactions (LeBlanc, Causey 2009).
Mucus and mucociliary currents are considered as a one defence mechanism in mare’s uterus even though the issue is not fully understood. Mucus protects the epithelia of endometrium and prevents the bacterial adhesion by binding pathogens to mucus blanket. Uterine folds and continually cervical folds facilitate the movement of mucus and participate in normal uterine drainage. Reasons which might result uterine folds to separate from each other and disturb normal drainage, include fluid accumulation or diminishing in endometrial fold size resulting from scarring, pathology or endocrine disruption. (Causey 2007)

1.1.4 Pathogens and their features

Bacterial uterine contamination during natural mating or AI origins from posterior part of mares genital tract and from the semen (Pyörälä et al. 2014). Streptococcus equi ssp. zooepidemicus and E.coli are most commonly isolated bacteria and are presented in most uterine infections (LeBlanc 2009).

One of the factor affecting the infection and pathogenesis are pathogens and their individual features (LeBlanc, Causey 2009). Biofilm organisms have inherent resistance against many antimicrobials agents including antibiotics (Donlan, Costerton 2002). This inherent resistance relies on physiological features of biofilm organisms and structural characteristics of biofilm (Donlan, Costerton 2002). One well-known biofilm producing bacteria connected to endometritis and damaging the endometrial tissue is P. aeruginosa (Wittenbrink 2012). Other biofilm producing bacteria include E. coli, Staphylococcus epidermis and Enterobacter cloacae (LeBlanc, Causey 2009). Also a plenty of fungi and yeasts are counted as biofilm producing pathogens (LeBlanc, Causey 2009).

Most commonly found fungi from the equine uterus are Aspergillus spp. and Candida spp. (LeBlanc 2009). Candida is a normal vaginal commensal and Aspergillus a hyphae producing mould (LeBlanc 2009). E.coli, Klebsiella ssp., Enterobacter ssp., P. Aeruginosa and some yeasts are connected to antimicrobial resistant endometritis (LeBlanc 2008).

1.2 Diagnosing

Clinical signs of endometritis are variable and different from case to case (LeBlanc, Causey 2009). Clinical signs are rarely seen, but sometimes mares may exhibit vaginal discharge or shortening in luteal phase (Woodward, Troedsson 2013). Also decreased fertility may be observed (Woodward, Troedsson 2013). If mare is not exhibiting normal estrus cycles or is
anestrus during cycling season, uterine inflammation should always be considered as a differential diagnose (LeBlanc 2008). Intrauterine fluid accumulation is seen especially after breeding (LeBlanc, Causey 2009). Other signs clearly indicating uterine inflammation are positive uterine culture and neutrophilic uterine cytology (LeBlanc, Causey 2009).

Basically- diagnose is based on a history of the mare, detecting the uterine inflammation and isolation of typical organisms (Causey 2006).

1.2.1 Reproductive examination of mare

Chronically infertile mare should always be evaluated as whole and perform a physical examination including the evaluation of musculoskeletal system (LeBlanc 2008). Mares history should be recorded as completely as possible as it offers valuable information about the possible causes for infertility (LeBlanc 2008). The history should include the age when first bred, previous foalings, treatments used around breeding, possible other medications used during mares performance career as well as breeding manner (AI vs. natural) and type of used semen (LeBlanc 2008). Difficult births, number of live foals and delivery of premature or large foals should be reported as well (LeBlanc, McKinnon 2011: 2622).

In reproductive examination transrectal palpation is included as a tool to identify the size, tone and position of reproductive organs (LeBlanc, McKinnon 2011: 2623). Cervical palpation is a routine and should be done every time to evaluate the length, width and consistency of cervix (Katila 2012).

Pelvic region and perineum of mare should be evaluated. Most of uterine infections develop when bacteria from caudal reproductive tract enter on anterior vagina. The need for vulvoplasty should be evaluated. The integrity of vestibulovaginal fold should be assessed as loss of this fold may lead to chronic uterine inflammation. Vaginoscopic examination is also one important part of clinical examination in infertile mare. Vaginoscopic examination gives the opportunity to visualize the vagina and external os of cervix and can reveal inflammation or fluid accumulation such as urine or pus. (LeBlanc 2008)

1.2.2 Ultrasound

Intrauterine fluid accumulation is one the main signs of a susceptible mare (Causey 2006). The ultrasound has significantly improved the diagnosing of endometritis and fluid retention
Some susceptible mares may exhibit free luminal fluid even before breeding but in most cases the fluid is detected post-breeding (Troedsson et al. 2008). Mares considered to be susceptible to PBIE should be monitored closely and ultrasound examination should be carried out at 6 and 24 hours post-breeding (Troedsson et al. 2008). It should be noticed that not all mares with subclinical endometritis have excessive fluid accumulation during the oestrus or after breeding (LeBlanc, Causey 2009). Ultrasonographical examination can reveal abnormal oedema patterns or short, thick, hyperechoic lines as an indicator of air or exudate (LeBlanc, Causey 2009). The retention of fluid can be graded according to the echogenicity and volume (Woodward, Troedsson 2013). Small amounts of intraluminal fluid post-breeding is normal, but if the fluid is highly echogenic (Woodward, Troedsson 2013) or the depth of visualized intrauterine fluid is more than > 2cm (Brinsko et al. 2003), it might indicate inflammation (See Figure 1.).

![Ultrasound examination revealing intraluminal fluid](Photo: Sinituulia Kivipato, 2018)

**Figure 1.** Ultrasound examination revealing intraluminal fluid. (Photo: Sinituulia Kivipato, 2018).

### 1.2.3 Culture and cytology

Two basic techniques in diagnosis of endometritis are cytology and culture which can be used in detection of uterine pathogens and neutrophils (LeBlanc, Causey 2009). Both
samples can be collected by guarded cotton swabs, cytological brush, low-volume uterine lavage or intrauterine fluid recovery with AI-pipette (Canisso et al. 2016: 470).

Even though uterine cytology gives strict evidence of uterine inflammation, it is also important tool in recognition of false positive bacterial cultures (Causey 2006). The basic diagnostic database should include both uterine cytology and culture (Ferris 2016: 485). When cytology reveals > 2 PMNs and bacterial culture is positive, it indicates that mare has endometritis (Riddle et al. 2007).

When evaluating the cytology, the sample is applied to the glass slide, air dried and stained with a modified Wright stain (Ferris 2016: 487). The average number of PMNs is evaluated with microscope, based on at least 10 high power field per slide (Ferris, 2016: 492). Cytological smear in the case of fungal infection may contain spores or in chronic infections, also hyphae (LeBlanc 2009). Cytological result may vary among different pathogens-Streptococcal infection is more likely to give positive cytology, than coliforms (Riddle et al. 2007). Number of PMNs can be graded; no PMNs/few PMNs- normal, 1-2 PMNs- mild inflammation, 3-5 PMNs -moderate inflammation, >5 PMS-severe inflammation (Ferris 2016: 492).

Bacterial cultures can be sent to laboratory or cultivated by the veterinarian. Suitable agars, which support the growth of uterine pathogens are MacConkey agar, chromogenic agar, trypticase soy agar with 5% sheep blood and in cases of fungal organisms Sabouraud agar (Ferris 2016: 486). Cultivation usually is continued 72h, even though initial results are already available in 12 to 24 hours (Ferris 2016: 487). In persistent inflammations if no aerobes are detected, the anaerobic culture might give valuable information (Causey 2006).

1.2.4 Biopsies

Endometrial biopsy has been used for decades as a main procedure for uterine health assessment and it still offers a mainstay diagnostic tool in histopathologic assessment of uterine health (Snider et al. 2011). Endometrial biopsy helps to identify the cause of infertility (LeBlanc 2008). Biopsies can be taken in both estrus and anestrus (LeBlanc 2008), but not during the pregnancy (Snider et al. 2011). The minimum historical data that submitting practitioner should provide includes mare’s age, the date of biopsy, estrus stage, breeding history, if necessary years barren and any relevant findings during reproductive examination (Snider et al. 2011).
In healthy endometrium neutrophil can be present in vessels and surface during estrus, but when found in tissue it indicates inflammation. Macrophages and lymphocytes are combined to chronic inflammatory process however few scattered lymphocytes can also be found in healthy endometria. Previous hemorrhage can be detected through a presence of siderocytes, macrophages which contain iron by-products. Plasma cells are not normally found in endometria but when present chronic process can be suspected. The presence of eosinophils usually is linked to some specific inflammatory process, for example fungal disease. (Snider et al. 2011)

Endometrial biopsy can reveal several specific conditions which can impact the mare’s ability to become pregnant, e.g. inflammatory changes, fibrosis, cystic glands, glandular hyperplasia/hypoplasia and lymphatic stasis (Kenney 1978). Also infectious agents such as bacteria and fungi which can be detected (Snider et al. 2011). Widespread periglandular fibrosis is connected to embryonic and fetal loss (Kenney 1978). Biopsy can be useful in certain mares e.g. mares with abnormalities in genital tract, known pyometra or mucometra, in all barren mares and mares with known history of embryonic or fetal death (Kenney 1978).

1.2.5 Endoscopy

Endoscopic evaluation of uterine lumen can also be used, but the use is usually limited to mares which may have uterine adhesions, focal infections, foreign bodies or retention of endometrial cups. Endoscopy can also reveal endometrial scarring and loss of endometrial folds and be useful in mares with history of not exhibiting normal estrus cycles. Endoscopic examination is most easiest to perform during diestrus or early estrus. (LeBlanc 2008; LeBlanc, Causey, 2009)

1.3 Treatment

The cornerstones of treatment are correcting the defects of uterine defence, controlling the post-breeding inflammation and neutralizing virulent bacteria (Causey 2006; LeBlanc, Causey 2009). If mare has pre-existing uterine infection it should be resolved before breeding (Troedsson et al. 2008). Traditionally, ecbolics and uterine lavage have been used in prevention and treatment of endometritis in order to remove inflammatory material and improve uterine clearance (LeBlanc, McKinnon 2011: 2628). However, when breeding chronically infertile mare the treatment should be based on a diagnose and the unwarranted
use of antibiotics, steroids and ecbolics should be avoided (LeBlanc 2008). Also, unnecessary treatments of reproductively normal mares should be avoided as it may potentially be detrimental (Troedsson et al. 2008).

1.3.1 Correction of anatomical defects

Anatomic defects in reproductive tract should be surgically corrected before breeding (Canisso et al. 2016: 472). Most commonly seen abnormalities that need surgical correction are lacerations in the perineal body, poor perineal conformation as well as poor opposition of vulvar lips (Canisso et al. 2016: 472).

Vulvoplasty should be considered if clinical examination reveals that mare is in risk to develop pneumovagina and/or aspirate feces into the vagina. If mare has > 2,5 cm of vulva open dorsal to the pelvis or cranial ventral tilt of > 20° vulvoplasty should be demonstrated as a preventive procedure. Vestibuloplasty should also be taken in to consideration if air enters in to the anterior vagina when vulvar lips are parted and mare has repeated bouts of chronic bacterial endometritis. (LeBlanc 2008)

1.3.2 Cervical failure

Some mares may exhibit excessive post-breeding fluid accumulation as the cervix is inadequately dilatated during the estrus. This concerns especially old mares, nervous young maiden mares and mares which are used as embryo donors without carrying foals to term themselves. In these cases, misoprostol or prostaglandin E can be applied on the cervix in order to maintain cervix open. Also manual stretching and catheter with inflated balloon have been used for cervix dilatation. (Canisso et al. 2016: 476-477)

1.3.3 Uterine lavage

Uterine lavage is frequently used method to manage post-breeding endometritis in mares (Canisso et al. 2016: 474). Lavage physically removes bacteria and other material from the uterus, that might prolong the uterine inflammation (Brinsko 2001). Lavage can be safely performed 4 hours post-breeding without effecting negatively fertilisation (Canisso et al. 2016: 475). Uterine lavage is useful in cases of infectious endometritis and can be used in combination with antibiotic therapy (Brinsko 2001).
Usually the choice of the used product is made by individual practitioner (LeBlanc, Causey 2009), but in generally theriogenologists prefer balanced polyionic fluids for lavage such as lactated Ringer solution (Scoggin 2016: 503). It is extremely important for practitioners to know that unreasonable usage of intrauterine antiseptics can cause damaging adverse reactions including inflammation, discomfort, necrosis and even reproductive sterility (Causey 2006). 0,05% povidone-iodine solution can safely be used in uterine lavage, but higher concentrations can cause serious reactions and inflammatory response to endometrium (Brinsko 2001).

Uterus is commonly flushed with 2-4L once or twice a day, but the used volume and frequency of flushes is dependent of the severity of endometritis (Scoggin 2016: 503). In fungal infections, if mare has not resolved the problem spontaneously during 1-2 cycles, the uterine lavage is recommended (LeBlanc 2009). Uterine lavage should be performed with proper disinfectant for 5-7 days (LeBlanc 2009).

### 1.3.4 Ecbolics

Systemic administration of ecbolic drugs should be used in combination with uterine lavage (Pasolini et al. 2016: 309). Most commonly used ecbolic agents are oxytocin and cloprostenol (Canisso et al. 2016: 475). Oxytocin may not be effective in all mares and in these cases cloprostenol might be more beneficial (LeBlanc, McKinnon 2011: 2629-2630). These drugs are used to avoid intrauterine fluid accumulation as they increase the uterine clearance, stimulate myometrial contractions and promote lymphatic drainage from the uterus (Canisso et al. 2016: 475). Long-acting oxytocin analog carbetocin has also become available recently but more data is still needed before specific recommendations for clinical practice can be provided (Canisso et al. 2016: 476).

### 1.3.5 Immunomodulators

The aim of using immunomodulators in susceptible mares is to modulate PMIE (Bucca et al. 2008). Bucca et al. concluded in their research that administration of dexamethasone at the time of breeding improved the pregnancy rates significantly in susceptible mares if they had 3 or more risk factors present (Bucca et al. 2008). Papa et al. achieved remarkable increase in pregnancy rates by administrating prednisolone before AI to mares with prior history of post-insemination endometritis (Papa et al. 2008). However, the use of
corticosteroids should always be based on patient selection because it may have side effects such as laminitis and muscle wasting (Scoggin 2016: 505).

Platelet-rich plasma (PRP) is also known to work as a immunomodulator and it has been researched in different fields of equine medicine. Reghini et al. carried out a research in interest to find out the effect of PRP to uterine inflammation in mares suffering from chronic degenerative endometritis. Treatment modulated the inflammatory response and reduced the influx of PMNs and intraluminal fluid accumulation in these mares. (Reghini et al. 2015)

1.3.6 Antimicrobials

In equine reproduction there is not much scientific data available concerning efficacy of different antimicrobial treatments (Pyörälä et al. 2014). This applies also to antimicrobial treatment of bacterial endometritis (LeBlanc 2008). Currently treatment recommendations are mostly based on clinical experience (Pyörälä et al. 2014). The antimicrobial agent should be chosen according to culture and sensitivity results (LeBlanc 2008).

1.3.6.1 Intrauterine antimicrobials

Chronic endometritis in mares requires local antimicrobial treatment (Pyörälä et al. 2014). Usually mares with bacterial endometritis are treated with intrauterine antibiotic 3-5 days during the estrus (LeBlanc, Causey 2009). However, treatment length should be individually tailored for each mare based on isolated bacteria, chronicity of infection, mares ability to clear intraluminal fluid and the history of mare (LeBlanc 2008). Causey prefers to avoid intrauterine antibiotics if flora changes from cycle to cycle or culture reveals mixed flora but no pathogens (Causey 2006).

Any products administered into the uterus needs to be water soluble and non-irritant (Katila 2016). According to this trimethoprim-sulfonamide, tetracyclines and enrofloxacin can’t be administrated into the uterus (Katila 2016). Suitable for intrauterine use are amikacin, ampicillin, ceftiofur, gentamicin, neomycin, potassium penicillin, ticarcillin and ticarcillin-clavulanic acid (LeBlanc 2009). However, it is recommended to keep 3rd generation cephalosporins including ceftiofur as reserve drugs and avoid the use (Katila 2016). Amikacin and gentamicin, which have low pH, need to be buffered before intrauterine use (Katila 2016; LeBlanc 2009). Ampicillin can also be irritable and the use is recommended at high dilutions (LeBlanc 2009). In fungal infections antifungal agents which can be
administered in to the uterus include clotrimazole, miconazole, nystatin, amphotericin b and fluconazole (LeBlanc 2009).

Multiple antibiotics and antiseptics should not be mixed together as the therapeutic value is unknown and use can be harmful (LeBlanc 2009). It is important to demonstrate uterine lavage before administration of intrauterine antibiotics in order to remove all material that might influence the efficacy of chosen antimicrobial agent (LeBlanc 2008; Pyörälä et al. 2014).

There are certain complications associated with intrauterine antibiotic treatment. Mares may fail to resolve the infection or treatment can cause serious endometrial irritation. Secondary bacterial or fungal infection is also known complication. When treatment is pointed to one pathogen it may allow multiplication of others, much more severe pathogen. (LeBlanc 2009)

**1.3.6.2 Systemic antimicrobials**

Systemic use is only option when antibiotic treatment is needed after ovulation (Pyörälä et al. 2014). Systemic use have certain advantages compared to intrauterine usage (Causey 2006). The use of systemic antibiotics is not reliant of estrus cycle and it also allows longer treatment periods (Causey 2006; LeBlanc 2009). Superinfections are also less likely with systemic administration of antibiotics than with intrauterine use as vaginal flora is not disturbed (LeBlanc, Causey 2009). For example Canisso et al. prefer to use systemic antibiotics when possible to treat endometritis as repeated intrauterine antibiotic infusions predispose the mare to fungal infection (Canisso et al. 2016: 472). Systemic administration minimizes the risk of contamination and it can also achieve minimum inhibitory concentrations in endometrium (Causey 2006). Even though the intrauterine use of trimethoprim and enrofloxacin is forbidden, both can be used systemically when needed as they provide high concentrations also in uterus (Katila 2016). Systemic antibiotics that are commonly used in treatment of endometritis include e.g. amikacin, ampicillin, ceftriaxone, enrofloxacin, gentamicin, potassium penicillin, procaine penicillin and trimethoprim-sulfamethoxazole (Canisso et al. 2016: 473).

Chronic infectious endometritis and defects in caudal reproductive tract are reasons to consider systemic use of antibiotics (LeBlanc 2008). In fungal infections, long treatment is many times needed and systemic administration may be required (Katila 2016). In fungal infections local treatment can be continued with per orally administrated itraconazole,
fluconazole or ketoconazole for two to three weeks more when necessary (Katila 2016). When systemic antimicrobial treatment is used to treat bacterial or fungal endometritis uterine lavage should also be performed for 3-5 days as exudate and bacteria needs to be removed (LeBlanc, Causey 2009).

1.3.6.3 Evaluation of antimicrobial treatment

The treatment is most commonly unsuccessful in cases of chronic gram-negative infections and fungal infections. In chronic infections uterus should be evaluated on the last day of treatment and continue the treatment if necessary. Treatment outcome is assessed at the next estrus. If outcome is undesirable, background of ineffectual treatment needs to be detected and corrected. Reasons that may lead to treatment failure are drug resistance, wrong dosage, inappropriate microenvironment and superinfection. Host’s impaired defense mechanisms and constant uterine contamination due to loss of anatomical barriers are also associated to treatment failure. (LeBlanc 2009)

1.3.7 Other intrauterine treatments

Mucolytics and solvents have been added to lavage solutions in order to facilitate uterine clearance and resolve exudate, mucus and biofilm (LeBlanc, Causey 2009).

Gores-Lindholm et al. concluded in 2013, that use of 3,3% N-acetylcysteine is not irritable and decreased the extracellular mucus thickness. In pluriparous mares, with history of mucus hypersecretion but no pathogens, the use of 3,3% N-acetylcysteine solution combined with uterine lavage, oxytocin and if necessary antibiotics, had potentially positive impact on pregnancy rates. (Gores-Lindholm et al. 2013)

Also buffered chelators may be beneficial, especially in cases of unmanageable bacterial or fungal infections (Scoggin 2016: 506). For example EDTA-Tris is known to potent antimicrobials, break up biofilms and dissolve exudate (Katila 2016). Lyle et al. treated eight reproductively normal mares with intrauterine buffered chelators and didn’t reveal any negative effect to endometrium in biopsies (Lyle et al. 2011). Chelators are used in large volumes to obtain bacteriosidic effect (Katila 2016) and can be combined with antimicrobial agents if needed (Lyle et al. 2011).
1.3.8 Management and feeding

Interaction between mare and stallion after breeding might have positive impact on uterine activity and clearance (Troedsson et al. 2008). Also exercise have suggested to be beneficial for uterine drainage as movement is associated to increase the intra-abdominal pressure and through that support uterine clearance (LeBlanc 2008).

There is also data available that omega-3 fatty acid supplementation might be beneficial to susceptible mares. In the research carried out by Brendemuehl et al. algae sourced N-3 fatty acid supplement was given per orally to susceptible and resistant mares after they had been inseminated with frozen semen. Autors reported significant reduction in post-breeding inflammatory response. (Brendemuehl et al. 2014)
2. AIM OF THE STUDY

The aim of the study was to observe the diagnosing and treatment protocols of equine infectious endometritis through a case series in one Estonian practice and bring forward possible limitations or deficiencies in this practice if discovered. Also, the infectious endometritis as a complex was in the interest - how many mares were infected, what was the outcome of treatment and were these mares able to become successfully pregnant and compare the statistics and findings to literature.
3. MATERIALS AND METHODS

3.1 Materials

The data was collected during breeding season 2017 and was based on one Estonian equine practitioners breeding datasheet. Observation of clinical practice and diagnostic procedures were done in spring 2018. Also, some additional treatment results were added in spring 2018, including follow-up of foalings and result from control sample.

3.1.1 Practice

Veterinarian works in ambulatory practice across the Estonia. There is no stationary clinic or laboratory in use. All the procedures are performed in studs. All needed equipment, e.g. microscope and laboratory accessories, are carried along. Veterinarian focuses on gynaecology and reproduction, so also challenging cases from other veterinarians are referred to this practice. Veterinarian provides reproductive management support, education on natural covering, artificial insemination services (e.g. fresh/cooled/frozen semen) and fresh embryo transfers.

3.1.2 Animals

In total 76 mares were included in the research. Their age varied between 3 to 19 years. These mares were mostly warmblood sport horses (74), in addition one riding pony and thoroughbred were included. In veterinarian’s database mares were divided in groups according to their previous reproductive status as aborted (5), barren (12), not bred (12), maiden (19) and foaled (28). The body condition was also determined before breeding season- in year 2017, 1 mare was fat, 4 mares were very thin, 9 mares were thin and the rest of the mares were in good body condition.

Mares were located across the Estonia in different studs and were divided in several counties, e.g. Saare county, Harju county, Viljandi county and other counties. However, regardless the different locations, according to veterinarian the management and feeding systems are quite similar. During the breeding season mares live outside and are kept on the pasture. Additional
feed (own produced grain/commercial feed), vitamins and minerals are given daily. If mare showed no signs of heat, but corpus luteum and follicle were found, cloprostenol was used to induce the oestrous cycle. Human chorionic gonadotropin (hCG) was used to induce ovulation, when timed ovulation was needed. It was used when mares were confirmed to be in heat and follicle > 3,5cm. Mares, bred with frozen semen, were monitored after every 12h to detect the ovulation. If fresh or cooled semen was used, monitoring was performed every 24h. Mares were vaccinated against equine herpesviruses.

In season 2017 several breeding methods were used. 20 mares were covered naturally. 11 mares were inseminated with fresh semen- the semen was collected and evaluated on the stud and mares were inseminated immediately without processing the semen. Three mares were inseminated with cooled, processed semen imported from Holland or France. Most of the mares (40) were inseminated with frozen semen produced in different European breeding stations. All semen batches fulfilled the requirements of European legislation. Two mares were not inseminated due to severe uterine problems.

### 3.1.3 Diagnostic procedures

In researched practice the diagnose was first based on transrectal ultrasound examination. When intraluminal fluid was detected it was measured - if there was any/more than 0,1 cm of intraluminal fluid, endometritis was suspected. The following treatment or diagnostic protocol was dependent on mare’s previous history, owner’s expectations and other findings (e.g. ovarial activity, consistence of vaginal mucus).

Mares were prepared for sampling with moist cleansing wipes and the whole perineal area was wiped. Alcohol was many times used in last wiping. Tail was usually wrapped, but not held up every time.

Endometrial samples for cytological examination were collected using an endometrial brush (Uterobrush; Medscand Medical, Malmö, Sweden) attached to a stainless steel device for use in cows and mares (European Patent EP2029026B1). Slides for cytological examination were prepared by rolling the Uterobrush onto a clean microscopical glass slide, left airdry and then stained with Diff-Quik (Dade Behring Inc, Germany). The number of PMNs per 10 fields was evaluated microscopically under x400 magnification. If > 1 PMNs was detected before insemination, cytology was estimated as positive. Samples were evaluated during the visits in studs.
For the bacterial culture, the sample was obtained with either double guarded swab (Minitube, Tiefenbach, Germany) or human tampon. Method was chosen depending on the amount of fluid detected by ultrasound evaluation. Tampon was used when the amount of fluid was remarkable. After obtaining the sample, the fluid from tampon was squeezed in to syringe. When sample was obtained with swab, the cotton stick was cut and placed in to Amies Transport Medium. Collected sample (syringe or cotton swab), was sent to the laboratory. Sensitivity was done every time.

In Estonia, the results from culture take usually 4-5 days. In most cases systemic antibiotics and ecbolics were given during the waiting period. Intrauterine treatment was started later and based on laboratory results.

### 3.2 Methods

Researched data were collected from veterinarian’s database, EquiBreedVet, which is a special reproduction management programme for equine practitioners. Also, laboratory reports from mares with infectious endometritis (9) were included in researched material. Cultures were sent to three different laboratories- Viljandi County hospital, Synlab laboratory in Tallinn and Estonian Veterinary and Food Laboratory (VFL). The results from cytology and some of laboratory results were based on personal communications with veterinarian.

Data collected from EquiBreedVet was processed with Microsoft Excel. Processed data included the name of the mare, age, body condition, reproductive status, endometrial fluid, collected samples (culture/cytology), treatment, pregnancy, breeding method and foaling in 2018 (if information was available). Orientation in practice was done in spring 2018. Practice in general as well as diagnosing and treatment decisions were observed during the visits with veterinarian.
4. RESULTS

4.1 Mares

Mares were investigated in different groups based on their clinical findings. Division of mares, age and pregnancy numbers from different groups is demonstrated in Table 1.

From 76 mares, 45 were considered healthy and had no signs of uterine infection before or after mating. When entering the breeding season 2017, mares were divided as foaled (17), maiden (14), not bred (10), barren (3) and aborted (1). Pregnancy rate in this group was 84,4 % as 38 mares become pregnant (18 fs+nc=unprocessed semen, 27 df+cs=processed semen). However, 5 of them aborted later. Age varied between 3 to 19, average was 8,9 years. Still, 20 of these mares were treated with corticosteroids around the insemination as considered to be in risk group (e.g. maiden, > 14 years old, bred with frozen or cooled semen).

From 76 mares, in 30 transrectal ultrasound revealed intraluminal fluid and were suspected to have uterine environmental problem. From these 30 mares, 9 were sampled to specify the diagnose of infectious endometritis. Out of nine sampled mares, eight had positive cytology and culture and were diagnosed with infectious endometritis (IE). One mare had only positive cytology, but no bacteria detected in culture. This mare was diagnosed as urometra and had no infectious endometritis. The status division in mares with uterine environmental problems (21 mares with intraluminal fluid+one with urometra) was foaled (9), barren (4), maiden (4), aborted (3) and not bred (2). Average age was 10,9 years. Pregnancy rate in these mares with uterine endometrial problems was 63,6% (6 fs+nc, 8 df+cs). Two from these aborted later.

Nine sampled mares were diagnosed with infectious endometritis (IE). Eight of these had intraluminal fluid accumulation and one had severe uterine oedema. Before entering the season 2017 mares diagnosed with IE, were classified as aborted (1), barren (5), foaled (2) and maiden (1). Age varied between 5 and 17 years, average was 11,7 years. Mares were inseminated with frozen semen (4), fresh semen (1) and by natural covering (2). Two of the mares were not inseminated during the season 2017 due to severe uterine infection. Pregnancy rate in this group was 44,4 % as four mares managed to become pregnant ( 3 df, 1 nc). No abortions were reported. The youngest, 5 years old mare, gave birth to clinically
premature foal (30 days earlier). From the rest of three pregnant mare, the result of pregnancy was unknown due the late expected foaling date in summer 2018.

The frequency of IE in study population was 11.8 % as 9 mares from 76 were diagnosed with IE during the breeding season 2017.

**Table 1.** Division of mares in season 2017. age and pregnancy rates

<table>
<thead>
<tr>
<th>Group of mares</th>
<th>Number of mares</th>
<th>Pregnancy rate</th>
<th>Average age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy mares</td>
<td>45</td>
<td>84.4 %</td>
<td>8.9</td>
</tr>
<tr>
<td>Uterine problems</td>
<td>22</td>
<td>63.6%</td>
<td>10.9</td>
</tr>
<tr>
<td>Mares with IE</td>
<td>9</td>
<td>44.4%</td>
<td>11.7</td>
</tr>
</tbody>
</table>

4.2 Pathogens and treatment of IE

Most of mares revealed in microbial culture *E.coli* and *Streptococcus Zooepidemicus ssp. equi*. Fungal infections were caused by *Candida sp.* (G, H). Only two mares were diagnosed with strict pathogens (C, I). One mare (C) culture revealed *E.coli, Enterococcus aerogenes* and *Streptococcus dysgalactiae* and another had *Pasteurella spp* (I). From four mares (A, C, F, D) *Taylorella equigenitalis* was excluded and all the results came out negative. In fungal infections (G, H), one mare resolved the problem, other developed unmanageable pyometra and was excluded from breeding.

Five mares received cloprostenol to diminish uterine oedema and accelerate the uterine clearance. Ovulation was induced with hCG in seven mares. Uterine lavage was demonstrated only in two mares. One of the mares received intrauterine acetylcysteine (I). Most of the mares received antimicrobials - systemic or intrauterine according to the laboratory results and sensitivity. Intrauterine cefazolin was used in five mares and one received penicillin. Systemic antibiotics included trimethoprim sulfonamide, doxycycline and enrofloxacin. Also, systemic antifungal agent, ketoconazole, was used. Dexamethasone was given to seven mares. Local misoprostol and cloprostenol as well as mechanical drainage system was placed to one mare (H) due to completely cervical stenosis (See Figure 2). Individual treatment protocols of each mare are demonstrated in Table 2.
Figure 2. Customized mechanical drainage system was placed for one mare (H) to succour fluid drainage, as mare suffered from cervical stenosis and uterine clearance was restrained. (Photo: Ulrika Tuppits, 2017)
<table>
<thead>
<tr>
<th>Mare</th>
<th>Age</th>
<th>Status</th>
<th>Pathogen</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12</td>
<td>Maiden</td>
<td>Streptococcus Equi sp. zoopidemicus</td>
<td>Cloprostenol (IM), Dexamethasone (IM), Cephazolin (IU)</td>
<td>Pregnant Foal not born yet</td>
</tr>
<tr>
<td>B</td>
<td>17</td>
<td>Barren</td>
<td>E.coli, normal microflora</td>
<td>Dexamethasone (IM), Cephazolin (IU), Sulfamerazin-Trimetoprim (PO)</td>
<td>Pregnant Foal not born yet</td>
</tr>
<tr>
<td>C</td>
<td>14</td>
<td>Barren</td>
<td>1. Streptococcus dysgalactiae 2. E.coli, Enterobacter aerogenes, Streptococcus dysgalactiae</td>
<td>Cloprostenol (IM), Dexamethasone (IM), Cephazolin (IU), Oxytocin (IM), Doxycyclin (PO)</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>D</td>
<td>5</td>
<td>Foaled</td>
<td>Streptococcus Equi sp. zoopidemicus</td>
<td>Cloprostenol (IM), Cephazolin (IU)</td>
<td>Pregnant Premature foal</td>
</tr>
<tr>
<td>E</td>
<td>14</td>
<td>Barren</td>
<td>E.coli</td>
<td>Cloprostenol (IM), Dexamethasone (IM), Cephazolin (IU), Ringer Lactate (lavage)</td>
<td>Pregnant Foal not born yet</td>
</tr>
<tr>
<td>F</td>
<td>9</td>
<td>Foaled</td>
<td>Streptococcus Equi sp. zoopidemicus</td>
<td>Dexamethasone (IM)</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>G</td>
<td>10</td>
<td>Aborted</td>
<td>Candida sp.</td>
<td>Oxytocin (IM), Flukonazole (PO)</td>
<td>Not inseminated Sampled in spring 2018, sample clean</td>
</tr>
<tr>
<td>H</td>
<td>14</td>
<td>Barren</td>
<td>E.coli Fungal infection, Candida sp.</td>
<td>Cloprostenol (IM), Dexamethasone (IM), Oxytocin (IM), Penicillin G (IU), Misoprostol (cervix, local) Subsequent cycle: Flukonazole (PO), Ringer Lactate (lavage)</td>
<td>Not pregnant Unmanageable pyometra, excluded from breeding</td>
</tr>
<tr>
<td>I</td>
<td>10</td>
<td>Barren</td>
<td>Pasteurella sp.</td>
<td>Oxytocin, Enrofloxacin (PO), N-Acetylcysteine (IU)</td>
<td>Not inseminated</td>
</tr>
</tbody>
</table>
During the cycle when problem was detected, mare was sampled, and the sample was send to laboratory. In this cycle mare was not treated with intrauterine antibiotics, but ecbolics (cloprostenol/oxytocin) and in some cases systemic antibiotics were used. Intrauterine treatment was demonstrated during the next heat with antibiotics or with systemic antifungals (e.g. fluconazole). The choice of antimicrobial agent was based on the bacterial culture and sensitivity. In fungal infections sensitivity determination was not done, as it is not possible in used laboratories.
5. DISCUSSION

It has been suggested that 10% to 20% mares either have endometritis or are prone to it (Scoggin 2016:500). This number was represented also in our study as 11.8% from all the mares included were diagnosed with IE. Based on a literature endometritis, subclinical and clinical, is one of the leading causes of infertility in broodmares (LeBlanc, Causey 2009). In this study statistics promoting this fact, are pregnancy rates between healthy mares and mares with IE. In healthy mares pregnancy rate was 84.4% and in infected mares 44.4%. Also, the mares with any kind of uterine environmental problems had lower pregnancy rate compared to healthy mares (63.6%). Even though there are several factors influencing the pregnancy rates e.g. breeding manner, quality and type of semen and individual properties of mare, the results also from this study promote the fact that endometritis has remarkable influence in mare’s fertility and uterine health influences the mare’s ability to become pregnant. Unfortunately, at the time when study was completed, some foaling records remained unknown. However, the pregnancy in these mares was already so long expected, that it could be counted in this study as foaling. To achieve full records from this kind of study the follow-up period should be longer.

Based on the literature *Escherichia coli* and *Streptococcus equi ssp. zooepidemicus* are two most commonly isolated bacteria and are presented in 50-80% of bacterial endometritis cases (LeBlanc 2009). Also this number was similar in this study, as culture revealed E.Coli/Streptococcus equi ssp. or both in 7/9 of mares with IE (77.8%). The average age of healthy mares was 8.9 years and mares with IE 11.7 years. Even though this difference is not outstanding it is in the same line with the literature as mares suffering from chronic infectious endometritis are usually described to be > 12 years old (LeBlanc 2008). This study didn’t collect the information concerning perineal conformation and unfortunately included only one pony and not Estonian native breeds at all. It might have been interesting to see the difference in vulvar conformation and incidence of infectious endometritis in ponies compared to horses.

Knowing the reproductive history of clinically infertile mare is relevant part of diagnosing process (LeBlanc 2008), which was revealed also in this study. One mare was imported to Estonia from Europe some years earlier and the exact history of mare was unknown. It was
known that mare might had aborted earlier or delivered a foal. Mare had no intraluminal fluid prior to natural covering, however after that, ultrasound revealed 2-3 litre of intraluminal fluid and mare showed clinical signs of uterine infection. Mare was suspected to have CEM, but test of Taylorella equigenitalis came out negative. Uterine culture revealed *E. coli, Enterobacter aerogenes* and *Streptococcus dysgalactiae*. Result was confusing as *Streptococcus dysgalactiae* is commonly known pathogen in bovine mastitis. Sample was re-checked and Streptococcus dysgalactiae was found also in second sample. It might have been *Streptococcus dysgalactiae sp. equisimilis* which have been isolated from horses and is potentially pathogenic (Pinho *et al.* 2016), however the strain was not specified in this case. Mare didn’t manage to become pregnant and the origin of infection stayed unclear.

One mare with IE managed to become pregnant but delivered a premature foal. Placenta was clean and no signs of infection at the time of pregnancy were detected. Mare was young but had history of previous foaling. Based on the literature bacterial uterine infection is most commonly connected to early fetal losses, though also birth of a septic neonate is reported (Causey 2006). In this case the causality between IE and premature foal was unclear and not determined. Sending placenta to the pathological evaluation might had been beneficial.

Compared to literature the diagnosing procedures in researched practice are considered as evidence based. Perineal conformation is evaluated, and ultrasound is used for initiate diagnosing. In literature > 2cm depth of intrauterine fluid during the oestrus is associated with mares susceptibility (Brinsko *et al.* 2003). In researched practice veterinarian uses even more strict ultrasound evaluation (> 0,1cm) and mares showing any intraluminal fluid were not bred before problem is solved. This is partly due to owner’s expectations and the fact that it is pointless to waste valuable sperm and cause unnecessary costs. Based on this study, in Estonia owner’s seem to have high expectations and successful breeding/insemination resulting pregnancy is expected. Cultures and cytology are examined together as recommended in literature (LeBlanc, Causey 2009; Riddle *et al.* 2007). Even more reliable result especially in subclinical cases can be achieved, when both culture and cytology are obtained with cytobrush (Overbeck *et al.* 2011). In this year veterinarian has already started to use cytobrush for both culture and cytology, so this change has been already done.

Even though the basic diagnostic procedure is under control, no endometrial biopsies were taken. One of the reasons why biopsies were not taken was the owners attitude towards this method. Most of the owners experienced biopsy as risky and expensive method, without any
remarkable benefits. In these cases, the communication between owner and veterinarian is extremely important. Veterinarian should explain the owner importance of this diagnostic method and clarify what information it can give. Even though there are possible complications in this procedure, it can give important information when detecting deep degenerative or inflammatory changes (LeBlanc, Causey 2009) and potentially reveal bacteria deeper in the endometrial tissue (Ferris, 2016: 486).

In diagnostic process cytology is important, rapid and inexpensive method to gain valuable information before the result from culture is available (Ferris 2016: 487). In researched practice all the equipment, e.g. microscope, needs to be carried along and obtained cytology is evaluated on the stud. At the same time, many of the mares were referred to veterinarian from other stationary equine breeding stations, where laboratory equipment was on the place and yet no cytology was done. In researched practice veterinarian attempts to get proper diagnose and not inseminate without treatment, even though clinical procedures in ambulatory practice might be challenging.

To minimize the potential contamination when sampling, it is recommended to wrap the tail and lift it up, wash the perineal area three times with non-residual soap and then rinse properly (Ferris 2016: 482). Unfortunately, in researched practice, in many studs there is no rinsing water or drainage nearby the examination stocks and use of water might be difficult. However, it might be possible to demonstrate washing and use bucket for rinsing.

The lack of laboratories in Estonia, especially operating during the weekends, sometimes slows down the diagnosing process of bacterial culture. Dispatching the sample during the weekend is also challenging. Equine reproduction season is busy, and the work is done during the weekends and sometimes even night. Due all this and distant locations of studs, it can take several days before obtained sample reaches the laboratory and the result is available. In equine reproduction practice rapid result is needed as prolonged and slow diagnosing process may lead to situation where mare has already ovulated, and the cycle is missed (Ferris 2016: 486). This year veterinarian in researched practice has decided to test sample cultivation herself as this enables more rapid results and hopefully gives the opportunity to start treatment earlier if needed.

Single dose of dexamethasone at the time of insemination is described to be safe and effective way to improve the pregnancy rates in risk mares (Bucca et al. 2008). Also, in this practice veterinarian prefers to use glucocorticosteroids preventively once at the time of
insemination in mares that are in higher risk, e.g. maiden mares, mares > 14 years old and in mares bred with frozen or cooled semen. Dexamethasone is also used in mares that exhibit intraluminal fluid before mating. Veterinarian has experienced that this has positive impact on pregnancy rates.

Fungal endometritis can result from intra uterine antibiotic treatment (LeBlanc 2009). Also, in this study, both mares which were later diagnosed with fungal endometritis, were treated with intrauterine antibiotics prior to diagnose. One mare had history of receiving intrauterine amoxicillin due to puerperal metritis and the second mare got intrauterine penicillin for treatment of endometritis. It is not clear if the infection was already existing in these mares before or was it result from the treatment. First mare was re-sampled in spring 2018 and result came out clean. In this year mare is again planned to be bred. Unfortunately in fungal infections the prognosis is hesitant and treatment failure and relapses are common (LeBlanc 2008), as seen in the second mare in this study which developed unmanageable pyometra and was no longer suitable for breeding. Overall, the use of antimicrobials in researched practice was reasoned and was based on culture and sensitivity which reflects good veterinary practice.

Only two mares with IE were treated with uterine lavage. As uterine lavage is reported to beneficial in treatment of endometritis (Brinsko 2001), it might be good to demonstrate lavage more often in the future and also add it to the treatment protocols when intrauterine (Brinsko 2001) or systemic antimicrobial treatment (Leblanc, Causey 2009) is used. Also, ecbolics could be used more often. However, the treatment decision is every time made based on the individual findings of each mare, so there is no such a treatment plan that would be suitable for all mares.
6. CONCLUSIONS

This study pointed out similar findings concerning equine infectious endometritis as presented in literature. Equine endometritis is a complex disease, which influences mare’s fertility and is more often problem in older mares. Pregnancy rates are lower in infected mares compared to those with normal uterine health. Diagnosing process may be challenging and should include proper reproductive examination as well as laboratory samples. Most commonly seen pathogens are genital commensals including *E. coli* and *Streptococcus equi ssp. zooepidemicus*. Also, fungal infections appear, and they may result from antimicrobial treatment. From diagnosing and treatment point of view, knowing the mare’s reproductive history is important.

In researched Estonian practice diagnosing and treatment protocols were evidence based, however some improvement could be done. Biopsy, even though it is not necessary in all infected mares, might be added in diagnostic protocol when necessary. Treatment procedures were considered as evidence based, antimicrobial agents were chosen according to results from culture and sensitivity. Uterine lavage never the less, could be performed more often and also when mare is treated with systemic or intrauterine antimicrobials.
SUMMARY

Equine endometritis, inflammation of uterine inner layer, is described as one of the major causes of infertility in horses. Some mares are more susceptible to endometritis than others. Healthy mare can clear up the uterus when predisposed to microbes, but in susceptible mares this ability is impaired which may lead to uterine infections. Predisposal factors include different kinds of anatomical and other physical changes in genital tract. In Estonia, based on the information from Equine database of Estonian Agricultural Registers and Information Board, approximately 700 foals are registered. According to this it is likely that endometritis is an issue concerning also the mare population in Estonian and is diagnosed and treated from behalf of Estonian veterinarians.

Study was based on one Estonian equine practitioner’s breeding datasheet gathered in season 2017. The aim was to observe diagnosing and treatment protocols of infectious endometritis in this Estonian practice, follow the treatment outcome and study the complex of this disease as whole. Veterinarian uses EquiBreedVet database, which is special management program for equine practitioners. The data was collected from program and processed with Microsoft Excel. From each mare collected data included the name of the mare, age, body condition, reproductive status in year 2017 (foaled, maiden, aborted, barren, not bred), endometrial fluid (possible sign of uterine pathology), collected samples (culture, cytology), treatment and was the pregnancy successful or not. Also breeding methods were reported (natural covering, artificial insemination fresh/cooled/frozen semen). Accompanying laboratory results were included in the study. Observation and orientation of researched practice and used diagnosing and treatment protocols was done in spring 2018 and at the same time it was possible to gain more information of mares from previous year, e.g. re-sampling result, foaling data.

In breeding season 2017 in total 76 mares were intended to be bred in this veterinarian practice. From those mares 45 were healthy and had no signs on uterine pathologies. Variable intra uterine pathologies and uterine problems were diagnosed in 22 mares but didn’t had infectious endometritis. During this breeding season nine mares were confirmed with laboratory results to have infectious endometritis. Based on the literature endometritis concerns 10-20% of broodmares, which was in same line in this study (11,8%). Infections
were mostly caused by genital commensals *E.coli* and *Streptococcus equi ssp. zooepidemicus*. Also, other pathogens were found – *Enterococcus aerogenes, Streptococcus dysgalactiae ja Pastorella ssp.* Fungal infection caused by *Candida ssp.* developed in two mares. Pregnancy rates in infected mares was considerably lower compared to healthy mares (44.4 vs. 84.4%). Based on the collected data was also found that infected mares were also older (8.9 vs. 11.7 years) which also mentioned in the literature.

Diagnosing and treatment protocols in this Estonian practice were similar for those presented in literature. Initial diagnose was based on clinical examination and ultrasound evaluation. When intraluminal fluid was detected or there were other findings which may had reflect uterine infection, the necessary laboratory sampling was performed. Cytology was done on the stud and if confirmed positive (polymorphonuclear neutrophils detected), sample was sent forward to some of Estonian laboratories for bacterial culturing and sensitivity determination.

In the cycle when problem was detected, no uterine medications were administrated when waiting for laboratory results. In that precise cycle mares were treated only with systemic antibiotics ecbolics (oxytocin, cloprostenol). During the next cycle mares were treated with intrauterine antibiotics or systemic antifungals. Antimicrobial treatment was based on sensitivity of pathogen which reflects good veterinary practice. In researched practice biopsy was not used in diagnostic protocols.

Pathological findings and frequency of infected mares were similar compared to literature. The difference in pregnancy rates between healthy and infected mares was expected. Study revealed that Estonian horse owners have high expectations for positive result from breeding. Based on this, it necessary for veterinarian to do accurate decisions in order to achieve the right and optimal time for breeding.

Study revealed some disadvantages in veterinarian’s work in this practice. Biopsy could be included in diagnostic protocols. Even though biopsy is not necessary in all infected mares, it is valuable diagnostic tool especially in more chronic cases and in infertile mares as it provides important information of uterine health. What concerns the treatment, the uterine lavage is used quite rarely, so it could be added to treatment procedures more often. Uterine lavage is important also in combination of antimicrobial treatment.
LIST OF REFERENCES


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HOBUSTE ENDOMETRIIDI DIAGNOOSIMINE JA RAVI – EESTI PRAKTIKA

ÜLDKOKKUVÕTE

Hobuste endometriit, ehk emaka sisekesta põletik, on kirjeldatud kui ühte peamist sigimatuse põhjust hobustel. Osad märädest on tundlikumad endometriidi suhtes kui teised. Terve mära on võimeline ise emaka puhastama, kui tema emaka valendikku satuvad mikroobid, kuid tundlikel märadel isepuhastusvõime on oluliselt nõrgem. See toob kaasa põletikulised seisundid. Soodustavateks teguriteks on suguelundite anatoomilised iseärasused ning muud suguelundite füsioloogilised muutused. Tuginedes Eesti Põllumajanduse Registrite ja Informatsiooni Ameti (PRIA) andmetele registreeritakse Eestis igal aastal umbes 700 varssa sünd. Sellele põhinevalt on tõenäoline et endometriit puudutab ka Eesti märade populatsiooni ja seda diagnoositakse ning ravitakse Eesti loomaarstide poolt.


Aastal 2017 käsitleti loomaarsti praksises kokku 76 sugumära, kelle eesmärk oli tiinestumine. Nendest 45 olid terved ning emakapatoloogiatele viitavaid muutusi ei
avastatud. Erinevad emakasisesed patoloogiad ja emaka probleemid avastati 22 märal, kuid infektsioosset emakaspilettiku nendel ei esinenud. Selle hooaja jooksul üheksa mära sai laboratoorselt kinnitatud infetsioosse endometriidi diagnoosi. Endometriiti esineb kirjanduse andmetel 10-20% sugumäradest, mis langeb kokku uuringutulemustega (11,8%). Infektsioonitekitajad olid peamiselt märade genitaaltrakti kommensaalide hulka kuuluvad E.coli ja Streptococcus equi ssp. zooepidemicus. Leiti ka teisi patogeene – Enterococcus aerogenes, Streptococcus dysgalactiae ja Pastorella ssp. Kahel märal tekkisid seeneinfektsioonid, mille põhjustaks olid Candida spp. Tiineestumine haigestunud märadel oli tunduvalt halvem kui tervetel märadel (44,4% vs 84,4%). Kogutud andmete põhjal leiti, et endometriidiga märad olid vanemad (8,9 vs 11,7a) nii nagu kirjanduses on kirjeldatud.


Eesti praksises patoloogilised leidud ning haigete märade osakaal vastas kirjanduses kirjeldatud. Tervetel märade tiinestumisega kinnitatud erinesid haigete omadest, mis oli samuti ootuspärane. Uuringu käigus selgus, et Eesti hobuseomanike ootused positiivsele seeemendustulemusel on väga kõrged, selle tõttu teeb loomaarst rangeid valikuid. Antimikrobiaalne ravim põhines tundlikkusele, mis on hea veterinaarse tava töötamise viis. Uuritud praksises biopsiat diagnoosimise meetodina ei kasutatud.


Eesti praksises patoloogilised leidud ning haigete märade osakaal vastas kirjanduses kirjeldatud. Tervetel märade tiinestumisega kinnitatud erinesid haigete omadest, mis oli samuti ootuspärane. Uuringu käigus selgus, et Eesti hobuseomanike ootused positiivsele seeemendustulemusel on väga kõrged, selle tõttu teeb loomaarst rangeid valikuid, millal ta otsustab seeemendada.

Uuringu käigus selgusid ka mõned puudused loomaarsti töös. Biopsia võiks lisada diagnoosimise skeemi. Sõltumata sellest, et biopsia ei ole vajalik iga patsiendi puhul, on meetodi abil võimalik saada olulist informatsiooni emakasisesest terveisest, eriti kui probleem on olnud pikemaaja ja sigimattuse põhus on teadmata. Ravimeetodite valiku
osas, võrreldes kirjanduses käsitletule, emakaloputusi teostati üsna harva, ehk nende osakaalu raviskeemis võiks suurendada. Emakaloputus on oluline ka siis kui antimikrobiaalne ravi on kasutusel.
APPENDIXES

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