

## **PERSISTENT MATING INDUCED ENDOMETRITIS (PMIE): AN EMERGING REPRODUCTIVE DISEASE IN THE MARE – ACUPUNCTURE INTERPRETATION AND THERAPY**

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### **Abstract**

Persistent Mating Induced Endometritis (PMIE) is a pathological condition of mares incapable of clearing the transient physiological inflammatory reaction produced in the uterus after artificial insemination. Fifty nine (59) estrus cycles of mares presenting this condition were randomly treated with Acupuncture (n=21), Oxytocin (n=25) or Placebo (n=13). The quantity of fluid was reduced in all group but Oxytocin reduced the amount of uterine fluid more than Placebo ( $p<0,01$ ) and Acupuncture more than Oxytocin and Placebo ( $p<0,01$ ). Both the mares treated with acupuncture and Oxytocin showed an increased pregnancy rate compared to Placebo mares though Acupuncture showed a significant increase of the pregnancy rates compared with Oxytocin (76,2 vs 20,0%). In our study, the treatment with acupuncture was able to reduce fluid in uterus and to increase pregnancy rates more than the other two groups ( $p<0.01$ ).

### **Introduction**

Mares develop a transient uterine inflammatory reaction to semen introduced during either natural cover or artificial insemination (Troedsson 2001). This inflammatory reaction is considered a normal physiological event after mating capable to remove excessive spermatozoa, seminal plasma and contaminants from the uterus before the embryo enters the uterus (Troedsson 2006). The condition of persistent mating induced endometritis (PMIE) occurs when the inflammation is not cleared within 24 and 36 hours of mating. In approximately 15% of broodmares, the system fails and the initial physiologic inflammation becomes a pathologic condition, with a detrimental effect on fertility (Zent 1998). Western medicine explanation for impaired uterine clearance refers to reduced myometrial activity in response to inflammation (Troedsson 1993), failure of the cervix to relax during estrus or insufficient lymphatic drainage. Mares can also be susceptible to PMIE due to poor perineal conformation or greater uterine mucus production during estrus. Myometrial activity was extensively investigated but the mechanisms able to impair contractility are still not completely understood. The effect of nitric oxide, which mediates smooth muscle relaxation, in delaying uterine clearance has been proposed (Alghamdi 2005).

TCM interpretation of endometritis refers instead to a variety of patterns that can occur in the female. Endometritis can be caused by a downward infusion of Cold and Dampness, Liver and Kidney deficiency, Qi-Xue deficiency or a stasis in Qi- Xue. Cold and Dampness affects primarily the lower burner, can cause presence of clear watery discharge from the vagina and is also noticeable with enteric symptoms and edematous limbs. Liver deficiency and Kidney deficiency can provoke general weakness, depression, exhaustion, lumbar pain and frequent urination. Stasis of Qi, which can eventually lead to Xue stagnation, appears with dry stools, constipation, fullness of thorax and abdomen and the mare shows restlessness and agitation. Mares are usually treated prior to insemination in order to prepare the mares to a fertile mating season. Therefore, the mare's uterus would be able to eliminate the excessive fluid in the uterus. Persistency of uterine fluid can create an inhospitable environment for the embryo when it descends into the uterus. Also, bacteria may find favorable growing conditions in mares with persistent inflammation. If the inflammation

becomes chronic, fibrosis may also develop and lead to scar tissue which will be definitely detrimental to the mare fertility.

Western medicine has proposed a wide variety of treatment aiming at controlling PMIE in susceptible mares, but no single treatment has been widely accepted as efficacious and they have varied considerably among practitioners over the years. Ecbolics, oxytocin or the long lasting carbetocin and prostaglandin are probably the most commonly employed alone or in association with uterine lavage with isotonic saline. Dexamethasone administered at breeding time was proposed as a safe and effective modulator of PMIE in susceptible mares (Bucca 2008) as well as treatment with NSAIDs (Rojer 2010). Administration of immune stimulants has also been promoted in recent years (Barton W.R. 2007). Mares can manifest lack of uterine clearing regardless the treatment performed maybe due to inappropriate uterine contractions or continuous formation of fluid because of inflammation. TCM was therefore taken in consideration by previous authors; Liu proposed the use of electro-acupuncture to increase uterine contractility in mares with delayed uterine clearance (Liu 2008) and Le Blanc suggested positive results with acupuncture for the treatment of retained fluid (LeBlanc 2006). Our study aimed to provide acupuncture treatment for the control of Persistent Mating Induced Endometritis (PMIE). Its efficacy was also compared to the treatment with oxytocin and with a placebo treatment.

### **Materials and methods**

The experimental design was performed in three consecutive breeding seasons between March 2008 and July 2010. During estrus, all mares were examined daily by rectal palpation and transrectal ultrasonography (7.5 MHz rectal transducer- Sonosite Micromaxx) to monitor follicular growth, uterine fluid accumulation and uterine edema. Uterine swabs for bacteriology (*Equi-Vet*®, Kruuse, Marslev, Denmark) were collected if vaginitis- cervicitis signs were detected. Only mares with a negative bacterial swab were used for the study. Subsequently, when a follicle of 35 mm in diameter together with uterine edema was detected, ovulation was induced with 2500 IU human chorionic gonadotropin (Vetecor® Farma Mediterranea, San Just Desvern, Spain). Fresh semen from stallions of proved fertility was used for the artificial insemination in proximity of the ovulation. Mares were scanned for detection of ultrasonographically visible uterine fluid at 24 hours from AI. If fluid was detected, its maximum diameter was measured using electronic callipers and scored as score 1: up to 2.0 cm in diameter and anechogenic; score 2: 2.0–3.0 cm in diameter and anechogenic; score 3: 3.0-5.0 cm in diameter and hyperechogenic; score 4: >5 cm and hyperechogenic. Mares that presented ultrasonographic signs of PMIE were randomly divided into 3 groups of treatment: acupuncture, oxytocin (20 UI ev) and placebo (saline solution, 10 ml ev). Mares were re-evaluated with ultrasonography at 24 hours after treatment for detection of permanence of fluid. If fluid was still present, mares were scanned every 24 hours until clear. Pregnancy rates at 15 days after breeding were also assessed. Acupuncture groups mares followed the TCM diagnosis by palpation, pulses type and tongue colors were also recorded. A standard protocol of acupoints for every mare was used using the acupoints *Baihuanshu*, *Yao Baihui* (acupuncture and moxibustion), *Taixi*. In addition, the acupoints *Pishu*, *Shenshu*, *Guanyuan*, *Guanyuanshu*, *Geshu*, *Gongsun*, *Huangmen*, *Huiyang*, *Jingming*, *Mingmen*, *Neiguan*, *Sanjiaoshu*, *Tai Yang*, *Zu Sanli* were added to the basic treatment depending on the condition of the animal. Only one treatment was provided and points were stimulated with acupuncture needles of 0.30 x 40 mm, 0.30 x 25 mm e 0.30 x 70 mm (Hwato®, Cloud & Dragon®, Huanqiu®, Cina). Two mares received acupuncture with 23G, 0.6 x 25 mm needles.

## Statistical analysis

Statistical analysis was performed with the program Stat Soft Inc, Tulsa, USA for Windows. All

data were normally distributed (TD student analysis). Differences between fluid time scores (0- 24

hours) were compared by ANOVA and with post hoc Tukey's HSD test. A p-value <0.01 was considered significant. All values are given as means  $\pm$  SE. Pregnancy data and comparison between endometritis syndromes were analyzed with Chi-square analysis and expressed as %.

## Results

During the three breeding seasons from March 2008 to July 2010, fifty nine (59) estrus cycles of thirty seven (37) mares were examined when signs of heat were manifested: thirty seven (37) estrous cycle displayed normal estrus characteristics while twenty-two (22) showed accumulation of fluid during estrus prior to insemination. Ultrasound scan within 24 hours of A.I. displayed evidence of PMIE signs which were scored as follows: score 1(n=20), score 2 (n=20); score 3 (n=16) and score 4 (n=3). Estrous cycles were randomly divided as follows n=21 were treated with Acupuncture, n=25 were treated with oxytocin (20 UI ev) and n=13 were treated with placebo (saline solution, 10 ml ev). All mares in the study had negative bacterial swabs. Among the 21 cycles treated with acupuncture, the acupuncture diagnosis showed that thirteen (13) of them manifested signs of Liver and Kidney deficiency; four (4) showed Qi and Blood Stagnation; three (3) of them displayed Damp Cold pattern. Both acupuncture and oxytocin groups significantly reduced the amount of fluid compared to placebo ( $p < 0,01$ ) within 24 hours of treatment but acupuncture treatment was more effective in reducing the amount of fluid respect all groups ( $p < 0,01$ ). Permanency of fluid for more than 24 hours after treatment was observed in one case (4%) in the acupuncture group cycles, in fourteen cases (56%) in the oxytocin group cycles and in eleven cases (84%) of the placebo group cycles. Nevertheless, the placebo group demonstrated statistically significant reduction of fluid ( $p < 0.05$ ) within 24 hours from the insemination. Both the mares in the acupuncture and in the oxytocin groups showed an increased pregnancy rate compared to the placebo group (76,2 % and 20,0 % vs 15,4 %). Acupuncture showed a significant increase of the pregnancy rates compared with oxytocin (76,2 vs 20,0%) ( $p < 0.01$ ). Oxytocin group didn't show a statistically significant increase in pregnancy rates compared to placebo group (20.0 % vs 15.4 %) ( $p > 0.05$ ). Mares presenting a Liver and Kidney deficiency and Damp- cold patterns showed a statistically significant increase in pregnancy rate compared to stagnation of Qi and Xue group ( $p < 0.05$ ).

## Discussion

In our study, which aimed to prove the efficacy of acupuncture treatment on PMIE, the intrauterine fluid reduction was more evident in the acupuncture treatment group compared to both oxytocin and control group. Oxytocin is dose dependant and tetanic contractions can occur if more than 25 IU of oxytocin are administered, leading to retention of uterine fluid (Maischberger 2008); in addition, ecbolics may not be effective in mares with impaired uterine clearance due to a possible defect in PGF2 $\alpha$  release at the oxytocin receptor or post-receptor level (Nikolakopoulos 2000). In our study tough we didn't use more than 20 IU of oxytocin, 84% of the mares showed retention of fluid after oxytocin treatment for more than 24 hours. In contrast, acupuncture was able to significantly reduce uterine fluid and increase pregnancy rate. The reduction of the fluid may be due to a complex

mechanism of regulations. As known, acupuncture can modulate the release of endogenous opioid which can directly reduce uterine contractility (Faletti 1992) or indirectly by modulating the release of potent endothelium-dependent vasodilator calcitonin gene related peptide (CGRP) and substance P at the spinal cord level (Collin 1993). These can be found in the myometrium smooth muscle throughout pregnancy and are involved in the regulation of smooth muscle activity in the female genital tract. CGRP is a potent inhibitor of spontaneous contractile activity in the oviduct and uterus, while SP induces contraction in human oviduct and uterus (Samuelson 1985). Interestingly, the expression of these neuropeptides in L<sub>6</sub>-S<sub>1</sub> nerves dorsal root ganglia, exactly where Yao Baihui acupoint is located, seems to be directly or indirectly influenced by estrogens and progesterone (Collins 2002). In our opinion, acupuncture is therefore able to modulate uterine contractions and this action may be also mediated by estrogens production which regulates the liberation of vasoactive substances in the uterus. Adequate research is therefore required for a better understanding of these correspondences and the hormonal involvement after acupuncture treatment. Prostaglandin production after acupuncture treatment is controversial: acupuncture showed to decrease prostaglandin production in primary dysmenorrheal human patients (Shi 2011) but demonstrated to be effectively increasing prostaglandins in secondary dysmenorrheal patients (Jin 2009). This indicates the balancing action of acupuncture and a possible receptor involvement. Our understanding of PMIE under TCM concepts describes it as a uterine Qi deficiency (Qi xu) with concomitant uterine fluid accumulation (tan). Our therapy was addressed to tonify uterine Qi and to remove Tan, therefore to promote embryonic implant. The protocol of points in our study was chosen accordingly to a basic and repeated acupuncture treatment composed of the acupoints *Baihuanshu*, *Yao Baihui* and *Taixi*. These points are commonly used in reproduction problems. *Baihuanshu* has a direct action in stimulating activity on the cervix and on the uterus for facilitating the elimination of fluid. *Yao Baihui*- a hundred meetings was warmed with moxa and was chosen for its direct yang influence on the uterus. *Taixi* was used to tonify Kidney Yin and Yang and to strengthen the kidney ability to receive Qi for responding at the Qi uterine deficiency (Longo 2011). Other points were also added to the previous protocol and used accordingly to each individual case: *Pishu* was chosen when signs of damp were present such as pale tongue or edema in the rear limbs, *Shenshu* was chosen accordingly to his function of dispersing dampness and tonify kidney Yin, *Guanyuanshu* were used in order to regulate the Lower Burner and to remove obstructions of the channel, *Yinlingquan* was chosen due to its immune stimulating function and its beneficial function on the Spleen action of transportation and transformation and of dampness dispersion, *Zu Sanli* was chosen for its tonifying action on Blood and Qi and for dispersing cold and dampness, *Guanyuan* was chosen for its strong action of tonification of the Kidneys function of dominating water, *Geshu* for its tonification action on blood, *Gongsun* and its coupled point, *Neiguan* were used for the opening action on the Chong mai for its direct action on the uterus, *Huangmen* was chosen for its dispersing functions, *Huiyang* was used for its action on lower orifices, *Jingming* for its direct stimulating action on the pineal gland, *Mingmen* was chosen for its influence in the water metabolism and when signs of cold were present, *Sanjiaoshu* for its regulating water function of the Triple Heater (Longo 2004). The treatment was provided once only to avoid excessive interference with uterine contraction at ovulation. The increased pregnancy rate in the acupuncture group compared to other groups (76,2 % vs 20,0 % vs 15,4 %) may also be imputed to the beneficial effect of the liberation of endogenous peptides on the animal wellbeing. Oxytocin was able to increase pregnancy rates compared to the control group (20,0 % vs 15,4 %) which highlights the ecobolics stimulating effect on uterine clearance, tough in our study the pregnancy rate of oxytocin treated mares wasn't statistically significant compared to the placebo group. Oxytocin efficacy is also dose dependent and some mares may not respond to ecobolic stimulation if their uterine contractility is impaired (Nikolakopoulos 2000; Maischberger 2008). Some differences in pregnancy rates can also be found within the acupuncture group. Interestingly, our data show that the mares presenting a Liver and Kidney deficiency and Damp- Cold pattern benefitted from the treatment of our chosen acupoints. These mares significantly increased their pregnancy rates

compared to the mares presenting stagnation of Qi and Xue ( $p < 0.05$ ). This shows that a condition like Qi and Xue deficiency is more severe and therefore may require more treatments in order to obtain a successful outcome. In our study the acupuncture treatment of PMIE symptoms associated with Liver and Kidney deficiency and Damp- Cold patterns in the mare appeared to be successful at reducing uterine fluid and increasing uterine Qi. The improvement in uterine clearance and in pregnancy rates obtained in our experiment are notable though similar studies should be applied to a bigger group of animals in order to achieve more consistent results.

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