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Article: DIAGNOSTIC AND THERAPEUTIC ADVANCES TO MANAGE THE EQUINE ENDOMETRITIS

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ABSTRACT:

Endometritis is failure of the uterus to clear foreign contaminants (i.e., bacteria, spermatozoa) resulting in inflammation of the inner lining of the uterus (endometrium). This is an important cause of reduced fertility and infertility in mares, and therefore is a major contributor to economic loss in the industry. Most cases of endometritis are the result of bacterial infections involving such bacteria as Streptococcus zooepidemicus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and less commonly Taylorella equigenitalis (which causes contagious equine metritis or CEM). Yeast and fungi (Candida spp. and Aspergillus spp., for example) can also infect the uterus. The normal mare uterus is protected from external contamination by the vulva, vestibule, vagina, and cervix, which function as physical barriers that block the passage of foreign material(s). Injury, anatomic abnormalities, or loss of structural function (e.g., post-foaling) can permit the introduction of microorganisms into the uterus, resulting in endometritis. In severe or persistent cases, a chronic endometritis can develop accompanied by degenerative changes of the endometrium, including fibrosis (scarring). This condition is usually noted in older, multiparus mares (those which have foaled more than twice). Semen also incites an inflammatory reaction in the uterus post-breeding. Almost all mares develop a transient, post-breeding endometritis regardless of breeding technique (natural or artificial insemination). Mares with persistent post-

breeding Endometritis (PPBEM) have an increased rate of embryonic loss and a lower overall pregnancy rate than those without the condition. To enhance conception rates, mares at high risk need optimal breeding management as well as early Adavnces diagnosis, followed by the most appropriate Adavnces treatment and Mangement.

KEYWORDS: *Equine, Endometritis, Ultrasonography, Uterine cytology, Uterine biopsy, Antibiotics Antifungal agents, Mechanical curettage, Chemical curettage, Uterine lavage, Immunomodulators, Bacteriology, Cytology, Buffered chelators, Mucolytics, Corticosteroids, Plasma therapy, Acupuncture therapy. Herbal therapy.*

INTRODUCTION:

The mare is one of the most delightful & frustrating creature encountered by vets. Equine endometritis is 3rd medical condition of horse after colic and respiratory track disorder.

Endometritis is failure of the uterus to clear foreign contaminants (i.e., bacteria, spermatozoa) resulting in inflammation of the inner lining of the uterus (endometrium). (Drost *et al.*, 2002) This is an important cause of reduced fertility and infertility in mares, and therefore is a major contributor to economic loss in the industry. Endometritis is a common cause of infertility in mares (Varadin, 1975), causing early embryonic death, and leading to shortened interestrus intervals (LeBlanc and Causey, 2009). Most cases of endometritis are the result of bacterial infections involving such bacteria as *Streptococcus zooepidemicus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and less commonly *Taylorella equigenitalis* (which causes contagious equine metritis or CEM). Yeast and fungi (*Candida* spp. and *Aspergillus* spp., for example) can also infect the uterus (Drost *et al.*, 2002). The normal mare uterus is protected from external contamination by the vulva, vestibule, vagina, and cervix, which function as physical barriers that block the passage of foreign material (s). Injury, anatomic abnormalities, or loss of structural function (e.g., post-foaling) can permit the introduction of microorganisms into the uterus, resulting in endometritis. In severe or persistent cases, a chronic endometritis can develop accompanied by degenerative changes of the endometrium, including fibrosis (scarring). This condition is usually noted in older, multiparus mares (those which have foaled more than twice) (Drost *et al.*, 2002). Semen also incites an inflammatory reaction in the uterus post-breeding. (Squires) Almost all mares develop a transient, post-breeding endometritis regardless of breeding technique (natural or artificial insemination). This inflammatory process may arise from repeated breeding, infusion of irritating compounds, aspiration of air or urine, bacterial infections that could be attributed to poor perineal conformation, delayed uterine clearance of bacteria and debris, or an inherent immunological deficit. Post-breeding inflammation is a normal physiological phenomenon and rapidly resolves in the normal mare (Katila, 1995). However, in some mares inflammation persists and the embryo is unable to survive. These so-called “susceptible mares”

have a defective reproductive defense mechanism that is unable to prevent infections (Hughes and Loy, 1969 and Peterson *et al.*, 1969). These defects allow bacteria to colonise the uterus and cause endometritis, which impairs embryo development. There are also highly pathogenic bacteria that will overcome the uterine defenses in healthy mares (Causey, 2006).The most common causes of endometritis in mares are nonspecific bacterial infections by uterine invasion of bacteria such as *Streptococcus zooepidemicus* or *Escherichia coli*. Other microorganisms can be involved, including bacteria such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Taylorella equigenitalis*, the causative agent of CEM. Yeast and fungi (e.g., *Candida* spp., *Aspergillus* spp.) can also infect the uterus resulting in an endometritis.

PREVALENCE

The prevalence of endometritis is estimated to be quite high (Varadin, 1975), accounting for the cause of infertility in 25-60% of barren mares (Dimock and Edwards, 1928; Bain, 1966; Collins, 1964; Traub-Dargatz *et al*., 1991; Morris and Allen, 2002). Approximately 15% of a Thoroughbred broodmare population developed post-mating induced endometritis (Zent and Troedsson, 1998). In a survey completed by 1149 equine practitioners, endometritis ranked in the top three medical problems in equine adult patients (Traub-Dargatz *et al.*, 1991).

IMPACT OF ENDOMETRTIS

Endometritis has a significant economic impact. Affected mares often have irregular estrous cycles, require intensive clinical breeding management, and need more cycles to become pregnant, incurring additional costs to the owner. One estimate of the cost of producing a Thoroughbred foal through its sale as a yearling is $85,142. This cost includes the cost of keeping a broodmare, stud fee, weanling cost, and yearling cost (Thalheimer and Lawrence, 2001). This estimated high expense is the result of low reproductive efficiency. Only about 50–60% of mares are expected to produce a live foal after a single breeding (Bosh *et al.*, 2009).

CLASSIFICATION OF ENDOMETRITIS

Based on the etiology and pathophysiology, endometrtis can be divided mainly into four groups:- (I) Contagious equine metritis (II) Persistent mating-induce endometritis (III) Chronic degenerative endometritis and (IV) Infectious endometritis:- (A) Acute endometritis, (B) Chronic endometritis and (C) Fungal endometritis (Davies Morel, 2008).

(I) Contagious equine metritis:- Etiology- *Taylorella equigenitalis* (Gram negative microaerophilic coccobacillus) is the causative organism of contagious equine metritis (CEM)

Transmission:- Stallions are the most common source & organism is transmitted mainly during mating. And by Artificial insemination. In untreated stallions, organism can persist for months or years in the; Urethral fossa and its associated sinus, Distal urethra, Exterior of the penis and prepuce, and Occasionally in the pre-ejaculatory fluid (www.cfsph.iastate.edu, 2009). Mare can carry

organisms asymptomatically after recovery from acute disease for months & maintain in it's clitoral sinuses and fossa, but a few carry it in the uterus. Foal (colt) born to infected mare can carry bacteria on the external genitalia and may become long term asymptomatic carrier.

Incubation Period:- The incubation period is 2 to 14 days. Most infections become apparent 10 to 14 days after breeding (www.cfsph.iastate.edu, 2009).

Clinical signs:- Stallion display no clinical signs. Mare develop metritis and temporary infertility but no systemic signs. Some subclinical infections show only a sign to return in estrus after a shortened estrous cycle. Mare also develop a mucopurulent vaginal discharge 1-2 week after breeding; in severe cases, the discharge is copious. The discharge is usually grayish-white in uncomplicated cases; mixed bacterial infections may result in a gray to yellow exudate. The discharge often disappears after a few days to 2 weeks. Most infected mares do not conceive. Variable degrees of endometritis, cervicitis and vaginitis can sometimes be found if the reproductive tract is examined, using a speculum. The infertility usually lasts a few weeks, and long-term effects on reproduction have not been reported.

(II) Persistent mating-induce Endometritis:- Mare in which post-mating endometritis fails to resolve within 48 to 72 hours are considered to have a persistent post-breeding endometritis. Affects 15-43% of Thoroughbred mares after natural cover (Zent *et al.*, 1998). Early embryonic death rates are 3 times (Malschitzky *et al.*, 2003).

Pathophysiology:- The deposition of semen, bacteria and debris in the uterus of the mare after breeding normally induces a self-limiting endometritis. The resultant fluid and inflammatory products are cleared by 48 hours post-cover. Mares that have impaired uterine defense and clearance mechanisms, making them unable to resolve this inflammation within the normal time. This persists beyond 48 hours post-breeding and causes persistent fluid accumulation within the uterus. A mare that is susceptible to PMIE is unable to clear such fluid by 96 hours and the resulting prolonged inflammation generates an embryo-toxic environment (Maischberger, 2008).

(III) Chronic degenerative Endometritis:- Endometrosis is a syndrome encompassing a group of degenerative changes, typically including periglandular fibrosis, cystic dilation of glands, and glandular necrosis or hyperplasia of the endometrium. It occurs in older mares that have been exposed to repeated inflammatory conditions or aging.

These severe changes can be a sequelae:-

- ✓ Vesico-vaginal reflux (urine pooling)
- ✓ Chronic bacterial contamination
- ✓ Delayed uterine clearance with inflammatory by-products remaining in the uterine lumen (Youngquist and Threlfall, 2007).

Pathophysiology:- The pathogenesis of endometrosis is not completely understood; However, associations between the presence of endometrial degeneration and angiotensin converting enzyme (an enzyme associated with fibrosis in tissues including heart, kidney, liver, and spleen) have been detected in the mare as well as modulations in fibroblasts and collagen IV. Microscopic changes of the blood vessels in the endometrium lead to reducing endometrial perfusion and drainage. The changes noted included elastosis and fibrosis of the intimal media and adventitial layers. (Youngquist and Threlfall, 2007) Increasing age and worsening biopsy grade significantly decrease pregnancy rates, increase the rate of pregnancy loss, and decrease the foaling percentage. Uteri exhibiting fibrotic changes result in a slower embryonic death because of lack of trophic support. Mares with extensive fibrotic changes may conceive, only to lose the pregnancy subsequently. It is considered that periglandular fibrosis adversely affects endometrial gland function, probably by separating the affected glands from their capillary supply. Mares with severe endometrial fibrosis had three times the fetal loss (combined EED and abortion) that mares with no fibrosis experienced. For each increase in severity on a four-step scale from no fibrosis to severe fibrosis, foaling percentage declined 22.8% (Scott, 2002).

(IV) Infectious endometritis

(A) Acute endometritis:- Physiological (normal) transient inflammation process post-mating. Characterized by an influx of neutrophils in the endometrium stroma and uterine lumen. Serves to clear the excessive spermatozoa, debris and inflammatory products from uterus before the embryo descends into the uterus at 4-5 days after ovulation.

(B) Chronic endometritis:- A major cause of infertility in older and multiparous mares which become contaminated either during breeding or due to anatomic defects of the perineal and vulvar region leading to pneumovagina, urovagina and fecal aspiration (Le Blanc, 1997). It is thought that in some of the mares with delayed uterine clearance, defective uterine immune defense mechanisms may also contribute to persistence of infection (Watson, 1998). **Etiology:-** Mare affected by Persistent mating-induced endometritis initially in the breedi uterine infection (Watson, 2000).

Pathophysiology :

With increasing age and parity

- Effective length of the vulva increases
- Abdominal musculature weakens
- Abdomen expands.
- Permitting the intestinal tract to sink deeper
- Pulling the anus forward. (Watson, 1987)

Poor perineal conformation

Suspensory attachments between the rectum and reproductive tract drag the vagina and vestibule forward over the ischium, resulting in a more horizontal vulvar cleft and reduced efficacy of the vestibular seal. This predisposes the mare not only to pneumovagina and bacterial contamination of the cranial reproductive tract but also urovagina. (Scott, 2002) Such perineal conformation allows repeated fecal contamination of the vulva and vestibule. Susceptibility to uterine infections is enhanced by the frequent supplementation of performance fillies with altrenogest (an oral progestin) or injectable progesterone products. (Hurtgn, 2006)

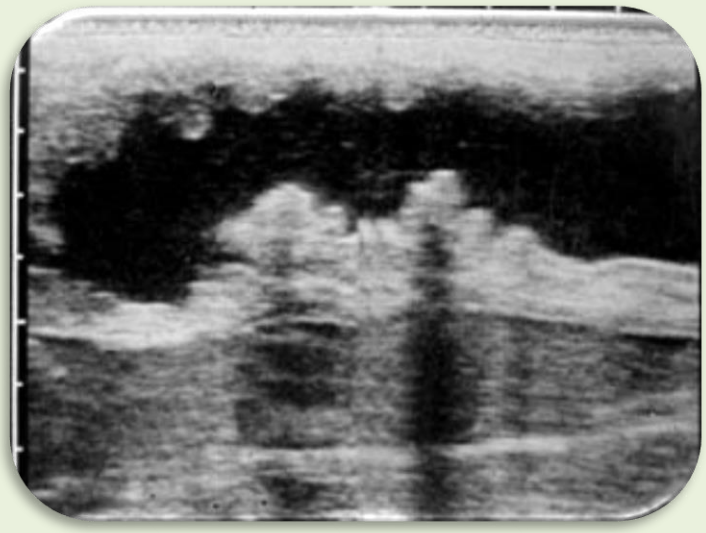
(c) Fungal endometritis:- Candida spp. & Aspergillus spp. are the most common fungal organisms associated with fungal endometritis.

- ✓ Poor conformation
- ✓ Repeated or lengthy intrauterine antibiotic treatment
- ✓ Excessive reproductive manipulation. (Blue, 1983).

DIAGNOSTIC AND THERAPEUTIC ADVANCES

Diagnostic Methods:- Rectal examination, Vaginal speculum examination, Ultrasonography, Uterine culture, Uterine cytology, Endometrial biopsy**, Rectal palpation:-**Rectal palpation may reveal a large relaxed uterus**, Vaginal-speculum examination:-** In vaginal speculum examination Vaginal vault and cervix appear hyperemic with or without the presence of exudates**,**

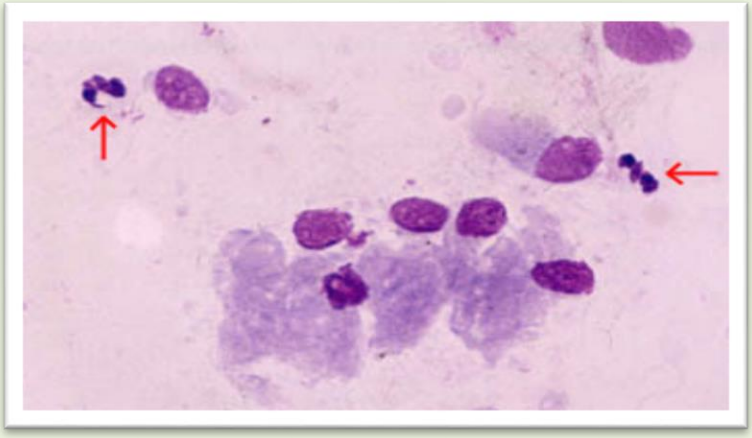
Ultrasonography:- Ultrasonography the uterus can be examined non-invasively for pathologic changes and to monitor therapeutic regimen (s). The three most common forms of uterine pathology detected by ultrasonography are accumulations of intrauterine fluid, air and debris. In PMIE Transrectal ultrasound examination at 24- 48 hours after breeding showing presence of free fluid in the uterine lumen(>15-20 mm in diameter) is diagnostic for the condition.



(Guevenc *et al.*, 2005), (Bucca *et al.*, 2008)

Uterine cytology:- Significance of the cytology its evaluated for the presence or absence of inflammatory cells, specifically neutrophils. Sample collection by guarded swab was fiix with

methanol & stain with hematological stains (Diff Quick). And results with Neutriphils (>5 netrophils / microscopic fields.) was considered as positive (Dascanio, 2003)



Inflammatory cells - Neutrophils seen at left and right with double nuclei (Pycock., 2003).

Endometrial culture

Uterine culture detect the pathogenic organism.

The combination of endometrial cytology smear and culture results for more accurate diagnosis

Culture Growth	Inflammatory Cell Presence	Diagnostic Determination
Negative	Negative	Mare can be considered "clean" and can be bred with no further diagnostic testing required
Positive	Positive	Mare has uterus most likely infected with the pathogen identified in the culture
Positive	Negative	Mare is unlikely to have an infected uterus, and the sample has probably been contaminated.
Negative	Positive	Mare has something irritating her uterus. In all likelihood, this is a pathogen that swabbing has missed. Further diagnostic work is required

Sensitivity and Specificity of culture, cytology & biopsy

Mares may also yield positive uterine cytologies with negative uterine cultures, and vice versa. According to the Riddle *et al.*, (2007) 36 % Culture positive to be negative on cytology. 65 % Cytology positive cases failed to yield pathogen. where as according to Nielson, 2005 Culture and cytology sample have 82 % sensitivity & Guarded swab has 77% sensitivity. so. A now day using biopsy techniques as a gold standard give more accurate results. Pathogens that were associated with uterine fluid were more likely to have neutrophils on cytology while pathogens not associated with uterine fluid, tended to be negative for neutrophils on cytology. These data indicate that not all uterine pathogens induce an acute neutrophilic response and support the finding that intrauterine fluid indicates acute inflammation and not necessarily bacterial infection. To determine the relative importance of culture and cytology, it is helpful to have a ‘gold standard’ for the presence or absence of disease against which the results can be compared, and thereby generate estimates of sensitivity

(per cent of disease-positive which testpositive) and specificity (per cent of disease-negative which test negative) (LeBlanc and Causey, 2009).

With this approach, Nielsen (2005) used the presence of neutrophil infiltration of the luminal epithelium and stratum compactum as a gold standard to indicate the presence of disease (endometritis) in 212 uterine biopsies. Against neutrophil infiltration, he compared culture of the endometrial biopsy tissue specimen, swabbing of the endometrium using a guarded culture swab and uterine cytology by smearing the biopsy on a slide. Of the three techniques (biopsy culture, swab culture and cytology), sensitivities were calculated to be 0.82, 0.34 and 0.77, and specificities 0.92, 1.0 and 1.0, respectively. What became apparent was that uterine culture, with a sensitivity of only 0.34, appeared to have a high degree of false negatives in detecting cases of endometritis, and that cytology or culture of a uterine biopsy was twice as sensitive as swab culture in predicting the presence of endometritis.

Techniques	Sensitivities (%)	Specificities (%)
Biopsy culture	0.82	0.92
Swab culture	0.34	1.0
Cytology	0.77	1.0

LeBlanc *et al.*, (2007) used the same gold standard as Nielsen (2005), but evaluated uterine culture, cytology and indices of inflammation obtained from low-volume uterine flushes, a technique originally reported by Ball *et al.*, (1988). It was hypothesized that more complete sampling of the endometrium might be obtained through such a technique.

Techniques	Sensitivities (%)	Specificities (%)
Uterine flush culture	0.71	0.86
Cytology	0.80	0.67

Using small volume lavage, sensitivity of culture was 0.71 (i.e. double that previously reported for uterine swab cultures), and sensitivity of cytology was 0.80. Specificity of flush culture and cytology were 0.86 and 0.67, respectively. Uterine flush cytology thus tended to under-report inflammation with 70% of positive cultures yielding a negative cytology (a clinical estimation of false positive culture). However, if lack of cytological debris and a clear efflux were added to the definition of a negative cytology, only 11% of positive cultures would have been clinically classified as false. Thus, additional indices available from the flush improved clinical detection of inflammation. Cloudy efflux was highly correlated with the presence of bacteria. The improvement in uterine flush culture sensitivity over swab culture was because of increased detection of E. coli in uterine flush. Isolation of E. coli was characterized by moderate to heavy debris observed on cytology. Therefore, uterine flush culture would appear to be helpful in detecting mares with endometritis because of E. coli.

In LeBlanc study on the basis of sensitivity and specificity it was revied that uterine flush culture, biopsy and cytology culture have hibh sensitivity for diagnosis of endometrtis in mares.

Pregnancy rate in relation to endometrial cytology

Uterine/ Endometrial cytology	Pregnancy rate (28 days of gestation period)
0-2 Neutrophiles / 400x field	60%
2-5 Neutrophiles / 400x field	36%
>5 Neutrophiles / 400x field	23%

Uterine biopsy

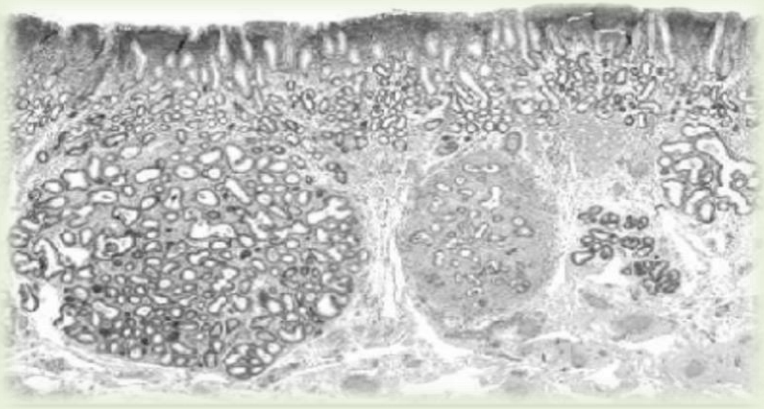
A uterine biopsy may be collected from a mare to evaluate the histology or microscopic anatomy of the uterine lining or endometrium. Endometrial biopsy samples are examined for the presence of inflammatory and degenerative changes (Christensen *et al*., 2009).

Endometrial biopsy grades (Christensen *et al*., 2009)

Biopsy Category	Degree Of Endometrial Changes	Predicted foaling rate (%)
I	None/ mild inflammation or degeneration	80-90
IIA	Moderate inflammation or degeneration	50-80
IIB	Moderate inflammation and degeneration	10-50
III	Severe inflammation and /or degeneration	0-10

- ✓ It is Important for diagnosis of chronic endometritis and endometrosis
- ✓ Sample fixed in Bouin's fixative & stained with H& E stain
- ✓ Interstitial leukocyte infiltration is the pathognomonic sign of endometrial inflammation, with neutrophils predominating in the acute process and lymphocytes in chronic cases.
- ✓ Periglandular fibrosis, lymphatic stasis, and endometrial cysts are characteristic signs of endometrosis.

Endometrial histopathology of endometrosis



Degenerative glands with periglandular fibrosis

Comparison of the biopsy and cytobrush techniques

Buczkowska *et al.*, (2014) carried out study on the comparision of biopsy and cytobrush techniques. Sample collected from 69 mare suspected for subclinical endometritis was smeared on sterile glass slides for cytological examinations and on culture media for microbiological examinations. They

compare bacteriological and cytological results obtained from the mare uterus using biopsy (EB) and cytobrush (CB) techniques and relating these findings to the presence of polymorphonuclear cells (PMNs) in endometrial tissue as the gold standard for detection of endometritis. Bacteriological cultures and cytological samples were classified as negative (no growth or mixed cultures of more than three microorganisms; <2% PMNs) or positive (pure growth of microorganisms; >2% PMNs) for endometritis.

Microorganisms	Cytobrush			Endometrial biopsy		
	Number of smears with negative cytology	Number of smears with positive cytology	Concordance between cytology and microbiology (%)	Number of smears with negative cytology	Number of smears with positive cytology	Concordance between cytology and microbiology (%)
β-hemolytic Streptococcus	0	5	5/5 (100)	0	7	7/7 (100)
α-hemolytic Streptococcus	2	0	0/2 (0)	3	1	1/4 (25)
E.coli	2	2	2/4 (50)	3	1	1/4 (25)
Fungi	0	8	8/8 (100)	4	5	5/9 (56)
Bacillus spp.	4	4	4/8 (50)	6	3	3/9 (33)
Corynebacterium spp.	0	2	2/2 (100)	0	2	2/2 (100)
Pseudomonas aeruginosa	0	1	1/1 (100)	1	0	0/1 (0)
Micrococcus spp.	0	0	0	0	2	2/2 (100)
Staphylococcus epidermidis	1	1	1/2 (50)	1	1	1/2 (50)
Candida + β hemolytic Streptococcus	0	3	3/3 (100)	0	3	3/3 (100)
Aspergillus + Staphylococcus aureus	0	0	0	0	1	1/1 (100)

In the given table the concordance between cytology and microbiology (%) using cytobrush techniques was almost similar to that of biopsy techniques with not much significant differences. So, On the basus of microbiological and cytological results obtained with no significant differences it was concluded that cytobrush techniques can be used in clinical practice.

Therapeutic measures:-It includes Uterine lavage, Antibiotics (Systemic & Intrauterine), Ecbolic, Antifungal agents, Mechanical curettage & Chemical curettage and Surgical correction of anatomical defects (Caslick's vulvoplasty).

Antibiotics (Systemic & Intrauterine)

Gentamicin, Amikacin, Ampicillin and Penicillin Ceftiofur are the most effective against the majority of pathogenic organisms. Antibiotics infused directly into the uterus daily for 3 to 5 days or more during estrus in volumes between 60-100 ml based on the assumption that this volume is optimal for coating the endometrial surface (LeBlanc, 2008).

Antibiotics	*Dose per treatment*	*Additional comments*
Amikacin	1-2 grams	Gram negative coverage
Ceftiofur sodium	1 gram	Gram positive and gram negative coverage

Ampicillin	1-3 grams	Only the soluble product should be used and should be diluted to decrease irritation, gram positive and *E.coli* coverage
Potassium Penicillin	5 million IU	Gram positive coverage (*streptococcus zooepidemicus*)
Gentamicin	1-2 grams	Requires buffering due to acidic nature; gram negative coverage
Ticarcillin	6 grams	Should be infused in 200 ml solution ; gram positive coverage with same *pseudomonas spp.*

Ecbolic (LeBlanc and Causey, 2009 and pycock *et al.*, 2007):- Prostaglandin E1 analogue (Misoprostol), Carbetocin, Oxytocine (10-20 IU /IV) and Cloprostenol (250 mg/im)

Treatment (drugs)	Removal of uterine fluid	Pregnancy rate
Oxytocin (10-20 IU, IM)	24%	19%
Carbetocin	100%	51%

(Reitzenstein *et al.*, 2002)

Synergistic effect of antibiotic and oxytocin on conception rates in endometritic mares

150 adult mares with the history of repeat breeding was reported for treatment of endometritis.out of which 135 adult mares showing bacterial growth and antibiotic sensitivity by swab culture and AST.

The following preparations of various antibiotics were used for treatment:- Gentamicin -12 ml, Chloramphenicol – 5 gm, Steptopenicillin 5gm + kanamycin -2 gm and Norfloxacin 10 ml

Conception rate in endometric mare treated with antibiotic alone or with oxytocin

Sr. No	Group	N	Conception rate	
			No.	%
1	Control	45	18	40.00
2	Gentamicin	8	5	62.50
3	Gentamicun+Oxytocin	10	8	80.00
4	Chloramphenicol	13	8	61.50
5	Chloramphenicol+Oxytocin	9	7	77.78
6	Streptopenicillin+ kanamycin	10	6	60.00
7	Streptopenicillin+ kanamycin +Oxytocin	14	12	85.71
8	Norfloxacin	12	7	58.33
9	Norfloxacin+Oxytocin	14	12	85.71

On the basis of study of Ahmad et.al, 2001,the overall conception rates was higher in mares treated with antibiotic + ecbolic agents so, it was concluded that effect of antibiotic along with uterogenic use have high conception in endometric mares.

Antifungal agents

Antifungal Agent	*Dose per treatment*	*Additional comments*
Amphoteracin B	100 - 200 mg SID	Dilute in greater than100 ml solution ;*candida spp.*,*Aspergillus* ., and *dimorphic* fungi coverage.

Clotrimazole	400 - 700 mg	*Candida spp.,Aspergillus* ., and dimorphic fungi coverage.
Fluconazole	100 mg	Acidic – may need to adjust pH ;*candida spp*., coverage
Miconazole	500- 700 mg	*Candida spp.,Aspergillus* ., and dimorphic fungi coverage.
Nystatin	0.5 - 2.5 million units	Dilute in sterile water*; candida spp*., coverage
Lufenuron	3 – 4 packets (270 mg each)	An inhibitor effect on chitin production of fungal elements

(leBlanc, 2008)

Mechanical curettage & Chemical curettage (Liu, 2011 and Troedson, 2008)

(a) Mechanical curettage:-Inducement of acute inflammation**,** Attendant movement of neutrophils and Serum-derived opsonins into the uterine lumen.

(b) Chemical curettage:- Dimethyl sulfoxide (DMSO), Hydrogen peroxide (h202),magnesium sulfate (mgso4), Streptococcus filtrate, Kerosene and Dilute disinfectants (0.2% povodine-iodine solution).

Uterine lavage

If Uterine fluid > 20 mm in diameter than 1 to 2 litres of warm, sterile saline/Ringer's solution + 5 mL Betadine (10% povidone-iodine) was used.Other solution use for lavage DMSO (10-20%), Vinegar (2%) can also used (LeBlanc, 2003). Therapeutic effects of uterine lavage are it timulate blood flow, Improve tonicity of uterus and help in removal of debris.

Effect of uterine lavage containing antibiotics alone or with uterotonic agents in PMIE in Arabian mares (Taha, 2007)

42 Arabian mares with PMIE are examine by rectal palpation ,vaginoscopy and cytology. The mares were qualified as PMIE due to accumulation of uterine fluid 1-2 post breeding. 36 mares treated and 6 mares used as a control. 36 mares were treated with uterine lavage + antibiotics (crystaline penicillin and steptomycin sulphate) 6 hours post breeding . After 8 hours mares were divided in three groups and give Inj.250 µg cloprostenol given s/c. Inj .oxytocine 20 iu, IM and No Hormonal treatment.

Pregnancy rate in Arabian mare after treatment of lavage containing antibiotics alone or with uterotonic agents groupes (PMIE)

Groups	*No.of mares*	*Pregnant (Pregnancy rate%)*
Uterine lavage	12	6 (50)
Uterine lavage + PGF2	12	10 (83.3)
Uterine lavage + Oxytocin	12	8 (66.6)
Control (untreated)	6	0 (0)
Total	42	24 (57.1)

On the basis of study it was concluded that pregnancy rate was high in mares treated with uerine lavage containing antibiotics along with ecbolic agents.

NEW ADJUAENT TREATMENT STRATEGIES

Immunomodulators (Rohrbach *et al.*, 2006)

(a) Propionibacterium acnes (Eqstin):- It enhance Cell mediated immunity.

(b) Mycobacterium cell-wall extract (Settle):- Normalize uterine inflammatory response, Uterine involution and Bacterial reduction in the uterus by a non-specific immunological response during endometritis

Efficacy and safety of SETTLE given after foaling and its impact on breeding efficiency at the Foal Heat (Fumuso *et al., 2003*):- The foal heat usually has a low fertility rate, with poor uterus involution, inflammation and contaminant microorganisms. SETTLE™ (Bioniche Animal Health USA, Inc., Athens, GA) a biological immunomodulator was shown to significantly reduce endometrial inflammation in Post Breeding Endometritis.a This study evaluated the efficacy and safety of SETTLE given after foaling and its impact on breeding efficiency at the Foal Heat. Eighty-one Argentina Saddle Horse mares were allocated to one of two treatments: (i) SETTLE i.v. -1.5 mg or (ii) placebo. All mares were ultrasound scanned at 15 days post ovulation. Twenty-five of them were swabbed for bacteriology and exfoliative cytology (PMN count) on the day after foaling (S1) and again seven days later (S2). Pregnancies: A significant higher proportion of mares were pregnant in the SETTLE group than in controls (P=0.0003, Fisher's test).

Bacteriology:- The bacteria and isolation frequencies are shown in the table. At S1, both groups had high contamination scores. At S2, almost all of the control mares were highly infected except three, which cultured negative. In the SETTLE group, most mares (11/13) were cultured negative. Whereas both groups did not differ at S1 (P>0.05), they did at S2 (P<0.05). While there was no significant variation between both samples (S1 v S2), in the control group, the effect of SETTLE resulted in a highly significant (P<0.001) difference between S1 and S2.

Cytology:- At S1 the controls had high cytology scores, which decreased at S2 (not significantly; Kruskall-Wallis test). The mares given SETTLE also showed a high score at S1 but they demonstrated a dramatic decrease (P<0.001) at S2. Whereas both groups did not differ in their cytology scores at S1, they did significantly at S2 (P<0.05).

	Placebo		*SETTLE*	
Pregnancy (n=81)	9/38 (24 %)		28/43 (65 %)	
	S1	S2	S1	S2
Bacteriology (n=25)	1-4(3)	0-3 (3)	0-4 (3)	0-0.2(0)
Cytology (n= 25)	12.4-60 (30.1)	0.8-35 (14.9)	12.8-60 (21.8)	0-0.25 (0.8)

It was concluded that SETTLE administered the day after foaling promoted (i) a significantly higher pregnancy rate (ii) a dramatic pathogen-independent decrease in bacterial contamination in the endometrium, (iii) a significant decrease in the number of endometrial inflammatory cells presented

at foal heat. Furthermore the SETTLE treatment has demonstrated to be a safe, intravenous, non-antibiotic therapy for nursing mares.

Buffered chelators

Tris-EDTA (Ferris *et al.*, 2014)

- ✓ Chelates calcium and magnesium, components of the cell wall of bacteria and fungi,
- ✓ Anti-microbial and anti-biofilm
- ✓ Most effective against biofilm produced by *P.aeruginosa & E. col*

EDTA-Tris (ethylene diamine tetra-acetic acid-tromethamine) is an agent that chelates calcium and magnesium, components of the cell wall of bacteria and fungi. EDTA-Tris has been shown to have broad-spectrum, anti-microbial and anti-biofilm properties and can even have synergistic effects when combined with certain antimicrobials. Its anti-biofilm properties have been demonstrated, and it appears to be most effective against biofilm produced by *P.aeruginosa & E. coli.* (Ferris *et al.*, 2014)

(a) Recommended use:- Infuse Tricide (8mM disodium EDTA dehydrate and 20mM 2-amino-2-hydroxymethyl-1,2propanediol 500-750 ml. Must lavage uterus 6-24 hrs post infusion (Leblanc and McKinnon).

Mucolytics

Acetylcysteine:- Act as Anti-inflammatory effects (antioxidant and reduces free radicals), Antimicrobial, Ati- fungal and also Decrease biofilm produced by certain gram-negative bacteria (E.Coli).

Acetylcysteine (NAC) is the N-acetyl derivative of the amino acid L-cysteine, a precursor in the formation of the antioxidant glutathione in the body. It has anti-inflammatory effects (antioxidant and reduces free radicals) and mucolytic effects. It is useful in removing mucus that may be abnormal in mares with PMIE, or infused prior to antimicrobial use for bacterial or fungal endometritis to remove mucinous debris and facilitate antimicrobial penetration and efficacy. It has been demonstrated that NAC decreased biofilm produced by certain gram-negative bacteria (E.Coli) (Inoue, 2013).

(a) Pre-breeding recommendation: Infuse NAC (33% solution, dilute 30 mL 20% solution into 150 mL sterile saline) 24-48 hours prior to breeding. Administer ecbolic agents (oxytocin 20IU IM every 6-8 hours but not within 4 hours of breeding), or uterine irrigation with sterile lactated ringers prior to breeding if debris is excessive. Studies have shown pre-breeding NAC infusion increased the per-cycle pregnancy rate in a group of barren mare (Gores-Lindholm *et al.*, 2013).

(b) Pre-antimicrobial recommendation:- Infuse 33% solution (150 mL volume) into the uterus 24 hours prior to antimicrobial treatment, and lavage prior to antimicrobial treatment.

Corticosteroids

(a) Acetate 9-alpha-prednisolone (0.1 mg/kg) twice daily (Dell'Aqua *et al.*, 2004)

(b) Dexamethasone (50 mg, IV) (Bucca *et al.*, 2008):- Single dose dexamethasone administered within one hour of mating and daily prednisolone administration given before and after mating have 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 postbreeding therapies (uterine irrigation, ecbolic 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).

Plasma therapy:- Platelet-rich plasma concentrates(IU) it Enhance opsonization process (Brinsko *et al.*, 2011)

Acupuncture Therapy:- Electro-acupuncture has also been used clinically to increase uterine contractility in mares with delayed uterine clearance.

Effect of Acupuncture Therapy in PMIE mare (Longo, 2004)

The experimental design was performed in three consecutive breeding seasons between March 2008 and July 2010. During estrus, all mares were examined daily by rectal palpation and transrectal ultrasonography (7.5 MHz rectal transducer- Sonosite Micromaxx) to monitor follicular growth, uterine fluid accumulation and uterine edema. Uterine swabs for bacteriology (*Equi-Vet*R, Kruuse, Marslev, Denmark) were collected if vaginitis- cervicitis signs were detected. Only mares with a negative bacterial swab were used for the study. Subsequently, when a follicle of 35 mm in diameter together with uterine edema was detected, ovulation was induced with 2500 IU human chorionic gonadotropin (Vetecor R Farma Mediterranea, San Just Desvern, Spain). Fresh semen from stallions of proved fertility was used for the artificial insemination in proximity of the ovulation. Mares were scanned for detection of ultrasonographically visible uterine fluid at 24 hours from AI. If fluid was detected, its maximum diameter was measured using electronic callipers and scored as score 1: up to 2.0 cm in diameter and anechogenic; score 2: 2.0–3.0 cm in diameter and anechogenic; score 3: 3.0-5.0 cm in diameter and hyperecogen; score 4: >5 cm and hyperechogen. Mares that presented ultrasonographic signs of PMIE were randomly divided into 3 groups of treatment: acupuncture, oxytocin (20 UI ev) and placebo (saline solution, 10 ml ev). Mares were reevaluated with ultrasonography at 24 hours after treatment for detection of permanence of fluid. If fluid was still present, mares were scanned every 24 hours until clear. Pregnancy rates at 15 days after breeding were also assessed. Acupuncture groups mares followed the TCM diagnosis by palpation, pulses type and tongue colors were also recorded. A standard protocol of acupoints for every mare was used using the acupoints *Baihuanshu, Yao Baihui (*acupuncture and moxibustion*), Taixi.* In addition, the acupoints *Pishu, Shenshu, Guanyuan, Guanyuanshu, Geshu, Gongsun, Huangmen, Huiyang, Jingming, Mingmen, Neiguan, Sanjiaoshu, Tai Yang, Zu Sanli* were added to the basic treatment depending on the condition of the animal. Only one treatment was provided and points were

stimulated with acupuncture needles of 0.30 x 40 mm, 0.30 x 25 mm e 0.30 x 70 mm (HwatoR, Cloud & DragonR, HuanqiuR, Cina). Two mares received acupuncture with 23G, 0.6 x 25 mm needles.

Groups n= 59	*Treatment*	*USG exanination after 24 hrs. (Decrese in Uterine fluid)*	*Pregnancy rate (at 15 days of breeding)*
n=25	Oxytocin (O)	O > P	20.0%
n=13	Placebo (P)	—	—
N= 21	Acupuncture therapy (A)	A> O & P	76.0%

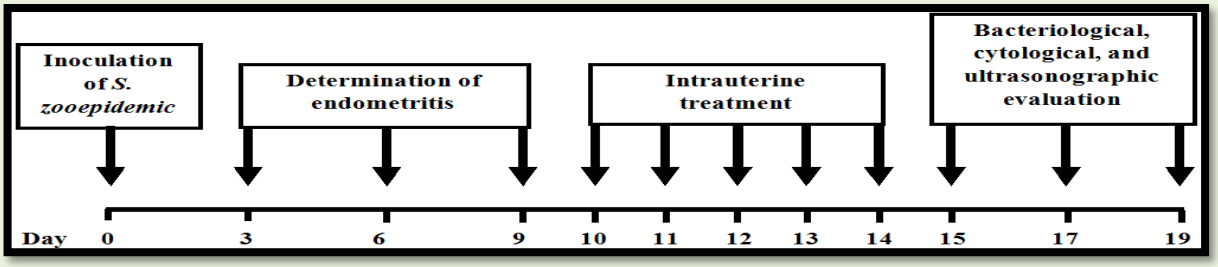
The quantity of fluid was reduced inall group but Oxytocin reduced the amount of uterine fluid more than Placebo (P<0.01) and Acupuncture more than Oxytocin and Placebo (P<0,01). Both the mares treated with acupuncture and Oxytocin showed an increased pregnancy rate compared to Placebo mares tough Acupuncture showed a significant increase of the pregnancy rates compared with Oxytocin (76,2 vs 20,0%). In this study, the treatment with acupuncture was able to reduce fluid in uterus and to increase pregnancy rates more than the other two groups (P<0.01).

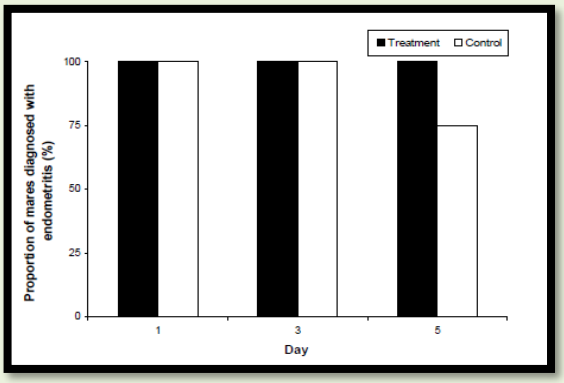
HERBAL THERAPY

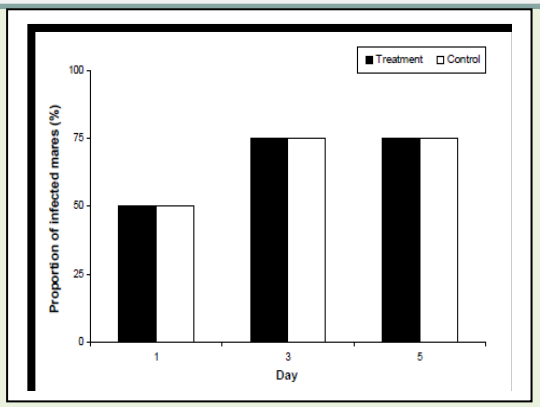
***Eucalyptus oil solution*:** Act as Antibacterial, antiviral, antifungal and antiparasitic effects (sadlon and lamson, 2010), Immunomodulatory (Serafino *et al.*, 2008) and Anti-inflammatory agent (Juergens *et al.*, 2004).

Therapeutic effect of intrauterine infusion of 6% *Eucalyptus globulus* oil solution

The study was conducted to evaluate intrauterine infusion of *Eucalyptus globulus* oil as treatment for mares with experimentally induced endometritis. Mares (n=8) were synchronized using two injections of PGF2α (10 mg) 14 days apart. All mares were inoculated with a live culture of 5 × 106 CFU *S. zooepidemicus* in the dioestrus phase after the termination of oestrus. The day of inoculation was considered as day 0 of the experiment. On days 3, 6 and 9, cytological and bacteriological specimens were collected to determine endometritis. Mares were randomly assigned to two groups on day 10. Mares in treatment (n=4) and control (n=4) groups received intrauterine 6% *Eucalyptus* oil solution (100 ml) and intrauterine saline solution (100 ml) for 5 consecutive days. Cytological, bacteriological, and ultrasonographic evaluations were implemented at days 15, 17 and 19 (1, 3 and 5 days after termination of intrauterine treatment, respectively).







(Bacteriological and cytological findings on day 15,17,19 show no significant changes in infection.so.the treatment was failed to treat the endometritis.)

The number of mares diagnosed with endometritis, the depth of their intraluminal uterine fluid and the number of infected mares did not change by the treatment (P>0.05). In conclusion, the 5-day intrauterine infusion of 6% *E. globulus* oil solution failed to treat experimental endometritis in mares.

OTHERS THERAPY

(1) Omega-3 (N-3) fatty acids, specifically docosahexaaenoic acid (DHA), have been shown to confer anti-inflammatory properties in many species by inhibiting the production of cytokines involved in acute and chronic inflammation. In mares it has been shown that daily supplementation with an algal N-3 fatty acid (14,400mg of N-3 fatty acids, 4,000mg DHA from micro-algal source) resulted in reduced post-breeding inflammation (reduced uterine fluid and neutrophils in the uterine lumen after 63 days of supplementation) in both susceptible and resistant mares (Brendemuehl *et al.*, 2014)

Recommended treatment:-

(2) Administer omega-3 fatty acids (15,000 mg N-3 fatty acids) daily, beginning approximately 2 months prior to the breeding season and maintain through gestation.

(3) Cationic Steroid Antimicrobial (CSA):- CSA is a class of peptides that are now being synthesized which have demonstrated broad spectrum antibacterial, anti-vial, and some anti-fungal properties. They cause depolarization of bacterial cell membranes and activate apoptotic pathways leading to cell death. They also have the benefit of having a decreased risk of bacterial resistance (Mari, 2014). There is currently a product on the market (Ceragyn uterine infusion) that has been shown to be no more irritating than saline when infused into the uterus of mares and contains a mimic CSA-13 to these antimicrobial peptides.

Recommended use for Ceragyn product:

1) Uterine lavage: Mix 60 ml with sterile saline or LRS and use as a pre- or post-breeding lavage.

2) Uterine infusion: Administer 60 ml as a uterine infusion to treat bacterial or fungal endometritis. (Maria R., 2014).

CONCLUSIONS:

Failure of the ability to physically clear inflammatory products and bacteria from the uterus is an important predisposing factor to equine endometritis. Endometritis is diagnosed by uterine ultrasonography, endometrial cytology, endometrial culture and biopsy. endometrial biopsy tissue or the efflux obtained from a small volume uterine flush is more sensitive in identifying uterine pathogens than is the culture swab method. Successful treatment of endometritis requires a lot of work and diligence on the parts of the veterinarian and owner. As pathogens induce different uterine responses and have developed different methods for evading the immune response, treatment containing use of antibioticsand and ecbolics along with uterine lavage and adjuvant therapy including immunomodulaters, mucolytics, buffered chelating agents& acupuncture therapy can improve success rate.

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