

Managing Aged Arabian Mares with Some Uterine Affections

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Abstract

A Subfertile mare cannot conceive when mated several times by a fertile stallion and thus is not pregnant at the end of the breeding season. Age of mare and her reproductive status are strongly associated with reproductive efficiency. Ultrasonography plays an important diagnostic role in evaluating the reproductive status of mares. Endometritis and endometrial cysts are the most common causes of subfertility that occurred in aged Arabian mares in this study. Twenty-seven (> 15 years old) Arabian subfertile mares had two forms of infertility, a non-infectious form (uterine luminal cysts) and an infectious form (endometritis) diagnosed through their examination by ultrasonography and uterine swabs. In the present work, the uterine bacterial pathogens isolated were *Escherichia (E.) coli* 4 isolates, comprising 41.1% followed by *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Proteus mirabilis*, 3 isolates, comprising 17.6% each isolated. Other *streptococci* were found at a lower frequency of 1 isolate, 5.8%. The study also revealed that Sulphadiazine/trimethoprim, Ciprofloxacin, Enrofloxacin, and Gentamicin were the most effective antibiotics inhibiting most of the uterine bacterial isolates in our study.

Keywords: Subfertile; Mare; Aged; Endometritis; Ultrasonography; Bacteria Antimicrobial agents.

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1. Introduction

Most losses due to uterine affections in mares are related to age and parity. All studies on the reproductive efficacy of mares concluded that the single most important factor contributing to subfertility in well-managed mares is increased mare age (>13 years) [1,2]. Also, in embryo transfer technology a lower embryo recovery rate and post-transfer pregnancy rate following the transfer of embryos were observed from aged Arabian mares than from younger mares [3]. for aged mares. Endometrial cysts are the most common form of non-infectious uterine disorders [4]. most affected mares are older than 10 years old and thus the incidence of cysts increases with age and parity, with 13% to 22% expected occurrence [5,6]. Bacterial endometritis is the main cause of subfertility in repeat-breeding Arabian mares [7]. with an expected occurrence of 25–60% of all breeds barren mares [8].

Reproductive ultrasonography is probably the most common and valuable diagnostic tool by which different uterine disorders are detected. It has been stated that intraluminal uterine fluid accumulation during diestrus is related to endometritis [9] and that ultrasonography can detect glandular and lymphatic cysts [10]. The treatment of bacterial endometritis has two modes of action uterine lavage followed by ecboic use and antibacterial agents [11,12].

The purpose of this review was to state the main causes and methods of diagnosis of mare uterine affections, the relation of age and kind of bacterial isolates to mare endometritis, and outline the effective treatment protocols in aged subfertile mares.

2. Material and Methods

2.1. Animals

This study examined data obtained during three breeding seasons (March to June), from 2020 to 2023, for 30 (n=30) aged (>15 years old) and subfertile pure Arabian mares selected and treated in different governmental and private equine farms in Egypt. The selected mares were considered subfertile based on previous breeding history (history of endometritis and or endometrial cysts); mares had been bred with fresh semen from a proven fertile stallion more than a year ago but failed to conceive, or due to advanced age (>15 years).

Mares were grouped according to their age into mares aged from 15 to 20 years (n=16) old and mares aged more than 20 years old (n=14).

2.2. Reproduction Examination and Sample Collection

2.2.1. Ultrasound

The ultrasonographic examinations were performed using a multi-frequency linear array transducer with 7.5 MHz central frequency with black and white 2D imaging using a Sonoscape digital ultrasound machine (Model A5 VET, China). Mares were examined by ultrasound to evaluate the reproductive organs and stage of the estrous cycle to determine uterine edema and intrauterine fluid grades. Also,

the echotexture, nature, and amount of uterine content were monitored.

Thirty aged infertile Arabian mares had two forms of infertility; non-infectious form (uterine luminal cysts) and infectious form (endometritis) total of twenty-seven, and three fertile old aged mares. Some cases suffer from two forms of infertility (Table 1).

Table 1: Total Number of examined mares according to age

| Age of mares | Total no. of examined mares | Total no. of normally reproductive mares | Total no. of reproductive disordered mares |
|------------------------|-----------------------------|--|--|
| 15 to 20 years old | 16 | 3 | 13 |
| More than 20 years old | 14 | 0 | 14 |

All examined mares suffered from endometritis and or endometrial cysts and had intraluminal uterine accumulated fluids with different grades. Intraluminal uterine fluid was graded by ultrasound scanning from I to III according to the degree of echogenicity as I (anechoic), II (hypoechoic with hyperechoic particles), or III (moderately echogenic).

2.2.2. Endometrial Swab

Mares were restrained in an examination stock, the tail was raped and the vulva and perineal region were washed with Betadine Shampoo and then dried with cotton. Using a long sterile disposable glove and sterile gel (mini tube), a double-guarded long uterine swab (Equine Culture Swab, mini tube, Germany) guided by hand was passed through the vagina into the cervix, then entered into the uterine body and kept in contact with the endometrium for a minimum of 20 seconds before being removed. The cotton swab was placed in transport media for bacteriological examination.

2.2.3. Uterine culture and Antibiotic sensitivity test

Uterine swabs were examined and cultured for detection of the presence of bacterial pathogens using standard culture methods of [13,14,15]. The isolated bacterial pathogens were subjected to the antibiotic sensitivity test using a disc diffusion test recommended by the National Committee for Clinical Laboratory Standards [16]. We used different antibiotic discs such as Cefipime, Sulphadiazine/trimethoprim, Ciprofloxacin, Enrofloxacin ceftifur, Amikacin, Gentamicin, Penicillin G, Streptomycin and Cefotaxim.

2.2.4. Bacteriological Examination

Sample collection: sterile cotton swabs were used in the collections of uterine samples by double-guarded swabs from 30 subfertile mares. The uterine samples were taken by double guarded swabs.

Enrichment of samples: [17] samples were inoculated into nutrient broth and then incubated at 37°C for 24 hrs. For isolation of *Salmonella*, the samples were inoculated into Selenite- F- broth and incubated at 37°C for 14-18 hrs.

Plating on solid media: [17] an loopful from the incubated nutrient broth tubes were streaked onto plates of nutrient agar, McConkey agar, Mannitol salt agar, and sheep blood agar media [18]. but for *Salmonella*, the incubated Selenite- F- broth was streaked onto Brilliant green agar media and S.S. agar media. Then all inoculated plates were incubated at 37°C for 24 – 48 hrs. The incubated plates were examined for morphology, hemolysis on blood agar and pigment production on nutrient agar, and also for characteristics on Mannitol salt agar, S.S. agar and Brilliant green agar media.

The selected colonies were picked and transferred into nutrient agar and incubated at 37°C for 24 hrs for further examination.

2.2.5. Bacteriological Identification

Smears obtained from isolated bacteria were prepared and stained with Gram's stain for microscopical examination.

Identification of the Gram-negative bacteria: the isolated Gram-negative organisms were subjected to further biochemical tests as follows; Catalase test, Indol test, Methyl red test, Voges- Proskauer test, Cytochrome oxidase, Citrate utilization test, H₂S production detection, Sugar fermentation test for (glucose, lactose, sucrose, dulcitol, sorbitol, arabinose, rhamnase and xylose), Motility test, Lysine and ornithine decarboxylation and Arginine dihydrolase test, Gelatin liquefaction test, and Nitrate reduction Urease test according to [17,19,20].

Identification of the Gram-positive bacteria (cocci): under a microscope, Gram-positive cocci were examined for cell arrangement. Some cells tend to form grape-like clusters or chains. Catalase test, Coagulase test, and hemolysis on blood agar were used to differentiate between staphylococci and certain streptococci.

2.2.6. Antimicrobial sensitivity test

[21], The organismal colony was transferred into sterile Muller Hinton broth mixed well and incubated at 37°C for 24 hrs. The turbidity of the incubated broth was matched to McFarland tube number 0.5. A sterile cotton swab was dipped into the broth, then streaked on Muller Hinton agar plate and left for 5-15 minutes to dry. The selected antibiotic

discs were placed onto the surface of the medium using sterile forceps, then incubated for 18-24 hrs at 37°C, and the inhibition zone diameter was measured. Results were categorized by using the breakpoints for sensitive (S), intermediate (NT), or resistant (R) recommended by the NCCLS for bacteria [22,23].

2.2.7. Treatment strategies for endometritis

During estrus, subfertile mares (with accumulated intrauterine fluid) were lavaged with 2 to 3 liters of physiological saline or Ringer's solution through a sterile large-bore catheter inserted only after thorough cleansing of the perineum. The lavage volumes are massaged throughout the uterus and are retrieved from the uterus by gravity flow and followed by 25 i.u. oxytocin injection for 2 to 3 days [24,25,26,27]. The antibiotic therapies used for the treatment of bacterial uterine infection were:

- Sulphadiazine- trimethoprim administrated systemically for 10 days during estrus [28].

- Enrofloxacin (Enroflx 5%) was used for systemic treatment at a dose 5.5-7.5mg/kg in 500 ml 0.9 normal saline slow intravenous infusion with 24hr intervals for 5 days during estrus [24,29].

- Gentamicin, 1000 mg to 2000 mg diluted with an equal volume of sodium bicarbonate in Ringer lactate solution to a volume of 60 ml used in other 3 infertile mares for intrauterine infusion after uterine lavage for 3 days during estrus [12,30] by uterine plastic catheter.

All treated mares were conducted for reproductive ultrasound examination after treatment to detect the efficacy of treatment. The absence of intrauterine fluid accumulation is a useful indication of treatment success and mares may be bred as soon as signs of inflammation have resolved [30].

3. Results

3.1. Incidence

3.1.1. Incidence of the different types of reproductively disordered mares

Among the total number of examined mares (n=30), there were 16 mares aged 15 to 20 years old and 14 mares more than 20 years old. Among the total number of examined mares (n=30), there were 3 (10%) normal reproductive mares and 27 (90%) mares found to have different types of reproductive disorders. This high percentage of abnormal reproductive mares was because all examined mares were aged more than 15 years old, thus age was the most important contributing factor to the etiology of **subfertility** in the examined mares (Table 2).

3.1.2. Incidence of different types of reproductively disordered mares among the total number of examined mares

The overall reproductive disorders in the whole examined mares (n= 27) were 17 (56.6%) infectious

endometritis cases and 10 (33.3%) non-infectious endometrial cysts cases (Table 3).

3.2. Ultrasound imaging of abnormal uterine findings

3.2.1. Bacterial endometritis

3.2.1.1. Sonographic imaging of endometritis

The incidence of uterine infection increased with age, thus all endometrial swab samples collected from 17 reproductively incapable elderly mares (>15 years) were isolated in the present work. The intraluminal uterine fluid accumulations were very typical of mares suffering from endometritis. All 17 infertile mares suffered from intraluminal fluid accumulation during estrus and were diagnosed with bacterial endometritis after uterine culture. one mare accumulated fluid during diestrus. Intraluminal accumulated fluid was graded by ultrasound according to echogenicity (concentration of inflammatory cells and debris) into grade I anechoic black, grade II hypoechoic with hyperechoic particles, grade III moderately echogenic and grade IV hyperechoic. The most detected one was grade I intrauterine fluid accumulation accompanied by bacterial endometritis. Grade IV uterine fluid was detected in one mare only and detected during diestrus (Figures 1, 2, 3 & 4).

3.2.1.2. Accumulated intraluminal fluid grades incidence

There were 9 mares diagnosed grade I intraluminal uterine fluid accumulation with percentage 33.3% among a total number of reproductive disordered mares (n= 27) and 52.9% from uterine infectious mares, 4 cases diagnosed grade II intraluminal uterine fluid accumulation with percentage 14.8% among a total number of reproductive disordered and 23.5% from uterine infectious mares, 3cases diagnosed grade III intrauterine fluid accumulation with percentage 11.1% among total number of reproductive disordered mares and 17.6% from uterine infectious mares and one mare diagnosed grade IV intraluminal uterine fluid with percentage 3.7% among total number of reproductive disordered mares and 5.8% from uterine infectious mares (Table 4).

3.2.2. Endometrial cyst

3.2.2.1. Sonographic imaging of endometrial cyst

Endometrial cysts are shown by ultrasound as immobile, single or multiple irregular fluid-filled nonechoic structures with varying sizes surrounded by a hyperechoic membrane within the uterine lumen (Figure 5&6). The endometrial cyst was diagnosed in 3 cases accompanied by endometritis.

3.2.2.2. incidence of endometrial cyst

The detection of the endometrial cysts which were multiple and largest size were found in older mares 15 yr. old. 10 cases with a percentage (33.3%) among the total no. of examined mares were imaged by ultrasound had uterine cysts and (37%) from reproductively disordered mares (Table 5).

Table 2: Incidence of the normal and reproductively disordered mares among the total number of the examined mares according to age

| Mares age group | Total no.of examined mare | Normal rep. mares | Abnormal repr. mares |
|-----------------|---------------------------|-------------------|----------------------|
| 15 to 20 y.o. | 16 | 3 | 13 |
| ➤ 20 | 14 | 0 | 14 |
| | 30 | 3 (10%) | 27 (90%) |

Table 3: Incidence of different forms of infertility of mares among the total number of examined mares

| Total no. of examined mares | Total no. of Repr. Disord. mares | Detected form of infertility | |
|-----------------------------|----------------------------------|------------------------------|------------------------|
| | | Non infectious | Infectious |
| | | Endometrial uterine cyst | Bacterial Endometritis |
| 30 | 27 (90%) | 10 (33.3%) | 17 (56.6%) |

Table 4: The incidences of uterine fluid grades occurrences among the total number of reproductive disordered mares and uterine infectious mares

| Total no. of Repr. Disorders Mares | Uterine infectious endometritis | Intraluminal uterine fluid | | | |
|------------------------------------|---------------------------------|----------------------------|-------------------------|-------------------------|-----------------------|
| | | Grade I | Grade II | Grade III | Grade IV |
| 27 | 17 (62.9%) | 9 (33.3%)* (52.9%)** | 4 (14.8%)* (23.5%)** | 3 (11.1%)* (17.6%)** | 1 (3.7%)* (5.8%)** |



Figure 1: Ultrasographic image of grade I uterine fluid: anechogenic.



Figure 2: Ultrasographic image of grade II uterine fluid: Hypoechoic with hyperechoic particles.



Figure 3: Ultrasonographic image of grade III uterine fluid: Moderately echogenic.



Figure 4: Ultrasonographic image of grade III uterine fluid: hyperechogenic

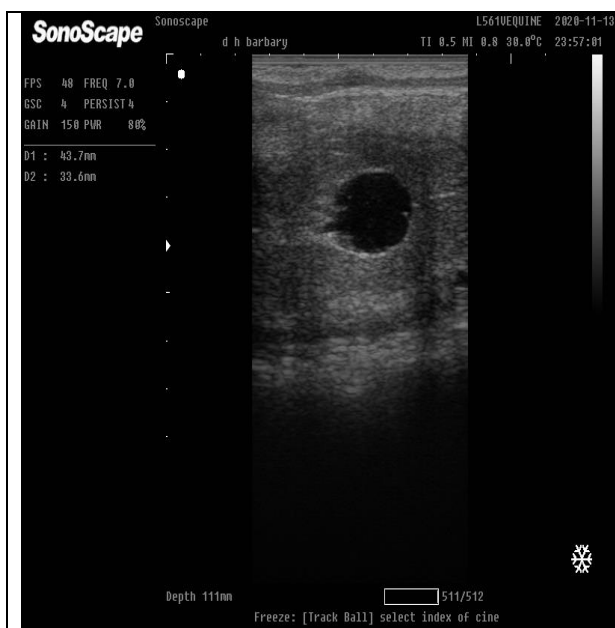


Figure 5: Ultrasonographic image of a single endometrial cyst within the uterine body

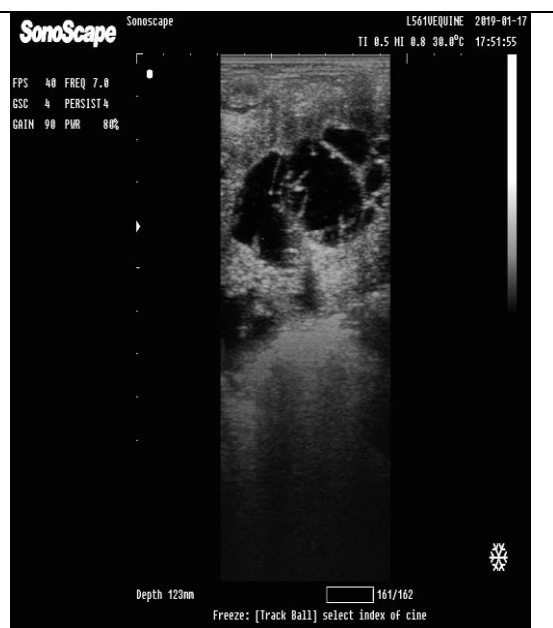


Figure 6: Ultrasonographic image of multiple endometrial cysts of a mare uterus

Table 5: Incidence of endometrial cyst occurrence among the total number of examined mares and reproductive disordered mares

| Total no. of examined mares | Total no. of reproductive disorders mares | Uterine cysts |
|-----------------------------|---|---------------------------|
| 30 | 27 | 10 (33.3%)* (37%)** |

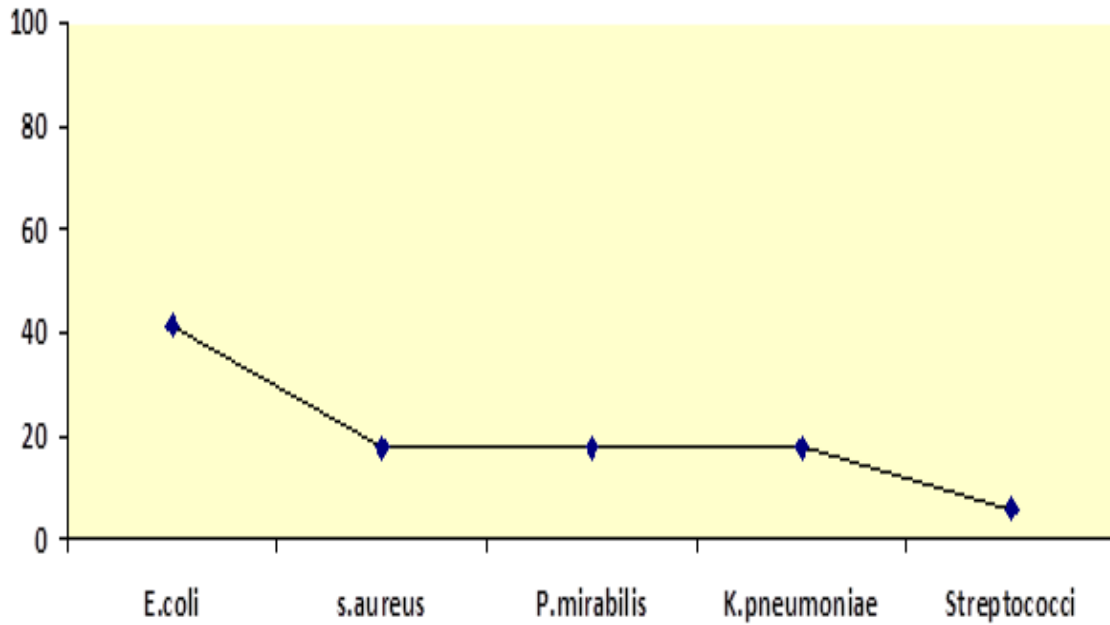


Figure 7: Frequency (%) of different types of bacterial isolation of the examined mares.

Table 6: presents an overview of the bacteria isolated and recovered from uterine infection mares

| Isolated bacteria (17 samples) | No. of times isolated | Frequency of isolation (%) |
|-----------------------------------|-----------------------|----------------------------|
| Escherichia coli | 7 | 41.1 |
| Staphylococcus aureus | 3 | 17.6 |
| Proteus mirabilis | 3 | 17.6 |
| Klebsiella pneumoniae | 3 | 17.6 |
| Streptococci | 1 | 5.8 |

Table 7: Presents an overview of the sensitivity patterns of the isolated bacteria to different antibacterial agents

| Antibiotics selected | <i>E. coli</i> | | | <i>klebsiella pneumoniae</i> | | |
|----------------------------|----------------|---|---|------------------------------|---|---|
| | S | I | R | S | I | R |
| Cefipime | | I | | | | R |
| Sulphadiazine/trimethoprim | S | | | | | R |
| Ciprofloxacin | S | | | S | | |
| ceftifur | | I | | | I | |
| Enrofloxacin | S | | | S | | |
| Amikacin | S | | | | | R |
| Gentamicin | S | | | S | | |
| Penicillin G | | | R | | I | |
| Streptomycin | | I | | | I | |
| Cefotaxim | | I | | | I | |

| Antibiotics selected | <i>S. aureus</i> | | | <i>Proteus mirabilis</i> | | | <i>streptococci</i> | | |
|----------------------------|------------------|---|---|--------------------------|---|---|---------------------|---|---|
| | S | I | R | S | I | R | S | I | R |
| Cefipime | | | R | | I | | | | R |
| Sulphadiazine/trimethoprim | S | | | S | | | | | R |
| Ciprofloxacin | S | | | S | | | S | | |
| ceftifur | | I | | | I | | | | R |
| Enrofloxacin | S | | | S | | | S | | |
| Amikacin | | I | | S | | | | | R |
| Gentamicin | S | | | S | | | | | R |
| Penicillin G | | | R | | I | | | | R |
| Streptomycin | | I | | | | R | | | R |
| Cefotaxim | | I | | | I | | | | R |



Figure 8: Cross section of uterine horn with hyperechoic accumulated intraluminal fluid



Figure 9: Cross section through uterine horn without intraluminal fluid during estrus after treatment

3.3. bacteriological exam of uterine swabs

3.3.1. Incidence and frequency of uterine isolated bacteria

17 mares (62.9%) from 27 reproductively disordered examined mares with uterine infections diagnosed by uterine swabs bacteriological exam.

3.3.2. Antibiotic sensitivity results of uterine isolated bacteria

Table (7) presents an overview of the sensitivity results of the isolated bacteria to different antibacterial agents. As for *E. coli* isolates, marked resistance was observed for Penicillin G with 100% of the isolates being none inhibited. Susceptibility was high for Sulphadiazine trimethoprim, Ciprofloxacin, Enrofloxacin, Amikacin, and gentamicin. Also, *E. coli* exhibited intermediate susceptibility to ceftifure and streptomycin. For *Klebsiella pneumoniae* pathogens were all highly susceptible to, Enrofloxacin, Ciprofloxacin and Gentamicin), while highly resistant to Sulphadiazine trimethoprim, Amickacin, and exhibited variable susceptibility to Streptomycin, ceftifur, and Penicillin G. The three *S. aureus* isolates were all highly and uniformly susceptible to Sulphadiazine trimethoprim, Ciprofloxacin, Enrofloxacin and Gentamicin of tested antibiotics. Intermediate sensitive to ceftifure, Amickacin, Cefotaxim and streptomycin. Also, *S. aureus* isolates were highly resistant to Penicillin G and Cefipime. The ciprofloxacin, enrofloxacin, Sulphadiazine trimethoprim, to Penicillin G and Gentamicin inhibited *Proteus mirabilis* isolates and marked resistance was observed for streptomycin and exhibited intermediate susceptibility to ceftifure. One isolate of *streptococci* was resistant to most tested antibiotics

In the present work, the bacterial pathogens isolated were *Escherichia (E.) coli* 7 isolates, 41.1% followed by *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Proteus mirabilis*, 3 isolates, 17.6% as same isolated. Other *streptococcus* was found at lower frequency 1 isolate, 5.8% (Figure 7 & Table 6).

except for Ciprofloxacin, and Enrofloxacin with 100% of the isolates being inhibited. A previous study [31] concluded that antibiotics with moderate or intermediate susceptibility to bacterial isolates were included as a susceptible group.

3.4. Sonographic imaging of uterine bacterial infection mares after treatment

Out of seventeen mares diagnosed by ultrasound and uterine swab culture as having bacterial endometritis 12 were cured (normal by ultrasound, no accumulation of intraluminal uterine fluid) after a single treatment. Meanwhile, the other 5 mares responded after multiple treatments, and then all cured 17 mares were subjected to an embryo transfer program.

Ultrasound image of uterine horn cross-section characterized by hyperechoic thickening of endometrial lining with echogenic accumulated intraluminal fluid but after complete treatment no accumulated intraluminal uterine fluid could be seen with thin normal endometrial echogenecity (Figure 8 & 9).

4. Discussion

Bacterial endometritis and endometrial cysts were important causes of subfertile mares in this study. In agreement with our study [8,12,10,32] stated that endometritis and uterine cysts are the most common cause of

uterine disorders and contributing most common factor to subfertility in the mare. In the present study, data was obtained from 30 aged (>15 years old) subfertile pure Arabian mares, in which 17 (56.6%) infectious endometritis included mares and 10 (33.3) noninfectious endometrial cysts included mares. Thus we concluded that Advanced maternal age remains a major predisposing factor for reduction of reproductive efficiency. Similar results were obtained by [33, 34,35,36] who showed that increased maternal age is a major factor in the reduction of reproductive performance and reduced pregnancy rate, also It is widely accepted that mare fertility begins to decrease after 13–17 years old [2,37]. diagnostic ultrasonography is an important noninvasive tool to monitor normal findings of the genital tract as well as to diagnose different types of reproductive disorders in our study, which is following [38,39] stated that ultrasonography compared with other diagnostic methods is one of the most noninvasive, rapid, harmless and accurate tools to diagnose early pregnancy, uterine pathology Also determining the stage of estrus cycle, status of follicles and corpus luteum in mares.

Accumulated intraluminal uterine fluid during the ovulatory period is the major recognized ultrasound feature on screen in uterine swab specimens collected from 17 subfertile older mares in the current study. On the same line, [12, 25,40] summarized that diagnosis of endometritis is based on reproductive examination including transrectal ultrasonography and laboratory investigation, also The first solely diagnostic sign of clinical endometritis by ultrasonography are intraluminal uterine fluid accumulation. In the current study all 17 mares suffered from bacterial endometritis diagnosed after uterine culture in which 9 cases (52.9%), 4 cases (23.5%), 3 cases (17.6%), and 1 case (5.8%) accumulated intraluminal uterine fluids among total number of uterine infectious mares were in grade I, II, II and IV respectively. Also, [25] stated that the ultrasonographic character of intraluminal uterine fluid is graded from I to IV according to echogenicity. It is [5,41] reported that endometrial uterine cysts increase with parity and age; most affected mares are older than 10 years. This supported the findings in the current study of an increased presence of uterine cysts in aged mares: among the total number of uterine-affected mares (n=27) there were 10 mares with incidences of 37% having endometrial cysts. As well as, [24] and [10] pointed out that the incidence of endometrial cysts in mares is quite high (up to 27%), being more common in mares older than 10 years. However, a higher incidence of endometrial cysts than our finding was detected by [6]. He concluded that 73.1% and 29.1% of the uterine cysts were diagnosed in mares between 7 and 14 years old and over 14 years, respectively. On the other hand, [42] concluded that most uterine cysts do not cause a clinical problem in mares.

Ultrasonography permits an accurate fast diagnostic tool for endometrial cysts in our finding which appeared as single and multiple immobile echolucent structures with hyperechoic well-defined borders with spherical to irregular shapes. These results are consistent with those obtained by [43] who mentioned that Uterine cysts were recognized ultrasonically as immobile anechoic fluid-filled structures varying in shape from spherical to long and oval with a single cavity or split into several cavities. The uterine cyst can be single or multiple and size can vary from a few

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millimeters to several centimeters [44]. In our work, 17 mares with a percentage (62.9%) of the reproductively disordered examined mares (n= 27 suffered from bacterial endometritis diagnosed by ultrasonography and uterine bacteriological examination. also, [32,45, 46] reported that bacterial endometritis occurs in 25–60% of barren mares and is considered a major most important cause of subfertility in the equine breeding industry. In the present work, positive correlations were found between age and the occurrence of uterine infection as the incidence of bacterial endometritis increased in older mares (> 15 years). Also, [12] mentioned that major losses due to bacterial endometritis are related to advanced maternal age. The bacterium most commonly isolated from the mare's uterus was *Escherichia (E.) coli* in our work 7 isolates. These organisms account for 41.1% of bacterial endometritis cases followed by *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Proteus mirabilis*, 3 isolates, 17.6% as same isolated. *Streptococci* have also been isolated from infertile mares at lower frequency 1 isolate, 5.8%. These results were very close to that obtained by [7,47, 48,49,50, 51] They found that *E coli* was the most common pathogenic microorganism isolated at a high frequency from mares with fertility problems and associated with repeat breeding with or without clinical symptoms of endometritis and that *Streptococci* are the fourth most frequent, which is not by [31] who stated that *streptococci* were the most important microorganism isolated from the uteri of problem mares followed by *E coli*.

In the current work *E. coli* was highly sensitive to Sulphdiazine trimethoprim, Ciprofloxacin, Enrofloxacin, Amikacin and gentamicin, and marked resistance was observed for Penicillin G with 100% of the isolates being none inhibited. also [31] stated that *E. coli* isolates were all highly sensitive to Enrofloxacin, Kanamycin, Gentamicin, and Trimethoprin/ Sulphamethoxazole of tested antibiotics, while highly resistant to Ampicillin and Penicillin. For *klebsiella pneumoniae*, marked resistance was observed for Sulphdiazine trimethoprim, Amickacin, but The highest susceptibility was observed for Ciprofloxacin, Enrofloxacin and Gentamicin and intermediate susceptibility to ceftifure, streptomycin Penicillin G in the current study.

A previous study [31] found that *S. aureus* isolates were inhibited by Amoxicillin/Clavulanic acid, Enrofloxacin, Gentamicin, and Rifampicin. These findings are in agreement with the results of the present study in which *Proteus mirabilis* and *S. aureus* isolates were susceptible to Sulphdiazine trimethoprim, Ciprofloxacin, Enrofloxacin, and Gentamicin. *Streptococcal* isolates were sensitive to Ciprofloxacin and Enrofloxacin only of tested antibiotics, on the other hand, [52,78], concluded that all *Streptococcal* isolates were sensitive to β -lactam antibiotics. The treatment protocol of 17 uterine bacterial infection mares in our work had two modes of action, fluid drainage and antibacterial activity. During estrus, subfertile aged mares were lavaged with 2 to 3 liters of warmed (42° to 45° C) normal saline or Ringer lactate solution and oxytocin 25 IU used complementary with uterine lavage until the efflux recovered is clear for 2 to 3 days which is similar treatment protocol stated by [25,26,27].

According to the result of bacteriological culture and type of antibiotic the systemic and intrauterine antibacterial

agents were used in our study. sulfadiazine and trimethoprim and Enrofloxacin were the antibiotics to which the majority of common uterine bacterial isolates had no resistance, also enrofloxacin and trimethoprim have a large volume of distribution and produce high concentrations in peripheral parts of the body including the uterus, and are thus efficacious also in systemic use [3]. Also, Ciprofloxacin and Enrofloxacin, were the only antimicrobials to which common endometritis pathogens had no resistance to uterine infectious Arabian mares [7]. Fortunately, this supports using an antibiotic enrofloxacin at a dose of 5.5mg/kg intravenous infusions to treat older mares infected with bacterial endometritis [54,55]. Also, in present work used sulfadiazine and trimethoprim systemically for at least 5 days [28,56]. Intrauterine infusion of an appropriate antibiotic (Gentamicin 1g) buffered with an equal volume of sodium bicarbonate as reported by [24,30] for 3–5 days during estrus performed in the current study after uterine irrigation which is following [12] who stated that infusions should be preceded by uterine irrigation to increase the therapeutic efficacy of antibiotic. Whereas, [24] showed that intrauterine infusion of antibiotics with or without prior uterine lavage is confirmed. In a present study about 60 ml of gentamicin buffered with an equal volume of sodium bicarbonate was infused with intrauterine which is similar to other studies reported by [57] who recommended a volume of approximately 30 to 60 ml to prevent expulsion of the drug but [58,59] prefer to infuse large volume 200 to 250 ml to ensure thorough coverage of the entire endometrium. Ultrasonographic disappearance of accumulated intraluminal fluid is a useful indication of treatment success and mares may be bred after signs of inflammation have resolved [30]. Also in the present study Out of seventeen mares subjected to treatment protocol as previously discussed 12 were cured (sonographically normal, no accumulation of intraluminal uterine fluid) after a single treatment, Meanwhile, the other 5mares responded after multiple treatments.

5. Conclusions

In summary, according to this study, Advanced maternal age (>15 years old) is an important factor in reducing reproductive efficiency with increased uterine susceptibility to infection in Arabian mare. Bacterial endometritis and endometrial cysts were the cause of subfertility, occurring in 90% of Arabian older mares in the present study. Reproductive ultrasound and bacteriological examination are necessary diagnoses of uterine affection in repeat-breeding mares. *E. coli* was the most frequently isolated uterine bacterial species in the current study. Ciprofloxacin and Enrofloxacin were the only antimicrobials that inhibit all uterine-isolated bacterial species in this study. Concerning Sulphadiazine trimethoprim the majority of common endometritis pathogens had no resistance. Systemic Enrofloxacin and Sulphadiazine trimethoprim treatment was suggested as the treatment of choice for infectious endometritis suffered mares of the present study.

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