TREATMENT AND PREVENTION OF ENDOMETRITIS IN MARES

Word count: 20482

Torge Adetunji Idowu
Student number: 01812027

Supervisor: Prof. dr. Peter Daels
Supervisor: Prof. dr. Bogado Osvaldo

A dissertation submitted to Ghent University in partial fulfilment of the requirements for the degree of Master of Veterinary Medicine

Academic year: 2020 - 2021
Ghent University, its employees and/or students, give no warranty that the information provided in this thesis is accurate or exhaustive, nor that the content of this thesis will not constitute or result in any infringement of third-party rights.

Ghent University, its employees and/or students do not accept any liability or responsibility for any use which may be made of the content or information given in the thesis, nor for any reliance which may be placed on any advice or information provided in this thesis.
Preface

I want to thank my Supervisor, Prof. dr. Peter Daels for the support and advice for writing this thesis. A special thanks to Machteld van Heule for always responding quickly, her effort, proofreading, constructive advice, and tips.
# Table of contents

1. Summary .............................................................................................................. 4
2. Introduction .......................................................................................................... 5
3. The healthy mare: Acute postmating endometritis ........................................... 6
   3.1. Anatomy of a healthy mare ........................................................................... 7
   3.2. Hormones and their effects on the uterus .................................................... 8
   3.3. Immune response of the uterus .................................................................. 9
4. Pathological Endometritis .................................................................................. 10
   4.1. Predisposing factors for endometritis ........................................................ 11
5. Persistent postbreeding endometritis ................................................................. 15
   5.1. Diagnosis of persistent postmating endometritis ...................................... 15
   5.2. Treatment of persistent postmating endometritis ...................................... 16
   5.3. Management of the mare with persistent postmating endometritis ......... 21
6. Infectious endometritis ....................................................................................... 22
   6.1. Bacterial infectious endometritis .................................................................. 23
   6.1.1. Diagnosis of chronic infectious endometritis ......................................... 24
   6.1.2. Treatment of chronic infectious endometritis ....................................... 29
   6.2. Fungal endometritis .................................................................................... 34
   6.2.1. Diagnosis of fungal endometritis ........................................................... 35
   6.2.2. Treatment of fungal endometritis ........................................................... 36
   6.3. Prevention and management of chronic infectious endometritis ............. 37
7. Chronic degenerative endometritis ................................................................. 39
   7.1. Diagnosis of chronic degenerative endometritis ....................................... 39
   7.2. Treatment of chronic degenerative endometritis ....................................... 39
8. Contagious equine metritis (CEM) ..................................................................... 41
   8.1. Diagnosis of contagious equine metritis (CEM) ........................................ 41
   8.2. Treatment of contagious equine metritis (CEM) ........................................ 42
9. Treatment of conformational abnormalities ...................................................... 44
   9.1. Caslick’s surgery ......................................................................................... 44
   9.2. Vestibuoplasty ............................................................................................. 46
   9.3. Perineal Body Transection ......................................................................... 47
   9.4. Episioplasty ................................................................................................ 47
   9.5. Urethral extension ....................................................................................... 48
   9.6. Surgery of cervical lacerations .................................................................... 48
10. Discussion ........................................................................................................... 50
Bibliography .......................................................................................................... 51
1. Summary

Equine endometritis is an inflammatory reaction to contamination present in the uterus' lumen (Christoffersen and Troedsson, 2017). Uterine contamination can result from different aetiologies, including pathogenic organisms, the effects of a pneumovagina and urovagina, and foreign objects (Brinsko, 2011b; Brinsko, 2011g). Self-contamination can occur due to suboptimal conformation of the reproductive tract, such as poor perineal and vulvar conformation but also inadequate closure of the cervix. In addition to these sources of contamination, external factors can contribute to uterine contamination, including low hygiene standards leading to contamination during the parturition, reproductive examination, or artificial and natural insemination (Brinsko, 2011a).

Endometritis is a complex issue because, in addition to the physiological response called acute postmating/postbreeding endometritis (APME), researchers also suggest that pathological endometritis can decrease the chance of normal development of the pregnancy (Dargatz et al., 1991). These types of endometritis are called “persistent endometritis” and can be divided into “persistent postmating endometritis (PPME)” and “chronic infectious endometritis (CIE)” (Brinsko, 2011a). Other endometritis types are endometriosis, previously called chronic degenerative endometritis, and contagious equine metritis (CEM) (Mair, 2013).

The clinical signs of the different types of endometritis are good indicators to establish a diagnosis. More specific symptoms, such as uterine fluid or abnormal edema, can be detected by ultrasonography or rectal palpation (Samper, 2009; Mair, 2013b). Additionally, cytological and bacterial analyses of uterine swabs or histological analyses of uterine biopsy are suitable diagnostic tests (Mair, 2013b). The proposed treatments include uterine lavage, systemic or local antibiotics, antifungal antibiotics, or using hormones like prostaglandin or oxytocin depending on the mare's reproductive status (Mair, 2013b; Dascanio; McCue, 2014c). It is essential to approach conformational abnormalities appropriately to treat and prevent the process of endometritis. For this purpose, different techniques are developed, including, amongst others, the Caslick’s surgery (Brinsko, 2011b).

This literature study aims to combine literature that employs diverse angles and approaches to studying endometritis in mares. In the following paragraphs, the mare’s physiological mechanism of maintaining a healthy endometrial environment will be discussed, followed by reviewing different endometritis types and their specific diagnostic, therapeutic and preventive possibilities.
2. Introduction

The science of equine reproduction is continuously evolving, established paradigms are being challenged, innovative methods are being developed, and just like in many other medical fields, opinions differ and context matters. Equine reproduction has become a professionalized sector where financial interests increasingly drive decisions. Still, there are emotional and ethical aspects as well, all of which a veterinarian needs to balance. Many different factors play a role in the success rate in equine reproduction. These factors can be minor or major, but they interact in a complicated way. One crucial factor is the fertility of the mare (Brinsko, 2011a). Subfertility or infertility can be caused by different factors, including abnormalities of the reproductive tract and uterine infections, leading to varying types of endometritis (Buczkowska et al., 2014). Optimal mare fertility requires a balance between allowing the natural endometrial immune response to react to exposure to allogenic material as well as preventing its progression to endometrial infections or other uterine abnormalities (Morris et al., 2020). Consequent and successful prevention and management of uterine diseases are critical elements of reproductive practice (Samper et al., 2007c).

Equine endometritis concerns those mares that show an inflammatory reaction to uterine contamination, which can be a significant cause of reduced fertility in equine reproduction. Although endometritis is a physiological process that is important during the development of a healthy pregnancy, endometritis also constitutes the number one cause of subfertility and the third most common disease affecting horses (Traub-Dargatz et al., 1991; Troedsson, 1999). Uterine contamination can lead to pathological types of endometritis. It may result from different aetiologies, including bacterial contamination due to a pneumovagina, a urovagina or the presence of foreign objects. External factors leading to endometritis include low hygiene standards during reproductive procedures like parturition, reproductive examination, natural and artificial insemination. Self-contamination can occur due to the mare's reproductive tract's suboptimal conformational characteristics, such as a poor perineal, vaginal, vestibulovaginal or cervical conformation (Brinsko, 2011a). Most uterine infections stay located in the endometrium and result in endometritis, but in rare cases, the infection can progress into deeper uterine tissues. When the infection spreads into the myometrium, metritis can develop, and in the worst case, it can result in perimetritis and peritonitis (Brinsko, 2011b).

The diagnosis of different kinds of endometritis consists of the combination of factors in the reproductive history, clinical signs, and diagnostic tools, including cytological and histological examination of the endometrium. Depending on the country and preference of the practitioner, treatments such as the use of ecbolics, uterine lavage with variable solutions and the administration of various medications such as antibiotics or anti-inflammatory products may be used. Further, there are multiple possibilities to correct conformational abnormalities (Brinsko, 2011b).
3. The healthy mare: Acute postmating endometritis

During natural service or artificial insemination, the semen is deposited directly into the uterus (Troedsson 1999), whereby the mare’s genital tract encounters substances like semen components of the stallion with all its natural or artificial by-products and possibly contamination by bacteria and debris (Brinsko, 2011b). A reproductively healthy mare shows a physiological transient inflammatory reaction called acute postmating/ postbreeding endometritis (APME/ APBE). According to Leblanc et al. (1994) and Brinsko (2011b), APME should be resolved within 24 to 48 hours in a reproductively sound mare. If this is not the case, the mare may be considered susceptible to endometritis (Leblanc et al., 1994). More recently, it is claimed that a mare is deemed susceptible to endometritis if she cannot resolve APME within 72 hours instead of 48 hours (Christoffersen et al., 2017). The acute uterine response peaks at about 6 hours, in which plasma proteins and neutrophils move into the uterine lumen. After about 12 to 24 hours, uterine cultures are usually negative (Samper et al., 2007j). This mechanism is vital for the arrival of the embryo, which will enter the uterus about 5 to 6 day after the ovulation. Therefore, a healthy and suitable uterine environment must be created (Brinsko, 2011a). In the past, it was accepted that the inflammatory response to breeding was due to bacterial contamination of the uterus, but now it is reported that the spermatozoa themselves are responsible for the acute inflammatory response (Samper et al., 2007g; Chandrasekharan, 2004).

The intensity of the reaction is dependent on the concentration and volume of the inseminate, to which highly concentrated (frozen) semen induces a more vigorous inflammatory response than fresh or extended semen (Samper et al., 2007g).

Clinical signs of acute postbreeding endometritis include fluid accumulation in the uterine lumen and slight endometrial edema, which can be detected by ultrasonography examination (Samper et al., 2007f). Small amounts of intrauterine fluid are often seen in reproductive active mares even in the absence of APBE, but most of the contents should be vanished by the moment of the embryo’s arrival (Brinsko, 2011a; Brinsko, 2011h). Sometimes discharge in small volume is appearing from the genital tract (Ferris et al., 2015).

The mare’s physiological mechanism (APBE) to ensure a healthy uterine environment consists of a combination of anatomic and mechanic factors and hormonal activity (Brinsko, 2011b). Different levels of various hormones lead to contractions of the myometrium (Samper et al., 2007j). Uterine contractions stimulate the elimination of excessive semen, bacteria, and inflammatory by-products after breeding, which could interfere with the embryo’s vitality and implantation (Christoffersen and Troedsson, 2017). The myometrium’s smooth muscle contractions are regulated by hormones like progesterone, prostaglandins, oxytocin, and estrogens (Brinsko, 2011a; Brinsko, 2011c).

Due to different predisposing factors, including conformational changes and a decreased uterine clearance, older mares are less resistant to infection and take longer to eliminate irritants from the uterus than young and healthy mares (Samper et al., 2007j).
3.1. Anatomy of a healthy mare

The first-line defence mechanism of a healthy genital tract is specifically designed to prevent uterine contamination and consist of physical barriers (Samper et al., 2007j). Therefore, three anatomic structures usually act like one-way gates to improve the efficiency of the uterine clearance. Starting from the outside, the first barrier is the vulva, followed by the vestibulovaginal sphincter (Mair, 2013a). Finally, the last anatomic structure is the cervix. These anatomic barriers are ideally situated to improve uterine clearance. They are positioned in a downward position towards the outside to work with gravity rather than against it (Brinsko, 2011e) and ideally prevent faeces, air, and environmental bacteria from ascending into the reproductive tract (Samper et al., 2007j).

Firstly, the vulva in a healthy and young mare should be in a nearly vertical position with at least 70% of the vulva ventral to the brim of the pelvis (Brinsko, 2011e; Brinsko, 2011g). This orientation allows the passing of faecal material without adhering to the vulva (Samper et al., 2007j). The muscular tone must be sufficient in sealing the genital tract to prevent aspiration of air and ascending contamination of the vestibulum or vagina (Brinsko, 2011g; Dascanio and McCue, 2014g).

Second, the vestibulovaginal sphincter creates the junction between the vestibulum and vagina. This structure acts as a protection to ascending contamination or aspiration of air (Brinsko, 2011e). The vagina of the mare is quite elastic but is usually collapsed except during breeding and foaling to prevent the passage of ascending contaminants (Brinsko, 2011e).

In addition to that, the cervix isolates the uterus from the external environment (Sjaastad, 2010). In natural breeding, the cervix will accommodate the expanded glans penis of the stallion at estrus to allow the deposition of sperm in the uterus. The cervix will close during pregnancy and diestrus to prevent ascending bacterial or fungal infections originating from the vagina or vestibulum (Brinsko, 2011b; Brinsko, 2011g). The cervix is very dynamic and internally lined by epithelium-secretory cells. The texture of the connective tissue is influenced by estrogen and progesterone. Cells, stimulated by estrogen, produce a thin, lubricating mucus during estrus and a thick mucus to occlude the cervix during diestrus (Brinsko, 2011e; Sjaastad, 2010).

The uterine wall consists of three layers which are the endometrium, myometrium and the perimetrium (Sjaastad, 2010). The endometrium is the innermost layer of the uterus and contains exocrine glands that produce nutritive secretions. Endometrial secretory activity is regulated by hormones like oxytocin, estrogen and progesterone (Brinsko, 2011c; Sjaastad, 2010). The myometrium contains an inner circular layer and an outer longitudinal layer of smooth muscle which are also affected by hormones from the ovaries and oxytocin (Sjaastad, 2010). The uterine wall contains lymphatic vessels which will drain the remaining uterine particulate material, edema and inflammatory cells after ovulation and cervical closure (LeBlanc et al., 1995).
3.2. Hormones and their effects on the uterus

Like nearly every natural process, APBE is likewise regulated by different hormones. In this case, hormones such as estrogen, prostaglandins 2α (PGF2α), progesterone and oxytocin are crucial elements in this hormonal complex playing an essential role in creating a suitable uterine environment. These hormones have direct and indirect effects on each other, on the mare’s reproductive tract characteristics and determine the uterine environment directly or indirectly (Brinsko, 2011c).

High progesterone levels will decrease the frequency of contractions, whereas low progesterone levels and a rise in estrogen will increase the contraction frequency. Additionally, prostaglandins will cause contraction of the myometrium by itself and the transition to estrus and subsequently lead to higher estrogen levels (Samper, 2009).

3.2.1. Estrogens
Firstly, estrogen is a hormone that plays an essential role in regulating the estrus cycle and the characteristics of the mare’s reproductive tract (Brinsko, 2011a). Production of estrogens occurs in the ovary, more specific in the granulosa cells of the developing follicle of cycling mares and the adrenal cortex (Brinsko, 2011a; Dascanio and McCue, 2014c). Amongst others, estrogen impacts the expression of the estrus, maturation of the reproductive tract, stimulation of follicular growth and secondary sex characteristics. When discussing endometritis, estrogens influence the reproductive tract, including cervical relaxation, improvement of the uterine circulation and uterine contraction (Sjaastad, 2010). Estrogen increases the resistance to uterine infections due to improved microbial clearance, resulting from a combination of the increased migrational capacity of neutrophils, neutrophil phagocytic or microbicidal ability and uterine physical clearance mechanisms. Estrogen is only useful in the absence of progesterone because progesterone has a dominant character on estrogens (Brinsko, 2011a; Brinsko, 2011c).

3.2.2. Progesterone
Another hormone is progesterone which is produced by the corpus luteum (CL) and the placenta. In the estrus cycle, secretion of progesterone culminates during diestrus because the corpora lutea are fully developed at this moment. The life span of the CL depends on the release of PGF2α from the endometrium, which will occur in bursts between 13 and 16 days after ovulation if no pregnancy is detected (Brinsko, 2011a; Brinsko, 2011f). Progesterone’s primary function is to create suitable conditions for the fetus’s growth and development in the uterus. Progesterone is dominant over estrogen and oxytocin and prevents estrus behaviour, suppresses uterine contractions by inhibiting the secretion of GnRH and prohibits ovulation of follicles. Also, it causes the cervix to close (Sjaastad, 2010).

3.2.3. Ecbolics
Furthermore, prostaglandin-F2α (PGF2α) and oxytocin belong to the group of ecbolics. PGF2α is a modified fatty acid synthesized and secreted from the endometrium during the second half of the luteal phase when no pregnancy is detected. Oxytocin secretion stimulates uterine PGF2α secretion, while pregnancy recognition substances inhibit its secretion. The presence of PGF2α will induce luteolysis.
and regression of the CL (Sjaastad, 2010). PGF2α will decrease the progesterone levels by luteolysis of the CL, leading to a transition to the estrus phase. PGF2α also causes myometrial contractions. A release of prostaglandins is part of the physiological reaction to uterine contamination (Brinsko, 2011a; Brinsko, 2011c).

Another ecbolic is oxytocin (Brinsko, 2011c). Oxytocin is released due to mechanical stimulation of the cervix and vagina. It initiates and stimulates myometrial contractions that need to be maintained for effective intrauterine fluid expulsion (Campbell & England, 2004; Risco et al., 2009).

### 3.3. Immune response of the uterus

A healthy mare reacts with an inflammatory response to uterine contamination. This reaction consists of the humoral defence mechanism and recruitment of polymorphonuclear cells (PMNs) for bacterial phagocytosis. The cell-mediated immunity is especially useful in confronting fungal endometritis. PMNs enter the uterus within 30 minutes after insemination, and the uterus produces fluid with chemoattractant properties for PMNs. Also, it is believed that spermatozoa initiate chemotaxis of PMNs through activation of complement (Nielsen et al., 2012). APBE usually lasts 24-48 hours and will ideally create an optimal uterine environment for the embryo’s arrival. The immune response reduces bacterial contamination of the uterus, improves pregnancy rates, and does not lead to illness symptoms in the mare (Bucca et al., 2008).

Seminal plasma has an immunomodulatory role in the endometrial response to spermatozoa. The presence of seminal plasma increases the expression of pro-inflammatory cytokines like IL-8 while suppressing others like TNF. Combined with spermatozoa, it facilitates the uterine clearance of polymorphonuclear cells by PMN chemotaxis, suppressing complement activation and phagocytosis. These effects will increase the elimination of contaminating bacteria, debris, seminal plasma, and excess spermatozoa from the uterine lumen (Brinsko et al., 2003; Troedsson et al., 1998). The seminal plasma has a low buffering capacity, and by-products rapidly accumulate in the plasma due to the high metabolic activity of the sperm cells. In frozen semen, the sperm concentration is higher, and during the freezing of sperm, seminal plasma is removed. The higher concentration and the absence of seminal plasma may be two reasons why the inflammatory response is more intense with frozen semen (Samper et al., 2007g).
4. Pathological Endometritis

In comparison to the physiological inflammatory response called APBE, it is reported that pathological endometritis can decrease the chance of normal development of the pregnancy due to a prolonged inflammatory reaction and fluid accumulation (Troedsson et al., 2001; Fumuso et al., 2007). The mare may even remain persistently infected on subsequent cycles (Canisso et al., 2020).

Pathological types of endometritis are called “persistent endometritis” and can be divided into “persistent postbreeding/ postmating endometritis (PPBE/ PPME)” and “chronic infectious endometritis (CIE)” (Brinsko, 2011b). The condition of a PPBE can predispose the mare to bacterial or fungal endometritis, leading to further degeneration of the endometrium (Morris et al., 2020). Other endometritis types are endometriosis, previously called chronic degenerative endometritis, and contagious equine metritis (CEM) (Mair, 2013b).

These main types of endometritis are associated with some significant consequences. They can lead to pregnancy failure, intrauterine adhesions, pyometra and other pathologies (Mair, 2013b). PPBE can lead to fluid accumulation within the uterine lumen because of delayed physical clearance of inflammatory material and additional increased fluid production. The pathological intrauterine fluid accumulation can adversely affect fertility by impairing spermatozoal motility and viability or inducing embryonic loss if the endometritis persists beyond day 5 or 6 postovulation when the embryo usually enters the uterine lumen. Also, PPBE can increase the sensitivity of the corpus luteum to PGF2α (Troedsson, 1997). Not only has the prolonged accumulation of fluid a negative impact on the spermatozoa and the embryo, but it is also shown that fluid that is retained in the uterus appears to lose its ability to support phagocytosis. In many cases, the fluid is free of pathogenic organisms and tests negative for bacterial growth and the presence of PMNs. Still, once the mare is bred, the fluid accumulation can be aggravated due to poor uterine clearance and contamination by bacteria. Bacteria can escape the immune reaction because neutrophils and bacteria will stratify to different suspension levels in the free fluid. Lymphatic drainage and mucociliary clearance are as well disrupted by intrauterine fluid accumulation. Nevertheless, chronic inflammation does not always require treatment because, in some cases, it does not appear to impair fertility as the early embryonic development can be supported and maintained (Samper et al., 2007g; Canisso et al., 2020).

Additional to the inflammatory reaction, other effects of endometritis can directly or indirectly lead to early embryonic death. The endometrial diseases are further classified as noninflammatory forms, including periglandular fibrosis and endometrial cysts, which have been considered an important factor in early embryonic and fetal death. The contact between uterine and placental tissue will be insufficient when the embryo is fixed in direct contact with a cyst or an area of fibrosis. Suboptimal communication will lead to inadequate nutrient exchange and hinder embryonic and fetal development (Samper et al., 2007e). Another noninflammatory form is a low progesterone production caused by the failure of maternal recognition of pregnancy due to uterine-induced luteolysis, fibrosis or cysts caused by endometrial irritation (Brinsko, 2011d). In this case, failure of maternal recognition of pregnancy causes
an inadequate block of the endometrial PGF2α-secretion with subsequent regression of the corpus luteum. The premature release of the PGF2α results in an early return to estrus (Samper et al., 2007g).

4.1. Predisposing factors for endometritis

Since the physiology that leads to a suitable uterine environment for the embryo is so complex, there are many factors along the way that can disturb this process and might lead to endometritis (Brinsko, 2011b).

The factors contributing to the delayed uterine clearance in susceptible mares can be summarized as decreased myometrial activity, decreased uterine lymphatic drainage or suboptimal reproductive conformation (Samper et al., 2007g). It is possible to divide these points of action into problems related to the anatomy, hormones, immune response, and anthropogenic factors (Brinsko, 2011b).

4.1.1. Anatomical predisposing factors

Every anatomical barrier must function efficiently to prevent ascending contamination of the genital tract. The vulva, the vestibulovaginal sphincter, the cervix and the uterus should have a correct conformation (Brinsko, 2011b). The following anatomical variations, often found in older maiden mares, can lead to contamination by bacteria, debris, and other foreign objects (Brinsko, 2011b).

Firstly, the perineum ought to be intact, and the anus should not be recessed (Brinsko, 2011e; Brinsko, 2011g). In older mares, the perineal conformation may shift inward, resulting in pulling the vulva onto the pelvic floor. This conformational abnormality can cause contamination of the vulva during defecation or the development of a pneumovagina (Samper et al., 2007j).

Next, the axis of the vulva should be vertical, with the vulvar labia brought together to create an efficient closure of the genital tract. Aspiration of air into the vagina can be found in older maiden or cachectic mares due to a suboptimal vulva conformation and cranial displacement of the anus (see figure 1). Other reasons for developing a pneumovagina include tearing or stretching of the vulvar seal or the vulvovaginal sphincter. If the dorsal commissure is greater than 5cm above the ischium, especially if the anus is sunken, the vulvar lips will not efficiently seal the genital tract. The vulvar lips can tip horizontally, so the vulva is no longer vertical (Brinsko, 2011a). The consequence of aspiration of air is a pneumovagina or commonly called “wind sucking”. A condition such as a pneumovagina includes the lumen of the vagina to be filled with air and distended. This will interfere with protecting the uterine environment because bacteria, debris and foreign objects are more likely to ascend into the genital tract (Brinsko, 2011a; Brinsko, 2011b).
Furthermore, if the vestibulovaginal seal is not efficient, there is also a higher chance of ending up with a pneumovagina due to negative pressure in the cranial vagina (Samper et al., 2007k and Brinsko, 2011a).

Another predisposing factor is urovagina which can be found in older maiden mares at times. An urovagina can evolve due to reflux of urine into the vagina during urination and can be caused by conformational changes that result from progressive descent of the vestibule and vagina into the abdomen due to repeated stretching of the tracts during pregnancy. The pooling of urine in the vaginal fornix can occur due to conformational changes where the urethral opening is positioned cranioventral to the brim of the pelvis and dorsal to the cranial portion of the vagina. The presence of urine can be irritating and contributes to inflammation and even endometritis, vaginitis, and cervicitis. Affected mares may drip urine from the vulva, leading to irritation of the skin of the tail, vulva, and inner aspect of the thighs (Brinsko, 2011a).

Finally, the cervix is the last barrier before entering the uterus and should close or open efficiently depending on the cycle stage (Brinsko, 2011a). Normally the cervix is closed during diestrus, which falls between day one and 14 or 15 (day 0 is the ovulation) of the estrus cycle. The second physiologic condition where the cervix is closed tightly is when the pregnancy is recognized between day 14 and 16 after ovulation until hours before parturition. These cervical changes are regulated by hormonal changes, including a decrease in progesterone and an increase in estrogens. Transluminal cervical adhesions can prevent the cervix from opening, and pericervical adhesions involving the portio vaginalis cervicis may prevent the cervix from closing adequately (Samper et al., 2007f). If the cervix cannot dilate enough during estrus, which is particularly the case in older mares, fluid can accumulate within the uterus because of insufficient clearance (Brinsko, 2011h). Cervical adhesions are the most common non-infectious abnormalities of the uterine cervix. These adhesions are internal scars made of fibrous tissue. This fibrous tissue forms an abnormal bond between parts of the cervix after trauma and can compromise the cervix’s ability to dilate during estrus or parturition (Samper et al., 2007f). This failure of cervical relaxation is one factor attributed to reduced fertility and could also lead to a pyometra development (Samper et al., 2007a; Dascanio and McCue, 2014f). If the cervix’s closure is inadequate,
ascending infections due to air, urine, debris, or other contamination sources are more likely to enter the uterus (Samper et al., 2007j). Cervical laceration or damage that can occur during parturition and often goes undetected can cause insufficient closing. The cervix can appear too short, persistently dilated, or adhered to the vagina. Most of the injuries are longitudinal tears in the cervical muscle, and sometimes more than one cervical defect can be detected (Brinsko, 2011g). Mares can remain fertile with anatomic imperfections in the vulva or vestibulovaginal sphincter, but less so with cervical damage (Samper et al., 2007j).

Older mares often show signs of a pendulous uterus, decreasing the efficacy of the uterine clearance (Scogging, 2015). This is an anatomical abnormality whereby the uterus has dropped ventrally within the abdomen (Dascanio et al., 2010). If the mare suffers from a pneumovagina or cervical incompetency, the uterus can be filled with air too, and a pneumouterus can be created (Canisso, 2016). Some conformational abnormalities can occur due to the foaling process. When talking about injuries between the shelf of the rectum and vestibule, the lacerations can be graded in different degrees. A third-degree perineal laceration or recto-vestibular laceration extends from the vestibule into the rectum’s lumen and through the perineal body. A recto-vestibular fistula is defined by a laceration through the vestibule’s dorsal aspect that perforates the rectum but does not disrupt the perineal body. A fistula can result in contamination of the vestibule and vagina with faeces. Sometimes the injury is not readily apparent because the perineal body is intact. Also, cervical lacerations can occur from the tearing of an insufficiently dilated cervix during delivery or dystocia (Brinsko, 2011g).

4.1.2. Uterine clearance
Another crucial factor in the maintenance of a healthy uterine environment is uterine clearance. The term uterine clearance includes mechanical mechanisms like myometrial contractions and lymphatic drainage but also cellular clearance which was discussed earlier (Leblanc et al., 1989).

Lymphatic drainage plays a vital role in the persistence of postbreeding inflammation and can be disturbed by endometrial fibrosis. Lymphatic lacunae are signs of lymph stasis and are common findings in endometrial biopsy samples taken from mares that are susceptible to endometritis (Samper et al., 2007e). For an efficient uterine clearance, the cervix must dilate, and the myometrial contractions must be maintained efficiently (Brinsko, 2011b).

A pendulous uterus, often seen in older maiden mares, can delay uterine clearance. Because of predisposing conformational changes and changes in the endometrium, older mares are, in general, more susceptible to postbreeding endometritis even if they have never been bred before (Dascanio et al., 2010; Stout, 2008).

4.1.3. Iatrogenic factors
The physical barriers are breached during natural or artificial insemination and other intrauterine procedures (Samper et al., 2007j). Intrauterine procedures that can be a source of contamination include
reproductive examination, artificial insemination, natural breeding, collection of samples for culture, cytology or biopsy, endoscopy, uterine lavage, and embryo transfer but also parturition or other procedures. Due to poor hygiene during veterinary interventions, it is possible to contaminate the genital tract. That is why it is essential to apply high hygiene standards during every act performed while interfering with the genital tract. It is recommended to execute an intrauterine intervention during estrus because the physiological uterine immunity and clearance is most efficient in this stage. When interfering with the uterine lumen during diestrus, it is recommended to administer prostaglandin afterwards to benefit from the natural uterine clearance during estrus. So, it is not only about working hygienically but also using the right products at the right time (Brinsko, 2011b).

Another example is the impact of intrauterine usage of disinfectants. Some disinfectants are known to kill neutrophils and to interfere with the cellular immune defence mechanism. That is why it is crucial to act on the principles of “bonus pater familias” (Brinsko, 2011b).
5. Persistent postbreeding endometritis

Endometritis may be the most significant cause of low progesterone concentrations during the first 30 days of pregnancy (Samper et al., 2007). Amongst others, persistent endometritis can be divided into persistent/chronic postbreeding endometritis (PPBE) and chronic infectious endometritis (Brinsko, 2011b). Chronic infectious endometritis can follow after PPBE if bacteria and other contaminants are inoculated in the uterus (Samper et al., 2007).

PPBE concerns those mares in which the physiological endometritis (APBE) cannot clean the uterus efficiently. If the body fails to resolve the inflammation within 48-72 hours, the case can be labelled as PPBE (Christoffersen et al., 2017). This sperm-induced inflammation may be a more important cause of subfertility or infertility in susceptible mares than infectious endometritis (Samper et al., 2007). The mare can show discharge from the uterus and have fluid in the uterus, although the clinical signs are not always present (Mair, 2013). Even if these clinical signs are absent in PPBE, there is a chance that the mare cannot become pregnant because the embryo enters the uterus 5 to 6 days after ovulation whilst the uterine environment is not suitable for the embryo yet (Troedsson, 1997).

5.1. Diagnosis of persistent postmating endometritis

The reproductive history, physical examination, and the clinical signs of endometritis are good indicators to establish a diagnosis. Still, there are more specific diagnostic tests, such as detecting the presence of uterine fluid and abnormal edema by performing ultrasonography or rectal palpation (Rasmussen et al., 2015). Additionally, cytological and bacterial analyses of uterine swabs and histological analyses of uterine biopsy are recommended to perform (Mair, 2013). It is advised to use cytology combined with culture and biopsy to diagnose endometritis (Dascanio and McCue, 2014). When discussing infectious endometritis, these methods will be explained more in detail.

Endometritis may be suspected in mares that show an abnormally short estrus cycle, subfertility, or free uterine fluid during diestrus detected on ultrasonography (Dascanio and McCue, 2014). The symptomatic external signs of endometritis are not always obviously visible. Sometimes chronic vaginal or cervical discharge can be seen, which can cause matting of the tail hairs or is visible at the ventral commissure of the vulva. When the cervix is open, or the horse suffers from a pyometra or metritis, exudate is most apparent (Brinsko, 2011).

Intrauterine fluid accumulation can be detected by using transrectal ultrasonographic examination (see figure 2). Often an enlarged uterus and endometrial edema scores higher than usual can be observed. Small amounts of fluid can be detected during estrus in the absence of endometritis and are not always linked with a pathology since pathogenic organisms are usually absent in uterine fluid during estrus unless the uterus has been contaminated. Regardless, any free fluid within the uterine lumen is considered abnormal and has a negative effect on embryonic survival (Brinsko, 2011). The character of uterine fluid can range from anechoic to hyperechoic, but there is no direct correlation between the echogenicity of the fluid and the severity of the abnormality. However, intraluminal fluid can be graded from I to IV according to the degree of echogenicity. When the fluid appears more echoic, it is more
likely contaminated with debris (Samper et al., 2007g). The cut-off merit of fluid accumulation in a healthy mare is 2 cm of intraluminal distention. More than 2 cm of distention is a sign of poor uterine clearance and is a good indicator associated with increased susceptibility to PPBE (Brinsko, 2011b).

![Fig. 2: Ultrasonographic image of intrauterine fluid.](image)

From Brinsko (2011b).

In acute cases, the endometrial edema score may be higher than usual (see figure 3) (Samper et al., 2007j). Deficient lymphatic drainage can result in abnormal endometrial edema patterns visible on ultrasound. It is also possible to detect the effects of aggressive types of endometritis like endometrial or uterine cysts, mainly originating from the lymphatic tissue (Brinsko, 2011b).

![Fig. 3: Ultrasonographic image of edema score 1 (on the left) and Edema score 3 (on the right).](image)

From Dascanio and McCue (2014).

5.2. Treatment of persistent postmating endometritis

Treatment-wise, many different options and choices are depending on the type and degree of endometritis. The challenge is to find a balance between interfering with the physiological defence mechanisms and using additional treatments. The natural endometrial defence mechanisms must be optimized, whereas an endometrial infection or other abnormalities must be prevented (Morris, 2020).

An effective way to influence the efficiency of uterine clearance is using hormonal mechanisms. Estrogen will increase uterine circulation, relaxation of the cervix, uterine contractions, and increased resistance to uterine infections in general. Usage of this hormone is not approved in horses in Europe,
but it is possible to increase the percentage of time that a mare is in estrus using prostaglandins 5 to 6 days after ovulation. On average, the mare comes into estrus between 2-4 days after prostaglandin administration (Brinsko, 2011c).

The most common practice is to combine the administration of ecbolics with an uterine lavage. The effects of oxytocin and prostaglandins on the mare can vary individually. Some mares only react to one of the ecbolics. Some mares respond to both equally, and some mares only react if the two hormones are combined (Dascanio and McCue, 2014c). Finally, exercising the mare has a positive impact on uterine clearance (Swift et al., 2019).

5.2.1. Oxytocin
Firstly, it is common to use an ecbolic like oxytocin to evacuate uterine fluid or other substances. The oxytocin dosage to stimulate and obtain myometrial contractions for 30-45 minutes is 20-40 IU IV or 20-40 IU IM (Brinsko, 2011c; Dascanio and McCue, 2014c). Doses higher than 40 IU result in tetanic, less effective contractions (Madill et al., 2002). Another beneficial aspect of oxytocin is that it stimulates prostaglandin release (Samper et al., 2007g). In Europe, it is possible to use carbetocin, which is oxytocin with a longer half-life of 17.2 minutes in the mare compared to oxytocin which has a half time of 6.8 minutes. A disadvantage is that carbetocin must be administered in a very high dose, making oxytocin more practical (Dascanio and McCue, 2014c). However, to date, there is no study comparing the ability of oxytocin and carbetocin to promote uterine clearance (Canisso et al., 2020). Oxytocin is often given in combination with a previous uterine lavage. Intravenously administered oxytocin results in increased uterine contractility for 20 to 50 minutes. Intramuscular administration has been shown to have a more prolonged effect on uterine contractions than administrating oxytocin intravenously. Still, it is not known whether the intramuscular or intravenous route is superior. Some practitioners administer oxytocin several times on a given day to create constant contractility of the uterus. Still, it is claimed that administration of more than 20 IU IV or administration more often than once every 4-6 hours induces spasmodic uterine contractions, which are likely to be unproductive in eliminating the fluid accumulation. It is crucial to ensure a patent cervix before usage because otherwise, the content can’t be expelled. The administration should be repeated for 1 to 2 days if the uterine lavage effluent remains cloudy or intrauterine fluid accumulation remains visible on ultrasound examination (Brinsko, 2011c). It is experienced that injection of 20 IU oxytocin intravenously once a day during estrus had no adverse effect on fertility (Brinsko, 2011c).

It is imperative to wait with oxytocin administration at least until 4 hours after breeding or insemination because the sperm colonization of the oviducts takes at least 4 hours (Brinsko et al., 1991). Generally, oxytocin administration near/ before the time of breeding is discouraged because it may decrease fertility. A possible reason is that semen could be expelled from the uterus before the sperm can access the oviduct. Also, a treatment of more than 2-3 days after ovulation is discouraged because the cervix will be less dilated (Brinsko, 2011c).
5.2.2. Prostaglandin

Another hormone belonging to the group of ecbolics is prostaglandin-F2α (PGF2α) (Brinsko, 2011c). Exogenous PGF2α is clinically used to either cause lysis of the corpus luteum or stimulate uterine contractions to expel fluid accumulations. The most common medications used are Cloprostenol (250 µg IM) and dinoprost tromethamine (Dascanio and McCue, 2014c). The effect on the uterus is significantly slower than the impact caused by oxytocin, but the uterus will contract for a longer time (Samper et al., 2007g). The contractions will be sustained for 2-5 hours when using PGF2α compared to 45 minutes when using oxytocin (Samper et al., 2007g; Brinsko, 2011c). When the mare was inseminated, it is not recommended to administer PGF2α after ovulation because it may harm the function of the corpus luteum (CL) (Brinsko, 2011c). When used after breeding, it is suggested to administer a dose of Cloprostenol of 250 µg given at 12 and 24 hours after breeding but not after ovulation (Samper et al., 2007g).

PGF2α is used to shorten the diestrus and bring the mare into estrus faster by inducing premature luteal regression, which can harm the pregnancy. The CL is not susceptible to PGF2α until five days after ovulation, so it is recommended to wait with the administration for one week after the ovulation is confirmed. PGF2α has a luteolytic effect on the CL to regress, and the mare comes in estrus. With this approach, mares with a mature CL will come in estrus on average 7 to 12 days after ovulation or 2 to 4 days after using PGF2α. The mares estrus has a positive effect on uterine clearance and defence mechanisms (Brinsko, 2011c). Side effects in using PGF2α are mild colic symptoms like transient sweating, abdominal cramping and diarrhoea (Dascanio and McCue, 2014c).

Prostaglandin-E2 (PGE2) can be applied topically to the external cervical os and within the cervical lumen to stimulate cervix relaxation during estrus. Insufficient relaxation is prevalent in older maiden mares. 2 mg of PGE2 is mixed with 2 to 4 ml of lubricating jelly and deposited in the cervical canal and external cervical os. Another possibility is to place 200 g to 1 mg of crushed tablets of PGE1 analogue misoprostol softened in a small volume of sterile saline solution or sterile lubricant into the external cervical os once daily (Brinsko, 2011c; Dascanio and McCue, 2014c). It will soften/ relax the cervix for better uterine drainage, but further investigations on the use of PGE in mares is warranted (Brinsko, 2011c; Dascanio and McCue, 2014c).

To summarize, ecbolic treatment combined with uterine lavage should be performed daily or sometimes twice daily in mares susceptible to PPBE after breeding, and the mare should be evaluated daily. Oxytocin administration is safe for 2 to 3 days after ovulation. PGF2α administration during the early postovulatory period/ 0-3 days after ovulation influences the development of the corpus luteum and lowers progesterone production during the following diestrus. After this period, the use of PGF2α could cause early embryonic death and is therefore not recommended after ovulation has occurred. The pregnancy rates of mares receiving PGF2α on the day of and one day after ovulation showed 50% lower pregnancy rates than mares receiving oxytocin or uterine lavage (Brinsko, 2011c).
5.2.3. Uterine lavage

Uterine lavage is a frequently used treatment before or after insemination, commonly combined with oxytocin (Vanderwall et al., 2003; Knutti et al., 2010). It can be used before breeding in those mares in which systematic treatment with ecbolics is not successful in reducing the intrauterine fluid accumulation (Brinsko, 2011b). Commonly used are 1-3 litres of warm (42-45°C) sterile crystalloid solutions such as lactated Ringer's solution or 0.9% saline solution (Dascanio and McCue, 2014m). A warm solution is used to prevent cramping by the mare and remove viscous fluids (Dascanio and McCue, 2014m). Uterine lavage helps physically remove microorganisms, debris, inflammatory cells, dead sperm, and mediators, negatively affecting the sperm before breeding or the embryo after breeding (Knutti et al., 2010). These substrates may also interfere with products such as antibiotics when administered into the uterus (Brinsko, 2011b). Additionally, uterine lavage can reintroduce viable neutrophils to reinstate an active degradation of microbes in an inactive inflammatory state (Canisso et al., 2020) and stimulate the uterine contractility through mechanical irritation of the endometrium (Brinsko, 2011b).

The mare may be lavaged during estrus one or more days before breeding. The procedure is performed preferably with lactated Ringer's solution and not 0.9% saline solution to increase pregnancy rates (Dascanio and McCue, 2014m). If used post breeding, just like with the administration of ecbolics, it should be used at the earliest 4 hours after insemination. After this period, the spermatozoa necessary for fertilization are present within the oviduct. The optimal time of treatment in highly susceptible mares was found between 4 and 6 hours, but the lavage is usually performed between 4 and 18 hours after breeding (Samper et al., 2007g). It is recommended to combine uterine lavage with ecbolics to improve uterine clearance of the fluid and contaminants (Brinsko, 2011b). Oxytocin should be administered intravenously during lavage, and after flushing, measurement of the recovered fluid and ultrasonographic examination of the uterus should be performed. It is essential to ensure that all the fluid has been recovered because the susceptible mare often has an impaired ability to spontaneously drain the uterus (Samper et al., 2007g).

To execute an uterine lavage, a catheter with a balloon is inserted in the uterus. The balloon is then distended with air after passage beyond the cervix to keep the catheter in place and seal the cervix to build pressure when the solution is brought into the uterus. Isotonic saline solution, lactated Ringer's solution, or other balanced salt solutions are generally used for uterine lavage. Usually, 1-3 L of the warmed solution will be inserted. Older and pluriparous mares may need larger volumes. It is possible to combine the uterine lavage with a uterine massage per rectum. The massage will distribute the solution within the uterus and stimulate uterine contractility (Brinsko, 2011b).

In general, the uterine lavage should be repeated for several following days until the effluent is clear or not turbid anymore. The therapy can be stopped when the effluent of the first flush of the day is normal in appearance. After the procedure is finished, it is advisable to control whether most of the fluid has been recovered. If any intrauterine fluid remains after treatment, it is advised to note its amount and re-examine at a later stage (Brinsko, 2011b).
Similarly, 500 ml of 0.02% diluted povidone-iodine solution can be used. It is essential not to use a too highly concentrated solution because there is complete inhibition of neutrophil motility at a concentration of 0.2% or more. In contrast, a concentration of 0.02% shows no depressive effect or random migration of neutrophils in vitro. Precautions should be taken when using a disinfectant, and they must be sufficiently diluted because some can be irritating to tissues. Lugol’s iodine and chlorhexidine should be chosen and used very carefully. If used inadequately, cases are reported with forming of transluminal adhesions of the tubular tract or the earlier mentioned negative effect on the neutrophils and cellular immune defence. The positive effects of disinfectants, when used correctly, are inducing an inflammatory response by the endometrium, which will generally ease off seven days after treatment. Good results in treating endometritis are reported, but because no controlled studies have shown superior efficacy of disinfectants over antibiotics or antifungal agents in treating endometritis, Brinsko (2011b) recommends avoiding their use except in certain instances.

5.2.4. More recent treatments
An alternative treatment for PPBE is N-acetyl cysteine (NAC), which is a mucolytic agent. NAC is currently used as an intrauterine infusion in mares with excessive uterine mucus production or in case of bacterial endometritis complicated by a biofilm (Leblanc, 2010; Troedsson, 2012). Other areas of application are treating respiratory diseases such as pneumonia, the pulmonary component of cystic fibrosis in humans, meconium impaction in both humans (Burke et al., 2002) and equine neonates, and meconium aspiration pneumonia in equine neonates (Morresey, 2008). It disrupts disulphide bonds linking mucin polymers (Sheffner 1963; Caissie et al., 2020). Additionally, N-acetyl cysteine has anti-inflammatory and antioxidant properties (Cazzola et al., 2017). An improvement in fertility rates of barren mares was observed by Gores-Lindholm et al. (2013) when treated with an infusion of N-acetyl cysteine (Gores-Lindholm et al., 2013). Also, intrauterine NAC administration decreased neutrophil numbers and COX-2 staining in uterine histological samples in normal mares. There are no adverse effects reported on the endometrium of a normal mare after infusion of either 3.3% or 5% solution (Gores-Lindholm et al., 2013; Melkus et al., 2013). Contradictory, Caissie et al. (2020) claimed that NAC’s use in mares affected by PPBE did not improve clinical signs. Additionally, endometrial biopsies of mares treated with NAC showed a more diffuse and severe neutrophil infiltration than the control group treated with saline solution. These different conclusions may be the result of other materials and methods which were used during the research. Caissie et al. (2020) used a small group of 9 mares (n=9) affected by PPBE. In contrast to that, Gores-Lindholm et al. (2013) and Melkus et al. (2013) used a normal or mixed group of mares, including healthy and affected mares. In the case of the research of Gores-Lindholm, the trial was performed in a mixed group of 20 mares (n=20: 10 barren treated, six fertile treated, and six fertile control). Caissie et al. (2020) state that normal mares are more likely to clear inflammatory products caused by NAC use than mares affected by PPBE and that PPBE-susceptible mares do not demonstrate normal uterine clearance. This factor may play a part in the different outcomes of the research. To make a definite conclusion, it is essential to use a larger group of mares affected by PPBE and a control-group to evaluate NAC’s effects (Caissie et al., 2020).
A study by Tsunoda et al. (2019) has shown positive effects of pentoxifylline in the treatment for PPBE. Pentoxifylline is a xanthine supplemented to semen extender and is known to improve tissue-blood flow and has an immunomodulatory character (Banihani et al., 2017). Also, pentoxifylline improves bacterial clearance by decreasing bacterial colonization, which will result in reduced tissue damage caused by neutrophils (Baumgartner, 2007). The uterine defence mechanism is stimulated and results in increased neutrophil migration to the uterine lumen about 6 hours after infusion. The increase in neutrophil migration promotes a more rapid endometrial recovery (Tsunoda et al., 2019).

5.3. Management of the mare with persistent postmating endometritis

Essential for successful breeding management in horses is identifying mares that need special care or attention because of susceptibility to PPBE. Before breeding the mares, it is essential to manage them to optimize uterine clearance of contaminants during and after the estrus of breeding. Every mare, especially older maiden mares, should be examined before breeding to detect factors that can harm reproductive success (Dascanio and McCue, 2014g). It is beneficial to make an early diagnosis to minimize significant damage to the endometrium (Brinsko, 2011b).

The patient's complete history should be obtained focusing on reproduction, including the current reproductive status. Is the mare pregnant, maiden, barren? How was the last parturition? When did she last foal? How many previous foals did she have? Were there any surgeries performed on the reproductive tract yet? How many cycles was she bred during the last season, and with which breeding technique (AI, natural, pasture breeding) and which stallion was used? Is the mare pregnant, and if so, when was the last date of breeding? Were any abnormal estrus cycles, uterine infections, embryonic losses, or abortions recorded in the past (Dascanio and McCue, 2014g)?

Focusing on the reproductive tract, the perineal and vulvar region should be evaluated for conformation, defects, inflammation, discharge, and other abnormalities (Dascanio and McCue, 2014g; Dascanio and McCue, 2014e). More information about the reproductive status and possible irregularities can be obtained by rectally palpating and performing an ultrasound on the reproductive tract. For example, most mares that are susceptible to PPBE accumulate uterine fluid more than 2 cm in height on ultrasound during estrus, which must be registered and managed (Brinsko, 2011b). A speculum should be used to examine the vestibule, the vagina, and the cervix (Dascanio and McCue, 2014n).

If there are any suspicions for endometritis or a chance of endometritis, the diagnostic procedures discussed in “diagnosis” should be used to decide which actions must be taken (Dascanio and McCue, 2014g; Dascanio and McCue, 2014i). During interventions related to the reproductive tract, it is crucial to minimize iatrogenic uterine contamination and limit the number of inseminations per cycle. Low bacterial inoculation in the stallion ejaculate is essential and excessive trauma to the genital tract must be prevented. All procedures should be performed under strict hygienic conditions (Brinsko, 2011b; Dascanio and McCue, 2014g). Other underlying problems, such as conformational abnormalities that can lead to pneumovagina or urovagina, should be corrected and managed in the right way (Brinsko, 2011b). The non-pregnant mare should be brought into estrus by using prostaglandin if an infection is
detected during diestrus. Susceptible mares should be free of uterine inflammation and fluid before breeding, and postbreeding inflammation must be aggressively controlled (Samper et al., 2007g).

Treatment for endometritis, removal of any uterine fluid and breeding of a susceptible mare is ideally performed before ovulation (Samper et al., 2007g; Samper et al., 2007j). Breeding should be performed only once within 24 to 48 hours before ovulation to use the natural clearance mechanisms during estrus. If natural service is performed, it may be beneficial to bring 30-50 ml of antibiotic-containing semen extender into the uterus immediately before mating to prevent uterine infections (Brinsko, 2011b). When artificial insemination is performed, it is possible to mix the semen of the stallion with an extender containing broad-spectrum antibiotics (Brinsko, 2011b).

Depending on the spermatozoa’s viability, it is beneficial to breed the mare 1 or 2 (or even 3) days before the anticipated time of ovulation. This way, the mare will be in a more prolonged state of estrus after insemination, which allows more time for drainage of fluid via an open cervix, and the natural resistance is more efficient. Another benefit is that it will enable more time to flush the uterus more than once before ovulation. The flush is more effective because the cervix will start closing after ovulation (Samper et al., 2007g). Also, the moment of insemination is critical. Susceptible mares may be bred only once per estrus cycle. If repeated inseminations are necessary, it is recommended to perform uterine lavage 4 hours after mating (Samper et al., 2007g).

The postmating therapy consists of uterine lavage, administration of oxytocin and, if necessary intrauterine antibiotic infusion. The presence of a stallion will stimulate the secretion of oxytocin in the mare and will have a beneficial effect (Samper et al., 2007g). Exercise of the mare has a positive effect on uterine clearance and draining of the uterus. The increased intra-abdominal pressure, which is associated with movement, will help evacuate uterine contents. This exercise can be realised by walking the mare. Bringing the mare in contact with a teasing stallion, oxytocin release is stimulated, and the mare will urinate more frequently, which positively affects uterine clearance (Samper et al., 2007g). In a study by Swift et al. (2019), who compared the effects of stall-rest, exercise, electroacupuncture and oxytocin and exercise on PBIE in susceptible mares, it was concluded that exercise and exercise in combination with oxytocin were the most efficient treatments against PBIE.

6. Infectious endometritis
Pathogens like bacteria and fungi can ascend into the reproductive tract due to poor hygiene, conformational abnormalities, and deficiencies in the mares’ defence mechanisms (Christoffersen et al., 2015).

Recently it has been shown that some pathogens can be present in a latent state, deep within the endometrial glands (Petersen et al., 2018). The pathogens may have been inoculated at a previous mating or ascended from the caudal genital tract. These latent pathogens can be activated by the physiological postbreeding inflammatory reaction triggered by any mechanical or chemical uterine irritation (Davis et al., 2013).
6.1. Bacterial infectious endometritis

It has been reported that 25%-60% of mares, which have failed to conceive, have been diagnosed with endometrial infections (Ferris et al., 2016). Sources of contamination can be breeding related or by any other manipulation of the reproductive tract. A pneumovagina or a poor perineal conformation are common underlying problems that can lead to ascending contamination (Samper et al., 2007). Aspiration of air and debris can lead to ascending infections if the mare’s uterine clearance mechanisms are defective, and the reproductive system is overwhelmed by the contaminants. The earlier mentioned conformational deficits can cause pneumovagina or ascending infections in case of failure of anatomical protection from the vulva, the vestibulovaginal sphincter or the cervix (Brinsko, 2011e; Brinsko, 2011g). The infections can also have a hematogenetic origin (Samper et al., 2007).

Venereal infections may include *Taylorella equigenitalis, Klebsiella pneumoniae, Pseudomonas aeruginosa* or any other pathogenic bacteria present on reproductive materials or the stallion’s penis. An endometrial infection can develop into chronic infectious endometritis and may be caused by the combination of potentially virulent bacteria and a weakened reproductive defence system (Canisso et al., 2020).

The most frequently mentioned aerobic organisms in literature are e.g. *Streptococcus equi* subspecies *zooepidemicus*, *Escherichia coli*, *Pseudomonas aeruginosa* or *Klebsiella pneumoniae*. Based on the literature, they cause up to 80% of the confirmed cases of endometritis in the mare. *Hemolytic streptococci, Staphylococcus spp.*, and other bacteria are sometimes detected but are more regarded as contaminants of the sample (Christoffersen et al., 2015). The role of *Mycoplasma, Chlamydia* and viruses is thought to be insignificant (Brinsko, 2011b).

The ascending bacteria and debris will result in chronic endometritis with infiltration of the endometrium with lymphocytes often accompanied by plasma cells, while B-cells and macrophages are uncommon (Rudolph et al., 2017).

Previously, it was believed that the uterine environment was sterile (Swartz et al., 2014) and that there was no “normal flora” in the healthy mammalian uterus, and any organism isolated from the uterus is a potential cause of inflammation (Samper et al., 2007). This claim was recently challenged by the publication of the Human Microbiome Project (2007), which has shown a unique microbiome present in the uterine cavity (Moreno and Franasaki, 2017). By using 16S rRNA sequencing, it is demonstrated that the uterine cavity harbours a unique microbiome. The uterine microbiome changes according to the stage of the estrus cycle and across studies. Proteobacteria are the predominant species in the physiological microbiome in fertile mares (Swartz et al., 2014). It is hypothesised that any shift in the population may lead to an increased risk of endometrial pathologies (Marth et al., 2018). The microbiome’s disturbance can be caused by any mechanical or chemical irritation of the uterus (Morris et al., 2020).
Bacteria can be divided into organisms situated within a biofilm and free-floating organisms (Costerton et al., 1978). Approximately 80% of bacteria isolated from the equine uterus can produce a biofilm (Ferris et al., 2014; Ferris et al., 2016). They can be the same species, but they are physiologically distinct from each other. Many Gram-negative bacteria like *Escherichia coli* or *Pseudomonas aeruginosa*, and *Streptococcus equi subspecies zooepidemicus* can produce biofilms. The location of the bacteria influences their susceptibility to treatment (Ferris, 2017).

A biofilm comprises an aggregate of microbial cells adherent to a surface embedded within a complex matrix of extracellular polymeric substances. This matrix can consist of microbial nucleic acids, proteins, polysaccharides, and lipids and host components (Stoodley et al., 2002). Whereas free-floating organisms are more likely to be detected and eliminated by the host immune system or antibiotic product, bacteria in a biofilm, however, are protected from these defence mechanisms. The bacteria within a biofilm can survive with depressed metabolism and lower replication rates than free-floating bacteria. Besides, bacteria in a biofilm may be 10-1,000 times more resistant to antibiotic treatment. Another strength of the bacteria within a biofilm is that antibiotics often only act against metabolic active and multiplying bacteria, leading to the destruction of the bacteria of the outer regions of the biofilm. Still, those deep within the biofilm may survive and become the nidus for the infection's recurrence (Olsen, 2015).

The characteristics of associated biofilm infections are typically difficult to diagnose and treat because traditional antibiotic therapy is often ineffective against the residing bacteria (Beehan et al., 2015). Thus, treatment includes biofilm disrupters in combination with antibiotics (Morris et al., 2020).

6.1.1. Diagnosis of chronic infectious endometritis

There are different indicators and diagnostic tools which can be used in combination when detecting chronic infectious endometritis. Specific facts in the reproductive history and findings on clinical examinations can indicate infectious endometritis. In combination with conformational abnormalities, these symptoms are good indicators that a mare may suffer from endometritis (Samper et al., 2007j). To confirm the diagnosis, diagnostic tools such as uterine cytological and bacterial analyses and uterine histological analyses are performed (Mair, 2013b).

6.1.1.1. Reproductive history and physical examination

The first clinical signs related to the reproductive history are abnormal reproductive events resulting from infectious endometritis. The estrus cycle may be abnormally short, and the mare often has a history of not becoming pregnant (Dascanio and McCue, 2014g).

External signs of endometritis are not always visible, but sometimes chronic vaginal or cervical discharge (see figure 4) can be seen (Canisso et al., 2016). Uterine edema and fluid accumulation are often detected on ultrasound at which the fluid can appear abnormal with an increase in echogenicity or less echogenic (Dascanio and McCue, 2014i).
A pneumovagina can be detected by recognizing foamy exudates appearing from the vaginal lips during the rectal examination. By using ultrasonography, it is also possible to visualize air in the uterus. Air mixed with uterine fluid is visible as white lines or hyperechoic flecks in hypoechoic fluid (Samper et al., 2007e).

Next, urine in the uterus, resulting from an urovagina, has a hyperechoic aspect due to urine crystals and mucus typically present in equine urine. The detection of placentitis can indicate conformational abnormalities, leading to ascending infections (Samper et al., 2007e).

When performing a vaginal speculum examination, cervical reddening may be visible due to the inflammation. In some cases, discharge coming out of the cervix is visible. It is possible to detect factors like an urovagina, debris, or pneumovagina, which predisposes endometritis development. The presence of a pneumovagina can be suspected if the typical sound of sucking air when placing the speculum is absent (Brinsko, 2011a).

By performing a digital examination of the cervix during diestrus, the cervix’s ability to close or open can be examined. If there is a suspicion of reproductive failure due to cervical laceration, adhesions, or cervical incompetence, the examination should be performed during diestrus when the cervix is typically closed due to progesterone’s working mechanisms. It is recommended to perform a cervical exam immediately after dystocia or other difficult births to evaluate the cervix and vagina for trauma (Dascanio and McCue, 2014n).

Finally, a transcervical endoscopic examination can be used to directly visualize the uterine cavity by insufflating the uterus with air or fluid to dilate the lumen. The endoscope is then advanced to the internal bifurcation and a uterine horn (Brinsko, 2011a).
6.1.1.2. Cytology and culture

Part of the routine breeding soundness examination in the mare is a culture of the uterus. It is done as part of a fertility examination or on request by a stallion owner before breeding (Dascanio and McCue, 2014k). It is also performed to set the foundation of efficient treatment and a positive outcome if a pathologic process is suspected (Brinsko, 2011a; Brinsko, 2011b).

To set up a treatment against pathogenic microorganisms, it is essential to know which bacteria or fungi are present in the uterus. Therefore, a sample of the endometrium must be cultured, and the sample must be cytologically or histologically analysed. Analysis of the culture and cytology go hand in hand in diagnosing uterine pathologies (Brinsko, 2011a; Dascanio and McCue, 2014j; Dascanio and McCue, 2014k). An antimicrobial susceptibility test should be carried out to select the right drug (Brinsko, 2011b). Samples are generally taken by using a swab or a brush. A low volume uterine lavage is indicated for an infertility workup or when suspecting an infection, although the previous uterine swab turned out to be negative (Dascanio and McCue, 2014j).

Uterine swabs can be taken during any stage of the mare’s cycle. Still, it is recommended to take a swab in early estrus when the cervix is relaxed, and the uterus is more resistant to infections (Brinsko, 2011b). Cultures may be performed in diestrus, but it is recommended to administer prostaglandins after the interference to lyse any luteal tissue and bring the mare into estrus (Dascanio and McCue, 2014j).

No diagnostic tool is faultless. Isolated infections, deep-seated infections, or infections early in their course can result in normal uterine cytology without inflammatory cells, and infection can still be present (Dascanio and McCue, 2014j). Importantly, a positive culture without any signs of inflammation does not indicate that the mare is infected. The reason is that the swab may be contaminated during the execution of swabbing by microorganisms originating from the perineum, vulva, vagina, cervix, or other locations. That’s why it is necessary to work very hygienically and subsequently lower the risk of contamination. Cleaning the mare’s hindquarters and using the right and sterile equipment like a guarded swab are the first steps for receiving a representative sample. To take intrauterine swabs, two common methods can be used. The first method is using a guarded swab which is protected with a sterile gloved hand while passing the vagina. After inserting the swab into the uterus and taking the sample, the swab must be retracted into the guard before removing the swab from the uterus. It is also possible to take samples of the uterus using a sterile vaginal speculum passed to the cervical os. A sterile guarded swab is then inserted into the visible cervix opening, and a sample can be taken. It is essential to consider that the value of uterine cytology is usually limited to documentation of acute or subacute inflammation, as neutrophils are a prominent luminal component of endometritis. More subtle changes of the endometrium are often not detectable with cytology. Thus, it is possible to miss the presence of chronic endometritis (Brinsko, 2011b). Sometimes a small number of neutrophils may be visible on a cytological sample that originates from possible blood contamination during the collection process (Dascanio and McCue, 2014k). If three or more neutrophils per five fields (400 magnification) occur during microscopy examination (see figure 5), the sample is considered positive for endometritis (Katila, 2016).
Standard uterine culture techniques do not always detect bacterial or fungal presence in a mare with infectious endometritis because of slow growth and difficulty culturing anaerobic bacterial or fungal organisms (Dascanio and McCue, 2014k).

Polymerase chain reaction (PCR) is a useful technique for detecting and identifying the genus and species based on the microorganisms' DNA material's presence and is thereby a valuable diagnostic tool (Dascanio and McCue, 2014f). The disadvantages of using PCR are, amongst others, that dead microorganisms are likewise detectable. This characteristic can give a false impression of the present microorganisms. Another detriment is that no antibiogram can be gained by the PCR information (Dascanio and McCue, 2014f).

A more sensitive detection method is to perform a small volume uterine flush with about 60 ml of sterile phosphate-buffered saline solution (Brinsko, 2011b). This method leads to better recovery of bacteria than sampling the endometrium with a guarded swab because the lavage is distributed throughout the entire uterus (Katila, 2016). The cons of using a small volume flush are the risk of possible contamination since the tubing is not guarded. Another negative fact is that performing and processing a uterine lavage sample requires more time (Dascanio and McCue, 2014k). The flush medium will be centrifugated, and the cells are harvested for cytology (Brinsko, 2011b).

Additional to swabbing the uterine lumen to bring the sample in culture, it is possible to examine the uterine cytology with a microscope. Therefore, the sample must be spread on the surface of a microscope slide and air-dried, fixed and stained with a stain such as Diff-Quick. Under the microscope, the stained cytology sample is examined for microorganisms and white blood cells. The condition of luminal epithelial cells can be investigated. Cytological preparations from mares with endometritis usually contain increased numbers of white blood cells, abnormal epithelial cells, and sometimes microorganisms can be detected. In contrast, a healthy endometrial cytological preparation contains healthy epithelial cells with a few or no white blood cells, no bacteria, fungi, or other organisms (Brinsko, 2011b). Candida spp., Aspergillus spp., and Mucor spp. are the most common yeast and fungal
organisms seen, which are more likely detected in cytologic specimens than in biopsy preparations (Brinsko, 2011b; Ferris et al., 2015; Bohn et al., 2014).

6.1.1.3. Histology
The definitive diagnostic method for (chronic) endometritis is an endometrial biopsy and contains a collection of samples of the endometrium for histological evaluation (Brinsko, 2011a). The sample can also be used for culture and cytologic evaluation. It is possible to perform the biopsy at any stage of the estrus cycle. Still, it is required to record the mare’s estrus cycle stage so that the pathologist can interpret the architecture accordingly (Dascanio and McCue, 2014b). It is said that the most representative sample is the one taken during diestrus because edema during estrus makes it less easy to analyse the sample optimally. The biopsy is collected with a stainless-steel uterine biopsy punch. It will give information about the endometrial condition and the horse’s potential as a broodmare. First, the biopsy forceps is inserted into the uterus protected/ guarded by a hand in a sterile glove (Brinsko, 2011a). The examiner generally removes the arm guiding the biopsy instrument from the vagina and inserts it into the rectum to manually aid in the device’s placement in the correct location (Dascanio and McCue, 2014b). The optimal site for taking a sample is the uterine horn base, where the embryo’s early development will occur (Brinsko, 2011a). After removing the biopsy punch, the endometrial specimen is placed in a suitable fixative, such as Davidson’s solution or 10% buffered formalin (Katila, 2016). The use of formalin as a fixative is suitable for histopathological and several immunohistological investigations. To subclassify lymphocyte populations, zinc-salt fixation is recommended because subclassification in fixed tissue samples has not been achieved on formalin-fixed tissue sections (Huth et al., 2014; Rudolph et al., 2017). It is essential that the sample is removed atraumatic with a needle rather than with a forceps to avoid damaging the endometrial anatomy (Brinsko, 2011a). A single biopsy collected from one site is generally representative of the entire endometrium. Still, collecting multiple samples may be advantageous to characterize the endometrium better when the mare has fertility problems. This procedure by itself has no recorded negative impact on the mare’s fertility. Nevertheless, the owner should be warned that the mare may have slight vulvar hemorrhagic discharge after the biopsy for the first 24 hours (Dascanio and McCue, 2014b).

In the laboratory, it is possible to evaluate the endometrial biopsy microscopically. Acute inflammation is characterised by the accumulation of polymorphonuclear leukocytes in the endometrial tissue (Dascanio and McCue, 2014b). Significant and long-standing infiltration of the endometrium with lymphocytes and plasma cells confirms the chronicity of the infection. These parameters give reasonable indications about the mare’s future potential as a broodmare (Brinsko, 2011a). By performing a uterine biopsy, abnormalities like periglandular fibrosis, cystic glandular distention, lymphatic distension, and acute and chronic inflammatory changes of the endometrium can be detected (Brinsko, 2011a).

In contrast to inflammation, fibrosis is a permanent and untreatable pathologic condition. To grade the mare’s potential as a broodmare, the endometrium is classified on a grading scale on biopsy characteristics from I to III. Minimal inflammation or fibrosis is graded with grade I and is essentially
normal, whereas grade III is characterized by severe inflammatory or fibrotic changes. Grade II is divided into subcategories IIA and IIB encompassing the pathological levels between grade I and III. Besides other reproductive factors, the chance of carrying a healthy foal to term by a mare decreases with each advanced grade of the grading scale of her biopsy characteristics (Dascanio and McCue, 2014b).

Biopsy evaluation is used to detect pathologies and can be used to monitor the patient’s response to therapy (Brinsko, 2011a; Brinsko, 2011b). The endometrium grade may improve after successful treatment on a subsequent evaluation (Dascanio and McCue, 2014b).

6.1.2. Treatment of chronic infectious endometritis

The treatment of chronic infectious endometritis (CIE) consists of combining ecbolics and uterine lavage like earlier discussed for PPBE. When performing a uterine lavage with crystalloids to treat CIE, it is crucial to keep in mind that some bacteria such as *Escherichia coli* can utilize lactate, present in lactated Ringer’s solution, as substrates for growth. Uterine lavage with crystalloid should not be used alone to treat CIE, but these solutions can be enriched with antiseptics such as povidone-iodine and hydrogen peroxide or vinegar. Additives such as mucolytics can be used to break biofilms. Nevertheless, it is unknown how these products affect the resident uterine microbiome and how these agents can restore microorganisms’ balance in utero (Knutti et al., 2010). Often anti-inflammatory medications are administered (Reilas et al., 2006). In combination with antibiotic therapy, the use of autologous plasma has been reported to improve pregnancy rates for lactating and barren mares (Pascoe, 2018). Specific for infectious endometritis, there are possible treatments like the use of antibiotics or antifungal drugs, which can be used intrauterine or systemic (Canisso et al., 2020). To lower the risk of ascending infections and improve the healing process, conformational abnormalities must be corrected (Dascanio and McCue, 2014e).

6.1.2.1. Anti-inflammatory products

The administration of non-steroidal anti-inflammatory drugs (NSAIDs) in susceptible mares or mares with persistent postbreeding induced endometritis is still controversial (Reilas et al., 2006). The inhibition of prostaglandin-endoperoxide synthase and the arachidonic acid cascade would decrease PGF2α production (Boerboom et al., 2004). Consequently, a decrease in PGF2α diminishes myometrial activity and will lead to delayed uterine clearance. Additionally, the administration of high and sustained doses of NSAIDs in pre-ovulatory mares has been shown to increase the rate of anovulatory hemorrhagic follicles. Still, this effect was observed when twice the labelled amount was administered (Cuevroat-Arango, 2011). On the other hand, the combination of NSAIDs administration with oxytocin has been shown to improve uterine clearance and reduce PMNs infiltration. Also, the labelled dose of NSAID has shown no interference with ovulation (Donnelly et al., 2019).

Adverse effects, including delayed uterine clearance and impairment of ovulation, have been related when non-selective NSAIDs were used (Friso et al., 2019). Firocoxib, a COX-2 selective inhibitor, has been described to reduce the post-breeding inflammatory response in mares treated during the periovulatory period without affecting ovulation rates (Donnelly et al., 2019). Vedaprofen, another
selective COX-2 inhibitor, has been reported to positively affect fertility in susceptible mares without interfering with the inflammatory score of the endometrium and intrauterine fluid accumulation (Rojer et al., 2010). Selective NSAIDs can be considered a safe and efficient treatment to reduce post-breeding endometrial inflammation in susceptible mares. It should be combined with conventional therapies, such as ecabolic administration or uterine lavages (Friso et al., 2019).

Another group of anti-inflammatory products are glucocorticoids. In susceptible mares, it may be indicated to modulate the post-breeding endometrial inflammatory reaction with glucocorticoid treatment to prevent excessive inflammation and glucocorticoids are routinely used (Papa et al., 2007). The products can be administered preventive but also therapeutic. The glucocorticoids will alter the expression of genes for the complement, cytokines, TNF, and other inflammatory components. Commonly used products include prednisolone or dexamethasone. A previous study demonstrated that single-dose dexamethasone administered within one hour of mating and daily prednisolone administration before and after mating had improved pregnancy rates in mares with uterine fluid (Bucca et al., 2008; Papa et al., 2008). A single injection of dexamethasone administered within one hour of mating (50 mg, IV; approximately 0.1 mg/kg) combined with routine post-breeding therapies, like uterine irrigation, ecabolic drugs and in some cases intra-uterine antibiotics, resulted in increased pregnancy rates in mares with a history of fluid accumulation after ovulation and in mares with cervical incompetence (Bucca et al. 2008). Treated mares exhibited decreased uterine edema, decreased intra-uterine fluid and an increase in uterine fluid clarity. It can be said that dexamethasone did not increase pregnancy rates in the general population. Still, pregnancy rates were increased in mares with three or more risk factors for susceptibility to endometritis (Papa et al., 2008).

6.1.2.2. Intrauterine and systemic antibiotics
Generally, standard antibiotic use is becoming less frequent in individually managed mares due to a better understanding of the pathogenesis, the proven effectiveness of oxytocin and saline lavage, and the social concerns about antibiotics' overuse. Another possible risk is the inducement of fungal infections by using antibiotics. Nevertheless, intrauterine antibiotics are used if oxytocin and uterine lavage are not leading to good results. If the mare suffers from chronic infectious endometritis and the same pathogens can be isolated from the uterus at estrus of different cycles, intrauterine antibiotics are recommended. Antibiotic treatment is discouraged if different isolates are recovered from separate cycles, often in susceptible mares. In those cases, the use of antibiotics could lead to persistent fungal infections. If used correctly, the antibiotic treatment can have good results, and it is seen that the use of oxytocin has an additive effect whilst using antibiotics (Samper et al., 2007)).

Ideally, the drug's use should be based on the susceptibility pattern obtained from a bacterial culture. If antibiotics are used intrauterine, the standard infusion dose remains at or above bacteriostatic or bactericidal concentrations for at least 24 hours. The most commonly administered antibiotics include β-lactam antibiotics, including ceftiofur, ampicillin and penicillin, and aminoglycosides like gentamycin and amikacin at usual systemic doses (Dascanio, 2011).
Organic material must be removed by performing uterine lavage before administering the antibiotics to keep the antibiotic potent. Aminoglycosides can be inactivated by the presence of organic material in the uterus. Next to the susceptibility pattern, another consideration for antibiotic selection is the antibiotic-neutrophil interaction. Antibiotics like polymyxin B or tetracycline can penetrate phagocytes and may cause enhanced intracellular killing of microbes. Penicillins, streptomycin and gentamicin don't pass easily into phagocytes. Intracellular ingestion of bacteria may also protect the bacteria from extracellular antibiotics' actions and allow some bacteria to continue multiplication within neutrophils (Brinsko, 2011b).

One benefit of using intrauterine antibiotics is that it is easier and cheaper to reach minimum inhibitory concentrations than systemic antibiotics. Therefore, higher local endometrium concentrations can be achieved with less product. Another advantage is that its use involves minimal systemic disturbance of the microbiome in other body systems (Dascanio, 2011; Leblanc, 2009). A disadvantage is that there can be insufficient concentrations in the uterus' deep layers. There is a risk of irritation and bacterial selection in the reproductive tract, such as the vestibulum (Samper et al., 2007j). Systemic antibiotics can be advantageous because minimum inhibitory concentrations in the endometrium can be achieved without inducing contamination to the uterus. The antibiotic penetrates the deeper layers more efficiently. Also, the antibiotic levels are less fluctuating if used systemically. Sometimes systemic antibiotics are chosen if it is impossible to perform an intrauterine infusion for various reasons. Another benefit of administering antibiotics systemically is that antibiotic levels can be maintained throughout diestrus. This is less convenient when antibiotics are used intrauterine (Samper et al., 2007j). Higher doses are required to result in the same levels in the endometrium and uterine lumen (Brinsko, 2011b). Another disadvantage when antibiotics are administered systemically is the possible risk of inducing complications such as diarrhoea, colitis, and systemic anaphylactic reactions (Dascanio, 2011). Brinsko (2011b) claimed that most practitioners prefer to use antibiotics intrauterine based on the earlier discussed advantages. More recently, after performing a questionnaire, Köhne et al. (2020) concluded that most practitioners administer systemic antibiotics, like trimethoprim sulfate and only occasionally, intrauterine antibiotics were used. Veterinarians in Germany were more likely to use systemic antibiotics, whilst practitioners from the USA, France or the UK more commonly used intrauterine antibiotics (Köhne et al., 2020).

Not all systemic antimicrobials can be administered in the uterus without some adjustment (Rodriguez et al., 2012). The acidic or alkalotic pH of some antibiotics can be quite irritating to the endometrium if they are not buffered. For example, gentamicin and amikacin must be buffered with sodium bicarbonate because they have an acidic pH. Enrofloxacin has an irritating alkalotic pH but has been less effective when buffered to a more neutral pH (Brinsko, 2011b). The systemic formulation of enrofloxacin can cause endometrium necrosis if administered intrauterine (Rodriguez et al., 2012). It is recommended to use enrofloxacin systemically if suggested to use this drug based on the susceptibility pattern. If administered with a dosage of 5 mg/kg twice daily, enrofloxacin creates endometrial levels above the minimum inhibitory concentration of many bacteria that can cause endometritis (Brinsko, 2011b).
The use of intrauterine antibiotics can be accompanied by using ecbolics, but it is not thoroughly studied in which interval the ecbolic should be administered. If administered too soon after infusion, it could result in the antibiotic's expulsion before its beneficial effects on the bacteria were exerted (Brinsko, 2011b). By infusing approximately 30 to 60 ml, excessive expulsion of the drug can be prevented. If using larger volumes (200ml), the chance of covering the entire endometrium is ensured. Rectal massage is recommended to distribute the drug throughout the uterus (Samper et al., 2007). If no fluid remains in the uterus after lavage, the antimicrobial infusion can be administered immediately after the lavage. If ecbolic treatment is desired, the ecbolic can be administered 4 to 8 hours later. This interval should allow the beneficial effects of the antimicrobial drug to be exerted before being expelled. When oxytocin is used, which will increase the uterine contractility for 30 to 50 minutes, the antimicrobial infusion could be performed 1 hour after lavage and ecbolic administration. PGF2α sustains the uterine contractions for about 2 to 4 hours. So, when PGF 2 is used, the antimicrobial infusion shouldn’t be administered during this interval (Brinsko, 2011b).

The usage of intrauterine antibiotic infusions on the day of breeding is not advised because many antibiotics show spermicidal properties when present in high concentrations. Again, it is critical to wait at least 4 hours after breeding before using intrauterine treatment in order to not hinder the oviductal colonization of the sperm (Brinsko, 2011b).

The opinions on the intrauterine infusion of antibiotics after breeding are divided. Studies evaluated that the efficiency of routine postbreeding antibiotic infusion is limited, and much data indicates that the practice is either of no benefit in improving pregnancy rates or even disadvantageous. The remaining solution within the uterus can harm embryonic survival (Brinsko, 2011b). It is debatable because mares susceptible to postbreeding endometritis have a reduced capacity of uterine clearance and impaired defence mechanisms. They cannot be compared with healthy mares in which routine administration of intrauterine antibiotics has no adverse effect on pregnancy rates when treated (Samper et al., 2007).

6.1.2.3. Treatment of biofilm associated infections
Bacteria within a biofilm may be protected from the hosts' immune system and the traditional uterine treatments for infectious endometritis. For effective treatment, the biofilm must be disrupted to enable the antibiotic and immune system to access the bacteria (Knutti et al., 2010). Therefore, antibiotics and non-antibiotic agents to disrupt the biofilm are often combined. Tris-EDTA can be added to most common antibiotics to disrupt biofilms without causing reduced activity of the antibiotic. Another alternative is Dimethyl sulfoxide (DMSO), known for its anti-inflammatory actions, the bacteriostatic effect on Staphylococcus aureus and Escherichia coli, but also for the capability to disrupt biofilms. Additionally, it has a synergistic effect on some antibiotics. Some reports have shown that DMSO can inhibit the bactericidal activity of ampicillin, kanamycin, and some quinolones (Mi et al., 2016).

Hydrogen peroxide is an acidic, water-soluble fluid with strong oxidative properties and can inhibit many enzymatic processes. Due to the production of oxidants, it can be cytotoxic at high levels, which can be beneficial to decrease biofilm biomass and reduce colony-forming units of bacteria such as Escherichia
coli and Klebsiella pneumoniae. Generally, catalase-negative bacteria such as Streptococcus equi subspecies zooepidemicus, Enterococcus faecalis and Clostridia are susceptible to hydrogen peroxide, but catalase-positive bacteria can challenge the efficiency of the product. This makes use of hydrogen peroxide discussable because bacteria such as Escherichia coli and Staphylococci spp. can utilize the enzyme catalase to break down hydrogen peroxide into hydrogen and water to protect themselves. Furthermore, Escherichia coli is capable of adaptation by undergoing DNA repair in response to hydrogen peroxide exposure. This mechanism can lead to the forming of mutations and the development of resistance (Min and Gu, 2003).

It must be mentioned that there are mainly in vitro studies for the treatment with Tris-EDTA and DMSO and their clinical efficacy is yet to be proven in controlled studies in vivo (Morris et al., 2020).

6.1.2.4. Irritating intrauterine solutions
Various intrauterine solutions can be used, but they all should be used carefully and correctly (Samper et al., 2007j). A chemical curettage of the uterus can be accomplished by infusing strongly irritating solutions such as diluted kerosene or magnesium sulfate solution. This will cause endometrial necrosis and induces an acute inflammation with the subsequent movement of neutrophils and serum-derived opsonins into the uterine lumen (Brinsko, 2011b).

Diluted povidone-iodine solution with the concentration of 5 ml of 10% povidone-iodine in 1L of saline can be used 4 hours post-breeding. Using this treatment, no adverse effects on pregnancy rates in reproductively sound mares are reported compared with a uterine lavage with saline. In in vitro experiments, it is reported that this treatment can be a benefit in treating against antimicrobial-resistant bacteria such as Pseudomonas aeruginosa (Samper et al., 2007j).

There are reports of inflammation, necrosis, discomfort, and reproductive sterility, which can occur if these intrauterine solutions are not administered and used correctly. The degree of reaction also is individually dependant on the mare (Samper et al., 2007j).

For example, a 50 ml infusion of 0,2% iodine solution is reported to induce acute haemorrhage, edema, necrosis and chronic fibrosis in endometrial biopsy specimen. Blistering and vaginal straining can be induced using 0,5% chlorhexidine-gluconate, whereas a 0,25% concentration of chlorhexidine-gluconate caused acute endometrial inflammation and degenerative changes. However, if used correctly, these solutions can positively affect the future evolution of the uterus. For example, scrubbing the clitoris with a 4% chlorhexidine solution followed by packing with nitrofurazone ointment is the recommended treatment for Taylorella equigenitalis. This procedure is combined with intrauterine administration of antibiotics like potassium penicillin for 5 to 7 days (Samper et al., 2007j). After holding the irritant in the uterus for 5 to 10 minutes, the uterus is flushed with lactated Ringer's solution, and emollient medication of the vagina for protection against scalding is administered. Following every day or every other day, a lavage should be performed to prevent forming of intrauterine adhesions (Brinsko, 2011b).
In New Zealand, kerosene is a commonly intrauterine used product to purge the uterine lymphatic glands during chronic bacterial endometritis. The kerosene removes mucus, cilia, cases endometrial epithelial cells and stimulates the endometrial glands' apical portions. This has a beneficial effect on the regeneration of the epithelium and mucociliary apparatus. The uterine inflammatory changes resolve within 21 days of treatment so that 50% of the treated mares conceived successfully despite their predicted foaling rate being under 50% based on the uterine biopsy scores (Bracher et al., 1991).

6.1.2.5. Platelet-Rich Plasma
Platelet-rich plasma is whole blood plasma with concentrated platelets and is commonly used to treat joints, bursae, skin wounds and soft tissue injuries in equine clinical practice (Pereira et al., 2019; Arguelles et al., 2008). The combination of autologous plasma and antibiotic therapy has been reported to improve pregnancy rates for lactating and barren mares (Pascoe, 2018). The use of plasma products has been directed towards immunomodulation rather than elimination of uterine pathogens. This may be because platelet-rich plasma produces different growth factors in vitro that may be useful in treating endometritis. An anti-inflammatory effect is demonstrated due to platelet-rich plasma's ability to suppress the expression of COX-2, metalloproteinase-3, TNF-a, IL-1, and vascular adhesion molecules (Mazzocca et al., 2013). It is shown that its use can strengthen the uterine defences of susceptible mares by enhancing the bactericidal activity against Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae (Burnouf et al., 2013; Alvarez et al., 2011; Moojen et al., 2008). These bacteria are known causes of equine endometritis (Canisso et al., 2016). Administration of platelet-rich plasma modulates the uterine inflammation to semen and reduces inflammation in mares resistant or susceptible to endometritis (Reghini et al., 2014, 2016). Also, intrauterine treatment with autologous conditioned serum reduced neutrophils' infiltration in mares (Ferris et al., 2014).

During plasma collection, heparin or citrate should be used as an anticoagulant. EDTA has an inactivating effect on the complement, and its use should be avoided. Autologous plasma is recommended because infectious agents and immunologic incompatibilities can be transferred when heterologous plasma is used, but still heterologous plasma brings positive effects and is mainly used. Plasma is infused by adding 50 ml to 100 ml to the uterine lavage once daily for 4 to 5 days. Uterine lavage can be combined with intrauterine plasma infusion to treat chronic infectious endometritis (Brinsko, 2011b).

It may only be beneficial in mares without a mechanical clearance problem because mares susceptible to endometritis do not possess a quantitative deficiency of immunoglobulins. Plasma can be used in mares that repeatedly fail to become pregnant but have no history of fluid accumulation. So, it is questionable if this treatment is effective in susceptible mares, and more studies are needed to verify the benefit of using plasma instead of other treatment strategies (Samper et al., 2007).

6.2. Fungal endometritis
The most common yeast and fungal organisms are Candida spp., Aspergillus spp., and Mucor spp. These infections are primarily superficial and usually react well to treatment. In some cases, the infection
can get deeper into the tissue and result in chronic deep endometritis, which responds less to treatment (Brinsko, 2011b).

Whilst treating a bacterial endometrial infection, fungal endometritis can develop. These infections often must be treated over a long period of time because the mare can become reinfected or remain infected. But fungal uterine infections are also reported in mares without previous history of treatment for endometritis. Immunosuppression, malnutrition, tissue trauma, endocrine dysfunctions, physiologic changes associated with pregnancy or growth, and changes induced by antimicrobial and immunosuppressive drug therapy are predisposing factors to fungal infections. More specifically, antibiotics, corticosteroid and chemotherapeutic agents have been reported to influence the immune function and promote opportunistic fungal infections. By eliminating competing bacteria within the vagina using antibiotics, fungal infections can develop (Samper et al., 2007i).

The most isolated fungi from the mare’s reproductive tract are Candida spp. and Aspergillus spp. (Dascanio et al., 2010). Candida albicans can adhere to vaginal epithelium better than other Candida spp. and represents the most common Candida spp. infections (Samper et al., 2007i). The effects of estrogens increase Candida spp's ability to adhere to vaginal tissue (Samper et al., 2007i). Aspergillus spp. has been reported to invade tissues, which makes therapy more challenging. Tissue invasion is more likely when macrophage function decreases because macrophages are particularly effective against Aspergillus spp. and the hyphal forms are too large for phagocytosis by polymorphonuclear cells (Samper et al., 2007i).

An essential part of the immune response combating fungi in the reproductive tract consists of humoral and cell-mediated immunity. It is reported that Candida spp. infections can disturb and disrupt the mucosal surface and T cells. Complement-dependent and complement-independent phagocytosis by macrophages may be interfered by immunodeficient states of the mare. A common predisposing factor in Candida spp. infections is neutropenia which leads to decreased leukocyte mediated phagocytosis (Samper et al., 2007i).

Candida spp. infections may be contagious and can be transmitted through skin-to-skin contact. The transmission is an essential factor when natural service is performed, although there are no cases reported to be caused by natural cover or artificial. Fresh semen, extended semen and urethral cultures from stallions have yielded positive fungal cultures (Samper et al., 2007i).

Increased endometrial damage can develop because of enzymes, including endotoxins, mycotoxins, and elastases, which appear in some types of fungi such as Aspergillus spp. (Samper et al., 2007i).

6.2.1. Diagnosis of fungal endometritis
Approximately 2% to 3% of all positive uterine cultures will be positive for fungi (Samper et al., 2007i). Clinical signs include purulent vaginal discharge, an excess of echogenic fluid within the uterine lumen and often mares with fungal endometritis have a history of chronic infertility (Scott, 2019).
The most common diagnostic tools for detecting fungal endometritis are cytological uterine specimen, uterine culture or uterine biopsy. The sample can be taken from the uterus, the vagina and clitoral fossa. On cytology, Candida spp. may be present as both yeast and hyphal elements using a Diff Quick stain and are often seen first (see figure 6). By using a culture, the presence of fungi can then be confirmed second. Blood agar has been proven to be a suitable agar for fungal growth, and the culture can be subcultured on Sabaroud's agar. The growth of fungi takes more time than bacterial growth. It is essential for an optimal treatment choice to isolate and identify the fungi for further classification to allow for antifungal drug sensitivities to be performed. Yeasts alone are associated with superficial infections, whereas hyphae are associated with more tissue invasive fungal forms, and these deep situated infections may be more difficult to resolve (Samper et al., 2007).

Fig. 6: Aspergillus fumigatus organisms (on the left) and Candida albicans organisms (on the right) in a uterine cytology sample.
From Dascanio and McCue (2014).

Fungal organisms are known to have a slow-growing nature in culture or are difficult to cultivate. Using the polymerase chain reaction (PCR), this challenge can be mitigated, making it possible to identify fungal DNA in these situations (Ferris et al., 2013). PCR test results can be available in six hours (Ferris 2013).

6.2.2. Treatment of fungal endometritis

The initial treatment addresses the elimination of the predisposing factors, such as a poor perineal conformation, immunosuppression and discontinues intrauterine infusions with antibiotics, combined with the administration of ecbolics and performing uterine lavages (Dascanio et al., 2010).

Studies report that imidazoles, triazoles, other azole derivatives and antifungal agents such as polyenes can be used to treat mares with fungal uterine infections. Polyenes are considered fungicidal drugs, and the azoles are fungistatic even though both antifungal agents have similar mechanisms of action. They act in the fungal membrane by binding ergosterol or inhibiting ergosterol biosynthesis (Giguère, 2013). More recently used products, such as fluconazole, are also effective. The products are administered topically because oral absorption from the gastrointestinal tract is inadequate for most drugs, except for fluconazole, whose absorption is very high (Samper et al., 2007).
Amphotericin B and azole are employed in the treatment of *Aspergillus spp.* infections (Samper et al., 2007i).

It may be indicated in refractory or recurrent clinical cases to combine some antifungal products to treat the fungal infection more aggressively. For example, one recommended therapeutic protocol involves a uterine lavage's performance followed by an intrauterine infusion of an antifungal product once daily for five days and concurrent systemic administration of a second antifungal agent for 21 days. Products like nystatin can be used intrauterine, and fluconazole may be used systemically, for example. Another method to increase the antifungal agent's potency is to mix it into a buffered chelator such as Tris-EDTA before uterine infusion (Hess et al., 2002).

A bacterial infection will often develop after the fungal infection is resolved because the bacteria may have a less competing environment in which to grow. Another factor to be aware of is that the vaginal microflora can be disturbed, allowing overgrowth of pathogenic bacteria to develop (Samper et al., 2007i).

Every reproductive tissue which may harbour fungi must be treated, including the clitoris, clitoral fossa, vagina, cervix, and uterus (Samper et al., 2007i).

A large volume uterine lavage is performed to remove any potential uterine fluid, decrease fungal and bacterial numbers, and expose organisms to antifungal solutions. It is possible for the lavage to use 2% acetic acids such as white vinegar or 0.1% povidone-iodine solution. A benefit of the uterine lavage is the stimulation of neutrophilic influx, which enhances fungal destruction. Oxytocin or prostaglandins may be used to stimulate uterine contractility. After that, the antifungal agents are administered into the uterine lumen, and antifungal antibiotics are placed in the vagina and clitoral fossa to remove potential reservoirs of fungi. Treatment should be limited to estrus duration, which is 5-7 days if prostaglandins were administered (Samper et al., 2007i).

The uterus' fungal infection can lead to significant endometrial damage if it has been present for longer than a few estrus cycles. A uterine biopsy is recommended to get more information about the endometrium after the infection is cleared (Samper et al., 2007i).

### 6.3. Prevention and management of chronic infectious endometritis

To prevent contamination and infection of the reproductive tract during reproductive procedures, it is essential to work as sterile as possible. Generally, high hygienic standards should include the hygiene of the breeding equipment, the stallion's penis and the technique of the various procedures from examinations to natural or artificial insemination (Samper et al., 2007j).

Before semen collection from a stallion, the stallion’s penis should be washed and dried to minimize contamination. In addition, it is common to use antibiotic containing semen extender for artificial insemination. The antibiotic will decrease bacterial growth in the gathered semen (Samper et al., 2007j).
Also, the mare’s hindquarters must be free of contamination when performing procedures that can bring bacteria or debris into the reproductive tract. The mare’s tail should be wrapped and pulled to the side, and the vulva should be cleaned and dried before insemination. For invasive procedures, the labia and the vagina should be washed alternating with povidone-iodine scrub applied with wet cotton and rinsed with clean water. The labia and clitoris are then dried (Samper et al., 2007e). Every predisposing factor should be managed in the correct way (Dascanio et al., 2010).
7. Chronic degenerative endometritis

Another and more degenerative type of endometritis is called endometriosis or chronic degenerative endometritis. This condition lacks the typical features of active inflammation and has a more degenerative histopathologic character. The predominant cells observed in the submucosal periglandular endometrial tissues are lymphocytes, plasmacytes and macrophages. Endometriosis development is age-related and develops during a mare’s advancing reproductive lifetime, but the age of onset can vary between individual mares. The histopathologic changes are fibrosis, lymphatic stasis, uterine sacculation, transluminal adhesions, and glandular or lymphatic cysts. They have a negative impact on the mare’s ability to carry a foal to term. Cystic glandular changes are thin-walled and contain fluid with lymphocytes, suggesting that they may be due to stromal fibrosis obstructing uterine glands. The glandular duct obstruction arises from several mechanisms, including the strangling effect produced by periglandular fibrosis, decreased myometrial tone and peristaltic activity associated with prolonged anestrus or age-related changes. Another reason may be epithelia hypertrophy, one of the first responses to periglandular fibrosis observed in the equine endometrium (Samper et al., 2007e).

7.1. Diagnosis of chronic degenerative endometritis

Chronic infiltrative endometritis is diagnosed by the presence of mononuclear cells (histiocytes/lymphocytes and plasma cells) in biopsy specimens (Samper et al., 2007c).

Endometriosis is diagnosed by a biopsy of the endometrium. A good indicator is the presence of degenerative glandular changes surrounded by lamellae of fibrous tissue or cysts lined by glandular epithelial cells. Sometimes periglandular, perivascular or diffuse stromal fibrosis and angiopathy are seen. Scattered in the stroma lymphatic lacunae may be seen, which are pools of tissue fluid. These lymphatic cysts can migrate into the uterine lumen when becoming larger and are attached to the endometrium by a lymphatic duct. The lymphatic cysts can be seen via ultrasonography when large enough, but it is unusual to sample a lymphatic cyst using conventional biopsy techniques (Samper et al., 2007c).

7.2. Treatment of chronic degenerative endometritis

There are no reliable and specific treatments described that can permanently alter or reverse the course of endometriosis (Samper et al., 2007c).

A possible treatment is the attempt to induce superficial endometrial inflammation, necrosis, and tissue loss. There is a chance that the regenerated endometrial histomorphologic architecture will be in a better state to support any subsequent pregnancies. The techniques include endometrial curettage and chemical irritants. If used incorrectly, these treatments may induce irreversible damage to the urogenital tract. If superficial mucosal or submucosal necrosis is induced, it can lead to intrauterine, cervical, or vaginal adhesions (Samper et al., 2007m).
The long-term effects are scarcely studied. It is recommended to only use these kinds of treatments in mares that have failed to respond to more traditional intrauterine treatments (Samper et al., 2007m).

There are different techniques to remove endometrial cysts resulting from endometritis and endometrial fibrosis. The endometrial cysts may be glandular or lymphatic and can range from a few millimetres to 20 cm. The removal can be performed using a snare, laser hysteroscopy, loop cautery, or uterine biopsy instrument. After removing the cyst, the mare should be treated with uterine lavage and intrauterine antibiotic infusion (Dascanio and McCue, 2014h; Dascanio and McCue, 2014o).

The best action is to attempt to prevent endometrial damage and irritation of any kind. It can be realized by optimizing the breeding management technique and minimizing uterine contamination (Samper et al., 2007m).
8. Contagious equine metritis (CEM)

Another type of endometritis called contagious equine metritis (CEM) is caused by *Taylorella equigenitalis*, a microaerophilic gram-negative coccobacillus and is mainly transmitted venereal between mares and stallions (Mair, 2013b). The organism’s transmission can occur during live cover, during artificial insemination or between stallions via contaminated equipment during semen collection (Dascanio and McCue, 2014a). The stallions act like carriers without symptoms. Clinical signs in mares can be grey mucopurulent discharge, variable degrees of vaginitis, cervicitis, and endometritis, leading to temporary infertility (Timoney, 2011). A short estrus cycle and cases of symptomless carriers are also reported (Mair, 2013b). Infertility and abortion are also reported in mares (Dascanio and McCue, 2014a). The discharge is mostly visible 2 to 10 days after breeding. The spread of CEM is controlled and must be reported to federal authorities (Brinsko, 2011b).

In some cases, the mare will carry the pregnancy to term and will act as a carrier. *Taylorella equigenitalis* has been recovered from the placenta of positive mares and the genitalia of colts and fillies due to transmission in utero or parturition. It is also reported that *Taylorella equigenitalis* can lead to abortion as the bacteria have been cultured from several sites in the aborted foetuses (Samper et al., 2007h).

8.1. Diagnosis of contagious equine metritis (CEM)

The clinical signs of CEM include a greyish vulvar discharge which typically disappears without treatment. Another sign is a shortened inter-estrus interval due to acute endometritis. Some mares show no clinical signs, become asymptomatic carriers and can become pregnant and carry the pregnancy to term (Samper et al., 2007h).

Mares are tested as part of a surveillance programme for CEM or during a disease outbreak. Horses imported from countries positive for CEM are required to go through quarantine and treatment protocol. The diagnostic test must be negative for *Taylorella equigenitalis* for the mare to be released from quarantine. In order to take representative samples to diagnose CEM, it is essential not to disinfect or scrub the locations where the samples are taken and not to treat the mare with systemic antibiotics for at least seven days before testing. If necessary, organic debris can be carefully removed with a disposable paper towel or by using minimal sterile water before taking samples. Swabs are collected from the clitoral sinus and clitoral fossa for bacterial culture, each with separate sterile swabs. If the mare is not pregnant and after collecting the samples for clitoral culture, a guarded uterine culture swab is collected under appropriate hygienic standards of the cervix and the endometrium. The samples should be placed into Amies transport media with charcoal and kept cool (Couacy-Hymann et al., 2019). They should be tested in the laboratory within 48 hours of collection. *Taylorella equigenitalis* may be detected by bacterial culture or serology (Dascanio and McCue, 2014a).

If the mare is treated due to positive testing, a 21 period must elapse before new samples can be taken for diagnostic testing (Dascanio and McCue, 2014a).
If stallions from countries positive for CEM must be tested, the stallion is brought to an approved facility with mares that can be bred as part of the testing process. A sterile swab is used to collect samples from the prepuce near the base of the penis, urethral sinus, fossa glandis and the distal urethra. Again, for every location, a separate sterile swab is used (Dascanio and McCue, 2014a).

Is the stallion positive on any culture, treatment is initiated before any test breeding. If the stallion is negative on all cultures, two CEM-negative mares are bred by live cover. The mares will subsequently undergo CEM-testing by collecting a blood sample for complement fixation testing and the clitoral area and cervix/ uterus cultures as described above (Dascanio and McCue, 2014a).

Another more recent diagnostic tool includes the use or an rtPCR (Real Time-PCR), which reduces the assay duration and increases diagnostic specificity and sensibility compared to performing a diagnostic culture or serology. Using rtPCR, a pre-breeding screening of a large number of mares is possible to improve biosecurity practices, especially with today’s international movement of horses (Albertine et al., 2020).

8.2. Treatment of contagious equine metritis (CEM)

Most mares will recover spontaneously, but some mares may become chronic carriers (Samper et al., 2007h).

Pre-foaling treatment for culture-positive mares may decrease the likelihood that the foal will become infected. Still, the foal must be tested after parturition and foals born to positive mares must remain under quarantine until they are tested negative for CEM.

For treatment, any smegma from the central clitoral sinuses is removed manually. The two lateral and the central clitoral sinuses are cleaned with 0,5 ml of a 2,5% squalene cleansing solution to remove retained smegma. Next, the clitoral sinuses are lavaged using a curved tip sterile syringe with approximately 5 ml of warm sterile saline to remove debris. The clitoral sinuses and clitoral fossa are cleaned and scrubbed with a 2% chlorhexidine solution, and the washed areas are rinsed with warm sterile saline. The clitoral fossa and sinuses are then packed with 0,2% nitrofurazone ointment or a silver sulfadiazine ointment. According to the AHPIS/ USDA timetable and instructions, the procedure of scrubbing and packing is repeated. Still, the clitoral sinuses’ infusion with cleansing solution only needs to be done on the first day of treatment. The treatment's efficacy must be evaluated with a complete culture series of the clitoris and cervix or endometrium (Dascanio and McCue, 2014a).

For the treatment of the stallion, the prepuce, penis, fossa glandis, and urethral sinus are cleaned with 2% chlorhexidine once a day according to the AHPIS/ USDA timetable and instructions. After cleaning, the structures are coated/ packed with 0,2% nitrofurazone ointment or a silver sulfadiazine ointment (Dascanio and McCue, 2014a).
Therapy of the acute stage of CEM is similar to routine endometritis treatment. The uterus is treated with a large volume uterine lavage of sterile physiologic fluids, oxytocin injections and intrauterine antibiotic infusions based on culture and sensitivity (Samper et al., 2007h). The current recommended treatment consists of an intrauterine antibiotic administration of 5 to 10 million units of potassium penicillin for 5 to 7 days combined with thoroughly scrubbing of the clitoris with 4% chlorhexidine solution (Samper et al., 2007h).
9. Treatment of conformational abnormalities

A few commonly used surgical procedures can be performed on mares with conformational changes which harm the reproductive tract. These surgical reconstructions can correct abnormalities like pneumovagina, urovagina, recto-vestibular lacerations and recto-vestibular fistulas and the causes of ascending infections to restore the mare’s fertility. The most common surgical procedures are vulvar reconstruction and procedures performed to correct vestibular and cervical abnormalities. A pneumovagina is caused by a cranially sunken anus and tipping of the vulva and can be corrected by performing a Caslick’s surgery or vulvoplasty. A urovagina can be caused by cranioventral deviation of the vagina. Before and during surgery, the practitioner should make sure that the mare is fit to have surgery performed and that the mare has been adequately vaccinated against tetanus. Perineal injuries can be caused by abnormalities of the foaling process (Brinsko, 2011b).

Before performing surgery, the endometrium should be biopsied for histologic examination to make a prognosis for the mare to carry a foal to term in the future. It may not be very lucrative to perform surgery if the mare has severe and widespread periglandular fibrosis and other negative endometrial changes because the mare’s ability to conceive and carry a viable foal to term will be lower (Brinsko, 2011g).

9.1. Caslick’s surgery

The standard surgical procedure to treat a pneumovagina and infertility (subfertility) caused by infection of the genital tract is the Caslick’s operation, also called vulvoplasty. A Caslick’s surgery is a commonly used procedure to provide permanent apposition and healing of the dorsal part of the right and left vulvar lips to prevent ascending of contaminants, air, and bacteria (Dascanio and McCue, 2014e).

The mare is fixed in a stock to perform this operation, cleaned appropriately and sedated if necessary. The dorsal aspect of the mucocutaneous margin of the labia is desensitized with lidocaine, mepivacaine or another local anaesthetic product (Brinsko, 2011g; Dascanio and McCue, 2014h). The local anaesthetic solution is subcutaneously injected at the dorsal commissure of the vulva and then infiltrated along the margin of each labium to below the base/ floor of the ischium. To determine the level at which the margins of the labia should be sutured, the fingers are pressed on either side of the vulva to locate the ischium. The labia are sutured slightly below the ischium floor to prevent the vulva from migrating too far cranially. The ventral portion of the vulva must remain spacious enough to allow adequate urination (Brinsko, 2011g). For this, approximately 3 cm (two fingers) should be left unopposed (Samper et al., 2007d). The space should also be fitted to the reproductive technique that the practitioner wants to use. In other words, the opening must be big enough for the stallion to insert the penis during mating or to insert a vaginal speculum if the mare is going to be bred via artificial insemination (Brinsko, 2011g).

After infiltrating the mucocutaneous margin of the labia, a thin strip of tissue (0.5 cm wide) is removed at the mucocutaneous junction of each labium (Brinsko, 2011g). It is essential not to remove the skin because a shortage of skin will occur during future repairs, leading to difficulties in the surgery (Samper et al., 2007d). The two strips are joined at the dorsal commissure (Dascanio and McCue, 2014p). This removal can happen with a scissor or a bistoury/ scalpel, but it has been shown that working with a
scissor will give better results (Brinsko, 2011g). If using a scalpel, a U-shaped incision is made rather than removing a strip of mucosa (Dascanio and McCue, 2014p). After removing the tissue, the labia are apposed with no. 0 or 00 nonabsorbable sutures. In Samper et al. (2007), it is recommended to use absorbable suture material like catgut because the material will be degraded by either pregnancy testing (15 days) or the next breeding cycle. Nonabsorbable or delayed absorbable materials may damage the stallion if the mare is bred by natural cover (Samper et al., 2007). Patterns mostly used are simple continuous (see figure 7), continuous interlocking and continuous horizontal mattress. The type of suture pattern is not the most important factor. Creating a good apposition without too much tension on the suture line is most important (Brinsko, 2011g). The sutures should be placed 0.5 to 1 cm apart and 0.5 to 1 cm from the mucosa's cut edge to result in good apposition (Dascanio and McCue, 2014p). 10 to 14 days after the procedure, the sutures should be removed if nonabsorbable material was used, and at this point, the wound should be healed (Brinsko, 2011g; Dascanio and McCue, 2014p).

Assuming the labia must be opened for breeding, vaginal examination, or foaling, they should be closed again when the large opening is no longer necessary (Brinsko, 2011g). The period of reopening should be as short as possible to minimize the risk of contamination of the vagina and cervix and thus prevent ascending infections from occurring (Samper et al., 2007d). The vulva can be opened partially if the goal is to facilitate breeding or uterine therapy in a non-pregnant mare. Is the mare pregnant and about to give birth, the Caslick must be opened 10-14 days before the due date or earlier if the mare shows signs of parturition (Dascanio and McCue, 2014p). If not opened before parturition, the passage of the foal can cause lacerations of the vulva. If it is necessary to keep the Caslick’s intact, it is crucial to keep a close eye on the mare that is about to give birth to open the vulva before the passage of the foal. Once a Caslick's surgery is performed on a mare, the mare must remain Caslicked for the remainder of her reproductive life and the Caslick’s surgery must be redone every time the vulvar lips were opened because of parturition or other procedures (Brinsko, 2011g).

A variation of the Caslick’s surgery is a breeder’s/ breeding stitch. It is a single, simple interrupted suture placed at the ventral aspect of the vulva to preclude the need to open the sutured labia if the mare must be bred. The suture material is commonly umbilical tape or heavy, polymerized caprolactam suture. The suture bite must be extended at least 1 cm abaxial to the margin of the labia, and the deep portion of the suture should be buried in the labial submucosa to prevent injuries on the stallion’s penis during breeding in case the mare should be bred with natural cover. A breeding roll can be used to limit the extension of the vulvar opening when the penis is inserted during natural service to avoid tearing of the tissue of the breeder’s stitch (Brinsko, 2011g).
9.2. Vestibuloplasty

In the case of a mare with a too sunken anus and perineal body deviated so far cranially and ventrally that the vulvar cleft assumes a nearly horizontal position over the ischium, it may not be possible to correct the pneumovagina or urovagina with a Caslick’s surgery. In this case, the perineal body must be reconstructed, which is called perineoplasty, perineal reconstruction or vestibuloplasty (Samper et al., 2007d).

During this procedure, an isosceles triangle of mucosa from the dorsal aspect of the vestibule will be removed. The mare is restrained and sedated, and the perineum is desensitized with epidural anaesthesia with lidocaine or mepivacaine (2%, 1-1.25 ml/100kg, 18-20 Gauge needle). The rectum is evacuated manually and then filled with gauze sponges or rolled cotton lint to prevent the passage of faeces into the surgical field. The tail is wrapped, and the mare is cleaned appropriately (Samper et al., 2007d). After that, the vestibule's dorsal aspect is exposed by retracting the labia laterally with a loose suture of umbilical tape or Backhaus towel clamp placed through the labia at the juncture of its dorsal one third and ventral two thirds. Also, the dorsal commissure of the vulva must be retracted dorsally and caudally with the same devices. On the dorsal aspect of the vestibule that lies directly below the anus, a point is marked to serve as the apex of a triangle of mucosa to be removed. The distance between this mark and the dorsal commissure of the vulva is marked on the mucocutaneous margin of each labium. A line between these two points on the labia serves as the mucosal triangle base to be removed. Now it is possible to imagine a triangle between these points. Using a scalpel or a curved scissor, the points of the triangle are connected, and the mucosa in this area is removed. Two or three nonabsorbable sutures are placed horizontally through the perineum in a line from the triangle's apex to the base. Stents (small rolls of gauze swabs) are set to prevent the sutures from pulling through the skin. The triangle is brought together in a vertical position by placing tension on the sutures (Brinsko, 2011g). Using the Caslick’s operation, the perineum and vulva's skin are closed (Samper et al., 2007d). The sutures should be examined regularly to see if any signs of tissue necrosis are visible caused by excessive tension on the sutures. If this is the case, the sutures can be removed daily or at alternate-day intervals to relieve pressure. If no necrosis or other problems are discovered, the sutures could be removed after 5 to 10 days (Brinsko, 2011g). Complete healing of the deep tissue takes 4 to 8 weeks, and sexual rest must be observed until the healing process is complete (Samper et al., 2007d).
If the surgery was successful, the perineal body's area returns the vulva to a more vertical position. The vulvar opening will stay big enough for breeding the mare with natural service. The sunken position of the anus can’t be corrected in this way and will remain unchanged (Brinsko, 2011g).

9.3. Perineal Body Transection

Another technique to correct pneumovagina is the Perineal Body Transection or Pouret’s operation. The attachments between the ventral terminal rectal wall and the dorsal vaginal wall are cut during this surgery, allowing the vulva to take a more normal vertical position. Simultaneously, the pull of the sunken anus on the dorsal vagina is reduced, but it doesn’t reduce the size of the vaginal opening.

The preparation of the surgery starts with the sedation of the mare and preparation of the perineum. 2% Xylocaine is administered epidural, and the perineum is draped. A transverse incision of 4 to 6 cm in the perineal skin is made midway between the ventral anus and dorsal vulvar commissure. The perineal muscles are bluntly dissected anteriorly for 8 to 14 cm until all muscles connecting the caudal rectum and anus are sectioned, and the vulva is vertically orientated. Although skin closure is recommended, it is also possible to let the wound heal by secondary intention because the suture wound frequently dehisces (Samper et al., 2007d).

After surgery, the mare shouldn’t be bred by natural service, but it is immediately possible to perform artificial insemination (Samper et al., 2007d).

9.4. Episioplasty

In severe anatomic abnormalities where the Caslick’s operation would be ineffective and in mares with extensive or repeated second-degree perineal lacerations, a procedure called Episioplasty is used. The operation is designed to restore some degree of function to the perineal body.

The preparations are similar to these of the procedure of the Caslick's (Samper et al., 2007d).

The first step of the surgery is removing a triangular portion of the dorsal vestibule from both sides. An increase in the perineal body's size and a decreased propensity of the vestibule to create negative pressures are created by apposition of the incision's ventral borders and extinguishing the potential space above. The ventral edges are apposed with a continuous absorbable suture, and the dead space is minimized with single sutures. After that, the mucocutaneous junction is closed like the Caslick’s procedure (Samper et al., 2007d).

Again, it is crucial to open the dorsal vestibule and vulvar lips before foaling to prevent perineal laceration (Samper et al., 2007d).
9.5. Urethral extension

There are different surgical techniques to correct a urovagina. Options include the caudal retraction of the transverse fold or the urethral extension (Brinsko, 2011g).

Performing a urethral extension, the aim is to create a tunnel that extends caudally along the floor of the vestibule from the external urethral orifice to the labia. Preparation of the mare includes systemic administration of a sedative and desensitization of the perineal region by carrying out epidural anaesthesia with lidocaine or mepivacaine (2%, 1-1.25 ml/100kg, 18-20 Gauge needle). The tail is then wrapped and tied dorsally, the perineal area is scrubbed with antiseptic soap, and sterile tissue retractors are used to expose the lumen of the vestibule and the urethral orifice. The next step is suturing the transverse membranous fold (hymen remnant) to the dorsal aspect of the vestibule, and the mucosa of the ventral surface of the transverse membranous fold is incised 1 to 1.5 cm dorsal of the urethral orifice so that it encircles the cranial half of the urethral orifice. Both sides of the incision are continued caudolaterally and slightly dorsally to create a mucosal extension. This extension is wider caudally than cranially to prevent the build-up of pressure during urination. For apposition, the incision is closed in three layers using no. 0 or 00 absorbable suture material. First, the incision is closed by suturing the right and left ventral edges of the mucosal incision in apposition with a continuous horizontal mattress suture pattern. As a result of this, the sutured mucosal edges will invert into the lumen of the extended urethra. Next, the submucosal tissue on the right and left side of the vestibule exposed by the incision are sutured by using a simple continuous suture pattern. The last layer to close is the mucosa. The right and left dorsal edges of the mucosal incision are closed with a continuous horizontal mattress suture pattern, which will evert the mucosal edges into the lumen of the vestibule (Brinsko, 2011g). After the procedure, a broad-spectrum antimicrobial drug which will be eliminated through the urine is administered for 3 to 5 days. Trimethoprim-sulfamethoxazole is a commonly used drug. A nonsteroidal anti-inflammatory drug like flunixin meglumine or phenylbutazone is administered for 12 to 24 hours after surgery to lower postoperative pain of the horse (Brinsko, 2011g).

Often the mare suffers from a urovagina in combination with a pneumovagina. In this case, a Caslick’s suture is also placed (Brinsko, 2011g). Some mares are instead treated with performing a vestibuloplasty, as described previously.

9.6. Surgery of cervical lacerations

Mares with cervical lacerations are usually given a poor prognosis for being a broodmare. Still, a cervical repair may not always be necessary because the dimension of the tears can differ. Repair is not required if the laceration does not extend beyond more than half of the cervix's length and when the internal cervical os remains efficient. However, cervical lacerations can lead to cervical adhesions (Samper et al., 2007b).

Evaluation of the cervix's patency is best done in estrus when a competent cervix must be dilated to allow passage through the cervix. It should be possible to pass a finger into the uterine lumen (Brinsko, 2011g).
To evaluate the cervix’s closure, it is possible to administer progesterone intramuscularly (Brinsko, 2011g) in America or Australia. In contrast, in Belgium, progesterone administration is limited to the oral route (Berkmanns, 2018). Alternatively, rather than administering medication, it is also possible to assess the cervix during diestrus. The administration of sedatives is discouraged because that may artificially alter the tone of the genital tract and prevent the accurate assessment of the cervical competency. It is vital to make sure the mare is not pregnant because cervical manipulation in a pregnant mare may cause abortion or ascending placentitis (Samper et al., 2007b). If the cervix is not efficiently closed, the cervix must be repaired. Because of scar tissue formed after fixing, the cervix will be incapable of dilating enough. During parturition, the scar tissue is usually disrupted, and it is required to repair the cervix again after birth (Brinsko, 2011g).

Cervical adhesions can be broken manually. This must be done daily to avoid reattachment or scar formation due to bleeding. It is recommended to apply a steroid-antibacterial cream to the cervical lumen daily (Samper et al., 2007b).

When it is not appropriate to break the cervical adhesions manually, it is also possible to treat the cervical abnormality surgically (Samper et al., 2007b). The mare should be operated when she is in diestrus or anestrus. If necessary, progesterone can be administered to bring the mare in diestrus. Before performing the surgery, the mare should be sedated, epidural anaesthesia should be administered, the tail must be wrapped, and the perineal and vulvar region must be scrubbed with an antiseptic soap. A two-bladed speculum that opens laterally is used to visualize the cervix. The cervix is retracted caudally by placing two retention sutures in the external cervical os on each side of the tear and then tying the suture to one side of the speculum base. With scissors, the mucosa is excised from the defect until the cervical musculature is identified.

By using no 0. or 00. absorbable suture, the cervical defect is closed in three layers. At the cranial end of the defect and continuing caudally to the external os, the first layer is closed using a continuous horizontal mattress pattern. The next layer to suture with a simple continuous pattern is the muscular layer. The muscular layer is the most critical layer to close, so it is essential to place the sutures in healthy tissue to ensure that the layer remains intact after healing. The outer and last mucosal layer toward the vaginal lumen is sutured cranially to caudally in an everting manner into the vaginal lumen by using a continuous horizontal mattress pattern. After closing the layers, the speculum’s retention sutures are removed, and the vagina and the external cervical mucosa are covered with an oily, antimicrobial solution. If necessary, the mare must receive a Caslick’s operation or vulvoplasty. A broad-spectrum antimicrobial drug can be administered for 3 to 5 days, and the mare should sexually rest for one month. Before breeding, the cervix should be examined again for competency and patency (Brinsko, 2011g).
10. Discussion

The potentially severe consequences and the highly diverse nature of different endometritis types stress the need for an appropriate approach to this problem by the veterinarian. Therefore, the advantages and disadvantages of the different diagnosis, treatment, and prevention techniques must be considered and specifically chosen for the individual mare.

Essential factors in approaching endometritis in practice are maintenance of hygienic standards and preventing ascending contaminations of the reproductive tract at all times. Self-contamination must be avoided by managing the reproductive tract's conformational abnormalities, and the uterine clearance mechanisms should be supported. Before the mare is bred, it is essential to detect individuals that may be susceptible to endometritis or require special care by detecting predisposing factors, symptoms of insufficient uterine clearance, or endometritis. To achieve this, the appropriate use of cytological and histological examination methods of the uterus is advised.

Commonly performed therapeutical procedures, including uterine lavage after, or if necessary, before insemination, together with the use of ecbolics like oxytocin, have shown promising results in increasing reproductive success. Depending on cytological or histological findings, it may be necessary to administer systemic or intrauterine antibiotics. Other products, including biofilm disrupters, anti-inflammatory products, irritating intrauterine solutions, or platelet-rich plasma, have shown positive effects if used appropriately.

Amongst others, further research into the treatment of chronic degenerative endometritis, N-acetyl cysteine use, and the comparison of disinfectants' efficacy over antibiotics or antifungal agents could advance veterinary reproductive practice.
Bibliography


