Theriogenology Insight: 5(3): 157-182, December, 2015
DOI Number: 10.5958/2277-3371.2015.00018.2

Different Approaches to Diagnose Uterine Pathology in Mares: A Review

Nafis I Assad[1*] and Anand Kumar Pandey[2]

[1]*Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences Hisar-125004, Haryana, India*
[2]*Teaching Veterinary Clinical complex, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences Hisar-125004, Haryana, India*

**Corresponding author: dnnafie2020@gmail.com*

Abstract

Uterine infections and associated endometritis is the most common cause of reduced fertility and infertility in broodmares. Uterine infections are inevitable during natural service, artificial insemination, foaling and reproductive tract examination. Mares affected by such conditions are usually the best performing mares with a proven record of fertility. These pathological conditions are therefore responsible for major economic losses in equine industry, as huge effort, money and manpower are required to manage and treat such conditions year after year. Several methods have been evolved to confirm the uterine pathology in mare. Not a single diagnostic method *per se* is sufficient to diagnose uterine pathology accurately. Rectal palpation and ultrasound examinations identify fluid in the uterus, suggestive of endometritis. Microscopic analysis of an endometrial swab or endometrial biopsy has great importance to detect the etiological agent of the uterine pathology. This review therefore, is an attempt to discuss different approaches to diagnose the uterine pathology along with their merits and demerits. The ultimate aim being prompt identification and treatment of affected mares so that the problem of infertility in mare can be reduced.

Keywords: Uterine pathology, infertility, endometritis mare, diagnostics

Infertility in mares has been subject to limited study and research as compared to cattle, in which much more basic research on reproductive physiology and pathology has been carried out in recent years. Of late, it did succeed in gaining attention of veterinary clinicians and researchers to whom it posed a challenge for many years and

in fact still continues to do so. Uterine infections have long been recognized as one of the major causes of reduced fertility in animals. These infections are often caused by opportunistic microbes and a variety of species of bacteria and fungi have been isolated (Ricketts *et al.* 1993, Dascanio *et al.* 2000, Riddle *et al.* 2007, Nafis *et. al.* 2012a, 2012b). Bacterial uterine infections inflict major losses on equine breeding industry occurring in 25-60 % of barren mares (Bain, 1966). Such losses can appear as failure to conceive, early fetal death, mid gestational abortion, placentitis, birth of septic neonates, post-partum metritis or delays in rebreeding. Endometritis has been identified as third most common disorder of adult horses after colic and respiratory tract diseases (Traub-Dargatz *et al.* 1991).

Uterine infections are inevitable during breeding and foaling in mares, where contamination may include coitus, parturition, reproductive examination etc. (Card 1997). Persistent mating induced endometritis (PMIE) is among major causes of infertility in broodmares with incidence of about 15% (Traub-Dargatz *et al.* 1991, Zent *et al.* 1998). PMIE along with Post Breeding Metritis (PBM) is still a major cause of economic loss in equine industry. The older multiparous mares that are more often affected by uterine infections are usually also the most valuable proven mares, so great effort and money are invested every year in managing and treating these conditions (Relias, 2001).

Owing to the gravity of economic toll that the uterine diseases amount to, it is imperative to diagnose them as early as possible, so that proper therapeutic and managemental measures are directed to treat and control them. Many approaches ranging from more basic to highly sophisticated techniques have been used to aid diagnosis of uterine diseases. Listed below are some of them:

1. Transrectal palpation
2. Vaginoscopy
3. Uterine cytology
4. Uterine culture
5. Uterine/endometrial biopsy
6. Ultrasonography
7. Uterine endoscopy
8. Endocrinology
9. Immunology
10. Karyotyping
11. Laparoscopy

Transrectal palpation

The reproductive tract is palpated per rectum, so as to identify its significant features, determination of stage of estrous cycle and identify potential problems. Transrectal

palpation has gained much popularity in large animals not only in cattle and buffalo; but is also routinely used in broodmare practice. Palpation is often performed in conjunction with Ultrasonography (USG), however manual palpation can often identify features of the tract that can't be detected by USG, e.g. uterine tone, consistency of ovarian follicles, sensitivity of ovary to touch, etc. (McCue, 2008). Diagnosis of pyometra depends on transrectal palpation of distended uterus and/or USG that reveals echo-dense fluid (Sheldon *et al*. 2006). Presence of poor uterine tone and free intrauterine fluid are associated with poor uterine clearance and persistent inflammation (LeBlanc, 1999, Brinsko *et al.* 2003). It is possible to evacuate the uterine contents during the procedure when the fluid accumulation has taken place in pendent parts of the uterus and cervix is open. In such cases a gentle massage of the tract per rectum does the trick. Transrectal palpation for delayed involution is not a good technique for evaluating uterine infection, owing to its subjective nature (Lewis, 1997, LeBlanc *et al.* 2002).

Vaginoscopy

Vaginoscopy is a rapid and simple technique, where a speculum is used for examination of vaginal vault, *os externus* of cervix and detects pathological conditions. Here a vaginal speculum is inserted into the vagina far enough to enable visualization of the cervix. With the aid of a light source evidence of a purulent or muco-purulent cervical discharge can be recorded. Mares with endometritis may have hyperemic cervix and/or a purulent discharge from the external os of cervix (McCue, 2008). In mares with closed cervix, uterine discharge can't be detected by vaginoscopy.

Vaginoscopy can be performed using autoclavable plastic, metal or disposable foil-lined cardboard vaginoscopes (Sheldon *et al*. 2006). Since, positive diagnosis is based on presence of cervical exudates, it tends to underestimate the proportion of animals that reveal a positive uterine cytology (Drillich *et al*. 2004, Kasimanickam *et al*. 2005). Vaginoscopy often fails to identify all animals that are at risk of poor reproductive performance. LeBlanc *et al*. (2002) reported that absence of uterine discharge by vaginoscopic examination was not truly indicative of status and severity of uterine inflammation. Miller *et al.* (1980) concluded that vaginoscopy is a more accurate method of detecting uterine infections than rectal palpation. It may be important to mention that in case of mares the uterine exudates are not as overtly expressed as in cattle and buffalo. Thus, vaginoscopy as a diagnostic tool for uterine infections in mares has failed to gain much popularity.

The vaginal mucus that is revealed by the vaginoscopy can be collected and scored, thus it can be used to estimate the severity of clinical endometritis (Murray *et al*. 1990, Sheldon and Noakes 1998, Williams *et al.* 2005). Different people have followed different scoring schemes. Table 1 shows vaginal mucus scoring system followed by Sheldon *et al.* (2006).

Table 1: Vaginal mucus scoring system (Sheldon *et al.* 2006)

Character of vaginal mucus	Score
Clear or translucent mucus	0
Mucus containing flakes of white or off-white pus	1
Discharge containing = 50% white or off-white muco-purulent material	2
Discharge containing = 50% purulent material usually white or yellow but occasionally sanguineous	3

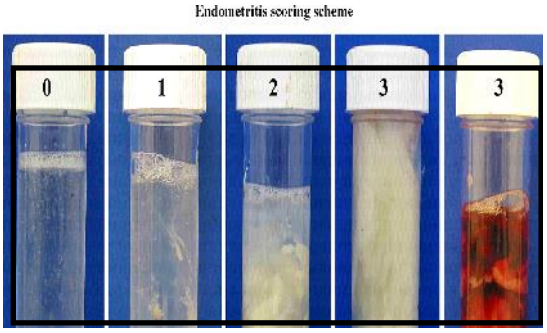

Fig.1: Scoring of vaginal mucus in accordance with the scheme followed by Sheldon *et. al.* (2006)

There has been some resistance to use of vaginoscopy because of perceived inconvenience and potential of disease transmission (LeBlanc *et al*. 2002). Barlund *et al.* (2008) reported that vaginoscopy is far superior diagnostic technique for endometritis but is underutilized by practitioners. This technique has gained much popularity in small animal practice. Sensitivity and specificity of vaginoscopy as reported by different workers is given below in Table 2.

Table 2: Sensitivity and Specificity of vaginoscopy

Sensitivity	Specificity	Reference
53.9%	95.4%	Barlund *et al.*(2008)
12.3%	90.2%	Drillich *et al*. (2004)
20.0%	88.0%	LeBlanc *et al*. (2002)

Uterine cytology

The role of Polymorphonuclear Neutrophils (PMNs) in equine uterine defense has been studied thoroughly and it was observed that delayed or impaired PMN function is responsible factor for mares unable to eliminate uterine infections. Polymorphonuclear Neutrophils (PMNs) seem to be of major importance for uterine function. Increased

numbers of PMNs have been observed in the uterine lumen during estrus, in early phase of puerperium and during infections for both mares and cows (Watson, 1988, Hussain, 1989). Uterine cytological smears from normal cycling fertile mare shows free epithelial cells without PMN infiltration (Knudsen, 1982; Brook, 1985). During uterine pathology, usually there is accumulation of intrauterine fluid and PMNs are the predominant cells found in these fluid accumulations (Zerbe *et al*. 2003). Determination of relative proportion of PMNs has been shown to be predictive of reproductive performance in post-partum animals (Gilbert *et al*. 2005). Endometrial cytology is a rapid and reliable diagnostic technique for detection of acute and chronic endometritis (Crickman and Pugh, 1986; Reiswig, *et al*. 1993). Uterine cytology provides a better guide to uterine infection than microbiological examination in mare (Digby, 1978). Pre-service uterine cytology can be used as a prognostic aid to predict fertility in mares (Ricketts and Alonso, 1991). A recent study by Riddle *et al*. (2005) has related uterine cytology findings to pregnancy results. Mares showing inflammation on cytology specimens were found to have low pregnancy rates than mares with normal cytological findings, irrespective of culture results. Further, in this study, endometrial cytology identified twice as many mares with endometritis as endometrial culture. Endometrial cytology has been described and related to bacteriological findings by several authors (Knudson, 1982; Brook, 1985; Nielson, 2005; Riddle *et al.* 2007). Moreover, it has been used both to diagnose inflammation in relation to persistent endometritis and in relation to post-breeding endometritis (Card, 2005).

Nielson (2005) in his study on uterine cytology found that endometrial cytology has a sensitivity of 77% when compared to the identification rate of PMNs from a histological preparation from the same animal. The same study showed a positive predictive value of 100 per cent and negative predictive value of 62% for endometrial cytology. This clearly indicates that uterine cytology has relatively high reliability in diagnosing endometrial inflammation, although false negative cases are to be expected. The authors observed a sensitivity of 71.42% and specificity of 69.23% for uterine cytology in the diagnosis of uterine infections in mare. The positive and negative predictive values and false positive and false negatives for uterine cytology as recorded by authors were 71.4% and 69.23% and 28.37% and 30.76%, respectively (Nafis, 2011). Uterine cytology provides direct evidence of uterine inflammation, and helps to identify false positive cultures. Consequently, uterine culture will provide ambiguous or misleading results if uterine cytology is not performed (Wingfield-Digby and Ricketts, 1982). Uterine cytology offers the advantage of ease of sample collection, low cost and rapid availability of results over the other methods.

Uterine cytological evaluation involves collection, processing and interpretation of cells lining the uterus and those exfoliated into uterine lumen. Samples for endometrial cytology may be collected using the following techniques:

(a) Double guarded swab (Brook, 1993; Card, 2005).
(b) Cytobrush (Bourke *et al.* 1997; Barlund *et al.* 2008; Oral *et al*. 2009).
(c) Low-volume uterine flush (Ball *et al*. 1988; Card *et al*. 2004; LeBlanc 2008; Nafis 2011).

Double guarded swab technique (DGS)

Here a swab guarded by two sheaths, an inner and an outer sheath is used. This method minimizes vaginal contamination. There are four types of swabs that are routinely used to obtain uterine material for cytological analysis: (i) Kalayjian uterine swab (ii) McCullough uterine swab (iii) Accu-CulShure system and (iv) Tiegland uterine swab (Dascanio *et al.* 1997). The end of double guarded swab is kept covered and free from lubricant as it is introduced into the reproductive tract. This DGS is then advanced into the uterus through cervix and the inner sheath is pushed through the outer sheath. The swab is moved forward to sample the endometrial cells using a rolling and pushing motion (for 1 minute). The examiners hand into the vagina is used to redirect the DGS into different areas of uterus. The sample swab is then retracted back into the inner sheath, which is pulled back through the outer sheath and entire assembly is removed as one unit. Cytology sample is prepared by rolling the swab on the sterile slide. The slide is then air dried and stained.

3.2 Cytobrush technique

The cytobrush with its handle guarded by a stainless steel sheath covered with a plastic sheath is lubricated and introduced into the vagina. Next a sleeved arm introduced into the rectum facilitates the passage of the instrument through vagina and cervix. At the external *os* of the cervix, the plastic sheath is perforated and the stainless steel sheath and cytobrush extension manipulated through the cervix and into the body of uterus, where cytobrush is rotated to obtain cellular material from adjacent endometrium. Leaving the stainless steel sheath in place the cytobrush extension is removed and rolled on glass slide. This allows to minimize the contamination from the other parts of tract viz cervix and vagina. The slide is air dried and stained for microscopic examination. Figure 2 below shows different varieties of cytobrushs that are used for uterine sampling.

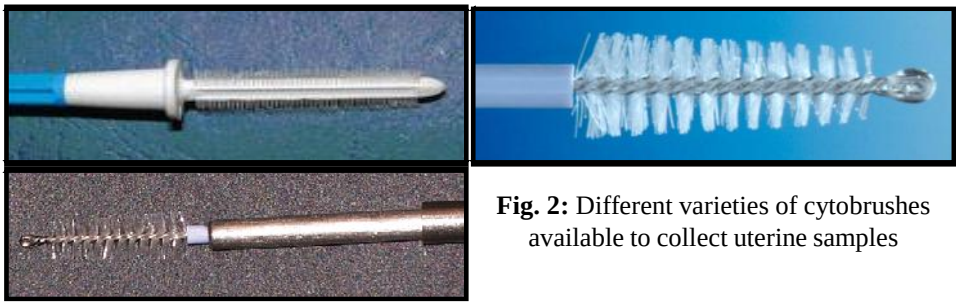

Fig. 2: Different varieties of cytobrushes available to collect uterine samples

Low-volume uterine lavage

A low volume 60–100 ml (even more) uterine lavage with Phosphate Buffer Saline (PBS) is performed by advancing an insemination pipette or Foley's catheter (Nafis 2011, Nafis *et. al.* 2012b) into the uterus, injecting the PBS and recollection of fluid after per rectal manipulation of the uterus. Then the fluid is aspirated back. Approximately 10% of the fluid is recovered (Card *et al.* 2004) and contents are emptied into a centrifuge tube. The slides are prepared by cryo-centrifuge technique. Sample is centrifuged at 400x*g* for 10 min. and pellet is re-suspended in 1 ml of PBS. Drops of suspension are put onto glass slides, air dried and stained for microscopic evaluation.

Although use of guarded swab is much popular due to ease of sampling, but not all mares with endometritis are identified, as the swab only comes in contact with 1-2 centimeter area of endometrium directly cranial to the cervix. However, low-volume uterine flush though more cumbersome than procedures utilizing guarded swabs, may prove valuable for conditions of subclinical endometritis in chronically infected mares (LeBlanc *et al.* 2007). Uterine flush technique was found to be superior over swab technique in cytological diagnosis of equine endometritis (Ball *et al.* 1988).

The stains used in cytology include: Giemsa stain (Berwal *et al.* 2006), Diff-Quick stain, which is a modified Wright's stain (Dascanio *et al.* 1997) and Hemacolor stain, which is a modified Wright-Giemsa stain (Santos *et al.* 2009).

Interpretation of uterine cytology

The parameters that are evaluated in uterine cytology include: (i) percentage of neutrophils (a minimum of 100 cells are counted using x1000 magnification); (ii) Identification of presence/type (rod, coccus, coccobaccillus) of bacteria/fungi; and (iii) Determination of presence/amount of debris. Dascanio *et al.* (1997) proposed identification of cell types also.

Neutrophils

There has been much lack of agreement with respect to interpretation of uterine cytology results. Different workers have followed different quantitative approaches for interpretation. While some believed that mere presence of PMNs in the sample would amount to uterine infection, others took a different opinion (Fig. 3 to 6). Various quantitative methods that have been followed by different workers, so as to recognize the animals as having positive uterine cytology are given in Table 3. While studying uterine cytology of mares Card (2005) grouped them as follows:

(a) $\leq$5% neutrophils no inflammation
(b) 5-15% neutrophils, mild inflammation
(c) 15-30% neutrophils, moderate inflammation
(d) >30% neutrophils were classified as cases with severe inflammation.

Table 3: Quantitative methods followed by different workers to interpret uterine cytology results

Parameter	Author	Parameter	Author
= 2% Neutrophils	Nafis (2011); Nafis *et. al* (2012a, 2012b)	=3-10% cells as Neutrophils	Crickman and Pugh (1986)
= 0.5% Neutrophils	Nielsen *et al*. (2010)	<15 endometrial cells per neutrophil	Ley (1986)
>8% Neutrophils	Barlund *et al.* (2008)	>5 Neutrophils in 10 fields	Brook (1985)
>5% Neutrophils	Card (2005)	>1 neutrophil per field (x1000)	Asbury (1984)
=1% per field (Neutrophil) × 400	Purswell *et al*. (1989)	Neutrophil:Epithelial cell ratio>10:1	Asbury (1984)
=2% cells are Neutrophils	Ball *et al.* (1988)	>1neutrophil in five fields (x240)	Knudsen (1964a)

Sensitivity and Specificity of uterine cytology has been worked out by many workers and has been summarized in Table 4 below:

Table 4: Specificity and Sensitivity of uterine cytology

Type of cytology	Sensitivity	Specificity	References
Flush cytology	71.42 %	69.23%	Nafis (2011)
Lavage cytology	92.3%	93.9%	Barlund *et al*. (2008)
Cytobrush cytology	12.9%	89.9%	Burland *et al*. (2008)
Flush cytology	80.0%	67.0%	LeBlanc *et al*. (2007)
Swab cytology	77.0%	100%	Nielsen (2005)

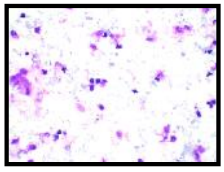

Fig. 3: Uterine cytological smear of an infertile mare showing epithelial cells with moderate neutrophilic infiltration (x400). Courtesy: Nafis, 2011

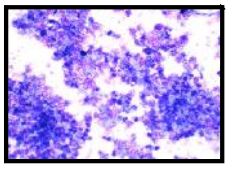

Fig. 4: Uterine cytological smear of an infertile mare showing heavy infiltration of neutrophils(x400). Courtesy: Nafis, 2011.

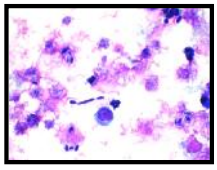

Fig. 5: Uterine cytological smear of infertile mare showing neutrophils, epithelial cells and a monocytes (x1000). Courtesy: Nafis, 2011.

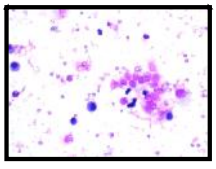

Fig. 6: Uterine cytological smear from a fertile mare showing epithelial cells, lymphocytes, monocytes and occasional neutrophils (x400). Courtesy: Nafis, 2011.

Bacteria and other micro-organisms

Bacteria are scored based on number of bacteria per high power field (x1000) in microscope. They may be found free or phagocityzed within neutrophils or macrophages. Same scoring system is used for yeast/fungi also if present. Bacteria are also classified as cocci, bacilli or coccobaccilli. If bacteria are seen another slide may be prepared for Gram's staining. Gram negative cocci are unlikely uterine pathogens, but bacilli and coccobaccilli should be Gram stained to determine if they are Gram positive or Gram negative (Maloufi *et al.* 2002). Scoring system for bacteria used by Card (2005) is shown in Table 5 below.

Table 5: Scoring system for bacteria (Card 2005)

Score	Number of bacteria	Score	Number of bacteria
1	No bacteria per 30 fields	4	2-10 bacteria per field
2	1 bacteria per 30 fields	5	4-50 bacteria per field
3	1 bacteria per 10 fields		

Fungal and yeast organisms are usually identified using Giemsa staining procedure. Lacto phenol cotton blue (LPCB) stain can also be used to demonstrate the fungal material in the smear.

Debris

Amount of debris on slide is scored based on proportionate amount of debris that covers a high power (x1000) field in microscope. The fluid collected after uterine flush has also been examined for efflux clarity and pH by LeBlanc *et al.* (2007). Efflux clarity is recorded by holding sample up to light and graded it as clear, cloudy and mucoid (clear with mucus stains). Card (2005) scored the amount of debris in the cytology slide on a 1-4 scale (Table 6).

Table 6: Scoring of debris on a cytology slide (Card, 2005)

Score	Amount of debris
1	< 25%
2	< 50%
3	< 75%
4	> 75%

For statistical analysis cloudy and mucoid types of efflux are graded positive for endometritis. Increase in pH of flush with *Streptococcus sp* and infection of *Escherichia coli* has been correlated with considerable amount of debris on cytological examination *(*LeBlanc *et al.* 2007*).* Depending upon the quality and quantity of cellular material,

bacterial contamination and amount of debris different people have interpreted the results differently (Table 7).

Table 7: Interpretation of uterine cytology as reported by Riddle *et al.* (2007) and Card (2005)

Interpretation by Riddle *et al.* (2007)		Interpretation by Card (2005)	
Description of smear	**Remarks**	**Description of smear**	**Remarks**
Epithelial cells + 0-2 PMNs/field	Normal cytology	Many epithelial cells + <5% PMNs + Few bacteria + Little debris	Healthy mare
Epithelial cells + 2-5 PMNs/field	Moderate infection	Many epithelial cells + >5% PMNs + Heavy bacteria + Heavy debris	Infected mares
Epithelial cells + >5 PMNs/field	Severe infection	Many epithelial cells + Few bacteria + Few PMNs	Questionable mares

Uterine culture

Uterine culture/microbiological investigations are of diagnostic value in detection of acute and chronic equine endometritis (Henry *et al*. 1982, Narwal and Monaga, 1994). Uterine pathogens can be cultured from representative samples collected from the uterine lumen. Samples may be obtained by using guarded culture swab (Blanchard *et al.* 1981) or low volume uterine lavage (Ball *et al.* 1988; LeBlanc *et al.* 2007; Nafis, 2011). In some instances uterine biopsies may be collected for uterine cultural examination (Nielsen *et al.* 2010). In the laboratory the sample is applied to culture media plates and incubated. Bacterial growth is usually evident within 24-48 hours, while yeast often requires several days to grow (McCue, 2008). Culturing the uterine low-volume flush (ULF) allows the quantification of bacterial growth. A fixed volume of uterine fluid is transferred with a loop onto an agar plate and spread evenly throughout the surface. After the incubation, colonies are counted. It is also possible to centrifuge the lavage fluid before culturing. The low-volume uterine flush technique has been shown to be more sensitive than swab technique in detecting bacteria (Ball *et al.* 1988).

Commonly used culture media are: Nutrient agar, Blood agar, Eosin-Methylene blue (EMB) agar and Muller Hinton agar (LeBlanc *et al.* 2007; Nielsen *et al.* 2010; Nafis, 2011) for bacteria. Laboratory growth medium routinely used for isolation of fungi is Sabouraud dextrose agar medium (2%) containing chloramphenicol antibiotic @ 0.5 µg/ml (Albihn *et al.* 2003, Nafis, 2011). Uterine cytology only provides the qualitative information about the mares' uterus, but diagnosis of nature of uterine infection to recommend suitable intra-uterine antibiotic therapy can only be established by culture of uterine flush (Bermudz *et al.* 1995, Zent *et al.* 1998).

4.1 Common bacterial and fungal isolates

Escherichia coli is primary bacterial pathogen that is isolated from uterus of problematic mares (Leblanc *et al.* 2007*). Streptococcus equi subsp. zooepidimicus* has been most commonly isolated during routine cultures in equine *(*Ricketts *et al.* 1993, McCue 2008*).* Most common uterine bacterial pathogens in endometritis mares as obtained by several authors (Collins 1964; Scott *et al.* 1971; Shin *et al.* 1979) include: β-haemolytic *Streptococcus* sp., *Escherichia coli, Staphylococcus* sp., *Pseudomonas* sp. and *Klebsiella* sp. β-haemolytic *Streptococcus* sp., *Escherichia coli*, *Staphylococcus* sp. and *Pseudomonas* sp. However, LeBlanc *et al.* (2007) reported *Pseudomonas auroginosa, Enterobacteria cloaca*, and *Staphylococcus aureus.* Authors (Fig 7 & 8) isolated *Corynebacterium* sp. *Escherichia coli* and *Streptococcus* sp. from a group of endometritis affected mares, of which Streptococcal isolates were most prevalent (33.33%), followed by *Corynebacterium* and *E. coli* (23.8 and 9.5 per cent, respectively) was isolated in mares (Nafis, 2011). *Aspergillus, Candida, Coccidiodes, Hansenula, Monosporium, Mucor, Paccilomyces* and *Trichosporon* were the commonest isolates obtained from endometritis mares by Pugh *et al.* (1986). Narwal and Monaga (1994) reported that *Aspergillus fumigates* was the most common uterine fungal pathogen in endometritis mares and also recovered *Aspergillus terreus, Aspergillus flavus, Aspergillus niger, Cladosporium cladosporoides, Rhizopus oryzae, Alternaria alternate, Mucor racemosus* and *Candida tropicalis*. Furthermore, fungal isolates reported to colonize the equine reproductive tract by several authors (Changappa *et al.* 1984; Petrites-Murphy *et al.* 1996; Dascanio *et al.* 2000) include: *Candida lusitaniae, Candida rugosa, Cryptococcus neoformans, Hansenula anomala, Hensenula polymorpha, Rhodotorula minuta, Rhodotorula rubra* and *Torulopsis candida*.

Fig.7: Blood agar plate showing growth of bacterial colonies after overnight incubation (white colonies). Courtesy: Nafis, 2011.

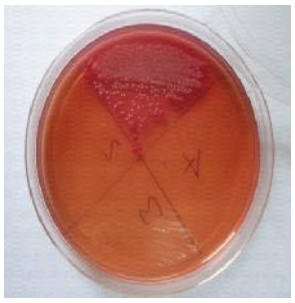

Fig. 8: McConkey agar plate showing growth of lactose fermenting bacteria (Pink colonies). Courtesy: Nafis, 2011.

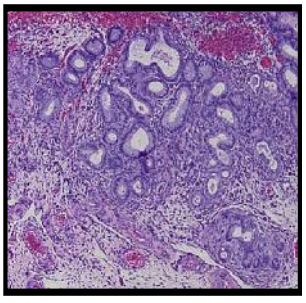

Fig. 9: Endometrial biopsy sample from a mare with chronic severe inflammatory and degenerative changes Courtesy: McCue 2008.

Interpretation of uterine culture

Samples are considered positive for growth only after four or more colonies of pathogenic organisms are isolated from them. *Bacillus* sp. *Micrococcus* sp. and α-haemolytic *Streptococcus* sp. are not considered to be pathogenic to equine uterus (Blanchard *et al.* 1981, Wingfield-Digby and Ricketts, 1982; Ricketts and Mackintosh, 1987; Nielsen, 2005, Riddle *et al.* 2007) and are recorded only if isolated in pure culture (LeBlanc *et al.* 2007). During routine identification of fungus yeast is not identified to species except for *Candida albicans* (McGinnis, 1980). Sensitivity and specificity of uterine culture as worked out by different researchers can be summarized as following in Table 8 below.

Table 8: Sensitivity and Specificity of uterine culture

Culture	Sensitivity	Specificity	References
Flush culture	100 %	100%	Nafis (2011)
Swab culture	34.0%	100%	Neilson (2005)
Biopsy culture	82.0%	92.0%	Neilson (2005)
Flush culture	71.0%	86.0%	LeBlanc *et al.* (2007)

Moreover, the positive and negative predictive values for uterine culture examination in the diagnosis of uterine infections in mares were 100 per cent for both (Nafis, 2011). Further, no false positive and false negative results were observed by authors for uterine culture technique.

Endometrial biopsy (EMB)

Endometrial biopsy is most definitive diagnostic tool for diagnosis of endometritis (Gilbert *et al.* 2005; Sheldon *et al.* 2006). It involves collection of a small sample of endometrium for histological examination. Kenney (1978) reported that collection of a single biopsy from one site is generally representative of the entire endometrium, although collection of multiple samples adds to advantage. Endometrial biopsy samples are examined for presence of inflammatory and degenerative changes. Acute inflammation is recognized by presence of PMNs in endometrial tissue (endometrial luminal epithelium, *Stratum compactum and Stratum spongiosum*).

Chronic endometritis is characterized by accumulation of mononuclear cells (mostly lymphocytes) in the endometrial tissue. Endometrial glandular degeneration is most often recognized by deposition of collagen in the form of fibrosis or scar tissue around endometrial glands. It represents a permanent untreatable condition. Sterile biopsies (95%) had negative cytology (Neilson *et al.* 2010). Thus it may be considered as a reliable diagnostic technique.

Interpretation of Uterine biopsy

Endometrial biopsies are classified on I to III grading scale based on histological characteristics as given below in the Table 9.

Table 9: Interpretation of uterine biopsy

Grade	Description of histology
I	Endometrium is essentially normal with minimal inflammation and fibrosis
III	Endometrium includes severe inflammatory and/or fibrotic changes (Fig. 9).
II	It is a broad category encompassing all the pathological levels between grade I and II

Grade III was associated with susceptibility to chronic uterine infections (Troedsson *et al.* 1993). Endometrial biopsies may also aid in reaching diagnosis of fungal infection by staining the sample with Gomori's methamine silver stain (Freeman *et al*. 1986). Ricketts and Alonso (1991) proposed that presence of 3 or more neutrophils per 5 fields (x400) may be considered as evidence of acute endometritis. Langer *et al.* (1997) reported the sensitivity and specificity of this technique to be 92% and 77%, respectively. However there are two demerits for this otherwise reliable diagnostic technique, viz. (i) the duration between sampling and laboratory results, which are usually more than a week, while practically a clinician cannot afford to wait for so long, and (ii) also histological examination only indicates that the animal has uterine inflammation, but does not provide information regarding etiology of the condition.

Ultrasonography

Diagnostic ultrasound is the non-invasive technique for imaging soft tissues and accurate assessment of the size, shape, position and texture of soft tissues in organs in different farm and pet animals. Its use has been demonstrated in different species of large domestic animals especially horses (Pycock, 2000, Godoi, *et al.* 2002 and Watson *et al.* 2003). The introduction of transrectal ultrasonography to evaluate the dynamic images of equine uterus has been a major stride in equine reproduction (Squires *et al.* 1988). Transducers with different frequencies have been used by different researchers *vis a vis* 3.5 MHz (Ginther and Pierson 1984), 5 MHz (Pierson *et al.* 1988) and 8 MHz (Oral *et al.* 2009). The latest development has been use of Doppler ultrasound for investigating uterine blood flow in mares (Bollwein *et al.* 2003). The authors used a linear array probe for transrectal ultrasound evaluation of broodmares, which operated at 5 and 7.5 MHz interchangeable frequency. For transrectal use the probe was put inside a finger of an examination glove along with some gel, so as to remove the air and ensure good contact between the probe surface and the glove lining. Initially used strictly for pregnancy detection, more recent applications in reproduction include: monitoring follicular changes and predicting ovulation, confirmation of ovulation,

evaluation of corpus luteum morphology, estimating stage of estrous cycle, detection of ovarian and uterine pathology, detection of twins and embryo reduction, determination of embryonic death and evaluation of testes and accessory sex glands in stallions (Squires *et al.* 1988). The application of ultrasound as a diagnostic tool to evaluate mare's uterus has been a major advancement. Although evaluation of uterine size can be performed by rectal palpation, however, more definitive measurements can be made by ultrasonography only. Increased size of uterus, thickening of uterine wall, and presence of cysts are all indications of uterine pathology. Ginther and Pierson (1984) described the ultrasonic appearance of uterus throughout the estrous cycle in mares. Following ultrasonic morphology was reported: diestrus (endometrial folds not distinguishable), estrus (prominent endometrial folds), and intermediate stage (folds moderately distinguishable). Squires *et al.* (1988) proposed a 0 to 3 scale for grading such folds, which assigned a grade zero to folds that are not discernible and three to extreme folding of endometrium. The prominence of endometrial folds during estrus, with small fluid accumulation, should not be considered pathological and does not require therapy (McKinnon *et al.* 1987).

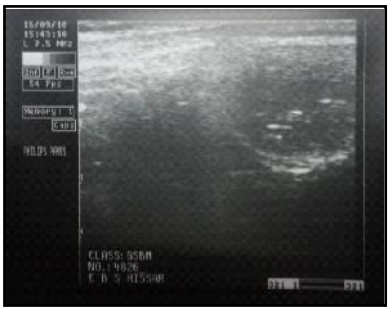

Fig. 10: Ultrasound view of right uterine horn of infertile mares showing small amount of echogenic (purulent) exudate along with non-echogenic fluid. Courtesey: Nafis (2011)

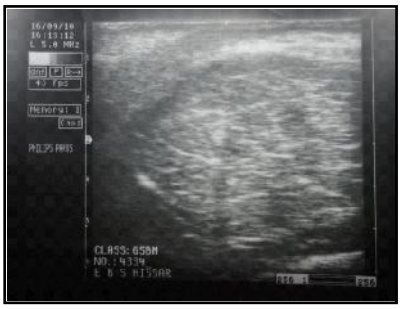

Fig. 11: Ultrasound view left uterine horn of an infertile mare showing voluminous purulent exudate with traces of echogenic exudates. Courtesey: Nafis (2011)

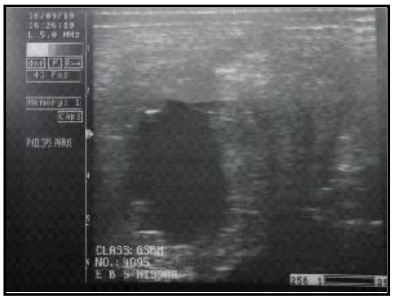

Fig. 12: Ultrasound view of uterine body of a infertile mare showing non-echogenic uterine exudates along with distinct wall thickening. Courtesey: Nafis (2011)

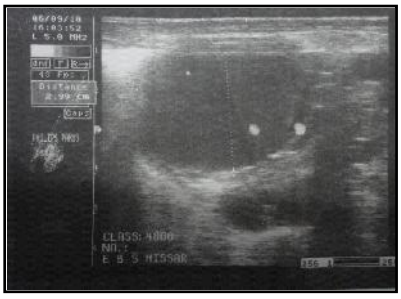

Fig. 13: Ultrasound view of right ovary of a fertile mare showing a large Graffian follicle at standing oestrus. Courtesey: Nafis (2011)

The presence of large quantities of fluid in mare's uterus during estrus and particularly diestrus, is suggestive of acute or chronic endometritis (Squires *et al.* 1988). Fluid present in the uterus is often associated with susceptibility to endometritis (Maloufi *et al.* 200; Brinsko *et al.* 2003). Highly fertile mares seldom have free intrauterine fluid after breeding (Burns *et al.* 2000). Characteristics of fluid can vary according to degree of inflammation and nature of debris (McKinnon *et al.* 1988a). Purulent discharges often are recognized as non-echogenic fluid with sprinklings of echogenic spots (Fig. 10 to 13). The more echogenic the fluid indicates more likely the fluid is contaminated with debris including PMNs (Squires *et al.* 1988). Presence of air in the uterus may also contribute to echogencity of the fluid. Transrectal ultrasonography allows the detection of even small fluid accumulations (Ginther and Pierson 1984) often causing unnecessary worrying among practitioners.

Interpretation of ultrasonography

Two things *vis-a-vis* uterine fluid and uterine wall thickness are considered during ultrasonography to determine uterine condition. Presence of persistent echo-dense intrauterine fluid at post-breeding evaluation suggests the onset of bacterial endometritis (Hurtgen, 2006). Kasimanickam *et al.* (2004) studied the intrauterine accumulation in terms of uterine size (diameter) at the base of uterine horn. Uterine diameter < 3 cm was interpreted as no endometritis and those with uterine diameter ≥ 3 cm as endometritis. However, Barlund *et al.* (2008) interpreted the ultrasonographic studies in mares on the basis of wall thickness and further categorized the animals as having endometritis where the uterine thickness was found to be ≥ 8 mm and no endometritis where thickness was < 8 mm.

Sensitivity and specificity of ultrasonography in diagnosis of uterine pathology, as worked out by different workers is summarized in Table 10 as follows:

Table 10: Sensitivity and Specificity of ultrasonography

Ultrasonography	Sensitivity	Specificity	References
Uterine fluid	86.66 %	50.00 %	Nafis (2011)
Uterine fluid	30.8%	92.8%	Barlund *et al.* (2008)
Uterine wall thickness	3.90%	89.2%	Barlund *et al.* (2008)
Uterine fluid	36.0%	94.0%	Kasimanickam *et al.* (2004)

Authors recorded the positive and negative predictive values for uterine ultrasonography in diagnosis of uterine infections in mares were 68.42% and 75.00%, respectively. Also the false positive and false negative results for this technique as observed by the authors were 31.57% and 25%, respectively.

Uterine endoscopy/hysteroscopy

The technique of direct visualization of the interior of the uterus using an endoscope is known as hysteroscopy. Use of endoscopy as a tool to work-up equine reproductive health was advocated more than two decades ago by Wilson (1984). Hysteroscopy has long been recognized as diagnostic modality in mares with reproductive diseases. This procedure allows direct observation of the reproductive tract as well as cytological, microbial and histological sampling of specific sites may also be done under visual control. Better visualization of cervix and uterus with endoscopy can improve sample collection procedures, thus augmenting other diagnostic procedures. Endometrial abnormalities observed with hysteroscopy include endometrial degeneration, endometrial cysts, intra-luminal fluid accumulation, trans-luminal adhesions, etc. (Bracher *et al.* 1992; Santschi, 2005). Uterine adhesions (Fig. 14) are very difficult to diagnose with procedures other than endoscopy. Inoue *et al.* (2002) reported a correlation between the appearance of small arteries under the endometrium (as found by endoscopy) with age and degree of endometriosis. Various surgical procedures can also be performed under hysteroscopic guidance e.g. endometrial cyst removal. Hysteroscopy can also be utilized to realize target specific intrauterine medication. This approach can be effectively utilized to evaluate the effect of local application of PGE_2 on tubal block. Direct application of medication to the affected area bypasses unfavorable pharmacokinetic profiles (like first pass effect) of most drugs, ensures high local concentrations, and allows assessment of integrity of the affected tissue (Lu and Morresey, 2006).

A flexible video endoscope interfaced with monitor and a recording device is routinely used for this procedure. The ability of the flexing endoscope to manure in all directions greatly enhances the operator's ability to examine the uterine milieu. The animal should be properly restrained for endoscopic procedure and more importantly should not be under progesterone influence (closure of cervix).

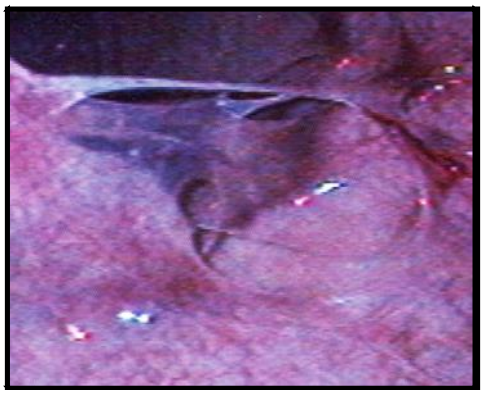

Fig. 14: Endoscopic view of intrauterine adhesions in mare (Courtesy: McCue 2008)

Endocrinology

Diagnostic endocrinology is a routine practice in brood mare management for over 2 decades now. Most commonly used assay is for progesterone (Douglas, 2004). Two often confusing terms are used in this pursuit, viz progestagens and progestins. While progestagens refer to metabolites of pregnanolone (P5) as well as progesterone (P4); by progestins is meant progesterone and its metabolites. In pregnant mares progesterone secreted by endometrial cups and secondary corpora lutea is present only through 120-150 days of pregnancy (Troedsson, 2007), and thereafter the predominant progestins are 5-á pregnanes and 17-hydroxy progesterone (Douglas, 2004). The source of these progestins is placenta. Thus progestin assay in pregnant mares may be a good diagnostic measure to evaluate the placental health. Rossdale *et al.* (1991) reported that mares with placental pathology may have increased plasma concentration of progestagens. Stawicki *et al.* (2002) suggested that measurement of repeated samples of plasma progestin concentration in mares with placentitis might be a useful method to identify mares that may abort or deliver prematurely. Studies conducted by Sheerin *et al.* (2003), found that mares that develop a chronic form of placentitis exhibited increased plasma progesterone concentrations, and those that develop acute placentitis and abort soon after infection show a rapid drop in plasma progesterone concentration.

Assaying the levels of progestins in serum of mares is done routinely to draw different conclusions. However more recently, in human monitoring programmes especially in post-menopausal women the focus has shifted to salivary levels of progesterone and other steroids. It is based on the maxim that salivary levels may represent the delivery of the hormone to the tissue membranes, where as serum concentration measures the quantity of hormone not yet delivered to the tissue (Gilson and Zava 2003). Authors could not find any report pertaining to measurements of salivary progestins in mares.

The fact that pregnant mare urine and blood had very high levels of estrogenic substances has been known since 1930s (Cox, 1975). The pregnant mare's serum and urinary estrogens during pregnancy reveal identical patterns. Many forms of estrogen are present in pregnant mare as free and bound fractions. Bound fraction of estrogen is represented by conjugated estrogens especially estrone sulfate, and free unbound estrogens include estrone, estradiol, equilin and equilenin. Assay for total estrogens is routinely used to assess pregnancy status of mare (Douglas, 2004). The estrone sulfate assay has been used to monitor fetal well being after 100 days of pregnancy in mare (Stabenfeldt *et al.* 1991). Douglas (2004) observed that during pregnancy between days 150-280, placentitis usually results in higher progestin and lower estrogen serum concentrations when compared to normal mares.

Relaxin is produced by equine placenta, and can be detected in peripheral blood from day 80 of gestation and throughout the pregnancy (Stewart *et al.* 1982). The role of relaxin during pregnancy though not fully understood, yet there is some evidence that

placental relaxin production is compromised in mares at risk of abortion (Stewart *et al.* 1992). Ryan *et al.* (1999) observed subnormal plasma relaxin concentrations in mares with abnormal pregnancies. As of now no test has been commercialized to estimate the equine relaxin.

The analysis of reproductive hormones in non-pregnant mare is most commonly performed to evaluate regression of corpus luteum (CL) and to diagnose ovarian abnormalities.

Immunology

Recently the concept of immunological infertility has been frequently discussed. The causes of the infertility arising from humoral immunity include anti-sperm antibodies (ASA), anti-zona pellucida antibodies (anti-ZP), anti-ovarian stroma antibodies; antibodies against seminal plasma and cauda epididymal extract (Risvanli, 2011). Other than gamete specific structures, infertility can develop due to immunity against GnRH, eCG etc. (Naz, 1999; Dalin *et al.* 2002). The commercialization of ELISA based assays for such antibodies promises highly in understanding the idiopathic causes of infertility in mares. These tests are yet to gain popularity among veterinary scientists and practitioners.

Identification of protein markers indicative of gestational and non-gestational diseases is a developing field in human and equine reproduction. In human beings, various markers of intrauterine growth retardation (IUGR) have been investigated, which include, Growth factors, Leptin, Vasoactive peptides like Insulin like growth factor I (IGF-1), Epidermal growth factor (EGF), Endothelin, angiotensin II, Tumor Necrotic Factor alpha (TNFá), Interleukin 8 (IL_8), etc. (Lu and Morresey 2006), however, these are lacking in field of equine practice.

Laparoscopy

Direct visualization of reproductive organs within the abdominal cavity may be performed using laparoscopy. Samples of tissue (e.g. ovarian biopsy) may be collected additionally for histological evaluation and medications may be topically applied to serosal surface of reproductive tract (e.g. PGE_2 onto the oviduct) using a laparoscope (Allen *et al.* 2006; McCue, 2008). However, due to more susceptibility of equine species to peritonitis as compared to other domestic animals, this approach has by and large failed to catch the imagination of equine practioners.

Karyotyping

Analysis of chromosome number and structure of chromosomes is termed as karyotyping. Karyotyping may be used as an important diagnostic test in evaluation of primary infertility in mares (Hughes and Trommershausen-Smith, 1977). The most commonly reported chromosomal abnormality of horse is XO gonadal dysgenesis, a

condition in which one of the sex chromosomes has been deleted, resulting in 63+X karyotype. Horses with XO gonadal dysgenesis develop as phenotypic females because of absence of a Y sex chromosome. Affected mares have small inactive ovaries and are infertile. Chromosome analysis on equine blood or tissue samples is currently only performed at Molecular cytogenetics laboratory, Maxwell H Gluck equine research centre of Texas A & M University (McCue, 2008).

References

Albihn, A., Baverund, V. and Magnusson, U. 2003. Uterine microbiology and antimicrobial susceptibility in isolated bacteria from mares with fertility problems. *Acta Veterinaria Scandinavica* **44**: 121-29.

Allen, W.R., Wisher, S., Morris, L., Lvowhurst, J.S., Hissuer, M.H. and Neal, H.N. 2006. Laparoscopic application of PGE_2 to re-establish oviductal patency and fertility in infertile mares, a preliminary study. *Equine Veterinary Journal* **38**: 454-59.

Asbury, A.C. 1984. Endometritis diagnosis in mare. *Equine Veterinary data* **5**: 166.

Bain, A.M. 1966. The role of infection in infertility in the Thoroughbred mare. *Veterinary Record* **78**: 168–73.

Ball, S.A., Shin, S.J., Patten, V.H., Lein, D.H. and Woods, G.L. 1988. Use of low-volume uterine flush for microbiological and cytological examination of mare‘s endometrium. *Theriogenology* **29**:1269-83.

Barlund, C.S., Carruthers, T.D, Walder, C.L. and Palmer, C.W. 2008. A comparison of diagnostic techniques for post-partum endometritis in dairy cattle. *Theriogenology* **69**: 714-23.

Bermudez, V., Sifontes, L., Navarro, N., Quintero, B., Moreno, J. and Burgos, M. 1995. Effects of intra-uterine infusion of sodium Ceftiofur on endometrium in mare. *Proceedings of American Association of Equine Practitioners* **41**: 261-63.

Berwal, S.S., Bugalia, N.S., Kapoor, P.K., Garg, D.N., Monga, D.P. and Batra, M. 2006. Cytological and microbiological evaluation of uterine flush of infertile and repeat breeder mares and post-treatment fertility. *Haryana Veterinarian* **45**(2): 15-17.

Blanchard, T.L., Cummings, M.R., Garcia, M.C., Hurtgen, J.P. and Kenney, R.M. 1981. Comparison between two techniques for endometrial swab culture and between biopsy and culture in barren mares. *Theriogenology* **16**(5): 541-52.

Bollwein, H., Mayer, R. and Stolla, R. 2003. Transrectal Doppler sonography of uterine blood flow during early pregnancy in mares. *Theriogenology* **60**: 597-605.

Bourke, M., Mills, J.N. and Barnes, A.L. 1997. Collection of endometrial cells in the mare. *Australian Veterinary Journal* **75**: 755-58.

Bracher, V., Mathias, S. and Allen, W.R. 1992. Videoendoscopic evaluation of mare’s uterus: II. Findings in subfertile mares. *Equine Veterinary Journal* **24**: 279-84.

Briskno, S.P., Rigby, S.L. and Varner, D.D. 2003. A practical method for recognizing mares susceptible to post-breeding endometritis. In: Proceedings of 49th conference of American Association of Equine Practitioners, New Orleans, LA. Pp: 363-365.

Brook, D. 1985. Cytological and bacteriological examination of mares' endometrium. *Journal of Equine Veterinary Sciences* **5**: 16-22.

Brook, D. 1993. Uterine cytology. IN: McKinnon AO, Voss JL. (Editors), Equine reproduction. Philadelphia, PA: Lea and Fibiger, Pp: 246-53.

Burns, T., Pierson, R. and Card, C. 2000. Subjective and quantitative assessment of endometrial changes in mares inseminated with Cryo-preserved semen. In: proceedings of the society of Theriogenology, San Antonio, TX.P. 47.

Card, C., Carley, S. and Green, J. 2004. Endometrial cytology in mares bred with frozen semen. In: Proceedings of 50th conference of American Association of Equine Practitioners, Denver, CO. Pp: 505-09.

Card, C. 1997. Current therapy in large animal theriogenology. Youngquist R S. (Editor) 1st edition. W B Saunders Co. pp: 151-153.

Card, C. 2005. Post-breeding inflammation and endometrial cytology in mares. *Theriogenology* **64**(3): 580-88.

Changappa, M.M., Maddux, R.L. and Greer, S.C. 1984. Isolation and identification of yeasts and yeast like organisms from clinical veterinary sources. *Journal of Clinical microbiology* **19**: 427-28.

Collins, S.M. 1964. A study of incidence of cervical and uterine infections in thoroughbred mares in Ireland. *Veterinary Record* **76**: 673-76.

Cox, J.E. 1975. Estrone and equilin in the plasma of the pregnant mare. *Journal of Reproduction and Fertility Suppl* **23**: 463-68.

Crickman, J.A. and Pugh, D.G. 1986. Equine endometrial cytology: A review of techniques and interpretations. *Veterinary Medicine* **7**: 650-56.

Dalin, A.M.O., Andresen, O. and Malmgren. 2002. Immunization against GnRH in mature mares: antibody titers, ovarian function, hormonal levels and estrous behavior. *Journal of Veterinary Medicine A physiology Pathology Clinical Medicine* **49**: 125-31.

Dascanio, J., Ley, W. and Schweizer, C. 2000. How to diagnose and treat fungal endometritis. Proceedings of annual convention of American Association of Equine Practitioners **46**: 316-18.

Dascanio, J., William, B.L. and Bowen, J.M. 1997. Hoe to perform and interpret uterine cytology. *Proceedings of annual convention of American Association of Equine Practitioners* **43**: 182-86.

Digby, N.J.W. 1978. The technique and clinical application of endometrial cytology in mares. *Equine Veterinary Journal* **10**: 167-70.

Douglas, R.H. 2004. Endocrine diagnostics in the broodmare: what you need to know about progestins and estrogens. Proceedings of society of Theriogenology, pp. 1-10.

Drillich, M., Kaufmann, T., Raab, D., Lenz, M. and Heuwieser, W. 2004. Comparison of new techniques for the diagnosis of chronic endometritis in dairy cattle. In: Proceedings of world buiatrics congress. 42.

Freeman, K., Rozel, J. and Slusher, S. 1986. Mycotic infections of the equine uterus. *Equine Practitioner* **8**: 34-42.

Gilbert, R.O., Shin, S.T., Guard, C.L., Erb, H.N. and Frajblat, N. 2005. Subclinical endometritis. Prevalence of endometritis and its effects on reproductive performance of dairy cows. *Theriogenology* **64**: 1879-88.

Gilson, G.R. and Zava, D.T. 2003. A perspective on HRT for women. *International Journal of Pharmacological Compounding* **7**: 330-38.

Ginther, O.J. and Pierson, R.J. 1984. Ultrasonic anatomy and pathology of equine uterus. *Theriogenology* **21**: 505-15.

Godoi, D.B., Gastal, E.L. and Gastal, M.O. 2002. A comparative study of follicular dynamics between lactating and non-lactating mares: effect of the body condition. *Theriogenology* **58**: 553-56.

Henry, M., Vandelplassche, M. and Bouters, R. 1982. A comparison of bacteriological, cytological and histological findings for evaluating of endometritis in mare. *Vlams Diergeneeskunding Tijdschrift* **51**: 498-512. *Veterinary Bulletin* (1983) **53**: 3488.

Hughes, J.P. and Trommershausen-Smith, A. 1977. Infertility in horse associated with chromosomal abnormalities. *Australian Veterinary Journal* **53**: 253-57.

Hurtgen, J.P. 2006. Pathogenesis and treatment of endometritis in mare: a review. *Theriogenology* **66**: 560-66.

Hussain, A.M. 1989. Bovine uterine defense mechanisms: a review. *Journal of veterinary Medicine* **36**: 641-51.

Inoue, Y., Ito, K., Terada, T., Nishimura, N., Hatazoe, T. and Sato, K. 2002. Degenerative changes in the endometrial vasculature of the mare detected by videoendoscope examination, in *Proceedings. American Association of Equine Practitioner* **46**: 325–29.

Kasimanickam, R., Duffield, T.F., Foster, R.A., Gratley, C.J., Leslie, J.S., Walton, J.S. and Jhonson, W.H. (2005). A comparison of cytobrush and uterine lavage techniques to evaluate endometrial cytology in clinically normal post-partum dairy cows. *Canadian Veterinary Journal*. **46** (3): 255-259.

Kasimanickam, R., Duffield, T.F., Foster, R.A., Gratley, C.J., Walton, J.S. and Jhonson, W.H. 2004. Endometrial cytology and ultrasonography for detection of subclinical endometritis in post-partum dairy cows. *Theriogenology* **62**: 9-23.

Kenny, R.M. 1978. Cyclic and pathological changes of the mare endometrium as detected by biopsy, with a note on early embryonic death. *Journal of American Veterinary Medical association* **172**: 241-62.

Knudsen, O. 1964. Endometrial cytology as diagnostic aid in mares. *Cornell Veterinarian* **54**: 415-22.

Knudson, O. 1982. A combined cytological and bacteriologic endometrial examination in the mare. In: Proceedings of 28th annual convention of American Association of Equine Practitioners. Pp: 51-54.

Langer, R.D., June, J., Pierce, B.A., Katherine, A., Hanlan, O. and Susan, R. 1997. Transvaginal ultrasonography compared with endometrial biopsy for the detection of endometrial diseases. *The new England Journal of Medicine* **377**: 1792-98.

LeBlanc, M.M., Magsig, J. and Stromberg, A.J. 2007. Use of low-volume uterine flush for diagnosing endometritis in chronically infertile mares. *Theriogenology* **68**: 403-12.

LeBlanc, M.M. 1999. The equine endometrium and the pathophysiology of endometritis. *Theriogenology* :78–81.

LeBlanc, M.M. 2008. When to refer an infertile mare to a theriogenologist. *Theriogenology* **70**: 421-29.

LeBlanc, S.J., Duffield, T.F, Leslie, K.E., Bateman, K.G., Keefe, G.P. and Walton, J.S. 2002. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. *Journal of dairy Science* **85**: 2223-2236.

Lewis, G.G. 1997. Uterine health and disorders. *Journal of dairy Sciences* **80**: 984-994.

Ley, W.B. 1986. Additional tips for endometrial cytology in mares. *Veterinary Medicine* **10**: 894.

Lu, K.G. and Morresey, P.R. 2006, Reproductive Tract Infections in Horses. *Veterinary Clinical Equine* **22**: 519-52.

Maloufi, F., Pierson, R. and Otto, S. 2002. Mares susceptible or resistant to endometritis have similar endometrial echographic and inflammatory cell reactions at 96 hours after infusion with frozen semen and extender. In: Proceedings of 48th conference of American Association of Equine Practitioners, Orlando, FL. Pp: 51-57.

McCue, P.M. 2008. The Problem Mare: Management Philosophy, Diagnostic Procedures, and Therapeutic Options. *Journal of equine veterinary science* **28** (11): 619-26.

McGinnis, M R. 1980. Yeast identification. In: Laboratory handbook of medical mycology. Academic press, Inc Ltd. Landon. GB. Pp: 337-11.

McKinnon, A.O., Squires, E.L., Harrison, L.A., Blach, E.L. and Schideler, R.K. 1988. Ultrasonographic studies on the reproductive tract of mares after parturition: effect of involution and uterine fluid on pregnancy rates in mares with normal and delayed first post-partum ovulatory cycle. *Journal of American Veterinary medical Association* **192**: 350-53.

McKinnon, A.O., Squires, E.L. and Voss, J.L. 1987. Ultrasonic evaluation of the mare's reproductive tract. Part II. *Compendium on continuing education for the practicing veterinarians* **7**: 472-82.

Miller, H.V., Kimsey, P.B., Kendrik, J.W., Derein, B., Doering, L. and Frenti, C. 1980. Endometritis of dairy cattle: Diagnosis, treatment and fertility. *Bovine Practitioner* **5**: 13-23.

Murray, R.D., Allison, J.D. and Gard, R.P. 1990. Bovine endometritis: comparative efficacy of alfaprotol and intrauterine therapies, and other factors influencing clinical success. *Veterinary record* **127**: 86-90.

Nafis, I.A. and Pandey, A.K. 2012a, Endometritis in mares due to coliforn and its successful treatment. *Indian Journal of Animal Reproduction.*, **33(1)**: 76-79.

Nafis, I.A. and Pandey, A.K. 2012b. Endometritis by *Corynebacterium* sp. in mares. *Indian Veterinary Journal.*, **89(10)**: 136-137.

Nafis, I.A. 2011. Microbiological, cytological and ultrasonographic studies in infertile mares and their therapeutic management. MVSc thesis. CCS Haryana Agricultural University-Hisar; India.125004.

Narwal, P.S. and Monaga, D.P. 1994. A comparison of bacteriological and cytological findings for evaluating endometritis in mare in mare. *Indian Journal of Animal Sciences* **64**: 1341-42.

Naz, R.K. 1999. Vaccine for contraception targeting sperm. *Immunological Review* **171**: 193-202.

Neilson, J.M., Troedsson, M.H., Pedersen, M.R. and Lehn-Jensen, H. 2010, Diagnosis of endometritis in mare based on bacteriological and cytological examinations of the endometrium: Comparison of results obtained by swabs and Biopsies. *Journal of Equine Veterinary Science* **30**:.27-30.

Neilson, J.M. 2005. Endometritis in mares: A diagnostic study comparing cultures from swab and biopsy. *Theriogenology* **64**: 510-18.

Oral, H., Sozmen, M., Serin, G. and Kaya, M. 2009. Comparison of cytobrush technique, vaginoscopy and trans-rectal ultrasonography methods for the diagnosis of post-partum endometritis in cows. *Journal of Animal and Veterinary Advances* **8**(7): 1252-55.

Petrites-Murphy, M.B., Robbins, L.A. and Donahue, J.M. 1996. Equine cryptococcal endometritis and placentitis with neonatal cryptococcal pneumonia. *Journal of Veterinary Diagnosis and Investigation* **8**: 383-86.

Pierson, R.A., Kastelic, J.P. and Ginther, O.J. 1988. Basic principles and techniques for transrectal ultrasonography in cattle and horses. *Theriogenology* **29**(1): 3-18.

Pugh, D.G., Bowen, J.M., Kloppe, L.H. and Simpson, R.B. (1986). Fungal endometritis in mares. *Compendium on continuing education for the practicing veterinarians* **8**: 173-77 and 180-81.

Purswell, B.J., Ley, W.B., Sriranganatahn, N. and Bowen, J.M. 1989. Aerobic and anaerobic bacterial flora in post-partum mare. *Equine Veterinary sciences* **9**: 141-44.

Pycock, J.F. 2000. Breeding management of problem mare (the old median mare syndrome). In Sampler J C. editor, Equine breeding management and artificial insemination. Philadelphia; WB Saunders. Pp: 222.

Rayn, P., Bennet-Wimbush, K., Vaala, W.E. and Bagnell, C.A. 1999. Relaxin as a biochemical marker of placental insufficiency in the horse: a review. *Pferdeheilkunde* **15**: 622-26.

Reiswig, J.D., Threlfall, W.R. and Rosol, T.J. 1993. A comparison of endometrial biopsy, culture and cytology during estrus and dioestrus in the horse. *Equine Veterinary Journal*. **23**(3): 240.

Rickets, S.W. and Mackintosh, M.E. 1987. Role of anaerobic bacteria in equine endometritis. *Journal of Reproduction and Fertility* (Suppl.) **35**: 343-51.

Ricketts, S.W. and Alonso, S. 1991. The effect of age and parity on the development of equine chronic endometrial disease. *Equine Veterinary Journal* **23**: 189-92.

Ricketts, S.W., Young, A. and Medici, E.B. 1993. Uterine and clitoral cultures. In McKinnon AO, Voss JL. (Eds.), Equine reproduction. Lea and Febiger, Malvern, PA, USA. P. 234-45.

Riddle, W.T, LeBlanc, M.M., Pierce, S.W. and Stormberg, A.J. 2005. Relationship between pregnancy rates, uterine cytology, and culture results in thorough bred practice in central Kentucky. In: Proceedings of 50th annual convention of American Association of Equine Practitioners. Pp: 51-54.

Riddle, W.T., LeBlanc, M.M. and Stormberg, A.J. 2007. Relationship between uterine culture, cytology and pregnancy rates in a thoroughbred practice. *Theriogenology* **68**: 395-402.

Risvanli, A. 2011. Reproductive Immunology in Mares. *Asian Journal of Animal and Veterinary Advances* **6**: 547-54.

Rossdale, P.D., Ousey, J.C., Cottril, C.M., Chavatte, P., Allen, W.R. and McGladdery, A.J. 1991. Effects of placental pathology on maternal plasma progestagens and mammary secretion calcium concentrations and neonatal adrenocortical function in the horse. *Journal of Reproduction and Fertility Suppl* **44**: 570-90.

Santos, N.R., Lamb, G.C., Brown, D.R. and Gilbert, R.O. 2009. Post-partum endometrial cytology in beef cows. *Theriogenology* **71**:739-45.

Santschi, E.M. 2005. Case presentation: uterine mass in a mare. *Compend Contin Educ Pract Vet* **27**: 229-35.

Scot, P., Daley, P., Baird, G.G., Sturgess, S. and Frost, A.J. 1971. The aerobic bacterial flora of reproductive tract of the mare. *Veterinary Record* **88**: 58-61.

Sheerin, P.C, Morris, S., Kelleman, A.A., Stawicki, R., Sheerin, B.R. and LeBlanc, M.M. 2003. Diagnostic efficiency of transrectal ultrasonography and plasma progestin profiles in

identifying mares t risk for premature delivery. American Association of Equine Practitioner, Focus in Equine Reproduction **1**: 22-23.

Sheldon, I.M., Lewis, G.S., LeBlanc, S. and Gilbert, R.O. 2006. Defining post partum uterine disease in cattle. *Theriogenology* **65**: 1516-1530.

Sheldon, I.M. and Noakes, D.E. 1998. Comparison of three treatments for bovine endometritis. *Veterinary Record* **142**: 575-579.

Shin, S.J., Lein, D.H., Aronson, A.L. and Nusbaum, S.R. 1979. The bacteriological culture of equine uterine contents, in vitro sensitivity of organisms isolated and interpretation. *Journal of reproduction and fertility* **27**(supplement): 307-15.

Squires, E.L., McKinnon, A.O. and Shideler, R.K. 1988. Use of ultrasound in reproductive management of mares. *Theriogenology* **29**: 55-70.

Stabenfeldt, G.R., Daels, P.F., Munro, C.J., Hughes, J.P. and Lasley, B.L. 1991. An estrogen conjugate enzyme immunoassay for monitoring pregnancy in the mare: limitation of the assay between days 40 and 70 of gestation. *Journal of Reproduction and Fertility Suppl* **44**: 37-43.

Stawicki, R.J., Ruebel, H., Hansen, P.J., Sheerin, B.R, O'Donnell, L.J. and Lester, G.D. 2002. Endocrinological findings in an experimental model of ascending placentitis in the mare. *Theriogenology* **58**: 849-52.

Stewart, D.R., Addiego, L.A., Pascoe, D.R., Haluska, G.J. and Pashen, R. 1992. Breed differences in circulating equine relaxin. *Biology of Reproduction* **46**: 648-652.

Stewart, D.R., Stabenfeldt, G.H., Hughes, J.P. and Meagher, D.M. 1982. Determination of the source of equine relaxin. *Biology of Reproduction* **27**: 17-24.

Traub-Dargatz, J.L., Salman, M.D. and Voss, J.L. 1991. Medical problems of adult horses, as ranked by equine practitioners. *Journal of American Veterinary Medical association* **198**: 1745-1747.

Troedsson, M.H., deMoraes, M.J. and Liu, I.K. 1993. Correlations between histologic endometrial lesions in mares and clinical response to intrauterine exposure with Streptococcus zooepidimicus. *American Journal of Veterinary Research* **54**: 570-72.

Troedsson, M.H. 2007. High risk pregnant mare. *Acta Veterinaria Scandinavica* **49**(Suppl 1): 1136-51.

Watson, E.D., Thomassen, R. and Nikolakopoulos, E. 2003. Association of uterine oedema with follicle wave around the onset of breeding season in pony mares. *Theriogenology* **59**: 1181-87.

Watson, E.D. 1988. Effect of ovarian steroids on migration of uterine luminal neutrophils and on chemokinetic factors in uterine secretions from mares. *Equine Veterinary journal* **20**: 368-70.

Williams, E.J., Fischer, D.P., England, G.C.W, Dobson, H., Pfeiffer, D.U. and Sheldon, I.M. 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the inflammatory response to endometritis in cattle. *Theriogenology* **63**: 102-117.

Wilson, G.L. 1984. Equine hysteroscopy: normal intrauterine anatomy. *Veterinary Medicine* **11**: 1388.

Wingfield-Digby, N.J. and Ricketts, S.W. 1982. Results of concurrent bacteriological and cytological examinations of the endometrium of mares in routine stud farm practice. *Journal of Reproduction and Fertility* **32**(supplement): 181-85.

Zent, W.W., Troedsson, M.T.H and Xue, J.L. 1998. Post-breeding uterine fluid accumulation in normal population of thoroughbred mares: A field study. *Proceedings of American Association of Equine Practitioners* **44**: 64-65.

Zerbe, H., Schuberth, H.J., Engel, K.E. and Leibold, W. 2003. Development and comparison of *in vitro* and in vivo models for endometritis in cows and mares. *Theriogenology* **60**(2): 209-23.