

## Diagnosis and Treatment of Endometritis with Intra-Uterine Infusion of A Solution of Honey 70% in Mares

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### Abstract

Our works on endometritis aims at showing the different methods of diagnosis and demonstrate the therapeutic efficiency of intrauterine infusions of honey 70% solution for the treatment of endometritis in the mare. During the breeding season 2015, our study involved a workforce of 119 mares, which were the subject of ultrasound examination in 1566, with an average of 13 passes per mare to determine ovarian status, pregnancy diagnosis and fertility.

Among the fifteen infertile mares, only seven had endometritis confirmed by ultrasound, and cytology (46.66%), the seven mares were divided into two lots: Three were treated with the usual protocols used at the stud (uterine Washing, hormone treatments and intrauterine infusion of an antibiotic) «Lot No 01," the four remaining cases received intrauterine infusions of honey 70% solution «Lot No. 02".

We conducted the ultrasound and cytological examinations for tracking cases of the two lots. After the first infusion honey, an increase in volume and echogenicity of uterine fluids has been found, and then a gradual decrease until the total disappearance between 2 to 8 days after the last infusion honey, with an improvement in ultrasonographic image of the uterus during the first diestrus and estrus after treatment, compared to control group protocols.

A cytological examination where there was a marked improvement in both the group treated with the honey, that is characterized by a total disappearance of inflammatory cells and fungi, with a richness of smear by endometrial cells compared to the case the control group.

These observations show that the honey has shown remarkable results concerning the drainage of uterine fluids, regeneration of endometrial cells with antifungal effect *in vivo*, As against the fertility of mares treated with honey was not improved, this seems to be due to the age of the mares (16-27 years) or other intrinsic factors (mares and stallions) or extrinsic factors (environment, power, etc.)

**Keywords:** Endometritis; Mare; Diagnosis; Infusion; Honey; Improvement

### Introduction

Endometritis is a major cause of infertility in the mare [1], the absence of removal of bacteria, sperm and post-breeding inflammatory exudate can lead to inflammation of the endometrium. In addition, susceptibility factors for endometritis that are: The conformational defects of the reproductive system, weakened myometrial contractions, impaired immune system, mucus overproduction, poor lymphatic drainage and mucociliary clearance and abnormal cervical function [2-4].

The diagnosis of endometritis is done by the detection of clinical signs: excessive intrauterine fluid, which is the most important sign of endometritis, abnormal vaginal discharge, and shortening of the estrous cycle. A positive endometrial cytology and / or culture positive endometrial biopsy confirmed with a positive diagnosis [3,4].

In subclinical cases, there is an absence of clinical signs, but sometimes a post-breeding endometrial edema and white lines between the folds of the endometrium can be observed in ultrasound examination [3].

According to LeBlanc in 2010, the usual treatments (post-breeding uterine lavage, oxytocin and intrauterine antibiotics), are not always sufficient because endometritis treatment goals are to correct the defects of the defense of the uterus, the neutralization of bacteria virulent and control the post-breeding inflammation.

Treatment with antibiotics yield variable results, they are often costly and cause delays in reproduction because the healing period [5]. Therefore, the treatment of endometritis should not only eliminate bacterial contamination but also promote drainage of endometrial glands and rapid resorption of the inflammation of the mucosa, which is not possible with the local antibiotic [6].

According to Jean-François Bruyas [7], if anti-infective treatments, are necessary they must be reasoned case by case, given the risks of antibiotic resistance because of the problems of choice of molecule

used, the route of administration, the therapeutic protocol, the type of infection, access of the antibiotic to the site of infection, the local tolerance of the pharmaceutical form chosen and the general tolerance of the antibiotic.

In Algeria, there are no statistics on the rate of endometritis, and it amounts to an absence of a diagnostic approach, consequently this problem allows increased infertility rate that has great health and economic impact.

following criticism of the usual therapeutic protocols mentioned above, the purpose of our study is to diagnose endometritis by different methods namely ultrasound examination, cytological, and test the effectiveness of an intrauterine infusion of a 70% solution of honey compared with conventional treatment regimens, hoping to achieve the objectives of the treatment of endometritis and to fight against the problems of antibiotic resistance.

The choice of honey is at the basis of the therapeutic properties of honey:

Anti-inflammatory, antioxidant, antibacterial, antiviral, antifungal, healing and stimulating the immune system [8-12].

Similarly, Vaillancourt have demonstrated the safety of an intrauterine infusion of a honey solution at 70% on the endometrium of the mare.

## Materials and Methods

Our study was conducted at the National Stud Chaouchaoua - Tiaret- Algeria, during the breeding season 2015. After a follow-up ultrasound examination 119 mares in the breeding goal tracking, fifteen mares had an infertility problem; seven among them had endometriosis confirmed by ultrasound, and cytology.

The seven mares were divided into two lots: Three mares (16-27 years) were treated with the usual protocols at the stud ie uterine lavage, hormone treatments and intrauterine infusions of antibiotics. "Lot No 01". The other four mares from 16 to 22 years (DHAYA, BOSRA, FATIA and TIFLETTE) were treated by intrauterine infusion of a honey solution 70% "Lot No 02"

## Botanical origin of honey

Forest honey, Boughaiden region, town of Had el Chekala, Wilaya Relizaine -Algeria, harvested in June 2014. The flora of this region has a multitude of species of trees, shrubs and many others. The most dominant plants: pine, fir, junipers, oaks, mastic (the pasticias) and some eucalyptus.

## Technical treatment

In our study, we seek to provide a standard treatment protocol with honey. Since this is the first test, we proposed the following protocol: During estrus, with a sterile insemination probe, three infusions of 50 ml of 70% honey solution to the uterine body, at intervals of 24 hours were given for each mare.

Ultrasound examinations were performed after each infusion of honey, to monitor uterine fluids and their echogenicity in the body and the two uterine horns, we took the largest diameter of these three places at each ultrasound up the total disappearance of the liquid (at three locations).

We used a degree from 0 to 3:

- \* Grade 0: anechoic liquid
- \* Grade 1: mild echogenicity
- \* Grade 2: moderate echogenicity
- \* Grade 3: high echogenicity

## Post-treatment diagnosis

In the case of endometritis, the uterine lining is not capable of receiving an embryo following damage caused by inflammation, so to ensure proper healing, we gave time to the endometrium to regenerate.

We diagnosed two healing methods that are:

Ultrasound examination: The presence of fluids during post-treatment diestrus is always a sign of endometritis, so any finding of fluids during this phase it is not a sign of healing. And the absence of liquid is not necessarily a sign of healing, due to the presence of subclinical endometritis. Any absence of liquids requires cytology during the first post-treatment estrus.

## Uterine cytology

**For the first batch:** The moment of cytological samples depends on the nature of the treatment.

**The second batch:** Cytological samples were taken in the first post-treatment estrus to benefit from progesterone impregnation of diestrus after the role of progesterone in the regeneration of the endometrium.

Any presence of inflammatory cells was seen as a sign of non-healing in the following cases:

- Neutrophils rate more than 5% of the entire blade (13).
- Significant presence of lymphocytic cells alone or together with other inflammatory cells.
- Presence of pathogens: Bacteria, fungi.

Any absence of inflammatory cells, endometrial cells in wealth and disappearance of pathogens it is a sign of improvement.

## Fertility control

The endometritis is one of the causes of non fertilization and early embryonic loss in our study, we project only mares that showed improvement on ultrasound and cytological plan.

Ultrasound examinations were performed using a branded ultrasound ISCAN "DRAMINSKI" connected to a linear sensor 5 to 7.5 MHz.

Cytological samples were taken by a human cytobrush attached to a gun Bovine artificial insemination, stained smears by the Papanicolaou technique.

## Results

### Results of the diagnosis of endometritis

	Lot n° 01			Lot n 02			
	Mare n° 01	Mare n° 02	Mare n° 03	DHAYA	BOSRA	FATIA	TIFLETTE
ultrasound diagnosis	Liquid grade 2	Liquid luteal phase	Liquid 5 mm Grade 0	Liquid 27 mm Grade 2	Liquid luteal phase	Liquid luteal phase	Liquid luteal phase
cytologic diagnosis	PNN rich and lymphocyte cells	PNN rich and lymphocyte cells	PNN rich and lymphocyte cells	Some yeasts, PNN,	lymphocyte cells	PNN rich and lymphocyte cells	Absence of inflammatory cells

**Table 1:** Ultrasound and cytological diagnosis of endometritis.

Ultrasound diagnosis confirmed endometritis in four mares in diestrus and cytology confirms the diagnosis by smear wealth per neutrophils and lymphocyte cells, except for one case the smear did not give more (Table 1). The three cases diagnosed in follicular phase had a uterine fluid retention confirmed by cytology.

## Results of treatment

### Lot N° 01

**The mare n° 01:** At the ultrasound examination, we found a uterine fluid retention 19 mm in diameter; we started treatment with an injection of oxytocin. 24 hours after the first injection of oxytocin, we found a decrease uterine fluid from 19 mm to 11 mm, at that time, we began the first mating.

48 hours post-breeding, purulent discharge was observed in the perineum, with an accumulation hyperechoic uterine fluids grade 2 "post-breeding endometritis".

We treated the mare with a uterine lavage using two vials of isotonic saline; where we have collected some of the liquid (cloudy liquid) a second injection of oxytocin and an injection of PGF2 alpha, with a second mating 4 hours.

A third mating 48 hours without passing ultrasound examination; 24 hours after the third projection in early diestrus, a significant accumulation hyperechoic liquid was observed, which remain during the diestrus.

**The mare n° 02:** This mare was treated with three injections of oxytocin at 24 hour intervals.

24 hours after the first injection of oxytocin, there was a complete disappearance of uterine fluids during the three days of treatment. Three days after the last injection of oxytocin, in post-ovulation, there was a recurrence of uterine fluids (luteal phase).

The cytological diagnosis after the first injection of oxytocin, shows the richness PNN and lymphocytic cells with the rarity of endometrial cells.

**The mare n° 03:** This mare was treated with four intrauterine infusions of 20 ml of sulfaprim + 50 ml of isotonic saline at 24-hour intervals.

At the beginning of treatment, there was not a very important fluid accumulation (05 mm), and during the four days of treatment, we did not observe fluid retention.

A resurgence of liquid was found during diestrus.

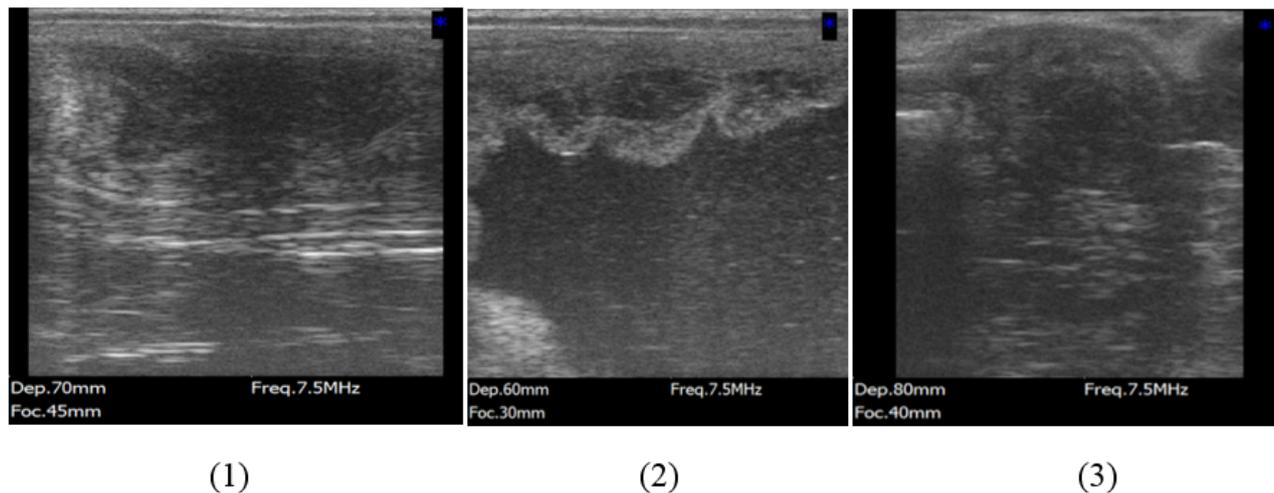
Cytology, during the first post-treatment estrus, the smear is rich in lymphocyte cells, and low in endometrial cells.

**Interpretation:** According to the results, we observed that the three protocols have not yielded positive results.

**Lot N° 02:** Results ultrasound during and after intrauterine infusions of honey 70% solution:

Depending on the results, we noticed a significant increase in the volume of fluids and uterine echogenicity 24 hours after the first infusion of honey in two mares (DHAYA and FATIA), and the other two mares (BOSRA and TIFLETTE) do not presented an increase of uterine fluids but strong echogenicity was found (Table 2).

There is a gradual disappearance of the liquid until no day eight after the third infusion of honey in the DHAYA mare, 24 hours after the second infusion in the BOSRA mare, three days after the third infusion in the mare FATIA and three days after the second infusion in the TIFLETTE mare (Figure 1).

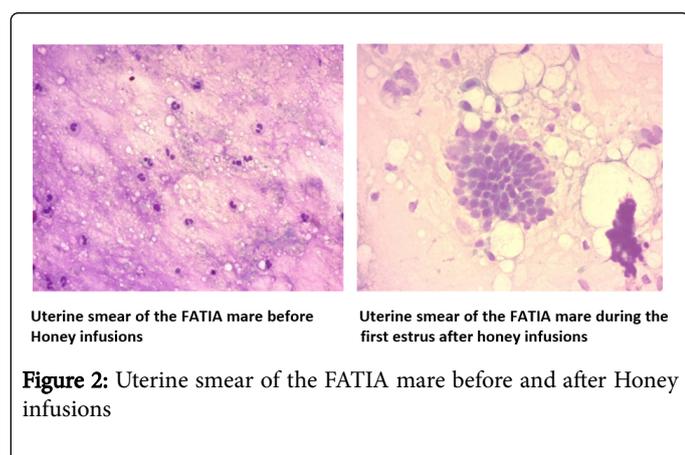


**Figure 1:** (1) Ultrasound image of endometritis the right horn of the DHAYA mare. (2) Ultrasound image of the right horn of the mare DHAYA 24 hours after the first infusion of honey. (3) Ultrasound image of the right horn of the mare DHAYA after treatment with honey.

### Diagnosis post-treatment

		DHAYA	BOSRA	FATIA	TIFLETTE
ultrasound diagnosis	During diestrus	No liquid	No liquid	No liquid	No liquid
	During the first estrus	Heat uterus, endometrial well edematous folds	Heat uterus	Anechoic fluid: the body and right horn	Heat uterus: the two horns. Anechoic liquid in the body
cytologic diagnosis during the first estrus		Rich in endometrial cells, disappearance of PNN, yeasts.	Unrealized following a traumatization of the mare.	Rich in endometrial cells, disappearance of PNN and lymphocyte cells	Smear rich PNN.

**Table 2:** Post-treatment diagnosis.



**Figure 2:** Uterine smear of the FATIA mare before and after Honey infusions

### Fertility control

According to the results, only two mares were diagnosed with positive pregnancy, but they showed embryonic mortality after 45 days in the DHAYA mare, and 21 days in the mare TIFLETTE.

### Discussion

According to the results obtained, we observed that the three protocols have not yielded positive results, this failure can be explained by:

- **The choice of treatment is random:** Do not correspond to the nature of endometritis (bacterial, fungal or noninfectious). There is no standard protocol: time, number of injections, and the number of intrauterine infusions. - With respect to intrauterine infusions of antibiotics, there is not a suitable choice of antibiotic used, safety on the endometrium and the dose administered.
- Operation of the treatment cycle without confirmation of cure, which may contribute to the spread of infection through the standards.
- **Inadequate assessment of healing:** Never use the disappearance of uterine fluids during the treatment period as a healing criterion, according to the results, there was a recurrence of uterine fluids during the first post-treatment diestrus.
- The protocols are incomplete because they do not cover all the objectives of the treatment of endometritis.

We noted that the infusion of honey has been accompanied by an increase in the volume of uterine fluids, which explains the osmolar effect of honey that has a liquid into the uterine call light from the

blood supply of endometrium, which promotes good blood circulation which is in stasis during inflammation. This has been demonstrated by Vaillancourt in 2009, that honey should also promote drainage of endometrial glands.

Of the four mares treated with honey, two did not show an increase in the volume of uterine fluid 24 hours after the first infusion of honey, which may be explained by rapid drainage of liquids.

After the first infusion honey, echogenicity uterine liquid has been increased in parallel with the volume of liquid, which confirms that the honey has caused a drainage liquid and inflammatory cells that are trapped in the blood vessels and endometrial glands due to congestion caused by inflammation.

A mare presented a total disappearance of uterine fluid 24 hours after the second infusion of honey, a mare with a maximum of 8 days after the third infusion honey. This disappearance of uterine fluids explains that honey has stimulated blood circulation and lymphatic drainage.

The two mares which showed high echogenicity of uterine fluids prior to treatment, drainage of uterine fluids took a little time compared to the other two mares that had echogenicity that do not exceed grade 1, which confirms the severity of inflammation in these mares. Even after the first infusion honey, in these mares, echogenicity uterine liquid was increased compared to other mares.

These findings have enabled us to confirm that during endometritis, there is a significant stasis of blood vascularization of the endometrium, which must be taken into consideration in the treatment of endometritis.

In four mares, there was an absence of uterine fluids during post-treatment diestrus with the uterus return to its normal size, it is a healing clinically to rule out the presence of subclinical inflammatory process we realized cytology during the first estrus after treatment, we found interesting results for both mares (DHAYA and FATIA), a total disappearance of inflammatory cells, fungi and richness in endometrial cells.

The wealth of uterine smear endometrial cells can be explained by the mitogenic effect of honey and the effect of progesterone during the period of rest (diestrus) (Figure 2).

At the ultrasound examination during the first post-treatment estrus, there was a marked improvement in the uterus of the two mares (DHAYA, BOSRA) which took the characteristic form of heat.

The TIFLETTE mare presented a retention uterine liquid with a rich smear neutrophils during estrus post-treatment, which is explained by a re-contamination of the uterus by the feces and urine during estrus following defective conformation of the vulva and perineum, this mare need surgical correction to prevent recurrences.

The use of honey diluted to 70%: - To facilitate its infusion - This dilution was tested for its safety on the endometrium by Vaillancourt in 2009; we have respected the basics of pharmacology, which is not to use treatment before testing its safety on the body.

The number of infusions of honey: Given the chronic endometritis four mares, and since this is the first test, we proposed a protocol of three intrauterine infusions at 24-hour intervals during estrus.

The infertility problems may be related to several factors but the most important is the age of the mare, the fertility decline gradually from the age of 12-13 years and the early embryonic mortality increases with age (14,15). Thus, during the breeding season 2015, we recorded several cases of embryonic mortality and not design, which were accompanied by a sudden change in the supply of mares, which can play an important role in infertility problems.

## Conclusion

The treatment of endometritis by intrauterine infusions of honey seems to be promising for improving the uterine drainage, liquid resorption, cytology improved post-treatment samples. Following these results, honey can be an alternative best to fight against the problems of antibiotic resistance and to minimize the cost of treatment.

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