# Title: Endometritis as a cause of infertility in mares.

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# Endometritis as a cause of infertility in mares.

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# ABSTRACT.

Endometritis is considered the most common cause of infertility in mares, which gives it greater importance in reproductive management, due to the large economic losses it can generate. Persistent inflammation/infection of the uterine mucosa or endometrium, causing a decrease in gestation rates.

The factors that must be taken into account on the part of the female, such as defects in anatomy and conformation, lymphatic drainage, myometrial contractions, vascular problems, etc., as well as on the part of the male, such as the semen that causes irritation and inflammatory reaction in the endometrium, are explained.

Endometritis is classified as water and chronic infectious endometritis, persistent riding-induced endometritis, endometriosis, endometritis caused by venereal infections. We also mentioned the different defence mechanisms that the mare presents in order to avoid this type of infection.

In this dissertation, we address the different types of endometritis and the causes of each one of them, as well as the different types of diagnostic methods that can be applied depending on the type and the most appropriate treatments for each one of them.

We must bear in mind that the diagnosis of endometritis should be made from a multimodal approach, based on clinical history, clinical signs, ultrasound, cytology and cultures, endometrial biopsy and histology.

The objectives of the treatment of endometritis are threefold, three main objectives: to correct the defects in the uterine defences neutralise pathogens and control post-cubrition inflammation. In addition to the above, reference is made to the indiscriminate use of antibiotics, which may be a key factor as a trigger for some endometritis such as fungal, especially in cases of mares after riding, as a prevention of the appearance of PMIE, and thus decrease the infertility that could generate.

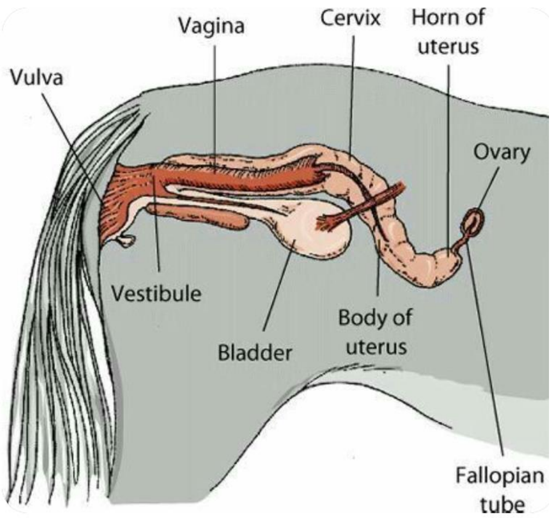
In order to be able to recognize the problems generated by this pathology and the signs it causes that help us to identify it, we must know the normal functioning of the reproductive physiology of the mares, in addition to reproductive anatomy as a method of identifying possible problems such as poor physical conformation of the reproductive system, as well as detecting problems in the oestrous cycle of the animal.

# 1. INTRODUCTION.

## 1.1. Reproductive anatomy of the mare.

The reproductive system is made up of two groups of organs: (1) those structures that are intrinsic to the reproductive tract (ovaries and tubular genitalia) and (2) those structures that are physically isolated from the reproductive tract but play a role in the regulation of reproductive events (e.g., pineal gland, retina, hypothalamus, pituitary gland) (Brinsko & Blanchard, 2011).

The reproductive tract consists of two ovaries and a tubular tract, including the paired oviducts and uterine horns, and a single uterine body, cervix, vagina, vestibule, and vulva. The lumen of the female reproductive tract is the only channel in the body that communicates between the abdominal cavity and the external environment. More than half of the reproductive tract lies within the abdominal cavity, with the remainder confined to the pelvic cavity (Brinsko & Blanchard, 2011). (FIGURE 1)




**(FIGURE 1).**

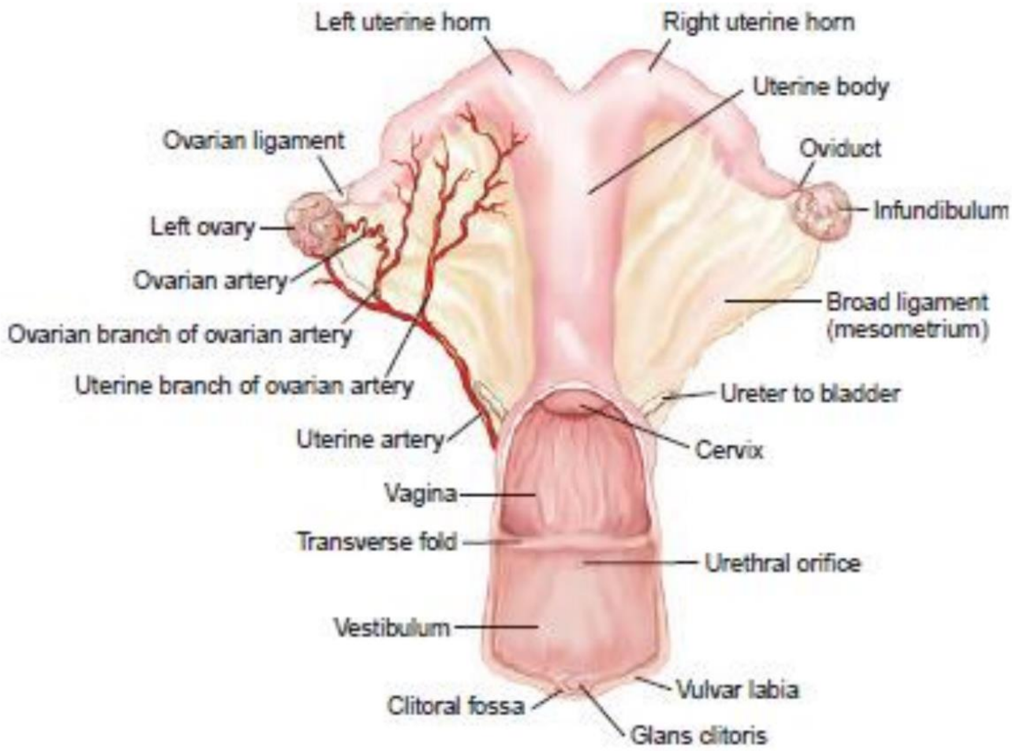
The reproductive tract can be thought of as a Greek Y-shaped tubular organ (Morel,2005). The tubular components are the oviducts, uterus, cervix and vagina (Senger, 2003).

The perineum, vulva, vagina, and cervix protect internal structures such as the uterus, oviducts, and ovaries, which are more delicate, and responsible for fertilization and embryonic development (Morel, 2005).

The anatomy of the female reproductive organs is strongly influenced by age, the reproductive state present at a given time and the previous reproductive history of the animal (Dyce, 2010).

When the ovum is discharged from the follicle at ovulation, it is received at the level of the ovarian bursa, which is thought to assist the passage of the ovum into the oviduct. The oviduct is responsible for movement of sperm and ova to a common site (the ampullary-isthmic junction) for fertilization. After fertilization, the developing equine embryo travels down the oviduct; and after 4.5 to 5 days, it secretes increasing amounts of prostaglandin E 2, which allows it to gain entrance into the uterus for gestational support. The uterus provides the proper environment for the embryo to develop further (Brinsko & Blanchard, 2011).

The cervix accommodates the expanded glans penis of the stallion at estrus to allow intrauterine deposition of sperm and closes tightly during pregnancy to prevent ascending bacterial or fungal infection from the posterior tract. The cervix also expands considerably at the time of parturition to accommodate passage of the foal. The caudal portion of the cervix projects into the lumen of the vagina. Longitudinal folds comprise the lining of the cervix and are continuous with the endometrial folds that line the uterine body. The cervix secretes two types of mucus: a thin mucus to lubricate the posterior genital tract in preparation for coitus and a more viscid mucus to help seal the cervical lumen during pregnancy. The vagina is a potential space that expands to permit penile and foal passage. A transverse fold (remnant of the hymen) overlies the external urethral orifice and is the anatomic division between the vagina, which is anterior, and the vestibule, which is posterior. The juncture between the vestibule and vagina is referred to as the vestibulovaginal ring. When closed, this ring restricts entry of air and debris into the upper tubular tract. The vulva is limited to the external opening of the tubular tract. (FIGURE 2)




**(FIGURE 2)**

## 1.2. Reproductive Physiology of the mare.

The mare, from the point of view of reproduction, is defined as a seasonal polyestric female. The sexual season is related to the length of the day, it is very manifest in spring and summer, it decreases or progressively throughout the autumn and ends up disappearing in the winter. Gestation lasts 11 months (Carvajal Ramos, 1998), and thus present their births in the most appropriate season for the survival of their offspring (Escobar, 1997).

It means that the mare presents repeated periods of "zeal" around March to September, which depend on the photoperiod, the environment, nutritional status, and race. They use the photoperiod to program if reproductive activity: ovulatory activity or estrous cyclicity on days with more hours of light and anoestrus with the reduction of the photoperiod (Escobar, 1997).

The interval between zeal is 21 days on average, varies between 18 and 24; but it is common to see in the period of riding that zeal reappears at shorter intervals. The zeal lasts an average of 6 days, it is shorter in that zeal that immediately follow the birth (3-4 days), and longer at the beginning of the sexual season (8-9 days). Ovulation is spontaneous and occurs 24-48 hours before the end of zeal. (Carvajal Ramos, 1998).

The regularity of the estrous cycle depends on a delicate balance of hormones produced by the pineal gland, hypothalamus, pituitary gland, ovaries, and endometrium (Blanchard, 2003).

The effect of the photoperiod is carried out by means of the hormone melatonin, secreted in the pineal gland during the dark hours (Diekman *et al;* 2002).

In the ovulatory season, the mare has oestrous cycles; they are repeated successively, as long as the mare does not conceive and conditions remain successively, as long as the mare does not conceive and the appropriate photoperiod (days with more daylight hours). To establish the interovulatory intervals, the hypothalamus-pituitary-gonad axis is stimulated, with the additional participation of the uterus. Stimulus leading to follicular growth with production of oestradiol, ovulation, formation of corpus luteum with production of progesterone and regression of the corpus luteum (Cortés-Vidauri Zimri *et al.,* 2018).

However, there are different pathologies that can affect the reproductive cycle reproductive cycle of equines, such as endometritis, a disease that is the third most common third most common medical condition in this species.

# 2. ENDOMETRITIS.

## 2.1. Introduction.

Generally equine production is affected by reproductive disorders, mares beings the most interest, since unlike the males they show clearly visible signs of these abnormalities. You can find infectious diseases such as; vaginitis, cervicitis, metritis, endometritis, among others (LeBlanc MM, 2010).

Which in turn leads to irregularities in the reproductive parameters of the female such as repetition oestrus, increase in the number of services by conception, anestrus, among others, resulting in economic losses, therefore, it is important to identify possible causes to minimize the risk of the presentation of the disease and provide practical solutions to this problem (M. M. LeBlanc, 2010).

Fertility varies markedly among mares. Apart from poor management and incorrect timing of mating, probably the most important reason for low pregnancy rates is endometritis/endometrosis. A survey of 1149 veterinarians in the United States ranked endometritis as the third most frequently occurring medical problem in adult horses (Traub-Dargatz *et al*., 1991).

"Equine endometritis" is often cited as one of the leading causes of infertility in the mare (Troedsson, 1999; Liu & Troedsson, 2008; LeBlanc, 2010; Troedsson & Woodward, 2016; Canisso *et al.,* 2020), also referred to as, persistent inflammation/infection of the uterine mucosa or endometrium (Gutjahr *et al*., 2000). And one of largest Equine reproduction problems, causing substantial losses economic (Concha-Bermejillo & Kennedy, 1982). Losses can present as conception failures, early embryonic losses, early foetal losses, mid-pregnancy abortions, placentitis, septic births, postpartum metritis or delays in return to cyclicity (LeBlanc & Causey, 2009).

The causes of subfertility In mares are varied and may or may not be Infectious, constituting for this reason a multifactorial phenomenon. However, pathologies affecting the uterus are the main cause of reduced fertility, being the third most important clinical problem after colic and diseases of the important clinical problem after colic and respiratory diseases (Traub-Dargatz *et al.,* 1991).

Uterine inflammation is considered the most important gynecological condition in horses, and is the most important cause of embryonic loss before 35 days of gestation in mares (Card, 2005). Where, infectious – inflammatory processes occupy 25% - 60% of the causes of infertility; of which endometritis has a prevalence of 30% (Causey, 2007).

Inflammation can be acute or chronic of the uterus modulated by the action of the local immune system or influenced by hormones, whether infectious or non-infectious in origin, being this disease one of the main causes of infertility in mares intended for breeding (Gallego *et al.,* 2018).

Since it leads to problems to embryo survival at the time of implantation in the uterus, as well as premature luteolysis by reason of high endometrial prostaglandin loads (Rodríguez, 2014).

It should be noted that in the mare it can occur physiologically as a response to covering or childbirth, and pathologically due to agents of infectious or non-infectious origin. In all cases, it is caused by the entry of an external agent, together with the uterine response to it (Morris *et al*., 2020).

The clinical signs for these two types of endometritis can be indistinguishable, except that the first type has microorganism(s) involved. In addition, to date, there are limited controlled studies comparing infectious vs. non-infectious endometritis in mares (Kotilainen *et al.,* 1994; Troedsson *et al.,* 1994). Mares with defective reproductive anatomy (e.g., poor vulvar conformation, torn vestibulovaginal sphincter, ventral sacculation of the uterus, impaired uterine contractility, cervix incompetence, and atrophied endometrium folds) are more prone to aspirate air or accumulate fluid or urine in the vagina and uterus, which make the mare simultaneously prone to infectious and non-infectious endometritis (Trotter *et al.,* 1988; Canisso *et al.,* 2016). Additionally, mares with competent immune response and functional anatomy of the reproductive tract are able to clear infections spontaneously (i.e., mares resistant to endometritis), whereas mares with a deficient immune response may be unable to combat the development of an infection or may have persistent inflammation (Fumuso *et al.,* 2003; Fumuso *et al*., 2007; Christoffersen *et al., 2012;* Christoffersen *et al.,* 2015).

The main factor on which the control of this pathology depends is the existence of defence mechanisms that eliminate uterine contents after mating or childbirth (Peña, 2011).

### 2.2. Mechanisms of defence of uterus in the mare.

The mare's uterus is kept free of contaminants by means of physical, immunological and functional lymphatic system mechanisms. The physical barriers that prevent microorganisms from accessing the uterus are the vulva, vaginal vestibule ring, and cervix. Immunologically, the complement system through its alternative pathway plays an important role in the immune cellular defence of the uterus (Couto & Hughes, 1985).

The uterine cavity is protected from ascending infection by several anatomic structures. The first line of defence in the prevention of contamination of the vagina and eventually the uterus is provided by the seal of the normal vulvar labia. The second physical barrier is the vestibulovaginal sphincter. And the third important anatomic barrier is the cervix. However, in some mares, compromised cervical function is observed, and its entity may remain open during anoestrus and even dioestrus. The most common cause of cervical incompetence is a lesion consequent to dystocia (Blanchard *et al.,* 2011).

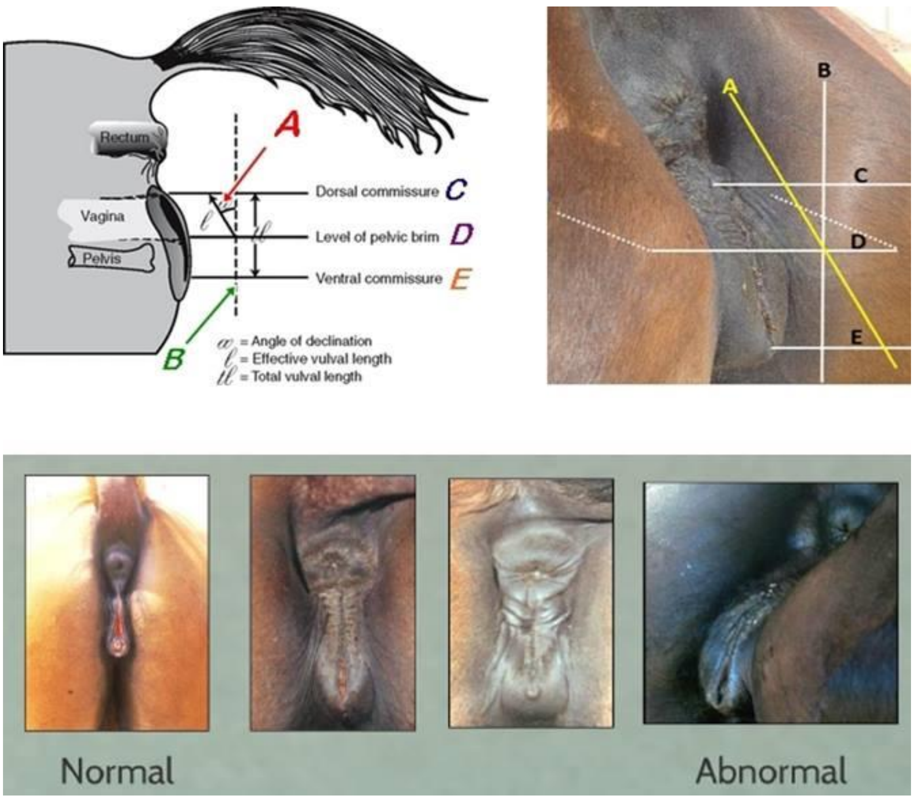
A plan for treatment and prevention of uterine infection should include a plan to reestablish normal barrier function. Surgical procedures such as episioplasty, vestibulovaginoplasty, and rectovaginal tear repair should be considered if indicated and reestablish the uterine contractility in the elimination of bacteria, fluid, and inflammatory products from the uterus after breeding (Liu IK *et al.,* 2008).

## 2.2.1. External barrier (anatomical conformation):

The uterine cavity is protected from ascending infection by several anatomic structures (Blanchard *et al.,* 2011). A good anatomical conformation of the anal, perianal, vulva, vaginal vestibule and cervix will prevent/decrease contamination at the uterine level.

- The first barrier against infection is the vulva, its angle in relation to the anus is important since when the animal defecates, its correct conformation prevents contamination of the reproductive tract by faeces. (Pycock, 2006).
- The second barrier is the vestibulovaginal fold, close to the vaginal entrance, between the labia and the cervix.
- The third barrier is the cervix, as an incompetence of this structure prevents drainage of inflammatory detritus in the process of endometritis or allows entry of the cervix of contaminants from the vaginal area into the uterus (McKinnon, 2009).

A plan for treatment and prevention of uterine infection should include a plan to reestablish normal barrier function. Surgical procedures such as episioplasty, vestibulovaginoplasty, and rectovaginal tear repair should be considered if indicated and reestablish the uterine contractility in the elimination of bacteria, fluid, and inflammatory products from the uterus after breeding (Liu & Troedsson, 2008). (FIGURE 3).




**(FIGURE 3)**

2.2.2. **Internal barriers (uterine defence mechanisms):** the three main defence mechanisms are:

- **Humoral response**: three types of immunoglobulins have been described in the genital tract of the mare:

  Immunoglobulins A, M and G (IgA, IgM, IgG), which are produced locally from plasma and endometrial epithelial cells or via uterine plasma supply. During oestrus females significantly increase IgA levels in the uterine environment, so that are much higher in the uterus than at the serum level. This phenomenon confers a special immune protection to the uterus in this phase of the cycle, in which the mare is more susceptible to bacterial contamination (Díaz-Bertrana, 2013).

- **Cellular response**: the presence of germs and sperm inside the uterus generates an inflammatory response characterised by increased blood and lymph vascularisation with leukocyte infiltration, mostly polymorphonuclear neutrophils (PMNs), into the uterine lumen, their main action being phagocytosis (Troedsson *et al.,* 2001).

- In addition to the aforementioned mechanisms as internal barriers, we have other **non-immunological mechanisms** such as: the increase of secretions, the opening of the cervix and the capacity of uterine contraction capacity during oestrus, due to the hormonal action mainly of prostaglandins, increase the drainage effect. This increase in secretions (uterine mucus), contains lysozymes, a slightly acid pH (6.4) and antimicrobial properties which confer a certain protection against bacterial contamination (McKinnon, 2009).

Fluid that remains stagnant in the uterine lumen, as time passes, inactivates complement proteins, which prevents neutrophils from being able to properly phagocytose bacteria. On the other hand, the free fluid separates the uterine folds, which will deprive the neutrophils of a surface on which to phagocytose bacteria (Troedsson, 1999).

### 2.3. Pathogenesis.

Generally, we know that endometritis is the result of the interaction between a causative agent, whether infectious or not, and the uterine defence mechanisms of the uterus. It is necessary to understand the diagnosis and treatment of this pathology, knowledge of its pathogenesis.
In the past, the condition was believed to be exclusively the result of bacterial contamination of the uterus and treatment strategies were focused on preventing bacteria from entering the uterus and on treating mares with signs of endometritis with antibiotics (Asbury *et al.,* 1993).

Fertility varies markedly among mares. Apart from poor management and incorrect timing of mating, probably the most important reason for low pregnancy rates is endometritis/ endometrosis. A survey of 1149 veterinarians in the United States ranked endometritis as the third most frequently occurring medical problem in adult horses (Traub-Dargatz *et al*., 1991).

Until relatively recently, mares were classified as resistant or susceptible to endometritis based on their ability to eliminate uterine infection within a certain period of time after challenge (Hughes & Loy, 1969; Peterson *et al*., 1969) and/or on their endometrial biopsy scores (Kenney & Doig, 1986).

Infection and disease depend on the adherence of pathogenic organisms to mucosa, colonisation or penetration of the epithelium and/or release of bacterial toxins leading to the development of uterine disease. The development of endometritis also depends on the balance between the immune response of the animal, and species and number of bacteria. When risk factors increase, it unbalances the balance developing the disease (Malschitzky *et al.,* 2007).

Disorders in the reproductive anatomy also play a very important role in the presentation of this disease, among them we can mention: deficiencies in myometrial contractions, inadequate lymphatic drainage, poor mucociliary activity, impaired cervical function, vascular degeneration, inflammation of the endometrium, poor perineal conformation. It has been reported that elderly mares may be susceptible to the disease (Ricketts, 2008).

Thus, we can affirm that endometritis is a pathology of multifactorial nature. Despite this, it can be classified, based on etiology and pathophysiology in four different categories: (1) endometriosis (chronic degenerative endometritis), (2) pathologies of sexual transmission, (3) persistent mount-induced endometritis and (4) chronic infectious endometritis (Troedsson *et al.,* 1995; Troedsson, 1997).

Therefore, additional causative agents have been identified, and has learned to separate uterine infections from "physiological" endometritis induced by reproduction, derived from uterine exposure to semen (Troedsson, 2006).

All reproductive events are associated with contamination. The coitus, parturition and the events that follow produce a great opportunity for microorganisms from the environment to migrate into the reproductive tract. External contamination also occurs because of poor perineal conformation associated with the general condition, anatomy and/or integrity of the internal reproductive organs. Despite the common introduction of pathogens into the uterus, persistent endometritis will occur in the mare fails to resolve the mare fails to resolve the inflammation (LeBlanc., 1998). The mares that could not clear the uterus natural infection were classified as susceptible to persistent endometritis (Troedsson, 2006).

### 2.3.1. Opportunistic infection endometritis.

Infectious endometritis plays a major role in equine subfertility (Wingfield Digby *et al.,* 1982; Riddle *et al.,* 2007). Microorganisms, including pathogenic or opportunistic bacteria and fungi, may gain access to the uterus during breeding. While the resistant mare should rapidly respond to the presence of microorganisms, inadequate immune response, and impaired uterine fluid drainage (e.g., due to pendulous uterus, tight cervix, or impaired myometrial contractility) may lead to infection (LeBlanc *et al.,* 2009; Overbeck *et al.,* 2011).

#### 2.3.1.1. Bacterial endometritis.

Bacterial endometritis is considered one of the most common causes of infertility in the mare, therefore, it acquires importance in reproductive management, due to the economic losses it can represent in equine exploitation (Causey, 2007).

The bacteria commonly identified in by results obtained from bacterial crops were: *E. coli, Proteus spp., Staphilococcus aureus, Sreptococcus spp., Pseudomona aeruginosa y Klebsiella pneumoniae* (Pacheco S, 2011).

Corresponding to the highest percentages of isolation in the crops represented in FIGURE 4:

| Bacterias aisladas de los hisopados uterinos de rutina* | | |
|---|---|---|
| **Bacterias Identificadas** | **No.** | **%** |
| ***Escherichia Coli*** | 48 | 54,54 |
| ***Proteus spp.*** | 9 | 10,22 |
| ***Staphilococcus aureus*** | 8 | 9,09 |
| ***Streptococcus*** sp, beta hemolítico | 5 | 5,68 |
| ***Pseudomonas aeruginosa*** | 2 | 2,27 |
| ***Klebsiella pneumoniae*** | 1 | 1,14 |
| * Porcentajes promedio en el periodo 2008-2010 | | |

**(FIGURE 4)**

At a rate of up to 50% of the endometritis cases, *S. zooepidemicus* is globally the most consid- erable bacterial endometritis agent in mares (Albihn *et al.,* 2003; Causey, 2006). This most commonly isolated pathogens from the uterus of mares, suffering from infectious endometritis associated with increased age, parity, and poor vulvar conformation (Causey, 2006; Hamouda *et al.,* 2012; Christoffersen *et al.,* 2015). Endometritis produced by *S. zooepidemicus* can origin from a reservoir of dormant bacteria residing within the endometrium, and not only as an ascending infection (Rasmussen *et al.,* 2013; Petersen *et al.,* 2015).

Generally, the most common bacteria isolated in uterine crops are *Streptococcus equi* subp. *Zooepidemicus* y *Escherichia coli* (Shin *et al*., 1979; Ricketts *et al*., 1993; Albihn *et al*., 2003), both commensal of the mucosa and considered as opportunistic pathogens (Shin *et al*., 1979; Ricketts *et al*., 1993; Riddle *et al*., 2007).

However, these bacteria can be introduced into the uterus by breeding because bacteria often are introduced into the uterus during coitus or AI (Tibary A *et al*., 2014). Other commensal bacteria isolated from reproductive tract of mare include *Actinomyces pyogenes, Proteus spp., Staphylococcus spp., and Citrobacter spp*. These bacteria are occasionally supported by cytologic of histopathologic evidence of concurrent inflammation. α-Hemolytic Streptococcus, Enterobacter spp., and Staphylococcus epidermidis are rarely causes of endome- tritis and should be considered simple contaminants (Troedsson *et al.,* 2011; Tibary A *et al.,* 2015).

### 2.3.1.2. Fungal endometritis.

Fungi are less commonly associated with endometritis (1–5%), and they may occur alone or in association with bacteria. Candida spp. and Aspergillus spp. are the most common fungal organisms isolated from the uterus of mares with endometritis. Fungal organisms isolated from the equine uterus include Aspergillus spp., several Candida spp., Cryptococcus neoformans, Fusarium spp., Hanse- nula anomala, Hansenula polymorpha, several Rhodotorula spp., Scedosporidium apiospermum, Saccharomyces cerevisiae, Trichosporon beigelii, Torulopsis candida, Acremonium spp., Actinomyces spp., Fusarium spp., Aspergillus fumigatus, Aspergillus glaucus, Aspergillus niger, Monosporium apiospermum, Monosporium spp., Aureobasidium pullulans, Mucor spp., Candida albicans, Nocar- dia spp., Candida ciferii, Paecilomyces spp., Candida famata, Penicillium spp., Candida guillermondii, Pseudallescheria boydii, Candida krusei, Rhodotorula glutinis, Candida lusitaniae, Rhodotorula minuta, Candida parapsilosis, Rhodotorula rubra, Candida pseudotropicalis, Rhodotorula spp., Candida rugosa, Candida stellatoides, Candida tropicalis, Torulopsis glabrata, Candida zeylanoides, Circinella spp., Trichosporon cutaneum, Coccidiodes immitis, Trichosporon spp., Cryptococcus humicolus, and Yarrowia lipolytica (Dascanio *et al.,* 2010).

Aspergillus and Candida are the most common genera, but other species have also been less commonly identified (e.g., Mucor sp) (Dascanio *et al.,*2010; Coutinho da Silva *et al.,* 2011; Beltaire *et al.,* 2012). It should be noted that fungal endometritis typically occurs as an opportunistic infection and has been identified after repeated use of intrauterine antimicrobials (Hinrichs, K *et al*., 1992; Stout, 2008; Coutinho da Silva *et al.,* 2011). They are yeast species that can be identified by direct microscopic examination by endometrial exfoliative cytology (Scofield DB *et al.,* 2013; Stefanetti V *et al.,* 2014).

Most mares presented with fungal endometritis have a history of previous bacterial endometritis. These mares usually undergo intense therapy that includes frequent uterine lavages and intrauterine infusion of antibiotics. The frequent uterine manipulation, associated with anatomical problems leading to pneumovagina, results in chronic contamination of the uterus. In addition, antibiotics drained from the uterus alter the normal bacterial flora in the vagina, predisposing to an excessive growth of opportunistic fungal organisms, making the vagina and external genitalia the primary reservoir for pathogens. Affected mares tend to be old and pluriparous with poor perineal conformation or maiden mares with cervical incompetence. Prolonged progesterone therapy may predispose mares to fungal endometritis because cervical drainage is decreased and uterine muscular activity and neutrophil function are altered (LeBlanc *et al.,* 2011).

Other factors contributing to fungal infections include the presence of a moist environment, exposure to a large number of fungi and the presence of necrotic focus as occurring with trauma, abortion, and retained placenta (LeBlanc *et al.,* 2011; Stefanetti V *et al.,* 2014).

The diagnosis of endometritis can be made by gynecological examination, rectal palpation, transrectal ultrasound, cytology and crops of the uterine contents (Card C, 2005; Aguilar J *et al.,* 2006).

Diagnosis is based on the presence of fungal elements and inflammatory cells in endometrial smears. Yeast appears as small, round, single cell, brown to black spores on cytological smears obtained from infected mares, whereas molds have long filamentous hyphae (LeBlanc *et al.,* 2011; Beltaire KA *et al.,* 2012). It has suggested that the hyphae are more invasive than yeast and, consequently, more difficult to treat and eliminate. Candida albicans can be present in both yeast and hyphal forms and therefore can penetrate deeper into the endometrium and/or growth intracellularly. However, in endometrial cytology and histology used to diagnose fungal endometritis, fungal elements only are visualized in the lumen of the uterus and not penetrate deeper in the endometrium (Beltaire KA *et al.,* 2012).

However, the difficulty in implementing a reliable diagnostic method has been a major challenge, causing false positives or false negatives, compromising the clinical evaluation of the animal and the therapeutic conduct of the veterinarian (Nielsen JM *et al.,* 2005; Overbeck W *et al.,* 2011).

### 2.3.2. Pyometra.

Pyometra is defined as the accumulation of mucopurulent material within the uterus (Asbury *et al.,* 2005), with or without corpus luteum, depending on the degree of lesion in the endometrium (Hughes *et al.,* 1979).

Pyometra can be caused by different causes. Including interference cleaning liquid is described from the uterus, obstruction of flow caused by the cervix closed by hormonal influence or cervical pathology such as fibrosis and adhesions, or secondary to trauma, such as experienced during dystocia (Vandeplassche M *et al.,*1979).

When the endometrium is severely damaged, with extensive loss of superficial epithelium, severe endometrial fibrosis, and glandular atrophy, it results in a prolonged luteal phase, due to the reduction of synthesis and release of prostaglandin F2a. In the mild endometritis, which presents accumulation of small amounts of fluid in the uterine lumen, premature release of prostaglandin F2a occurs and consequently luteolysis. The endometrial histopathology of acyclic mares revealed severe endometrial injury with almost complete erosion and atrophy (Hughes *et al.,* 1979).

Progesterone from a persistent CL can close the cervix. Similarly, exogenous progesterone administered continuously cleaning the uterine avoided. Endometrial cups have been observed as a cause of pyometra where no fetuses or fetal membranes were found within the uterine lumen (Vandeplassche M *et al.,* 1979).

Intrauterine accumulation of purulent material was observed in mares with open cervix (Kennedy PC *et al.,* 1993). These mares may have an innate resistance to a reduced endometrial infection, which can progress to a pyometra (LeBlanc MM *et al.,* 2011). The most common organism associated with pyometra in the mare *is S. zooepidemicus, E. coli, Actinomyces spp., Pasteurella spp., Pseudomonas spp., Propionibacteri- um spp., and Candida rugosa* (Abou-Gabal M *et al.,* 1977; Hughes JP *et al.,*1979). Mares with pyometra have vulvar discharge when the cervix is relaxed or open. The pus is often creamy, it may be variable, with higher volumes associated with, watery, thin gray appearance. Smaller volumes are associated with pus cheesy exudate thickened. Rarely are clinical signs of systemic disease (Hughes JP *et al.,*1979).

The cervix represents the third and last barrier between the uterus and the external environment in the reproductive tract of the mare (Brinsko SP *et al.,* 2011).

### 2.3.3. Venereal Infectious endometritis.

Sexually transmitted diseases are those acute infections which are induced after mating mares with stallions which are inapparent penile carriers of Taylorella equigenitalis, certain unspecified serotypes of Pseudomonas aeruginosa, and Klebsiella pneumoniae capsule types 1, 2 and 5 (Platt *et al*., 1977).

Among viral diseases that can be transmitted venereal are equine arteritis (EAV) and equine herpesvirus 3 or equine coital exanthema virus, and one is a protozoan infection (T. equiperdum) (Timoney PJ, 1998; Timoney PJ *et al.,* 2011).

T. equigenitalis is a highly contagious pathogen which caused infertility in Thoroughbred mares in the UK in 1977 (Powell *et al.,* 1978).

Although *K. pneumoniae, P. aeruginosa, and S. zooepidemicus* are commensal organisms of penis and prepuce in the stallion, in cases of disturbance as a result of indiscriminate washing of the penis or too frequent washing with a surgical scrub or detergent soap. Under such circum- stances, there is the potential for colonization of the penis and prepuce with the mentioned bacteria. These organisms, which rarely produce clinical disease in the stallion, can give rise to endometritis with reduced fertility in susceptible mares, especially in mares with a preexisting defect in uterine clearance (Lu K *et al.,* 2007).

Transmission of these bacteria occurs venereally, either natural breeding or AI with infective semen (Clement F *et al.,* 1994; Timoney PJ *et al.,* 2011). *T. equigenitalis* is sensitive to several antibiotics and treatment is highly successful (Ricketts, 1996*). P. aeruginosa* and *K. pneumoniae* tend to be much more intractable to treatment (Ricketts, 1999; Wingfield Digby, 1999).

#### 2.3.3.1. Contagious equine metritis (CEM).

CME is a venerea disease that was first described in 1977 (Crowhurt, 1977). It spreads by direct o indirect contact (Crowhurst, 1977; Platt *et al*., 1977; Ricketts *et al.*, 1977; Powell, 1981), being more likely transmission by natural mount.

I can also be transmitted through the use of contaminated materials (vaginal speculums, forceps, obstetric sleeves, etc.) so it can also be transmitted by IA if you do not have the appropriate hygienic-sanitary measures. CEM is a transmissible, exotic, venereal disease caused by *T. equigenitalis*. This bacterium is closely adapted to the specific environmental conditions of the equine genital tract, whereby it is the only venereal transmissible bacterial agent in horses (Timoney PJ *et al.,* 2011).

The main vector of transmission of this disease is the stallion asymptomatic (Duquesne *et al.,* 2007). The preferred areas of settlement being the urethral fossa, urethral sinus, distal urethra, the external surface of the penis and the foreskin. However, mares do show a clinical picture of urethral fossa and urethral sinus (Heath & Timoney, 2008).

Initial exposure to the disease usually results in infertility. An infected mare may fail to conceive or abort. The disease frequently is associated with an endometritis (Timoney PJ *et al.,* 1977; Timoney PJ *et al.,* 2011). Abortion due to *T. equigenitalis* infection is very rare (Nalashiro H *et al.,* 1981).

The infertility is temporary (only a few weeks), with adverse effect on a mare's fertility. Persistence of *T. equigenitalis* in the reproductive tract of a chronically infected mare not interfere with the maintenance of the birth of a healthy foal (Timoney & Powell DG, 1982; Timoney *et al.,* 2011). The mare, though asymptomatic, is still infectious and can remain a carrier for several months (Timoney & Powell DG, 1982; Timoney *et al.,* 2011; Breuil MF *et al.,* 2015).

#### 2.3.3.2. Equine viral arteritis (EVA).

Equine viral arteritis (EVA) is a contagious viral infection affecting both mares and stallions. Prevalence of EVA ranges from 1–3% in Quarter Horses to 7% in Arabians and Thoroughbreds and to 80% in Standardbreds. Mares clinically affected with EVA may show fever, limb edema, anorexia, depression, inflammation around the eyes, nasal discharge, skin rash, and abortion. The significance of EVA infection in stallions is that certain strains can develop carrier status in 30–60% of infected stallions. Carrier stallions can transmit the virus by natural breeding or AI with fresh, cooled, or frozen semen. Mares bred with EVA-infected semen may lead to outbreaks of abortion and deaths in foals (Timoney & McCollum WH, 1993).

### 2.3.4. Persistent mating-induced endometritis.

Endometritis is a normal physiological event after mating, but if the inflammation persists, the resulting environment is not compatible with establishment of pregnancy.

The inflammation is often, but not always, accompanied by accumulation of intrauterine fluid. Intrauterine fluid accumulation in mares was first reported by Knudsen (1964). Using rectal palpation, however more recently transrectal ultrasonography has been employed to detect intrauterine fluid (Ginther & Pierson, 1984; Adams *et al.,* 1987; Allen & Pycock, 1988; Allen, 1991; Pycock & Newcombe, 1996b).

Clinically, mares are classified as susceptible or resistant to persistent mating-induced endometritis (PMIE) based on their ability to eliminate this inflammation/infection within 48 hours of insemination. In addition, a poor vulvar conformation or a pendulous uterus are anatomical defects that favour endometritis. Age it is also determining factor, because at older age, the tendency to develop endometritis increases correspondingly (Troedsson *et al*., 2014; Woodward *et al*., 2015; Troedsson *et al*., 2016; Canisso *et al*., 2020).

Historically, mares have been classified as being susceptible to persistent, mating induced endometritis (PMIE) because of advanced age, increased parity (Asbury AC *et al.,*1993), presence of chronic inflammatory changes within the endometrium (Troedsson MHT *et al.,* 1993), presence of intra-uterine fluid at diestrus (Troedsson MHT, 1995), failure to clear an intra-uterine bacterial challenge (Troedsson MH *et al.,* 1993), or their ability to clear intrauterine radiocolloid within 2 h. The presence of more than 2 cm of uterine fluid during estrus, has recently been considered a useful predictor of susceptibility to PMIE (Brinsko SP *et al.,* 2003).

Inflammation of the endometrium is caused by a response to exogenous materials introduced directly into the uterus at breeding, as components of the semen, extender in the case of AI, bacteria, and other debris (Troedsson *et al.,* 2001; Troedsson *et al.,* 2011). PMEI can be caused by infectious agents (bacteria and fungi) or by non-infectious agents such as spermatozoa. All mares show this transient uterine inflammatory response within 30 minutes after natural mating or artificial insemination (Troedsson *et al*., 2014; Woodward *et al*., 2015; Troedsson *et al*., 2016; Canisso *et al*., 2020).

Two hormones, prostaglandin E 2 and oxytocin, regulate myometrial contractions after the influx of neutrophils into the uterine lumen and their phagocytic activity after opsonization of the target (Troedsson MH, 1999). This uterine defence mechanism reaches a pick at around 6–12 hours post-mating or AI (Katila T, 1996). In normal mares, most of the inflammatory products are cleared by physical uterine mechanisms within 48 hours after breeding, and the infection is cleared before the embryo leaves the fallopian tube and enters to the uterus on about days 5– 6 post-ovulation (Betteridge KJ *et al.,* 1982), the uterine inflammation has to be under control by 96 hours post-ovulation to maximize survival of the embryo (Troedsson MH, 1999). A susceptible mare with persistent post-mating endometritis is unable to clear such fluid by 96 hours, and the resulting prolonged inflammation generates an embryo-toxic environment. In addition, premature lysis of the CL is caused by prostaglandin E 2 and subsequent progesterone deficiency, all contribute to embryo mortality and infertility (Rambags BPB, 2003).

### 2.3.5. Endometriosis (chronic degenerative endometritis).

Endometrosis is a chronic degenerative condition of the endometrium and is thought to be irreversible (Kenney & Doig, 1986; Allen, 1993). Currently it has demonstrated that seasonal or cyclical endocrine changes do not influence the progression of this disease, but it is the inflammation itself, that is, endometritis is what activates the process. It is also influenced by the number of pregnancies (repeated inflammation) and by the age of the mare (Allen, 1993).

Degenerative fibrosis can be the result of normal aging processes or may be the end-product of a life of continuous reinfection: anyway, healing uterine mucosa causes infertility in older mares. However, the initial cause behind endometriosis remains unknown (Woodward *et al.,* 2012).

Problems related to weaken reproductive structures can cause urinary retention, a condition in which the mares do not empty completely urine, especially during estrus. Thus, the retained liquid can cause inflammation and prevent conception. The use of antibiotics is not always good. One of the disadvantages of using antibiotics is the fact that kills good and bad bacteria, leaving the animal with no natural protection against future bacterial or fungal invasions. The definitive diagnosis can only be achieved by biopsy, which shows degenerative histological changes in the uterus (LeBlanc MM *et al.,* 2011).

### 2.3.6. Chronic infectious endometritis.

Chronic infectious endometritis is more common in older mares and primiparous mares with poor perineal conformation. The predisposing factors for chronic infections in mares include self-repeated contaminations, cervix fibrosis, cervical tears or adhesions, and/or “mare older maiden” syndrome and mares in which holes uterus well below the edge of the pelvis. The organisms most frequently isolated in cases of chronic infectious endometritis include the same of acute *(S. zooepidemicus, E. coli, K. pneumoniae, P. aeruginosa, and Candida or Aspergillus)* (LeBlanc *et al.,* 2011).

Chronic cases of bacterial endometritis are those that have been treated traditionally as described for acute infections and are refractory to treatment. Current explanations as to why these cases are refractory to treatment are bacteria are protected by a biofilm from antibiotic exposure; antimicrobial resistance develops during treatment; or the mare become re-infected with the same genus of bacteria. Due to these issues, the management of mares with chronic infections is often much more intense from both a diagnostic and therapeutic perspective (Ball *et al.,* 1988; LeBlanc *et al.,* 2007).

Long-term chronic, refractory infections typically involve yeast and/or gram-negative bacteria (LeBlanc *et al.,* 2011). *S. zooepidemicus* is one of the most frequently pathogens isolated from uterus of mares suffering infectious endometritis. As described previously, it has the capacity to cause chronic infection latent deeply reside within the endometrial tissue (Christoffersen M *et al.,* 2015).

Clinical experience would show that mares which are affected by PMIE initially in the breeding season, can develop into mares with chronic uterine infection. Alternatively, mares which have no previous history of PMIE can present with a uterine infection. The major pathogens involved in equine endometritis are *Streptococcus zooepidemicus*, *Escherichia coli*, or yeasts (Dimock & Edwards., 1928) although anaerobes may play a role (Ricketts & Mackintosh, 1987).

Of these organisms, *S. zooepidemicus* is by far the most common and accounts for around 66% of infections. This organism is part of the normal microflora of horse skin and is a common contaminant of the uterus after mating. Whether infection is established or not depends on the efficacy of the mare's uterine defence system. E. coli is more frequently recovered from mares with anatomical defects of the perineal and vulvar region which predispose mares to pneumovagina and faecal contamination (Le Blanc, 1997).

The definitive diagnosis is by biopsy should show endometrial infiltration with lymphocytes and plasma cells. Chronic infectious endometritis is more common in older mares and primiparous mares with poor perineal conformation. Chronic inflammation may lead to fibrosis and perigland- ular mucus production by inadequate uterine epithelial or endometrial glands. If the elasticity, viscosity, or changes in the amount of mucus or if the cilia are damaged or denuded endome- trial epithelial cell, uterine drainage will be diminished, which contributes to the accumulation of fluid and infertility (LeBlanc *et al.,* 2011).

Endometrial biopsy for histopathology to evaluate the presence or absence of endometritis can be helpful when clinical and bacteriologic findings are inconclusive (LeBlanc & Causey 2009). Additionally, culture and cytology can be performed on the uterine biopsy sample and has been shown to be more diagnostic than traditional collection from a double guarded device (Nielsen, 2005; Nielsen *et al*., 2010, 2012).

## 2.4. Diagnostic.

Merocrine endometrial secretion and edema is normal during estrus (Tunon *et al.,* 1995), but the secretion should be effectively drained via lymphatics and the cervix. Both these draining mechanisms are dependent on functional myometrial contractions (Guyton, 1991).

Endometritis in the mare rarely gives evidentiable symptoms, they are prolonged in time and become chronic. If they are not very severe, females have normal cycles, so many times, if an in-depth study of the mare is nor made, they go unnoticed (Carvajal Ramos, 1998).

Distinctive clinical signs of endometritis include as mentioned, the accumulation of intrauterine fluid but also vaginitis, vaginal discharge, short intervals between heats, cytology or uterine biopsy with neutrophilia and positive crops; all clearly indicate inflammation of the uterus. Although the diagnosis can often be made easily, in some cases endometritis can be difficult to detect (LeBlanc & Causey, 2009).

The diagnosis of endometritis entails a multi-modal approach coupled with a detailed clinical history. Endometrial crops, cytology, and biopsy are the most common tools employed to diagnose endometritis in mares (Nielsen, 2005) (FIGURE 5).

Summary of common tools used to diagnose endometritis in mares.

| Technique | Approach and Applications | Limitations |
|---|---|---|
| Ultrasound | Used as a screening tool to detect the presence, amount, and appearance of IUF, which can be suggestive of endometritis. | Not all mares affected by endometritis, particularly chronic endometritis, accumulate IUF. The amount and echogenicity of fluid can be useful to direct the need for additional diagnostic techniques and therapeutic regimens. |
| Cotton-tip swab | Fast, user-friendly, and inexpensive approach to collect samples for culture and cytology. Results in combination with cytology can be used to dictate therapeutic approaches | Only a small segment of the uterus is sampled, and thus, focal infections not generating a diffuse endometrial response can be missed. In comparison with cytobrush, fewer cells are recovered, and cells are slightly compressed, making the evaluation more difficult |
| Cytobrush | Fast, user-friendly, and inexpensive approach to collect samples for culture and cytology, although it is more commonly used for cytology. | Only a small segment of the uterus is sampled, and thus, focal infections can be missed. Bacteria in biofilm may not be detected. |
| Low-volume uterine lavage | The whole surface of the uterus can be sampled for culture and cytology, and thus this technique is more utilized for the diagnosis of challenge and chronic endometritis. The recovered fluid can be centrifuged or allowed to decant before cytological evaluation. | There is a risk of contamination with commensal microorganisms of the caudal reproductive tract. It requires at least one well-trained clinician and an assistant. An excessive amount of fluid can overdilute the sample and cause a false-negative and may challenge the cytological evaluation. Mares with a pendulous uterus can have poor fluid recovery. |
| Endometrium biopsy | While this approach is primarily used for histological evaluation, endometrium biopsy is a sensitive and specific approach to diagnose endometritis in mares by histological evaluation and culture of the biopsy. Particularly useful for deep endometrium infection. Results may guide the treatment strategies employed. | It requires a biopsy, which is a minor procedure but still invasive. It also requires well-trained laboratory personnel capable of performing cultures and histological evaluations |

**(FIGURE 5)**

1. **Endometrial cytology** can be used to assess the type and proportion of inflammatory cells regarding endometrial epithelial cells present in the uterine lumen. In addition, cytology can occasionally detect the presence of bacterial colonies, hyphae, yeast, and urine crystals (Ferris *et al.,* 2015). Specimens for endometrial cytology can be obtained with a simple or double-guarded cotton-tip swab, cytobrush, or low-volume uterine lavage (Cocchia, N. *et al.,* 2012; Bohn, A.A. *et al.,* 2014; Ferris *et al.,* 2015). Cytobrush and low-volume uterine lavages yield superior diagnostic samples than cotton-tip swabs (Cocchia, N. *et al.,* 2012; Walter, J. *et al.,* 2012).

Are used to diagnose the presence of pathogenic microorganisms and inflammatory cells in the uterine lumen respectively (Riddle *et al*., 2007). The problem it appears when one of the tests is positive and the other negative. Pathogens that are associated with the presence of intrauterine fluid were more likely to present neutrophils on cytology while pathogens not associated with the presence of intrauterine fluid tend to shed negative results for neutrophils on cytology. These data indicate that all uterine pathogens induce the development of a neutrophilic response acute in the same way and that the discovery that the presence of intrauterine fluid indicates acute inflammation and not necessarily bacterial infection. Other possible causes of an acute neutrophilic response include pneumovagina, reflux of urine into the uterus, the presence of semen and excessive production of endometrial mucus (LeBlanc, 2010).

2. **Endometrial crops** should always be collected before any uterine or vaginal procedure to avoid potential contamination. Endometrial culture can be done via double-guarded cotton-tip swab, low-volume uterine lavage, or biopsy (Bain, 1966; Ball *et al.,* 1988; Nielsen, 2005; Nielsen *et al.,* 2010; Nielsen *et al.,*2012). After bacterial or fungal bacterial insolation, an antimicrobial susceptibility test should be performed to determine the most appropriate antimicrobial to treat the infection. In addition, PCR has been gaining popularity in clinical practice to identify bacteria and fungi in endometrium samples. Results can be available in 6 h, while the final culture and sensitivity results are pending (Ferris *et al.,* 2013).

In determining the relative importance of crops and cytology, it is useful to have a "gold standard" for determining the presence or absence of disease and that has a high sensitivity (percentage of patients testing positive) and specificity (percentage of healthy animals, which are negative to the test (LeBlanc & Causey, 2009).

Samples are collected in the same way as for cytology. The interpretation must always take into account possible false positives or false negatives. False positives are generally due to contamination of instrument, the external genitalia and the vagina, so it is important to use protected techniques so as not to carry over from other areas of the reproductive tract. False negatives are associated with inadequate sampling of the endometrium (LeBlanc & Causey, 2009).

Thus, in the microbiological crops is positive and the cytology shows more than visualises two neutrophils per field at 400x magnification (2cells/fieldx400), the diagnosis of endometritis is considered positive (Riddle *et al.,* 2007).

3. **Endometrial biopsy** also can be used as a diagnostic tool for endometritis, as well as for a proxy for putative ability of mares carrying a foal to term (Kenney, R.M., 1975; Doig *et al.,* 1981; Ricketts & Alonso, 1991; Nielsen *et al.,* 2012) (FIGURE 6).

The histological parameters assessed are as follows:

- **Inflammatory pattern**: it can be acute, with predominance of PMNs, affecting the stratum compactum or the luminal epithelium, or it may also be chronic, characterised by the presence of lymphocytic infiltration and, occasionally, plasma cells, eosinophils and mast cells. Cytological assessment of the endometrium should be based on the PMN count in relation to the epithelial cells, establishing the threshold set at 2% PMN for the scraping and 1% PMN for the biopsy smear, indicating that the latter have a higher sensitivity (Kozdrowski *et al.,* 2015).

- **Fibrosis pattern:** it must be taken into account that fibrosis is irreversible, becoming an important limiting factor of the mare's reproductive capacity.

- **Presence of endometrial cysts and glandular cysts:** these are structures that accumulate fluid, of glandular or lymphatic origin, the latter have serious consequences by altering the endometrium, in some cases even the myometrium. They alter the fixation of the embryonic vesicle and placentation.

- **Presence of lymphatic lacunae**: these are derived from the lymphatic ductus of the lamina propria and may contain eosinophilic material within them (Doig & Waelchli, 1993). Their role in this disease is unknown, but is unfavourable for carrying a pregnancy to term, especially if palpable by PTR.

Alteraciones histopatológicas uterinas según categorización de Kenney y Doig (1986)

| Grado | Alteraciones histopatológicas | Lesiones | Índice de preñez (%) |
|---|---|---|---|
| I | - Sin cambios patológicos | Ausente | 80-90 |
| IIA | - Infiltración inflamatoria difusa en estrato compacto<br>- Focos inflamatorios dispersos y frecuente en estrato esponjoso<br>- Compromiso de ramas glandulares<br>- Nidos fibróticos cada cuatro campos ópticos<br>- Atrofia parcial de endometrio | Leve | 50-80 |
| IIB | - Focos inflamatorios diseminados en epitelio luminal, estrato compacto y esponjoso<br>- Fibrosis severa<br>- Un nido fibrótico por campo<br>- Dilatación glandular quística<br>- Atrofia diseminada | Moderado | 10-50 |
| III | - Fibrosis periglandular generalizada<br>- Cinco nidos cada cuatro campos ópticos<br>- Inflamación severa<br>- Endometrio hipoplásico | Severo | <10 |

**(FIGURE 6)**

A grading system was developed by Kenney and Doig, where the endometrium is assessed for glandular distribution, inflammatory cells, lymphatic lacunae, and fibrosis and then graded on a scale of I–III (Kenney *et al.,* 1986). As these aspects of endometrial degeneration are associated with a predisposition to endometritis, a biopsy score combined with the mare's clinical history can be used to predict fertility (Kenney, R.M., 1975; Ricketts & Alonso, 1991).

Biopsy proves to the best diagnostic method in equine endometritis according to the publidhed literature, it is able to determine any type of endometritis as it examines both inflammatory ans degenerative processes (Overbeck *et al*., 2011).

4. **Ultrasound,** in addition to facilitating follicular monitoring, ultrasound allows the identification of small amount of intrauterine fluid, which is not possible by rectal palpation alone (McKinnon, 1988; Malschitzky *et al*., 2004). In addition, in those females that present the pathology in its subclinical form, the presence of abnormal edema patterns can be evaluated (Samper, 2009).

Ultrasound can show an increase or decrease in the amount of fluid, the characteristics of the fluid, the presence of air, as well as the degree of endometrial edema. It is a very important diagnostic tool as the presence of intrauterine fluid appears to negatively influence the fertility of the POC, especially when it is detected post-servicially (McKinnon, 1988; Malschitzky *et al*., 2004).

## 2.5. Treatment.

The objectives of the treatment of endometritis are threefold, three main objectives: to correct the defects in the uterine defences neutralise pathogens and control post-cubrition inflammation (LeBlanc & Causey, 2009).

The use of intrauterine antibiotics has been the method of choice for the management of infertility problems by infectious causes in mares. In practice, intrauterine therapy is recommended instead of systemic treatment (LeBlanc & Causey, 2009).

While antimicrobials are necessary to treat infectious endometritis in mares, these drugs are also often used with no clear medical indication, an example of which is the common single antibiotic dose intrauterine infusion following breeding (Zent *et al.,* 1998). The irrational use of antimicrobials has led to the rapid development of antimicrobial resistance. Therefore, the proper identification of microorganism(s), in addition to sensitivity to antimicrobials, is paramount to treat endometritis and prevent the development of antimicrobial resistance successfully.

If the culture is from one of the bacterial genus known to be pathogenic in the equine uterus treatment is always warranted (LeBlanc & Causey, 2009). However, there is a long list of bacteria with questionable pathogenicity, these organisms may be associated with an infection when in heavy growth, and with evidence of clinical disease (positive cytology, intrauterine fluid, or history of short cycling). The therapeutic plan is to aid the uterus in clearing infectious agents and inflammatory debris (LeBlanc, 2010).

Because the most common bacterial isolates in the mare's reproductive tract are *Streptococcus spp, Escherichia coli, Klebsiella sp, Pseudomonas sp, and Staphylococcus sp* (LeBlanc *et al.,* 2007; LeBlanc & Causey, 2009; Beltaire *et al.,* 2012; Walter *et al.,* 2012; Canisso *et al.,* 2016), the most common antimicrobials used to treat endometritis include β-lactam (e.g., ceftiofur, ampicillin, penicillin) and aminoglycosides (i.e., gentamicin and amikacin) (Dascanio, 2011).

This is accomplished by surgically correcting anatomical defects, improving physical drainage after insemination, reducing the length or modulating the inflammatory response to insemination and inhibiting bacterial growth. Post-breeding inflammation is most commonly treated by improving physical clearance of uterine fluid with uterine irrigation followed immediately by administration of either oxytocin (10–25 IU i.v. or i.m.) or cloprostenol (250 lg i.m.) (Brinsko *et al*., 1990; LeBlanc *et al.* 1994; Troedsson *et al.,* 1995; Combs *et al*., 1996; Pycock & Newcombe, 1996; Rasch *et al*., 1996; Knutti *et al*., 2000; Pycock, 2009).

It is interesting that *Streptococcus zooepidemicus and Escherichia coli*, the two most common isolates from mares with endometritis, were reported to be highly resistant to common antimicrobials (Benko *et al.,* 2015).

Fungal infections are difficult to treat and are largely caused by indiscriminate use of antibiotics. The prognosis for mares with fungal endometritis is generally unfavourable, because they tend to recur. The main reasons for treatment failure are resistance of uterine colonising forms to intrauterine therapy and recontamination with a reservoir located in the caudal reproductive tract (Abou – Gabal *et al*., 1977).

Treatments for fungal endometritis are not well elucidated, as there is a lack of controlled studies on fungal endometritis in mares and the pharmacokinetic and pharmacodynamics of these drugs in the reproductive tract. The initial treatment should address the predisposing factors (e.g., poor perineal conformation, immunosuppression, discontinue intrauterine infusions with antibiotics) in combination with uterine lavages and ecbolic drugs. Elimination of predisposing factors for endometritis can sometimes restore the fertility of these mares (Stout, 2008; Dascanio *et al.,* 2010).

Therapy for fungal endometritis involves treating the active infection via methods such as uterine lavage with dilute acetic acid or dilute povidone-iodine, plus systemic and/or intrauterine infusion of anti-fungal agents, in addition to correction of predisposing factors that could result in treatment failure. Administration of more than one anti-fungal agent may be indicated in refractory or recurrent clinical cases. Uterine lavage is indicated to remove retained fluid, reduce organism load, kill fungal organisms, and remove biofilm. It may also be beneficial to apply topical antifungal medication to the vagina and clitoris as these areas may act as a reservoir or nidus for reinfection. Ideally, selection of an antifungal agent would be based on results of susceptibility tests for each case of fungal endometritis (Coutinho & Alvarenga, 2011) (FIGURE 7).

ANTIFÚNGICOS

| **Droga** | Dosificación | Notas |
|---|---|---|
| **Nistamina** | 500.000U | Utilizado principalmente frente a las infecciones causadas por *Candida Albicans*. Se debe de diluir en 100 – 200ml de agua estéril, lo cual produce una suspensión que se aplicará por infusión intrauterina. |
| **Anfotericina B** | 200mg | Frente a las infecciones por *Aspergillus* spp, *Candida* spp, *Histoplasma* spp o *Mucor* spp. Se diluirán los 200mg en 200ml de agua estéril. |
| **Clotrimazol** | 700mg | Utilizado para el tratamiento de infecciones causadas por *Candida* spp. Generalmente se infunde después del lavado uterino. |
| **Miconazol** | 200mg | Es el más eficaz frente *Candida* spp. Diluir en suero salino estéril de 40 – 60ml antes de la infusión. |
| **Fluconazol** | 100mg | Administrar diariamente durante 5 – 10 días. |

*Antifúngicos para el tratamiento de la endometritis* (modificada de Díaz – Bertrana, 2012).

Common anti-fungal drugs used to treat mares suffering from fungal endometritis.

| Drug Class | Therapeutics | Mechanism of Action | AMR |
|---|---|---|---|
| Polyenes (e.g., amphotericin B, natamycin, and nystatin) | Fungicidal or fungistatic, broad-spectrum against *Candida spp, Aspergillus spp*, and *Mucor spp* | Binding to ergosterol in the membrane to disrupt the cell wall | Rare; the only mutant fungus enhances synthetic pathways for alternative sterols that replace ergosterol in the cell membrane |
| Imidazoles (e.g., clotrimazole, ketoconazole, miconazole) | Broad-spectrum activity against *Candida* spp | Inhibition of ergosterol synthesis in the fungal cell membrane by inhibiting the enzyme 14-α-demethylase, ultimately increasing cellular permeability and cell leakage | Resistance is found in filamentous fungi and after prolonged therapeutic regimens |
| Triazoles (e.g., fluconazole, itraconazole) | Potent anti-*Aspergillus* activity | Blockage of cytochrome P450–dependent enzyme C-14-α-demethylase (necessary for the conversion of lanosterol to ergosterol) | Resistance involves a single-point mutation in the cyp51A gene, which encodes for 14-α sterol demethylase |

Adapted from Giguère [270] and Beltaire et al. [49].

**(FIGURE 7)**

Uterine lavage is recommended in mares with excessive intrauterine fluid accumulation (e.g.,>2 cm depth) and high ultrasonographic echogenicity (Brisko *et al.,* 2003). Crystalloid solutions such as lactated Ringer's solution (LRS) and 0.9% saline are most commonly used to lavage mares' uteruses (Vanderwall & Woods, 2003).

These solutions can be enriched with anti-septics (e.g., povidone-iodine and hydrogen peroxide), vinegar to change the uterine microbiome in cases of fungal endometritis, and additives to break biofilm such as mucolytics (e.g., N-acetylcysteine, dimethyl sulfoxide, ethylenediaminetetraacetic acid-2-amino-2-hydroxymethyl-propane-1,3-diol alone or in combination with Tris; disodium ethylenediaminetetraacetate dehydrate-2-amino-2-hydroxymethyl-1,3-propanediol). Despite the wide use of these products in the treatment of endometritis, it is unknown how they affect the resident uterine microbiome and how these agents can be used to restore the balance of microorganisms *in utero.* Uterine lavage helps by physically removing microorganisms, debris, inflammatory cells and mediators, and dead sperm from the lumen, which can be detrimental for the sperm before breeding or the embryo after breeding (Brisko *et al.,* 2003; Vanderwall & Woods, 2003; Knutti *et al.,* 2010).

Not all endometritis responds to lavage uterine and antibiotic treatment. The flaws in treatment may be due to a ongoing contamination of the uterus due to anatomical abnormalitie s of the reproductive tract, neutralization of antibiotics by exudate uterine or by the presence of biofilm produced by bacteria. This biofilm is a mucoid substance produced by bacteria and fungi that allow their colonisation on the surface or the endometrium, isolating them from the uterine lumen and preventing penetration and contact with antibiotics, creating a protective and resistance mechanism (Wooley *et al.,* 2004).

Bacteria such as *pseudomonas aureoginosas, staphylococcus, E. coli* are major producers of biofilm in the uterine lumen.

Agents mucolitics and chelating substances most commonly used in endometritis treatments include:

- DMSO (30% solution). Equivalent to 33cc of a mixed 99% DMSO solution with 64cc saline solution) (Santos *et al*., 2003; Frazer *et al*., 1988; Ley *et al.,* 1989; Potz *et a*l., 1967).
- EDTA-tris solution (250cc 3.5 mM EDTA-tris) (Youngquist *et al.,* 1984; Kirklan *et a*l., 1983; Farca et al., 1997).
- N-acetylcysteine (30cc of 20% N-acetylcysteine in 150 cc solution physiological) (Estany *et al.,* 2007; Duru *et al.,* 2008; Leblanc, 2012).
- Kerosene (50 cc) (Bracher *et a*l., 1991).
- $H_2O_2$ (1% solution), equivalent to 20 cc of 3% hydrogen peroxide in 60cc lactate Ringer's solution) (Cardone *et al*., 2003; Dolezel *et al.,* 2010)

# 3. CLINICAL CASES.

**Case 1**

## 3.1. Animal profile

| | |
|---|---|
| **NAME** | AIRE (CARIÑOSA) |
| **SPECIE** | EQUINE |
| **BREED** | CRUSADE |
| **AGE** | 20 YEARS Y 5 MONTHS |
| **GENDER** | FEMALE |
| **MICROCHIP NUMBER** | 1001000072405010000 2643 |
| **HISTORY NUMBER** | 2245/16 |

## 3.2. Material and methods

Clinical history.

- On 06/04/2021 she was checked for vaccination and reproductive control, as her owner commented that she has been in heat for a long time. She is in good body condition, with normal body constants and in the ultrasound we observed manifest cartwheel, right ovary (D.O.) with small inactive follicles, left ovary (I.O.) with preovulatory follicle of 40mm. and given her history we recommend an ovulation induction which was performed on 07/04/2021.

- After one month, a control ultrasound scan is carried out because she has continued to be in marked oestrus after ovulation induction. There was no marked cartwheel, uterine oedema, I.O. with a 42mm pre-ovulatory follicle (in the same ovary as last time), D.O. with a corpus luteum. Uterine lavage is recommended but not possible as the cervix is not wide open.

- On 07/05/2021 she was re-evaluated and it was observed that she had a large oedema in the uterine corpus compatible with grade 4 endometritis. Intrauterine lavage was performed and 20cc of gentamicin was administered intrauterine.

- A week later, on 12/05/2021, she underwent an ultrasound scan to check the oestrous cycle. The owners told us during the visit that she still showed signs of oestrus but she was calmer. The ultrasound scan showed slight oedema in the uterus but to a lesser extent compared to the last time when it was compatible with endometritis. The D.O. has two small follicles. The I.O. has a small follicle and a patent corpus luteum. A vaginal examination is performed to check the degree of dilatation of the vaginal cervix, but it is very closed. A follow-up plan is made to see how she is progressing.

- On 25/05/2021 she contacted us again because since the last ovulation induction she was calmer, but at the weekend she was very jealous again and could not even work with other horses. A physical examination was carried out where all the constants were within range, in addition to observing clear signs of heat and how she is more nervous with other horses. In the ultrasound we observed slight oedema in the uterus, D.O. with preovulatory follicle of 37mm, I.O. inactive.

Treatment: an attempt is made to place an intrauterine ball but it is not possible, as the neck is very closed. She was given 1cc of prostaglandin and will be reviewed the following day.

- On 26/05/2021 we do a vaginal exploration and see that the cervix is more open but we still cannot place the ball.

- On 29/10/2021, the owners tell us that they have seen the mare's tail spotting, but yesterday they saw that she had a whitish-purulent discharge. She is a mare whose cycle is monitored due to irregular oestrus.

A general and reproductive examination of the mare is carried out and the following results are obtained:

| PHYSICAL EXAM | |
|---|---|
| **BODY CONDITION** | 5/9 ideal weight |
| **DEHYDRATION AND MUCOUS MEMBRANES** | Hydrated, pink (normal). |
| **LYMOHONODS** | Normal |
| **HEART RATE** | Tachycardia 48 ppm |
| **RESPIRATORY RATE** | 16 rpm |
| **TEMPERATURE** | 38,1ºC |
| **HECES** | Normal |
| **URINE** | Little |
| **MOTILITY** | Normal in all 4 quadrants |

A transrectal ultrasound was performed and it was observed that the bladder was quite full and there was some content in the uterus.
In addition, there is a lot of purulent content coming out of the vagina with considerable density. The differential diagnosis, based on clinical signs, is pyometra, endometritis IV.

Treatment: She was given 1cc of IV oxytocin, uterine lavage with 1.5 L of saline and 20cc of gentamicin. In addition to 2 ml of Oxytocin for IM injection 1cc for Saturday and Sunday.

- On 08/11/2021 she underwent a reproductive ultrasound examination. We are told that she has not had any discharge since the last wash. On ultrasound: on palpation the D.O. is larger and more consistent, ultrasonographically it is not active, there is a corpus luteum, the I.O. is not active either. There is intrauterine anechogenic content with inflammation of the endometrial wall (endometritis).

Treatment: Uterine lavage is attempted but the cervix is very closed, and the lavage tube can be passed. She is given 1.1cc of prostaglandin to bring her into oestrus and dilate the cervix.

- After 5 days, she was admitted to the HCV for pyometra. In the anamnesis we were told that she was not seen in heat (after 5 days of induction), she again had a lot of purulent discharge and was seen to be uncomfortable.

She arrives calmly at the HCV, with normal vitals and no stained tail or hindquarters. Our differential diagnosis is still pyometra, grade IV endometritis and grade IV endometritis.

**Diagnostic tests at the HCV:**

- **Ultrasound:** the body of the uterus is distended, and ultrasound shows some echogenicity. D.O. with a pre-ovulatory follicle of 37.2 mm, I.O. without activity.
- **Vaginal examination:** The cervix is not well observed.

**Treatment:**

- **Uterine lavage:** the cervix is very closed, an attempt is made to dilate it manually and a crushed misoprostol tablet is placed locally in the cervix with 3cc lidocaine to try to dilate the cervix. The cervix is dilated and allows the uterine probe to enter, it begins to expel purulent material (approximately 4l), and the uterus is washed with running water passed through the hose to dilute the contents considerably and wash the uterus well. The contents are not foul smelling, but thick. It is washed completely and once the liquid obtained is clean, 20cc of intrauterine gentamicin is administered. 7cc fnd.

**Follow-up plan:** Leave at HCV for ultrasound monitoring and serial washings for several days.

**Therapeutic plan:**

| DATE | DETAILS |
|---|---|
| **16/11/2021** | **Ultrasound**: D.O. with preovulatory follicle of 43mm, I.O. inactive.<br>Endometrial inflammation and fluid more anechogenic than yesterday in the body of the uterus.<br><br>**Uterine lavage:** the cervix is more dilated today, there is some brownish content without bad smell, in smaller quantity than yesterday, about 200ml.<br>Washing is done with the hose and then with saline. 20cc of intrauterine gentamicin. |
| **17/11/2021** | **Ultrasound:** the pre-ovulatory follicle of the D.O. is deforming and involuted, the I.O. is inactive. There is less |

| | |
|---|---|
| | content in the uterus and less inflammation in the endometrium.<br><br>**Uterine lavage**: the cervix is more closed; misoprostol and lidocaine are administered locally.<br>After introducing 500 ml of saline solution, a small amount of brownish-black content with some density is extracted, in a smaller quantity than yesterday.<br>Washing is done with water from a hose and the last with sterile saline. 20 cc intrauterine gentamicin. An attempt is made to place the intrauterine ball but it is too large and does not dilate the neck sufficiently.<br><br>3cc fnd IV. |
| **18/11/2021** | **Ultrasound:** CL in D.O., I.O. inactive, the cervix is well observed, unlike the previous days when the uterine dilatation was not well observed. There is still content in the uterus, but less quantity and it is more echogenic.<br><br>**Uterine lavage**: the lavage catheter can still be inserted; local misoprostol one tablet is inserted. Some of the brownish-black contents are removed, first washed with a hose and some of the contents with traces of blood are removed. The last washing is done with sterile saline and 20cc of gentamicin is administered intrauterine.<br><br>15cc doxycycline v.o. |
| **19/11/2021** | She is lively and with appetite. Vitals normal. She has a slight yellowish vulvar discharge.<br><br>**Ultrasound:** there is a little more intrauterine content than yesterday, the endometrium is still inflamed. D.O. with corpus luteum, I.O. inactive.<br><br>**Lavage is performed:** as it is difficult to enter the uterus, misoprostol is applied to help dilate the cervix. Without administering fluid, some brownish content is extracted with traces of bleeding, without bad odour. Washing is done with water, 20cc intrauterine gentamicin is administered.<br><br>Hospital Discharge. The mare is started on oral doxycycline, 15 cc for 20 days. |

- On 20/04/2022 a visit was made for internal deworming and vaccination and a check-up was carried out. The treatment with which she was discharged went very well and she regained normality in her cycles, in addition to not presenting purulent discharges.

# 4. DISCUSSION.

Endometritis a major cause of subfertility in the mare and has a significant economic impact on the equine industry (Dimmock, 1939; Collins, 1964; Bain, 1966; Hughes *et al*., 1966; Ricketts, 1975; Gordon & Sarlin, 1978; Kenney, 1978; Doig *et al*., 1981; Concha-Bermejillo & Kennedy, 1982; Traub-Dargatez *et al*., 1991).

Thus, we can affirm that endometritis is a pathology of multifactorial nature. Despite this, it can be classified, based on etiology and pathophysiology in four different categories: (1) endometriosis (chronic degenerative endometritis), (2) pathologies of sexual transmission, (3) persistent mount-induced endometritis and (4) chronic infectious endometritis (Troedsson *et al.,* 1995; Troedsson., 1997).

Due to the importance of this pathology, it is considered necessary for the veterinarian to act quickly to obtain a diagnosis, treatment and prognosis as soon as possible, in order to avoid the worsening of the case, as well as its chronification.

Defects in genital anatomy, myometrial contractions, lymphatic drainage, mucociliary clearance, cervical function, as well as vascular degeneration and inflammatory ageing underlie susceptibility to endometritis (LeBlanc & Causey, 2009).

Distinctive clinical signs of endometritis include as mentioned, the accumulation of intrauterine fluid but also vaginitis, vaginal discharge, short intervals between heats, cytology or uterine biopsy with neutrophilia and positive crops; all clearly indicate inflammation of the uterus. Although the diagnosis can often be made easily, in some cases endometritis can be difficult to detect (LeBlanc & Causey, 2009).

In addition to taking into account the factors on the part of the mare, males must be evaluated to avoid the transmission of venereal diseases. Semen plays an important role in this pathology, since they generate an inflammatory response in the endometrium that occurs after mounting or AI. Hence, PMIR is of great importance as the main cause of infertility in mares.

The diagnosis of endometritis can be made by gynecological examination, rectal palpation, transrectal ultrasound, cytology and crops of the uterine contents (Card C., 2005; Aguilar J *et al.,*2006).

Biopsy proves to the best diagnostic method in equine endometritis according to the publidhed literature, it is able to determine any type of endometritis as it examines both inflammatory ans degenerative processes (Overbeck *et al*., 2011).

The objectives of the treatment of endometritis are threefold, three main objectives: to correct the defects in the uterine defences neutralise pathogens and control post-cubrition inflammation (LeBlanc & Causey 2009).

Treatment of infectious endometritis due to latent bacteria, biofilm, or fungal organisms can be difficult. It is therefore important to understand the pathophysiology of the causative agents of the different types of endometritis, in order to be able to choose a treatment protocol appropriate to each case.

The current trend to avoid indiscriminate use of antimicrobials in mares will serve to prevent the development of new therapies or alternative therapies to the use of antibiotics to treat endometritis in mares, due to the resistance of pathogens to them.

## 5. CONCLUSION.

- In mares, reproductive efficiency is lower than in other domestic species, hence the importance of reproductive assessment.
- Endometritis is the main cause of infertility in mares and is a pathology that causes inflammation/infection of the endometrium.
- It is a multifactorial pathology caused by both physical problems and poor conformation of the reproductive anatomy, hindering drainage as a physiological inflammatory response to mating or AI.
- There are a variety of diagnostic methods that can be used at field level by an equine veterinary clinician such as ultrasonography.
- It is important to take into account various factors that could affect our fertility, such as nutrition, environment, physiological processes, anatomy and genetic factors, which could decrease fertility.

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