

## SOME BACTERIOLOGICAL AND IMMUNOLOGICAL STUDIES OF ENDOMETRITIS IN MARES BEFORE AND AFTER ALTERNATIVE TREATMENTS USING PLATELETS RICH PLASMA (AND/OR) ANTIBIOTICS

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### ABSTRACT

Bacterial endometritis is the most major cause of mare infertility. This study was carried out to investigate the efficiency of using alternative ways of treatment for endometritis by using selected antibiotics or platelet-rich plasma (PRP) or combinations between them. From a total of 46 mares, 39 suffered from endometritis (G1) and 7 were fertile mares (G2). The most commonly isolated bacterial species was *E. coli* (12 isolates, 30.77%) *S. aureus* (9 isolates, 23.07%), *K. pneumoniae* (8 isolates, 20.51%) followed by *Strept. equi* (7 isolates, 17.95) as single infection and *Strept. equi*+ *E. coli*+ *K. pneumoniae* (3 isolates, 7.7%) as mixed infection. All isolates were susceptible to the widely used Amikacin and Gentamicin. Before the beginning of treatment, (G1) was subdivided into three groups: 13 mares treated with selected antibiotic (G3), 13 mares treated with PRP (G4), and 13 mares treated with a combination of antibiotics and PRP (G5). Our study recorded a significant increase in nitric oxide (NO), haptoglobin (HB), and malondialdehyde (MDA) levels in (G1) compared with (G2), with significant decrease in total antioxidant (TAC). We found that the most effective result was detected in G5, which recorded marked decrease in bacteria isolates, significant decrease in NO, HB, polymorphonuclear neutrophils (PMN) and MDA levels. In addition to increase conception rate (92.3%) compared with other groups, we concluded that using selected antibiotics with PRP in the treatment of endometritis was effective in decreasing uterine infection and have a great role in improving immunological status and elevate conception rate.

**Keywords:** Mares; endometritis; PRP; bacteriological; immunological.

### INTRODUCTION

Endometritis is the most critical problem and regarded as the third most common disease affecting horses and causing poor reproductive efficiency in

mares (Lorenzo *et al.*, 2021). The uterine infections lead to subfertility in up to 25%–60% of mares fail to conceive causing significant losses to the equine breeding industry. (Albihn *et al.*, 2003), so it is important to detect mares with uterine infections and to choose an effective antibiotic therapy based on microbiological cultivation. (Benko *et al.*, 2015). The most common bacterial pathogens that cause uterine infections include *Streptococcus equi subsp. zooepidemicus*, *Escherichia*

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*coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, (Robert and Gilbert, 2014), Which reduces the ability of the mare's uterus to contract for the removal of fluid and contaminants from the uterus, resulting in a breakdown in the innate immune system as well as a reduced response to pathogens in the uterus and contributing to the development of bacterial endometritis (Ryan, 2017).

Uterine lavage (UL) is one of several methods used to treat endometritis in addition to collect uterine samples for cytological and microbiological examinations. (LeBlanc *et al.*, 2007 and Christoffersen *et al.*, 2015). Antibiotic resistance and its impact on animal health have received a lot of attention in the last years. Furthermore, antibiotic therapy has a number of complications; including hypersensitivity, direct toxicity, and antibiotic-induced immunosuppression, this is the reason for the requirement of new strategies. Endometritis in mares has traditionally been treated with a combination of anti-inflammatory medications, uterine lavage, and antibiotics. (Canisso *et al.*, 2020). The inability of mares with endometritis to respond to conventional therapy, as well as the rise in antibiotic-resistant bacteria, led to the development of alternative treatments. (Scoggin, 2016). Platelet-rich plasma (PRP) is defined as autologous blood plasma containing platelets, which are small, anucleated cell fragments (2 to 3  $\mu$ m in diameter) containing several growth factors and cytokines, neutrophils, stem cells, and numerous proteins with antibacterial and fungicidal properties.

Currently, treatment with Platelet-rich plasma (PRP) therapy is a new therapeutic option for mares due to it contains a high concentration of multiple growth factors and has anti-inflammatory, tissue regeneration, and antimicrobial properties. (Soares *et al.*, 2020), so it can improve pregnancy rates, and reduce inflammatory responses (Pasch *et al.*, 2021).

Furthermore, Platelets store and release a variety of growth factors to promote wound healing under normal physiological conditions (Pietrzak *et al.*, 2005), in addition to promoting the proliferation of a range of cell lines, such as epithelial cells and fibroblasts, so they have been used for wound repair (Pietramaggiore *et al.*, 2006). So, it has become a prominent unconventional therapy in veterinary medicine as a means of avoiding such problems. Treatments that appear to control, if not suppress, the immune response in order to minimize uterine inflammation following pregnancy. (LeBlanc and Causey, 2009). So the giving of PRP to mares with chronic endometritis during breeding reduces the intrauterine inflammatory response. (Reghini *et al.*, 2015) and intrauterine PRP therapy has immunomodulatory and antimicrobial properties (Lorenzo *et al.*, 2021). Total antioxidant capacity (TAC) is a commonly used analyte for determining the antioxidant state of biological samples and can be used to evaluate the antioxidant response to free radicals produced by a specific disease. (Camila *et al.*, 2016). Oxidative stress, caused by an increase in free radical production in cases of endometritis, with a decrease in (TAC) (Kaya *et al.*, 2017) causes oxidative damage to cellular membrane lipids, proteins, and DNA. 8-hydroxy-2'-deoxyguanosine (8-OHdG) is one of the most common forms of free radical-induced oxidative lesions, it has been widely used as an oxidative stress biomarker. The biomarker 8-OHdG has long been used to assess the impact of endogenous oxidative damage on DNA (Athanasios *et al.*, 2009).

The aim of this study is to evaluate alternative ways of treatment for endometritis and detect the bacteriological and immunological changes associated with different treatments, in addition to resolving endometrial inflammation by restricting the bacterial contamination and improving uterine physical status.

## MATERIALS AND METHODS

### 1. *Animals and grouping:*

This study was performed on 46 mares (at Agricultural Research Association, Samer Farm For Purebred Arabian Horses, Cairo-Alexandria Desert Road) with age ranging from 5 to 18 years and weighing between 300 and 400 kg.

### 2. *Ultrasonography:*

Ultrasonography was performed by using real time B-mode scanners (Sonoscape - China) which equipped with 4-7.5 MHz frequency linear-array rectal transducer for diagnosis of endometritis; determine the thickness of uterine wall and accumulation of uterine fluid.

### 3. *Sampling:-*

**A- Uterine** swabs for bacteriological examination and sensitivity test from 46 mares.

Before collecting the uterine swabs, Mares' external genitalia were washed twice with iodo povidone 0.1% and dried. A sterile cotton swabs with double protection introduced in the uterus via the cervix by a hand protected by a sterile rectal glove to reduce the risk of contamination according to Markes *et al.* (2013)

**B- Low** volume uterine lavage samples for immunological tests were applied according to LeBlanc *et al.* (2007).

### 4. *Bacteriological examination:-*

Each uterine sample (swab) was inoculated into brain heart infusion broth, and then was incubated at 37°C for 24 hours before being cultured on specific media as sheep blood agar, Macconkey agar, and Mannitol salt agar for bacteriological examination. A loopful from the incubated nutrient broth tubes were streaked on to plates under aseptic condition and the suspected colonies were smeared according to Quinn *et al.* (1994).

### During microscopical examination:

Smear from isolated bacteria were prepared and stained with Gram's stain. Biochemical identification of the recovered isolates: the isolated gram- negative organisms were subjected to further biochemical identification using different biochemical tests according to Quinn *et al.* (2002) as follows: oxidase test, Catalase test, Sugar fermentation test for (glucose, lactose, sucrose, dulcitol, sorbitol, arabinose, rhomnose and xylose), Motility test, Urease test. Gram-positive cocci were examined microscopically for cell arrangement. As some cells tend to form tetrads or grape like clusters or chains, further testing was done to definitely differentiate staphylococci from certain streptococci. The following tests were used: Catalase test, Coagulase test and Hemolysis on blood agar.

### 5. *Antibiotic sensitivity test:*

All bacterial pathogens isolated from uterine swabs were subjected to antibiotic sensitivity tests. On the basis of results effective antibiotic therapy was used for treatment of mares.

The antibiotic susceptibility test was determined by 11 antibiotic discs which used in the disc diffusion technique, and the results of sensitivity were read according to the standard criteria of the Clinical and Laboratory Standards Institute (CLSI, guide, 2010). The method was used to detect antibiotic susceptibilities of Ampicillin/sulbactam (A/S 10 µg/mL), Amikacin (AK 30 µg/mL), Ciprofloxacin (CIP 5 µg/mL), Cefotaxime (CTX 30 µg/mL), gentamicin (GEN 10 µg/mL), Enrofloxacin (ENR 5 µg/mL), Kanamycin (K30 5 µg/mL), tetracycline (TE 30 µg/mL), Penicilline (GEN 10 µg/mL), Imipnem (Imp10 µg/mL) and Levofloxacin (LE 5 µg/mL).

### 6. *Preparation of PRP:-*

The platelet-rich plasma (PRP) was prepared according to Carmona *et al.* (2006). Briefly, 100 mL blood samples

from each animal's jugular vein were taken in tubes containing 3.2% sodium citrate as an anticoagulant. After that, the blood samples were homogenised and centrifuged at 120× g for 10 min. After centrifugation, the top half of the plasma was removed from the centrifugation tubes, and the remaining plasma was aspirated with a sterile syringe transferred to another plastic tube without anticoagulant, and re-centrifuged again at 240× g for 10 min., finally, the supernatant was discarded, and the remaining portion was used as PRP. The plasma was kept at 20–25°C in an isothermal box for 1 hour before being used.

### **7. Grouping and Protocol of treatment:-**

The mares were selected according to the history of repeated breeder. At first the mares were examined via rectal palpation and trans rectal ultrasonography (SonoScape -China), to define the condition of the uterus. Depending on the results of the ultra-sonographic examination and bacteriological culture (46) mares were firstly divided into two main groups, G1 (39) mares suffered from endometritis and G2 (7) normal mares. Before the beginning of treatment G1 subdivided into 3 groups (according to method of treatment) as follows:-

G3 (13), mares treated with selected antibiotics.

G4 (13), mares treated with PRP.

G5 (13), mares treated with a combination of antibiotics and PRP.

### **Protocol of treatment:**

According to the regime of treatment, intrauterine infusions were given to mares with 50 mL of fresh PRP only or with chosen antibiotic only (which gave positive result in sensitivity test) or with a combination of them.

PRP is administered intrauterine via a one-way sterile rubber uterine catheter IMV (Instruments de Médecine Vétérinaire) into the uterine body. Then, the uterus transrectally massaged to facilitate the

distribution of the fluid evenly throughout the uterine lumen. Each prepared plasma is injected into the same animal from which it was taken.

### **Antibiotics for Intrauterine treatment:**

The antibiotic used in our study as resulted from antibiotic sensitivity test which act a powerful local treatment antibiotic in mares with bacterial endometritis was Amikacin (250 mg/ml) I/U injection 1-2 grams buffered with equal volume of sodium bicarbonate and diluted with sterile saline solution to a volume of 50 ml used in infected mares for intrauterine infusion for 3–5 days during estrus (LeBlanc and Causey, 2009 and Causey, 2007). After the course of treatment uterine swab and lavage were collected for evaluate the efficiency of treatment and determine the mares that ready for breeding.

The course of treatment was repeated in the beginning of next estrus, for mares that failed to conceive after first estrus, and so on till fourth estrus. During ultra sonographical examination absence of fluid accumulation is useful indication of treatment success and mares may be breed (Causey, 2007).

### **8 .Immunological examination:**

**A-** Measurement of nitric oxide (NO) concentration: It was assessed according to Rajarman *et al.* (1998).

**B-** Detection of lysozyme concentration: Lysozyme assaying was done according to Schultz, (1987).

**C-** Cytological smears:

For evaluation the presence of polymorph nuclear cell (PMN) in briefly, the uterine samples were centrifuged at 750 ×g for 10 min, and then discard the supernatant; the remaining pellets were re-suspended and smeared onto microscope slides. Stained all slides using a Diff-Quick stain kit (Sysmex, Japan), according to the manufacturer's instructions, all slides were examined under

a microscope (400× magnification). The numbers of epithelial endometrial cells and PMNs were counted (up to 200 cells per slide), the percentage of PMNs present was calculated (Kasimanickam *et al.*, 2004).

#### D-determination of haptoglobin (Hpt):

It was measured by commercial kits (ELISA Kit) (SunRed).

#### E-Determination of total antioxidant capacity TAC:

It was measured by commercial kits according to Trachootham *et al.* (2008).

#### F-Determination of Glutathione peroxidase (Gpx):

According to Paglia and valentine, (1967)

#### G-Determination of 8-Hydroxy-desoxyguanosine (8-OHdG):

It was measured by commercial kits (ELISA Kit) (SunRed)

#### H-determination of Malondialdehyde (MDA):

It was measured by commercial kits according to Ohkawa *et al.* (1979).

### 9. Conception Rate:-

The conception rate was calculated according to Ahmed *et al.* (2021).

Conception rate= number of non-retained to heat of mare / total number of breeding mare x 100

## RESULTS

### 1. Bacteriological examination

The total number of examined mares was (46) as showed in Table (1), the bacteriologically positive samples were 39 (84.8%) and bacteriologically negative samples were 7 (15.2%). The most frequently isolated bacteria from 39 mares that had uterine infection shown in table (2), single infection recorded in 36 mares (92.3%) were *E. coli* (12 isolates, 30.77%) followed by *S. aureus* (9 isolates, 23.07%) followed by *K. pneumoniae* (8 isolates,

20.51%) followed by *Strept. equi* (7 isolates, 17.95 %) and as mixed infection in 3 mares (7.7%) was *Strept.equi E.coli K. pneumoniae* (3 isolates, 7.7%).

As shown in Table (4) Group treatment by Antibiotic only (G3=13 mare) bacteria isolated before treatment was *E.coli*, *S.aureus*, *K. pneumoniae* *Strept.equi* and *Strept.equi+ E.coli+ K. pneumoniae* (30.7%-23.1%-23.1%-15.4%-7.6)

respectively and after treatment was (7.6%-7.6%-0%-0-7.6%).Group treatment by PRP only (G4=13 mare) bacteria isolated before treatment were *E.coli*, *S.aureus*, *K. pneumoniae* *Strept.equi* *Strept.equi+ E.coli+ K. pneumoniae* (30.7%-23.1%-15.4%-23.1-7.6%) respectively and after treatment were (7.6 %-23.1%-15.4%-7.6-7.6%) respectively .Group treatment by PRP + Antibiotic (G5=13 mare), bacteria isolated before treatment were *E.coli*, *S.aureus*, *K. pneumoniae* *Strept.equi* *Strept.equi+ E.coli+ K. pneumoniae* (30.7%-23.1%-23.1%-15.4-7.6%) respectively and after treatment were (0%-7.6%-0%-0%-0%).

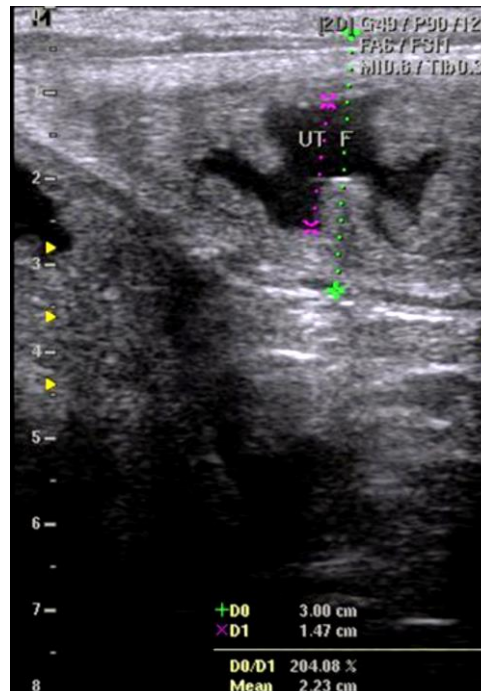
### 2. Antimicrobial susceptibility testing

All the bacterial isolates were subjected to antibiotic sensitivity testing against eleven commonly used antibiotics based on the Clinical and Laboratory Standards Institute (CLSI) guide, as shown in table (3) the antibiotics which most of isolates were susceptible included Amikacin (100 %), gentamicin (100%), Ciprofloxacin (100%), followed by Levofloxacin (94,9%), Enrofloxacin (94.8%) Ampicillin/sulbactam (89.7%), Imipnem (87.2%), and resistant against penicillin (100%), tetracycline (87.2%), Cefotaxime (79.5%), Kanamycin (66.7%). In our work amikacin, gentamicin, ciprofloxacin, enrofloxacin, and levofloxacin were the most potent antimicrobial agents inhibiting most uterine bacterial isolates *E. coli*, *S. aureus*, *k. pneumoniae*, *Strept. quie* and act as useful antibiotics treatment in mares with bacterial endometritis.

### 3. Ultrasonographic finding of the uterus of mares suffered from endometritis

During an ultrasound reproductive evaluation, all uterine infected mares had

intrauterine fluid accumulations. The uterine lumens contain abnormal fluid accumulation in addition to thickness of the wall of uterus as show in Fig.1



**Figure1.** Shows the presence of uterine fluid (UT F) of 1.47cm and the endometrial folds and the uterine body diameter of 3.00 cm

### 4. Immunological examination:

Our results recorded significant increase ( $p < 0.05$ ) in NO, LY, PMN, HB, (8-OHdG) and MDA levels ( $20.65 \pm 2.38$ ,  $122.16 \pm 7.29$ ,  $10.34 \pm 0.954$ ,  $14.54 \pm 1.14$ ,  $1.06 \pm 0.0717$ ,  $9.6 \pm 0.266$ ) respectively in mares suffered from endometritis (G1) compared with healthy ones (G2). Although, there were significant decrease ( $p < 0.05$ ) of TAC and peroxidase (GPX) levels ( $0.771 \pm 0.0347$ ,  $77.97 \pm 5.03$ ) respectively.

Mares treated with antibiotics only (G3) recorded significant decrease ( $p < 0.05$ ) in levels of NO, LY and PMN ( $14.47 \pm 0.784$ ,  $118.59 \pm 7.29$ ,  $6.07 \pm 0.75$ ) respectively compared with G1 and G5 (in nitric oxide and lysozyme levels) and in comparison with G1, G4 and G5 (in PMN %). While HB ( $11.69 \pm 1.33$ ) significant increase ( $p < 0.05$ ) compared with G1 only. (OHdG-8) and MDA recorded significant increase ( $p < 0.05$ ) ( $0.917 \pm 0.0367$ ,  $9.34 \pm 0.28$ )

respectively compared with G5 (in case of OHdG-8), and G4 and G5 (in case of MDA.

(G4) showed significant decrease ( $p < 0.05$ ) in PMN% ( $5.02 \pm 0.469$ ) compared with G1, G3, G5, (8-OHdG) ( $0.786 \pm 0.0522$ ) compared with G1 and G5 and MDA ( $7.66 \pm 0.407$ ) concentration compared with (G1) and G3 with non-significant decrease in HB and NO Levels.

Mares treated with both selected antibiotics and PRP (G5) recorded significant decrease ( $p < 0.05$ ) in NO, lysozyme, PMN and (8-OHdG) ( $11.16 \pm 0.42$ ,  $61.99 \pm 2.99$ ,  $2.18 \pm 0.218$  and  $0.486 \pm 0.0385$ ) respectively compared with G1 and other treated groups, while HB level showed significant decrease ( $p < 0.05$ ) ( $9.67 \pm 0.455$ ) compared with G1 only. TAC enzyme levels showed significant increase ( $p < 0.05$ ) ( $0.978 \pm 0.0471$ ) compared with G1 and G4, while MDA recorded significant decrease

( $p < 0.05$ ) ( $7.59 \pm 0.463$ ) compared with G1 and G3.

### 5. Conception Rate

The obtained result in Table (6) showed that conception rate in G3 (the group treated with antibiotics only) was 76.9%. (where 5 mares got pregnant after first estrus, 3 after second estrus and 2 after third estrus), while in G4 (group treated with fresh PRP only) was 38.5% (where no mare got pregnant in first estrus and one mare got pregnant in both second and third estrus and 3 mares in fourth estrus), conception rate in G5 (group treated with fresh PRP and

selected antibiotic) was 92.3% (where 9 mares got pregnant after first estrus, 3 in second estrus).

### 6. Statistical Analysis:-

The collected data were analyzed using IBM SPSS version 25 software package (SPSS, IBM, Chicago, IL, USA) and presented as mean  $\pm$  SEM (standard error of the mean). The relationships between different sets of data were examined by performing analysis of variance (ANOVA), Tukey's honest significance test, and post hoc analysis. Values of  $p < 0.05$  were considered statistically significant.

**Table (1): Bacteriological examination of Mares uterine samples.**

Total number of examined mares (46)		
	No	%
Bacteriologically positive samples	39	84.8
Bacteriologically negative samples	7	15.2

**Table 2:** Incidence of bacteria Isolated from examined uterine swab samples in examined mares.

The Isolated Bacteria from (39) examined mares		
Isolated bacteria	No. of isolated bacteria	Percentage of isolated bacteria (%)
<b>Single infection</b>	<b>(36)</b>	<b>(92.3)</b>
<i>E.coli</i>	12	30.77
<i>Staph.aureus</i>	9	23.07
<i>K. pneumoniae</i>	8	20.51
<i>Strept.equi</i>	7	17.95
<b>Mixed infection</b>	<b>(3)</b>	<b>7.7)(</b>
<i>Strept.equi, E.coli and K. pneumoniae</i>	3	7.7
<b>Total</b>	<b>39</b>	<b>100</b>

**Table 3:** The Antibiotic Sensitivity tests for different bacteria isolates.

Antibiotic discs	Sensitive (S)		Resistance (R)	
	No	%	No	%
	<b>Ampicillin/sulbactam (A/S 10 µg/mL)</b>	35	89.7	4
<b>Amikacin (AK 30 µg/mL)</b>	39	100	0	0
<b>Ciprofloxacin (CIP 5 µg/mL)</b>	39	100	0	0
<b>Cefotaxime (CTX 30 µg/mL)</b>	8	20.5	31	79.5
<b>Gentamicin (GEN 10 µg/mL)</b>	39	100	0	0
<b>Enrofloxacin(ENR 5 µg/mL)</b>	37	94.8	2	5.1
<b>Kanamycin (K30 5 µg/mL)</b>	13	33.3	26	66.7
<b>tetracycline (TE 30 µg/mL)</b>	5	12.8	34	87.2
<b>Penicilline(GEN 10 µg/mL)</b>	0	0	39	100
<b>Imipnem(Imp10 µg/mL)</b>	34	87.2	5	12.8
<b>Levofloxacin (LE 5 µg/mL)</b>	37	94.9	2	5.1

**Table 4:** The Effect of different treatment regime on bacterial isolation from infected mares.

No of mares (39)	Percentage of Isolated Bacteria before and after treatment																			
	<i>E. coli</i> (12)		<i>S. aureus</i> (9)		<i>K. pneumoniae</i> (8)		<i>Strept.equi</i> (7)		<i>Strept.equi+ E.coli+ K. pneumoniae</i> (3)											
	Before	after	Before	after	Before	after	Before	after	Before	after										
	No	%	No	%	No	%	No	%	No	%										
<b>Antibiotic only (13)G3</b>	4	30.7	1	7.6	3	23.1	1	7.6	3	23.1	0	0	2	15.4	0	0	1	7.6	1	7.6
<b>PRP Only (13)G4</b>	4	30.7	1	7.6	3	23.1	3	23.1	2	15.4	2	15.4	3	23.1	1	7.6	1	7.6	1	7.6
<b>PRP+ Antibiotic (13)G5</b>	4	30.7	0	0	3	23.1	1	7.6	3	23.1	0	0	2	15.4	0	0	1	7.6	0	0

**Table 5:** Some immunological measurement of uterine lavage of examined Mares.

immunological tests	Positive control G1	Negative control G2	Antibiotic treated group G3	PRP treated group G4	Antibiotic and PRP treated group G5
<b>NO (uM/ml)</b>	20.65±2.38 <sup>b,c,e</sup>	9.74±0.358 <sup>a,c,d</sup>	14.47±0.784 <sup>a,b,e</sup>	18.78±1.06 <sup>b,e</sup>	11.16±0.42 <sup>a,c,d</sup>
<b>Lysozyme (µg /ml)</b>	122.16±7.29 <sup>b,c,e</sup>	58.45±6.05 <sup>a,c,d</sup>	118.59±7.29 <sup>a,b,e</sup>	132.07±12.98 <sup>b,e</sup>	61.99±2.99 <sup>a,c,d</sup>
<b>PMN%</b>	10.34±0.954 <sup>b,c,d,e</sup>	1.71±0.142 <sup>a,c,d</sup>	6.07±0.750 <sup>a,b,d,e</sup>	5.02±0.469 <sup>a,b,c,e</sup>	2.18±0.218 <sup>a,c,d</sup>
<b>HB (µg /ml)</b>	14.54±1.14 <sup>b,c,e</sup>	8.71±0.433 <sup>a,d</sup>	11.69±1.33 <sup>a</sup>	12.56±0.652 <sup>b</sup>	9.67±0.455 <sup>a</sup>
<b>(OHdG-8) ng/ml</b>	1.06±0.0717 <sup>b,d,e</sup>	0.489±0.0335 <sup>a,c,d</sup>	0.917±0.0367 <sup>b,e</sup>	0.786±0.0522 <sup>a,b,e</sup>	0.486±0.0385 <sup>a,c,d</sup>
<b>TAC mM/ml</b>	0.771 ±0.0347 <sup>b,e</sup>	1.1±0.0559 <sup>a,c,d</sup>	0.822±0.0503 <sup>b</sup>	0.766±0.0371 <sup>b,e</sup>	0.978±0.0471 <sup>a,d</sup>
<b>Gpx mU/ml</b>	77.97±5.03 <sup>b</sup>	116.44±5.57 <sup>a,c,d,e</sup>	78.41±4.74 <sup>b</sup>	80.45±3.03 <sup>b</sup>	82.58±1.6 <sup>b</sup>
<b>MDA nmol/ml</b>	9.6±0.266 <sup>b,d,e</sup>	7.2±0.242 <sup>a,c</sup>	9.34±0.28 <sup>b,d,e</sup>	7.66±0.407 <sup>a,c</sup>	7.59±0.463 <sup>a,c</sup>

Superscript letters (a, b, c, d, and e) indicate significant differences ( $p < 0.05$ ) within the same row (between tested groups).



**Table 6:** Correlation between alternative treatment and conception rate.

Treatment cases N: (39) mares	Total Number of conceived mares	First Estrus	Second Estrus	Third Estrus	Fourth Estrus	conception rate after end of treatment (%)
Antibiotics G3 (13)	10/13	5	3	2	-	76.9
PRP G4 (13)	5/13	0	1	1	3	38.5
PRP + Antibiotic G5 (13)	12/13	9	3	-	-	92.3

## DISCUSSION

The most common cause of infertility is bacterial endometritis, which causes significant losses in the equine breeding economy (LeBlanc, 2012). Infectious endometritis is a prominent cause of infertility in horses; up to 25%–60% of mares that are unable to conceive have a bacterial uterine infection (Canisso *et al.*, 2020). In the present study, 46 mares examined, by ultrasound and uterine culture, 39 mares were shown to have bacterial endometritis. The most frequently isolated bacteria were *E. coli* (30.77%) followed by *S. aureus* (23.07%) followed by *K. pneumoniae* (20.51%) followed by *Strept. equi* (17.95 %) as a single infection in 36 mares and *Strept. equi*+ *E. coli* and *K. pneumoniae* (7.7%) as mixed infection in 3 mares. The obtained results recorded that *E. coli* was the most prominent pathogenic microorganism isolated from the uteri of infected mares and most considerably microorganism linked with fertility problems and repeat breeding in the mare. Although, the *Streptococci* are the fourth most frequent bacteria. These results agreed to that obtained by Ghasemzadeh *et al.* (2004). Parallel with our results, Frontoso *et al.* (2008) recorded that *S. aureus* was the second pathogenic microbe obtained from the affected mare. This microorganisms cause reproductive issues in the equine uterus.

From the immunologically point of view, the obtained result indicated an increase in NO and lysozyme concentration in mares suffer from endometritis (G1) compared with healthy mares (G2) this results agreed with Inas *et al.* (2019) and Woodward *et al.* (2013). NO is releases by inducible nitric oxide synthase (iNOS) in many cell types, including macrophages, neutrophils, and resulted from the release of inflammatory signals (Griscavage *et al.*, 1996).

Moreover prolonged increase in NO activity causes in smooth muscle relaxation and a reduction in myometrial activity which are resistant to uterine clearance, all of which contribute to the myometrial function could be related to the increased intrauterine accumulation of uterine fluid and increase nitric oxide (NO) accumulation (Alghamdi *et al.*, 2005). Following the same pattern haptoglobin and PMN concentration showed significant increase in (G1) compared with (G2) this due to heavy infestation of bacteria, so the body release large number of PMN to the uterus to overcome the infection as well as, the antimicrobial activity of PMN lead to the formation of NETs as a complementary mechanism to eradicating bacteria. Canisso *et al.* (2016) mentioned that presences of high level of bacteria in endometritis mare causes PMN penetration into the luminal epithelium and stratum compactum is used as a reference standard for endometritis diagnosis. On the other hand, healthy horses' blood plasma already contains

haptoglobin (Hp), and its content rises during inflammation (Jacobsen and Andersen 2007). Hsiang *et al.* (2009) demonstrated that HB is synthesized by neutrophils, parallel of this finding, some authors observed a brief increase of HB concentration after intrauterine infusion of high dose of *Escherichia coli* (Segabinazzi *et al.*, 2017). Moreover high Hp levels have been suggested as a possible sign of latent subclinical endometritis (Krakowski *et al.*, 2011).

Also, the obtained results TAC and GSH concentration recorded significant decrease in (G1) compared with (G2) while 8-OHdG and MDA enzyme showed significant increase. This results agreed with Inas *et al.* (2019) and Baithalu *et al.* (2017) who explain the decrease in total antioxidant to the over consumption of the total antioxidant during the protection against the invading organism. Bacterial infection sufficient to overwhelm the antioxidant defense uterus and cause cell damage, resulting in reductions in serum TCA and Gpx activities and an increase in MDA concentration. parallel with this fact, Any oxidative imbalance resulting in the accumulation of oxidants will cause oxidative damage to cells, such as changes in cellular macromolecules, lethal changes in genetic materials, such as DNA, which will increase the rate of cell death and, as a result, an increase in deoxyguanosine (8-OHdG), which is one of the most common forms of free radical-induced oxidative lesions and has been widely used as a biomarker for oxidative stress and a pivotal marker for measuring the effect of endogenous oxidative damage to DNA (Athanasios *et al.*, 2009).

Liu and Troedsson, (2008) and LeBlanc and Causey (2009) found that using of intrauterine antimicrobial infusions is powerful in reduction of bacterial endometritis. In current study this supported the using of chosen antibiotics this is based on bacteriological culture results. Amikacin and gentamicin were the

most effective antimicrobial drug in our study, eliminating the most of uterine bacterial isolates. *E. coli*, *S. aureus*, *K. pneumoniae*, *Strept. quie* and this antibiotic act a helpful local therapy in mares with endometritis, mares with strong infections in their endometrium, making them resistant to conventional treatment (Petersen *et al.*, 2015). Currently, a popular nontraditional treatment is platelet-rich plasma (PRP) which becoming an important therapeutic usage in mare due to it contain high concentration of various growth factors, could improve pregnancy rates and decrease inflammatory response (Pasch *et al.*, 2021). This byproduct has been used for its anti-inflammatory, tissue regeneration, and antimicrobial effects (Soares *et al.*, 2020).

In the present study, the results showed mares treatment with combination of selected antibiotic and PRP (G5=13 mare) recorded marked decrease in percentage of bacteria isolate (*S. aureus*) in addition to complete elimination of single infection (*E. coli*, *K. pneumoniae*, *Strept. equi*) and also give good effect on mixed infection this results due to the powerful effect of antibiotic in reduction and elimination the uterine bacteria, in Addition to bactericidal activity of PRP against *S. aureus*, *E. coli*, and *K. pneumoniae* (Cieslik *et al.*, 2009). So parallel with this results the obtained results recorded significant decrease in NO, lysozyme and PMN concentration compared with other groups, this due to the combination between PRP as anti-inflammatory role and antibiotics as anti-bacterial effects for eliminate the causative agent of infection and Improving the inflammatory state of the uterus (Kim *et al.*, 2014, Mazzocca *et al.*, 2013 and Monika *et al.*, 2016). Following the same pattern, TAC recorded significant increase, while MDA and (8-(OHdG) recorded significant decrease compared with other groups (Metcalf *et al.*, 2012). As a results of the effects of combination of both antibiotic (which eliminate most pathogen) and PRP (which contain proteins such as fibrinogen,

growth factors, cytokines as anti-inflammatory and antimicrobial agent) these growth factors activate the propagation of several cell lines, including epithelial cells and fibroblast, Platelets which used for healing process (Pietramaggiore *et al.*, 2006). Also this group recorded marked increase in conception rate (92.3%) where most treated mares become pregnant after second estrus, these due to removal of the causative agents and reduce the inflammation of uterus and repairing effects of PRP. This result agreed with Pasch *et al.* (2021) who recorded that intrauterine infusion with PRP could enhance pregnancy rates by reducing the post-breeding inflammatory response, and Pascoe *et al.* (1995) who recorded that the integration of autologous plasma with antimicrobial medication improves mare pregnancy rates compared with antibiotics alone. As well as, treatment with PRP increased uterine cell reproduction, improve tissue regeneration and participated in the regulation of the estrous cycle (Marini *et al.*, 2016) because they contain numerous proteins, several growth factors (GFs) and cytokines stored in cytoplasmic granules. The physiologic actions of the content of PRP which, including GFs, macrophages, neutrophils, and several hundred of proteins, which promote re-epithelization, and regeneration of the tissue, in addition to its fungicidal and antibacterial effects (Jang *et al.*, 2017 and Sills *et al.*, 2018). So finally we observed that combination between PRP (which has anti-inflammatory role and assist tissue repair) and antibiotics (which have anti-bacterial effects and can eliminate the causative agent of infection) give best results compared with others ways of treatment, in addition to decrease of excessive use of antibiotics, decrease the period till the mares become pregnant and improve pregnancy rates with improvement of immunologically results, so we had the benefits of using antibiotic in facing and eliminating the pathogen and using intrauterine PRP therapy has immunomodulatory and antimicrobial

effect which agree with Lorenzo *et al.* (2021).

## CONCLUSION

We concluded that using a combination of PRP and selected antibiotic therapy in the treatment of endometritis in mares is effective in modulating the uterine immunity, eliminating bacterial contamination, impaired uterine inflammatory response, and increasing pregnancy rate. In addition to the advantage of the PRP (as a nontraditional treatment) which we consider, compared to other immune-modifying drugs, is less expensive, easier to use, and has no side effects. So, we advise the use a combination of PRP and a selected antibiotic in treatment of endometriosis in mare.

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### بعض الدراسات البكتريولوجية و المناعية عن التهاب بطانة الرحم في الافراس قبل وبعد استخدام طرق بديلة لعلاج باستخدام البلازما الغنية بالصفائح الدموية و/ او المضادات الحيوية

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التهاب بطانة الرحم الجرثومي هو السبب الأكثر شيوعاً لضعف الخصوبة عند الأفراس ويعتبر ثالث أكثر الأمراض قد تصيب الخيول. أجريت هذه الدراسة للتحقق من كفاءة استخدام طرق بديلة لعلاج التهاب بطانة الرحم الجرثومي باستخدام المضادات الحيوية المختاره أو البلازما الغنية بالصفائح الدموية أو توليفات فيما بينهما للوصول الي افضل خيار للعلاج اجريت هذه الدراسه علي عدد ٤٩ من الافراس، ٣٩ منها مصاباً بالتهاب بطانة الرحم الجرثومي (مجموعه ١) وعدد ٧ من الفراسات الغير مصابه بالتهاب بطانه الرحم (مجموعه ٢). اظهرت الدراسات البكتريولوجيه ان البكتيريا الأكثر عزلاً هي اي كولي (١٢ عزله، ٣٠,٧٧٪) تليها استاف اورييس (٩ عزلات ، ٢٣,٠٧٪) تليها الكليسييله لرئوية (٨ عزلات ٢٠,٥١٪) تليها استربت اكواي (٧ عزلات ، ١٧,٩٥٪) كعدوى مفرده بالاضافه الي الكليسييله لرئوية ، الاكولي والاستربت اكواي (٣ عزلات ، ٧,٧٪) كعدوى مختلطه، وكانت جميع العزلات حساسة لاستخدام أميكاسين وجنتاميسين وإنروفلوكساسين وسبيروفلوكساسين. المضادات الحيوية الـ ١١ التي تم اختبارها ، ولكن لوحظ مقاومة البنسلين (١٠٠٪ من العزلات).

قبل البدء في العلاج تم تقسيم الفراسات المصابه بالتهاب بطانه الرحم الي ثلاث مجموعات (علي حسب طريقه العلاج) (مجموعه ٣) تحتوى علي ١٣ فرسه وتم علاجها باستخدام المضادات الحيوية المناسبه ، (مجموعه ٤) تحتوى علي ١٣ فرسه وتم علاجها باستخدام البلازما الغنية بالصفائح الدموية واخيرا (مجموعه ٥) تحتوى علي ١٣ فرسه تم علاجها باستخدام علاج مكون من مضاد حيوى وبلازما. سجل الفحص المناعي لدينا زيادة كبيرة فى ، مستويات أكسيد النيتريك، الليوزيم، العدلات النووية متعددة الأشكال المالوندايد ، الهابتوغلوبين ، انزيم الهيدروكسي دى اوكسى جونوسين باضافه الي انخفاض معنوى في نسبه مضادات الأكسدة الكلية فى الافراس المصابه بالتهاب الرحم بالمقارنه بالمجموعه الغير مصاب

فيما يتعلق بالمعالجات المختلفة ، وجدنا أن النتيجة الأكثر فعالية كانت في المجموعه الخامسه المعالجه ب مزيج من المضادات الحيوية والبلازما الغنية بالصفائح الدموية، حيث سجلت الدراسه انخفاضاً ملحوظاً في نسبه العزلات البكتيريا المختلفه بالاضافه الي نقص معنوي ملحوظاً في نسبة حمض النيتريك ، الليوزيم، الهابتوجلوبين، العدلات النوويه متعدده الاشكال، المالوندايد بالاضافه لانزيم الهيدروكسي دى اوكسى جونوسين ، مع زياده معنويه في تركيز مضادات الاكسده. كما زاد معدل الحمل الي (٩٢,٣٪) مقارنة بالمجموعات العلاقيه الاخرى ، لذلك خلصنا إلى أن استخدام المضادات الحيوية المختارة مع البلازما الغنية بالصفائح الدموية في علاج التهاب بطانة الرحم كان فعالاً في تقليل عدوى الرحم ولها دور كبير في تحسين الحالة المناعية ورفع معدل الحمل في الافراس.