Infectious endometritis in Arabian mares: an updated clinical investigation of uterine microbial isolates, antimicrobial sensitivities and fertility in Egypt

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Abstract

The present study aimed to survey the prevalence of microbial isolates in the uterus of sub-fertile Arabian mares and to identify their existing antimicrobial sensitivities. In addition, to evaluate the efficacy of antimicrobial therapy based on the fertility outcomes determined by sonographic pregnancy diagnosis. Double guarded uterine swabs were collected from (barren) Arabian mares during mid-estrus. Based on the laboratory findings of microbial culture and antimicrobial sensitivity, mares were treated, inseminated and tested for pregnancy two weeks after ovulation. The predominant microbial isolates were Beta-hemolytic streptococci (BHS) (38.9%), Escherichia coli (E. coli) (33.3%), Staphylococcus aureus (S. aureus) (20.4%) and Candida albicans (C. albicans) (7.4%). Moreover, the sensitivity of BHS and E. coli isolates to cefepime was significantly higher (P< 0.05) than the rest of the antimicrobials (57.14% and 55.56%, respectively). However, S. aureus isolates did not display a significant difference regarding their antimicrobial sensitivities, its sensitivity to amoxicillin-clavulanic acid was the highest (36.37%). On the other hand, fluconazole was the favored antifungal in this study (100 % sensitivity). The pregnancy outcomes were variable depending on the type of microbial isolate and antimicrobial treatment; for instance, cefepime resulted in the highest pregnancy rates in BHS and S. aureus mare groups (66.67% and 100%, respectively). Interestingly, amikacin achieved the highest pregnancy rate in the E. coli group (66.67%). In conclusion, BHS is the dominant uterine isolate in Arabian sub-fertile mares. For most cases of bacterial endometritis, cefepime may be the antibiotic of choice and fluconazole may be the effective therapy in the case of C. albicans infection. However, standard diagnostic and treatment procedures should be used to minimize the emergence of antimicrobial resistance.

Keywords: Arabian mares, cefepime, endometritis, uterine swabs

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Introduction

With over one million Arabian horses registered in 62 countries around the world, this breed of horses is considered to be a great animal resource requiring advanced breeding management to obtain newborn foals especially from the sub-fertile mares of unique genetic potential (WAHO, 2014). Endometritis is the most significant cause of subfertility in mares and the third most prevalent disease of adult horses -as ranked by equine practitioners (Traub-Dargatz et al., 1991, Gutjahr et al., 2000). The risk of endometritis is often associated with pregnancy failure due to premature luteolysis and/or early embryonic mortality (LeBlanc and Causey, 2009), leading to substantial economic and genetic loss in the equine breeding sector, estimated by 25-60% of barren mares requiring intensive clinical care and additional cycles to achieve pregnancy, which eventually means additional costs incurred by the equine owners.

Simply, endometritis is classified into acute infectious, chronic infectious, persistent breeding-induced and chronic degenerative endometritis (LeBlanc, 2010). Beta-hemolytic streptococci (BHS), Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Pseudomonas aeruginosa (P. aeruginosa) and Klebsiella pneumoniae (k. pneumoniae) are the most frequent uterine microbial isolates reported in equine endometritis studies. These potentially pathogenic organisms are generally acquired by the equine uterus during unhygienic gynecological procedures during vaginal examination, natural or artificial breeding processes, during and after parturition and as a result of the failure of physical barriers against infection i.e. pneumovagina (Tibary et al., 2014; Albihn et al., 2003; Szeredi et al., 2003; Frontoso et al., 2008).

Most equine practitioners want a quick microbiological diagnosis that will enable them to treat the mares while they are still in estrus. Since accurate antimicrobial therapy based on microbial culture and subsequent testing of antimicrobial sensitivity is considered a time-consuming strategy, even when performed in professional microbiological laboratories, practitioners often prefer a simple but long-term harmful approach involving the use of broad-spectrum antimicrobial agents, depending on the results of relevant studies or depending on their own experience (Maddox et al., 2015; Benko et al., 2015). These irresponsible antimicrobial administration policies between the 1970s and 1990s contributed to the emergence of microbial resistance to a broad spectrum of antimicrobial agents, such as penicillin and tetracycline. In addition to the continuous changes of antimicrobial sensitivities, the prevalence of uterine microbial isolates can differ over time as well as from one horse population to another (Mateu et al., 2008).

In general, endometritis may be diagnosed with transrectal ultrasound, transvaginal or uterine sampling using (swab, cytobrush, low-volume lavage, biopsy) for microbiological, cytological and histological evaluations (Katila, 2016). In 1924 Dimock and Snyder were the first to publish microbiological results after swabbing the mare uterus, this uterine sampling technique was established over time until it became a common practice in equine reproductive management (Dimock and Snyder 1924; Spilker et al., 2017; Katila, 2016).

While most of the previous equine endometritis studies have been conducted in thoroughbred mares, limited studies have been reported in the Arabian breed. To the best of our knowledge, most of the available data regarding the efficacy of endometritis treatment depends on the negative microbial culture post-treatment irrespective of the fertility results. We hypothesize that a comprehensive clinical study of equine endometritis from diagnosis to the post-treatment pregnancy assessment is more reliable. Therefore, the present study aimed to survey the prevalence of microbial isolates in the uterus of subfertile Arabian mares and to identify their existing antimicrobial sensitivities. In addition, to evaluate the efficacy of antimicrobial therapy based on fertility outcomes sonographic pregnancy diagnosis. Such data may serve as updated guidelines for the management of infectious endometritis in Arabian mares.

Materials and Methods

Mares selection: Sixty-seven Arabian broodmares (mean age 8.1 years ± 3.7) were admitted to a private equine reproduction clinic in the Giza governorate in the northern part of Egypt for breeding purposes from June to July during the 2017-2019 breeding seasons. They had a history of subfertility (barren mares, inseminated by fertile stallions for 2-3 estrous but failed to conceive). All mares were kept in straw-bedded boxes with permanent access to hay, grain and water, and were turned out daily to pasture. All mares were subjected to the standard transrectal ultrasound scanning using real-time B-mode scanners (Esaote Mylab30-Netherlands) equipped with 5-7.5 MHz frequency linear-array rectal transducer) to check the mares’ reproductive tracts, ovarian dynamics as well as uterine status to determine the optimal time for sample collection, induction of ovulation and fresh semen artificial insemination (AI). Mares displayed abnormal intrauterine fluids (echogenic and or ≥ 2 cm in diameter) or abnormal patterns of uterine edema to follicular development.

Study design: The schedule for reproductive management and sampling of the mares is shown in Fig. 1. After a routinely performed transrectal palpation and ultrasonography -if abnormal intrauterine fluids or edema were detected- uterine swabs were collected in mid-estrus for microbial examination. Mares were rested to the next estrus where they were treated according to the sensitivity results 1-3 days before AI. Mares were induced for ovulation then inseminated artificially by cooled semen from a fertile stallion 24h later. Ultrason sound check for ovulation confirmation was performed 48h from induction (un-ovulated mares were excluded from the study n=13). In total fifty-four (54) mares were included in the present study and they were checked for pregnancy.

Collection of the uterine samples: Double guarded uterine swabs (Minitüb GmbH, Germany) were
collected during the mid-estrus (moderate uterine edema with 36.8 ±2.8 mm large follicle diameters), according to Dascanio (2014). Briefly, after removing the feces from the rectum, the tail was wrapped and tied out, the perineal region washed with a non-residual soap and rinsed with fresh water at least 3 times to remove all visible debris and the region was dried with disposable paper towels, afterwards, wearing a clean, inverted rectal sleeve with a sterile glove over the top was used and a sterile lubricant was applied to the knuckles, thumb, and dorsal wrist area, the tip of the swab was held and covered in the palm, and using a slight rotating motion, the hand passed into the vagina till enabling palpation of the external cervical os, the index finger passed gently into the external cervical os followed by the uterine swab. After passing the cervical canal the cotton swab itself pushed forward through the outer then the inner guards to be contacted with the endometrium then gently rotated for 10–15 seconds. Finally, the swab was retracted back inside the inner guard then into the outer guard and the device was removed from the mare and the swab directly immersed in commercial transportation media.

Figure 1 Schedule for the reproductive management and sampling of the mares

**Microbial culture and antimicrobial sensitivity testing:** In the laboratory, all samples were incubated at 37 °C for 18-24 h, then a loopful of each sample was cultivated on blood agar (with 5 % defibrinated sheep blood), MacConkey agar and Sabouraud dextrose agar. Bacterial growth on blood and MacConkey agar plates were assessed and identified over 24-48 h of incubation at 37 °C. No visible growth plates were incubated at the same conditions and re-examined for another 24 h (Nielsen, 2005). Colonies were identified for their hemolytic behavior and morphological appearance, suspicious colonies were collected and studied microscopically in a Gram-stained film before being moved to semisolid agar to be subjected to further identification according to Quinn et al., (2002).

The emergence of two or more colonies of any species revealed a uterine infection existing. Sabouraud dextrose agar plates were incubated at 27°C and fungal growth was assessed and identified after 24 h of incubation. Plates that had no growth were incubated at 27°C and re-examined for another four days (Quinn et al., 2002). Antimicrobial sensitivities were determined for all isolates by the standard disk diffusion test on Müller Hinton agar (with 5 % defibrinated sheep blood).

**Intra-uterine treatment protocols:** All mares were sexually rested to the next estrus cycle until obtaining the laboratory results and for treatment, all mares were exposed to uterine lavage (1-2 liters of warm sterile saline solution (NaCl, 0.09%) before intrauterine infusion of the antimicrobial of choice according to the sensitivity outcomes. All treatments were infused into the uterus 1-3 times during estrus. The following antimicrobials were applied during estrus after reconstitution to (20-50 ml sterile saline): Cefepime (1gm), amoxicillin clavulanic acid (3.1 gm), ampicillin (1gm) and fluconazole (250 mg). Regarding, gentamicin (2 gm), levofloxacin (30 ml) and amikacin sulfate (2 gm) they were buffered with 10 ml of 8.4 % sodium bicarbonate before being used (Ferris, 2017; Derbala 2013).
**Ovulation induction and artificial insemination:** Intramuscular injection of human chorionic gonadotropin (2500 IU Epifasi ® EIPICO, Egypt) induced mares with ≥35 mm follicles, normal endometrial edema / fluids and accessible cervix. After 24 h, these mares were inseminated using approximately 10-20 ml of fresh semen with > 50% progressively motile sperm and > 300 million sperm/dose of insemination. 24 h after insemination, the mares were checked for confirmation of ovulation and uterine fluid accumulation by ultrasonography; the First pregnancy check was performed on the day 15th of ovulation, while the second pregnancy check was performed on day 35 (± 2 days) after ovulation.

**Statistical analysis:** Data variables including microbial isolates, antimicrobial sensitivities and pregnancy rates were analyzed by the Statistical Package for Social Sciences (IBM® SPSS) version 26.0 using Chi-squared (χ²) test and (P < 0.05) was considered statistically significant.

**Ethics approval:** All experimental protocols were approved by the Animal Care and Ethical Use Committee of Faculty of Veterinary Medicine, Cairo University (VET. CU 16072020166).

**Results**

The prevalence of uterine microbial isolates: All 54 samples yielded positive pure culture growth, which was subsequently isolated and identified. A total of (92.6%) of isolates were bacterial while the rest (7.4%) were fungal isolates (C. albicans). In twenty-one mares (38.9%), the BHS was isolated. Furthermore, E. coli was isolated in eighteen mares (33.3%), while eleven mares (20.4%) had S. aureus in their uterine sample (Fig. 2). All treated mares displayed a normal intrauterine environment (edema and or fluids) during heat and post insemination.

**Antimicrobial sensitivity of microbial isolates and the post-treatment pregnancy rates:** The sensitivity of BHS isolates toward cefepime was significantly higher (P< 0.05) than toward gentamicin, levofloxacin and ampicillin-sulbactam (57.1%, 19.1%, 14.3% and 9.5%, respectively). The pregnancy rate was higher in cefepime treatment (66.7%) than gentamicin, levofloxacin (50%, 33.3%, respectively), while there were no pregnancies after ampicillin- sulbactam treatment (Fig. 3).

Similarly, the sensitivity of E. coli isolates towards cefepime was significantly higher (P< 0.05) than towards amoxicillin-clavulanic acid, amikacin, and gentamicin (55.6% vs. 16.7%, 16.7% and 11.1%, respectively). Interestingly, amikacin treated mares revealed the highest pregnancy rate in comparison with gentamicin and cefepime (66.7% vs. 50% and 40%, respectively), while no pregnancy was recorded after using amoxicillin-clavulanic acid (Fig. 4).

In addition, S. aureus isolates did not display a significant difference in their antimicrobial sensitivity, although sensitivity to amoxicillin-clavulanic acid was the highest (36.4%) compared to levofloxacin, cefepime and ampicillin-sulbactam (27.3%, 18.2% and 18.2%, respectively). Surprisingly, cefepime treatment achieved a full pregnancy rate (100%) compared to (66.7%, 50% and 50%) upon using levofloxacin, ampicillin-sulbactam and amoxicillin-clavulanic acid, respectively (Fig. 5). Finally, however, 100% of Candida albicans isolates were sensitive to fluconazole, only 50% of the mares became pregnant after treatment.

**Figure 2** The prevalence of uterine microbial isolates in the sub-fertile Arabian mares.
Figure 3  β-hemolytic streptococci sensitivity and pregnancy rates after treatment.

Figure 4  Escherichia coli sensitivity and post-therapy pregnancy rates.

Figure 5  Staphylococcus aureus sensitivity and pregnancy rates post-treatment.
Discussion

Uterine microbial isolates and the trend of their antimicrobial sensitivity increases the need for continuous microbiological surveillance and antimicrobial testing in both human and animal reproductive medicine (Pisello et al., 2019). As such, this study was conducted to identify the most prevalent microbial isolates and their sensitivity to antimicrobial in the uterus of sub-fertile Arabian mares, in addition, to track the outcomes of pregnancy after treatment of these mares using the recommended antimicrobial for each case.

Microbial culture is necessary to provide an etiological diagnosis of infectious endometritis. Since the beginning of the last century, swabs from the endometrial surface have been used in the diagnosis of uterine infection (Dimock and Snyder, 1923). The sampling techniques have improved over time and the use of a guarded swab to collect uterine samples for cultivation has become standard practice (Spieler et al., 2017). Bacterial uterine infection was considered the major etiology for reproductive loss in mares (Buczkowski et al., 2014). Comparable with Baranski et al., (2003) and Frontoso et al., (2008) findings for positive bacterial cultures (66.2 % and 49%, respectively), our results showed that the majority of the animals sampled (92.6%) were positive for bacteriological growth, while mycological cultures found that (7.4 %) of the mares were positive for fungal growth. This percentage was close to reported by Dascanio et al., (2001). It is suggested that the spread and overuse of antibiotics may cause this variance (De Graef et al., 2004).

BHS and E. coli have been identified as the most isolated microorganisms of mares’ uterine swabs and it is quite clear that both microbes are widely involved in female reproductive diseases. In addition, it has been reported that the predominance of BHS or E. coli over the other microbial isolates has usually varied in previous studies which may be due to the variance of the mares breed (thoroughbred or Arabian) as well as the mares’ reproductive status (fertile or sub-fertile). In the current study, we found that BHS was the most frequently isolated microorganism from the uterus of sub-fertile Arabian mares while E. coli was the second most frequent isolate. Likewise, the findings of related studies in Arabian mares (Hamouda et al., 2012) and thoroughbred mares (Frontoso et al., 2008) reported BHS followed by E. coli were the dominant and most often isolated microorganisms in uterine swabs of sub-fertile mares.

Ibrahim et al., (2015) reported the dominance of E. coli over BHS isolates (33.3% vs. 21.4%, respectively) in uterine samples of sub-fertile Arabian mares. In addition, Albihn et al., (2003) reported that E. coli was isolated from 67% of swabs while BHS was only isolated in 20 % of sub-fertile thoroughbred mares’ uterine swabs.

Another retrospective study on 8,296 cases of bacterial endometritis mainly in thoroughbred mares revealed the presence of E. coli followed by BHS in 29% and 28% of uterine swabs respectively (Davis et al., 2013).

Christoffersen et al., (2015) reported that BHS is responsible for deeper uterine infections, while E.coli is mainly found in the superficial layers of the endometrium, so the discrepancy of findings among the studies might be due to the variance in sampling techniques, laboratory procedures and study management.

This study showed that cefepime (fourth-generation cephalosporin) was significantly effective against BHS and E. coli, whereas S. aureus isolates exhibited moderate cefepime sensitivity. Derbala (2013) and Barbary et al., (2016) reported that BHS, E. coli and S. aureus isolates from the uterus of sub-fertile Arabian mares were mainly sensitive to ciprofloxacin, levofloxacin and enrofloxacin.

On the other hand, Frontoso et al., (2008) reported that the most effective antimicrobials for BHS, E. coli and S. aureus were amoxicillin-clavulanic acid and enrofloxacin. In addition, the highest sensitivity recorded by Benko et al., (2015) was towards marbofloxacin and cefotaxime for isolates from the uterus of sub-fertile thoroughbred mares.

Indeed, there is a clear emergence of microbial resistance to traditional antimicrobials, so most uterine microbial isolates are more susceptible to the newest antibiotic generations. This may explain the variance in antimicrobial sensitivity over time between the different studies.

In the study, following treatment using the recommended antimicrobial, the highest pregnancy rate of BHS and S. aureus groups was in that treated by cefepime. Interestingly, amikacin achieved the highest pregnancy rate in the E. coli group. However, BHS and E. coli isolates showed a bit of sensitivity toward ampicillin-sulbactam and amoxicillin-clavulanic respectively, none of the treated mares became pregnant after using either antimicrobial. These findings are in complete agreement with Abou El-Amaiem et al., (2016) who reported that cefepime treatment for equine endometritis achieved the best conception rate (87.5%).

Flucanazole can be administered intrauterine, orally or intravenously. It is favored as it exhibits high water solubility, limited protein binding, superior tissue distribution, has a long half-life (40 h) and few side effects (Giguère, 2006). Furthermore, another study showed a successful endometrial fluconazole concentration after daily oral administration (Scofield et al., 2011).

All the yeast (C. albicans) isolates showed complete (100%) sensitivity toward fluconazole while (50 %) of them got pregnant. On the other hand, Beltaire et al., (2012) recorded a (75%) sensitivity of yeasts isolates against fluconazole, while no reports regarding the pregnancy rate after fluconazole treatment in mares were found.

In conclusion, BHS and E. coli are the main uterine pathogens reported in many studies but the predominance of one of them over the other in addition to their antimicrobial sensitivities differs over time, horse breed and population. In the current study, BHS was the dominant uterine isolate in Arabian sub-fertile mares. For most cases of bacterial endometritis, cefepime may be the antibiotic of choice, and fluconazole may be an effective therapy in the case of...
C. albicans infection. However, standard diagnostic and treatment procedures should be used to minimize the emergence of antimicrobial resistance. Studies are required to closely monitor the prevalence of uterine microbial isolates and their antimicrobial sensitivity in sub-fertile mares.

Conflict of interest: The authors declare that they have no conflict of interest.

References


of fluconazole following oral administration. Clin Theriogenology. 3:356.