The use of electrochemically activated saline as a uterine instillation in pony mares

C H Annandale[a*], M L Schulman[a] and R D Kirkpatrick[b]

ABSTRACT

Twelve pony mares were randomly assigned to either a control or a treatment group and inseminated with fresh, raw semen from a single stallion of known fertility in a cross-over trial design. Pregnancy was diagnosed by transrectal ultrasound 12–14 days post-ovulation and then terminated by administration of a luteolytic dose of cloprostenol. Treatment mares received a uterine instillation of 100 mℓ of electrochemically activated (ECA) saline 4–12 hours post-insemination. Control mares received no treatment post-insemination. Per cycle pregnancy rate was 58.3 % in the control group and 50 % in the treatment group. There was no statistical difference ($P = 1.000$) in pregnancy rate between the 2 groups. The principles of ECA and applications of ECA saline are discussed.

Key words: electrochemical, endometritis, fresh semen, mare, saline.

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INTRODUCTION

Endometritis in mares is of considerable clinical significance in equine practice. It has been cited[4] as the 3rd most common medical condition in mares. LeBlanc[7] classifies endometritis into 3 categories: 1) persistent mating-induced endometritis (PMIE), 2) chronic infectious endometritis and 3) sexually transmitted endometritis. In mares, both natural breeding and artificial insemination initiates a uterine inflammatory response[14]. Current thinking holds that transient uterine inflammation following breeding is a normal physiological process assisting evacuation of the uterus of bacteria, dead sperm and excess seminal plasma[20]. Normal mares can be expected to clear uterine inflammation within 12 hours post-breeding[6]. Persistence of inflammatory products occurs in mares with impaired uterine motility and function[15,19] and may lead to accumulation of fluid in the uterus and increased early embryonic death rates[18]. The latter consequence results in a lower overall pregnancy rate and hence reduction of persistent post-breeding uterine inflammation post-breeding could potentially yield considerable clinical and economic benefits.

Common therapeutic strategies for endometritis include: 1) intrauterine therapy *via* uterine lavage, irrigation and infusions of varying temperatures using saline, plasma and other solutions with or without the addition of antimicrobial agents (antibiotics and iodinate compounds), pH-altering compounds and other agents including colostrum, plasma and disinfectants[3,9,13,21,22]; 2) systemic ecbolics, principally oxytocin and prostaglandins[17]; and 3) systemic antimicrobials[16]. These therapeutic modalities are used alone or in various combinations. The combination of uterine lavage, antibiotic infusion and an ecbolic agent is possibly the most commonly applied strategy in current practice[17]. Some concern about antimicrobial resistance has led to numerous investigations into therapeutic modalities that can replace antimicrobial agents. Electrochemically activated saline holds promise in this regard[10].

Electrochemical activation (ECA) is a novel refinement of established electrolytic procedures for the electroactivation of aqueous solutions. The original reports claim it has applications in agriculture, dermatology, dressing and cleaning of wounds and disinfection of instruments[1,8]. During ECA of water, a dilute saline solution is 'activated' by passing through a cylindrical electrolytic cell in which the anode and cathode chambers are separated by a permeable membrane. Two separate streams of activated water are produced: 'anolyte' with a pH range of 2–9 and an oxidation-reduction potential (ORP) of +400 to +1200 mV and 'catholyte' with a pH of 12–13 and an ORP of about –900 mV. 'Anolyte' is an oxidising agent due to a mixture of free oxidising radicals and has an antimicrobial effect[12], while 'catholyte' is reducing with surfactant properties and is an antioxidant. Some of the oxidant species in 'anolyte' are ClO; ClO^-; HClO; OH^-; HO_2^-; H_2O_2; O_3; $S_2O_8^{2-}$ and $Cl_2O_6^{2-}$. The use of the oxidising solution 'Anolyte' as an antimicrobial agent is well established[11,12] and the solution has been extensively assessed for its mammalian toxicity profile without any adverse evidence of acute or chronic deviations from the norm[1,8]. 'Anolyte' may assist abolishment of the inflammatory process post-mating *via* its free oxidising radicals and hence contribute to the establishment of a favourable uterine environment. The effect of activated physiological saline on pregnancy rates of mares is as yet unknown.

The purpose of the present study was to determine and evaluate the effects of post-breeding intrauterine infusion of electrochemically activated saline on the per cycle pregnancy rate in pony mares.

MATERIALS AND METHODS

Animals

Twelve Nooitgedacht pony mares, an indigenous South African breed, were used. The mean age of the mares was 6.9 years (range: 3–20 years). Mares were fed grass hay and were on mixed pasture fields. They were all bred to a single Nooitgedacht stallion of known fertility as part of routine practical teaching of undergraduate students. The trial was conducted during the summer of 2005.

Experimental design

A randomised, prospective, cross-over model was used. At time of breeding, mares were randomly allocated to a treatment group ($n = 12$) or a control group ($n = 12$). Mares from the control group

[a]Section of Reproduction, Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.

[b]Radical Waters, PO Box 6482, Kyalami, 1685 South Africa.

*Author for correspondence.
E-mail: henry.annandale@up.ac.za

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were re-allocated to the treatment group at the next breeding and *vice versa*.

Breeding management

The 12 mares selected for the trial were evaluated for breeding soundness during dioestrus prior to breeding. Breeding soundness evaluation included transrectal ultrasound examination, using an Aloka SSD-500 ultrasound machine and a 5 MHz linear array transducer (Axim (Pty) Ltd., Midrand, South Africa) and guarded endometrial swabs (Minitüb, Tieffenbach, Germany) for cytology. Any cytology sample positive for signs of inflammation was also submitted for microbiological culture.

All mares were teased daily. Mares showing positive signs of oestrus behaviour were identified and their genital tracts evaluated by transrectal palpation and ultrasonographic examination. Mares were bred when the predicted pre-ovulatory follicle had reached a minimum diameter of 35 mm. Semen was collected by artificial vagina. Aliquots from each ejaculate were evaluated for sperm concentration, individual progressive motility in a modified Kenney's motility diluent at 35 °C and normal sperm morphology using eosin-nigrosin-stained smears. Mares in both groups were inseminated into the uterine body with fresh, raw semen within 15 minutes of collection. The dose for insemination was standardised at 500 million progressively motile sperm. The insemination volume ranged from 10 to 20 mℓ. Mares were only inseminated once per cycle.

At the time of insemination mares were randomly allocated to either the control or treatment group and 1500 IU of hCG (Chorulon®, Intervet, Isando, South Africa) was administered by intravenous injection to all mares to induce ovulation. All mares were examined ultrasonographically until ovulation was verified. Any intraluminal fluid accumulation was also recorded. Mares in the treatment group were additionally examined by transrectal ultrasound 4–12 hours after insemination and the presence of intraluminal uterine fluid recorded. At this time, mares in the treatment group received an intra-uterine infusion of 100 mℓ of electrochemically activated saline at ambient temperature. The solution was generated less than 24 hours previously and deposited directly into the uterine body using an appropriate sterile plastic pipette (Minitüb, Tieffenbach, Germany), preceded by standard aseptic preparation of the perineum. No additional intrauterine or parenteral treatments were given to any of the mares during the course of the trial, regardless of either the accumulation of fluid or the treatment group allocation.

Pregnancy was diagnosed by transrectal ultrasound examination of the uterus 12–14 days after ovulation. After recording of pregnancy status, a luteolytic dose of cloprostenol (Estrumate®, Schering-Plough, Isando, South Africa) was administered by intramuscular injection and the mare was returned to the teasing programme. The same procedure was repeated during the course of the ensuing oestrus.

Statistical analysis

The pregnancy results in the groups were compared with the Fischer's exact test to account for the small numbers of subjects in each group. A 2-sided *P*-value of 0.1 was taken as statistically significant.

Preparation of ECA saline

The oxidant 'anolyte' solution was produced in an electrochemical cell with a current of 5–7 A and a voltage of 24 V, yielding electric field intensity at the interface between the electrode surface and electrolyte of about 10^5 V/cm. An influent salt solution (2.5 g/ℓ NaCl) was electrolysed in the denominated chamber of the electrochemical cell. The resultant oxidant ECA saline was generated to have a pH of 7.4, and was bottled in a sterile container and delivered for utilisation in the trial within 24 hours of production.

RESULTS

The pregnancy rates for the mares in the 2 groups are presented in Table 1. The pregnancy rates were similar for the 2 groups and no statistically significant difference could be demonstrated (P = 1.000). In the control group, 7 pregnancies (n = 12; 58.3 %) were diagnosed. In the treatment group 6 pregnancies (n = 12; 50 %) were diagnosed. The cumulative pregnancy rate was 54.2 %. Four mares failed to conceive during the course of the trial. These 4 mares included 1 mare that consistently had post-breeding uterine fluid accumulation. None of the other mares had any post-breeding fluid accumulation.

DISCUSSION

Our results demonstrate no detrimental effect of post-breeding instillation of electrochemically activated saline upon the per cycle pregnancy rates of pony mares. The cumulative pregnancy rate and the per cycle pregnancy rate for the treatment and the control group in this study is lower than published reports for fertility with fresh semen AI[5]. This can in part be explained by the inclusion of 4 mares that failed to conceive, irrespective of treatment group allocation, over the course of the trial. Included among these 4 mares was 1 mare that had suffered a traumatic injury to the hock joint and underwent arthroscopy 1 week post-ovulation. It is possible that the anaesthetic and surgical procedure could have influenced her pregnancy status, but this remains undetermined. Accumulation of fluid post-breeding can account for failure to conceive in the mare in which it occurred, but the reason(s) for pregnancy failure in the other 2 mares were not determined. Removal of the 4 mares that failed to conceive during the course of the trial yields pregnancy rates of 87.5 % (7/8) and 75 % (6/8) for the control and treatment groups of mares, respectively. This conforms favourably to published results.

While numerous studies have looked at post-breeding instillations as a treatment for PMIE, the focus of most of these studies have been on the effect of the agent on the endometrial histology or the bacteriological population in the uterus[2,13]. The study reported here focused exclusively on pregnancy rate as the outcome after post-breeding instillation. The clinical efficacy of ECA saline as an irrigation agent in mares with endometritis and its antimicrobial effect against equine uterine pathogens associated with endometritis warrants further investigation.

Table 1: **Pregnancy rates for mares in control and treatment groups.**

Group	Pregnancy rate
Control	58.3 % (7/12)
Treatment	50.0 % (6/12)

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