Effect of Adding Autologous Plasma to an Intrauterine Antibiotic Therapy after Breeding on Pregnancy Rates in Mares

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ABSTRACT

During the 1988 breeding season, 1341 breeding cycles were evaluated on 905 Thoroughbred mares (141 maidens [M], 204 barren [B], 560 lactating [L]) aged 2-22 yr. The effect of the following variables on early pregnancy and foal production were analyzed using a general linear model system: age, mare status, uterine culture and cytology, uterine biopsy score, Caslick's operation, treatment, cycle, and farm nutrition.

Mares were randomly allocated to one of three treatment groups. Group 1 mares were controls (40 M, 50 B, 140 L). Mares in groups 2 [51 M, 84 B, 210 L] and 3 [50 M, 70 B, 210 L] were infused once 12-36 h after breeding with either procaine penicillin, neomycin sulphate, and 20 ml sterile normal saline (group 2) or with procaine penicillin, neomycin sulphate, and 90 ml of autologous plasma (group 3). Lactating mares treated with plasma and antibiotics had a significantly higher (p < 0.05) early pregnancy rate per cycle (14-16 days postbreeding) than did control mares (group 1) and mares treated with antibiotics alone (group 2) (67 ± 4.8%, 53 ± 5.4%, 57 ± 4.4%, respectively). Neither antibiotics (group 2) nor plasma and antibiotics (group 3) affected the pregnancy rate per cycle in maiden mares. In the barren mares, results tended (p < 0.09) to show that autologous plasma and antibiotics (group 3) after breeding was more effective than no treatment (group 1, 75 ± 7.9% vs. 66 ± 6.1%) or antibiotics alone (group 2, 67 ± 5.7%). This study shows that the addition of autologous plasma to an antibiotic therapy used after breeding improves pregnancy rates per cycle for lactating and barren mares.

INTRODUCTION

Uterine infection is a common cause of subfertility in mares. During breeding, the uterus is frequently contaminated with bacteria [Dimmock, 1939; Kenney & Ganjam, 1975]. Most normal mares can clear this contamination within 96 h and remain free of infection before the embryo descends into the uterus [Hughes & Loy, 1969]. In young healthy mares the pregnancy rate in the oviducts is as high as 96% [Ball et al., 1986], and embryo recovery from young fertile mares is as high as 80% [Squires et al., 1982; Pascoe et al., 1985]. Older multiparous mares, however, often cannot clear uterine contamination completely, resulting in uterine infection [Peterson et al., 1969]. This is reflected in the general population of broodmares, in which Woods et al. [1987] reported early pregnancy rates per cycle of 50-60%, suggesting that up to 20% of embryos could be lost in the second week of pregnancy prior to detection with ultrasound 14-16 days after breeding.

The mechanisms to clear uterine infection are believed to be a combination of local uterine antibody-mediated immunity [Kenney & Khalfeel, 1975; Asbury et al., 1980; Mitchell et al., 1982; Williamson et al., 1983; Widders et al., 1984, 1985], cellular phagocytic elimination of bacteria [Asbury et al., 1982; Cheung et al., 1985; Liu et al., 1985; Asbury & Hansen, 1987], and physical clearance of products from the uterus following an infection [Evans et al., 1986; Troedsson & Liu, 1991]. Uterine secretions collected from mares susceptible to endometritis have significantly less opsoninizing function than uterine secretions collected from young healthy mares. Phagocytosis by polymorphonuclear neutrophils (PMN) is adversely affected by uterine secretions from susceptible mares [Troedsson et al., 1993a]. Intrauterine plasma therapy has been advocated as a pre- and postbreeding treatment in susceptible mares because it contains IgG, complement, and various proteins [Asbury, 1984]. Thus plasma may play a role in uterine defense by providing additional opsoninizing factors for phagocytosis by PMNs. Reports on the efficacy of intrauterine plasma infusions as a potential method of improving pregnancy rates are conflicting. Intrauterine plasma therapy has been...
shown not to change the endometrium [Colburn et al., 1987; Adams & Ginther, 1989]; however, it has been shown to clear Streptococcus zooepidemicus infection from the uterine lumen [Troedsson et al., 1992].

The purpose of this study was to determine whether the addition of autologous plasma to a standard broad-spectrum antibiotic therapy used after breeding would increase the pregnancy rate per cycle in maiden, barren, and foaling mares.

MATERIALS AND METHODS

A study was conducted during the 1988 breeding season on 905 Thoroughbred mares (141 maidens [M], 204 barren [B], 560 lactating [L]) aged 2–22 yr in Southeastern Queensland, Australia. The mares were maintained on 17 broodmare farms that were visited on alternate days for routine fertility management. All mares were maintained on natural grass and pasture crops (barley and oats in early spring, and lucerne and millet in late spring and summer) and were supplemented with grain and lucerne, oaten, or barley hay.

Maiden and barren mares were examined at the onset of the first detected estrous period and lactating mares at the first estrous period after foaling. All mares had a rectal and vaginoscopic examination, uterine culture, uterine cytology, and uterine biopsy performed on the first cycle, and again on subsequent cycles if they were not pregnant. Uterine biopsies were taken either 8–24 h before breeding or 12–36 h after breeding. Biopsies were given an endometrial score according to the Kenney grading system [Kenney, 1978].

On the first examination, mares were randomly allocated to one of three treatment groups. Group 1 mares (40 M, 50 B, 140 L) were controls. Group 2 mares (51 M, 84 B, 210 L) were infused once 12–36 h after breeding with $6 \times 10^6$ IU procaine penicillin (Norocillin SA; Heriot Agvet, Roseville, Victoria, Australia), 4 g neomycin sulphate (Neomycin Sulphate BP; Delta Veterinary Laboratories, Hornsby, NSW, Australia), and 20 ml sterile normal saline. Group 3 mares (50 M, 70 B, 210 L) were infused once 12–36 h after breeding with $6 \times 10^6$ IU procaine penicillin, 4 g neomycin sulphate, and 90 ml of autologous plasma. All mares were bred by natural service once per cycle and given hCG (2500 IU, i.v.) 12–24 h prior to service. The stallions were washed with either water or soap and water.

The first cycle for lactating mares was divided into three periods: foal heat (5–14 days after foaling); short cycle (16–27 days), which occurred when mares in foal heat were given prostaglandin $F_2 \alpha$ 6 days after ovulation; and 30-day heat (27–35 days), which was defined as the next natural heat period following foal heat.

Ultrasonography (Technicare 210, 5 Mhz; Pittman Moore, Englewood, CO) was used to determine pregnancy. When multiple pregnancies were located, manual embryo reduction was used (between 14–16 days postbreeding) to reduce the number of pregnancies to one [Pascoe et al., 1987]. The mares were scanned on 3 occasions: first at 14–16 days after breeding, second at 30 days after breeding, and third at 45 days after breeding. Early pregnancy rate per cycle was defined as the presence of one or more embryonic vesicles at 14–16 days after breeding divided by the number of mares mated each cycle for each group. The subsequent foaling rate per cycle was defined as the foals born in 1989 and recorded in the Australian Stud Book (Vol. 37, 1991; Australian Jockey Club, Randwick, NSW, Australia) divided by the number of mares mated each cycle for each group.

Plasma Preparation

During estrus, blood (600 ml) was collected from the jugular vein of each group 3 mare in a TUTA Pack (single blood collection pack; anticoagulants: citric acid and sodium citrate; Tuta Laboratories, Lane Cove, NSW, Australia) and chilled (6°C) for 12 h to allow settling of red cells. The plasma was poured off in 90-ml aliquots into sterile bottles and frozen ($-20^\circ$C). Sufficient plasma was stored to allow treatment for 3 breeding cycles.

Statistical Analysis

A total of 1341 breeding cycles were evaluated to observe the effect on early pregnancy rates per cycle and foal production per cycle of the following variables: age, mare status, uterine infection (based on culture and or cytology), Caslick's operation, treatment, cycle, farm nutrition, and biopsy score.

Data were analyzed by means of a general linear model system [SAS Institute, 1989]. The model included the main variables of mare status, cycle, age, treatment, Caslick's operation, farm nutrition, biopsy score, and cervical
**TABLE 1.** Early pregnancy rates for 560 lactating mares infused in utero postbreeding with plasma and antibiotics (group 3) or antibiotics alone (group 2), or left untreated (group 1).

<table>
<thead>
<tr>
<th>Mare cycle</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
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<tbody>
<tr>
<td></td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td><strong>1st cycle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foal heat*</td>
<td>53 ± 5.4'</td>
<td>80</td>
<td>57 ± 4.4'</td>
</tr>
<tr>
<td>Short cycle*</td>
<td>57 ± 4.4'</td>
<td>30</td>
<td>63 ± 4.2'</td>
</tr>
<tr>
<td>30-day heat*</td>
<td>57 ± 5.6'</td>
<td>30</td>
<td>64 ± 5.1'</td>
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<tr>
<td><strong>2nd cycle</strong></td>
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<tr>
<td>3rd cycle*</td>
<td>50 ± 4'</td>
<td>4</td>
<td>42 ± 6'</td>
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<td>4th cycle*</td>
<td>--</td>
<td>20</td>
<td>5</td>
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</tbody>
</table>

*Lactating mares bred between 5 and 14 days after foaling.
*Lactating mares given prostaglandin F₂α 6 days after ovulation on foal heat.
*Natural heat period following foal heat.
*Expressed as percent only due to insufficient mare numbers.
*Values within rows with same superscripts are different (p < 0.05).

RESULTS

In lactating mares, the use of autologous plasma and antibiotics (group 3) after breeding significantly (p < 0.05) increased the early pregnancy rate per cycle (Table 1) and the subsequent foaling rate per cycle (Table 2) compared to those of the control group (group 1) and the antibiotic group (group 2). The early embryonic loss rate per cycle, 6% (34 of 560; 14–46 days of pregnancy), or the fetal loss rate per cycle, 5.6% (31 of 526; 45 days to foaling) were not significantly different. Uterine biopsy score was not a useful indicator of pregnancy outcome as 96% of the lactating mares had a grade 2 endometrial score.

The use of antibiotics (group 2) or plasma and antibiotics (group 3) did not affect the early pregnancy rate or subsequent foaling rate in maiden mares. In the barren mares, treatment tended (p < 0.09) to show that autologous plasma and antibiotics (group 3) after breeding was more effective than no treatment (group 1; 75 ± 7.9% vs. 66 ± 6.1%) or antibiotics alone (group 2; 75 ± 7.9% vs. 67 ± 5.7%). Ninety-five percent (194 of 204) of the mares had been barren for only one year, and 98% of the mares biopsied had either grade 1 (5%) or grade 2 (93%) endometrial score.

**TABLE 2.** Foaling rates for 560 lactating mares infused in utero postbreeding with plasma and antibiotics (group 3) or antibiotics alone (group 2), or left untreated (group 1).*

<table>
<thead>
<tr>
<th>Mare cycle</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
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<tr>
<td></td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td><strong>1st cycle</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Foal heat*</td>
<td>46 ± 5.6'</td>
<td>80</td>
<td>49 ± 4.7'</td>
</tr>
<tr>
<td>Short cycle*</td>
<td>47 ± 4.8'</td>
<td>30</td>
<td>54 ± 4.1'</td>
</tr>
<tr>
<td>30-day heat*</td>
<td>49 ± 6.2'</td>
<td>30</td>
<td>57 ± 4.8'</td>
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<tr>
<td><strong>2nd cycle</strong></td>
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*Lactating mares bred between 5 and 14 days after foaling.
*Lactating mares given prostaglandin F₂α 6 days after ovulation on foal heat.
*Natural heat period following foal heat.
*Values within rows with same superscripts are different (p > 0.05).
*Data expressed as mean ± SEM.
DISCUSSION

From these results, it appears that the addition of autologous plasma to standard antibiotic therapy improves early pregnancy rates per cycle and subsequent foaling rates per cycle in lactating mares. For lactating mares, the early pregnancy rate per cycle (54–57%; group 1) was similar to pregnancy rates reported in commercial brood mares [Woods et al., 1987]. The mean pregnancy rate per cycle for the antibiotic treatment (group 2) tended to be higher. The addition of autologous plasma to the antibiotic treatment increased the pregnancy rate per cycle to 67% for foal heat breedings and 75–78% for breedings on other cycles (Table 1), which was higher than pregnancy rates (42.7%) previously reported [Baker et al., 1993].

Pregnancy rates per cycle in maiden mares were not improved by the use of antibiotics or plasma treatments. Maiden mares had an early pregnancy rate per cycle of 76 ± 6%, which was similar to reported pregnancy rates in young mares [Squires et al., 1982; Pascoe et al., 1985]. Intrauterine infusion of autologous plasma and antibiotics (group 3) after breeding in lactating mares was followed by pregnancy rate per cycle similar to the pregnancy rate per cycle in maiden mares.

Mares with a history of persistent endometritis that were treated unsuccessfully with antibiotics for repeated cycles did become pregnant after intrauterine treatment with autologous plasma [Asbury, 1984]. The uterus responds to contamination, semen, and infection by an influx of PMNs, antibodies, and complement. Mares susceptible to uterine infection secrete the same or enhanced concentrations of antibodies into the uterine lumen [Asbury et al., 1980; Mitchell et al., 1982]. Although it has been suggested that mares remain susceptible to infection due to dysfunctional PMNs [Cheung et al., 1985; Liu et al., 1985; Watson et al., 1987], a recent study demonstrated that in susceptible mares the ability of uterine PMNs to respond to chemotaxis and phagocytosis was fully functional as long as there was no contact with toxins from uterine secretions [Troedsson et al., 1993a]. Asbury [1984] proposed that the opsonins in plasma may be a key factor in clearing uterine infections. The addition of autologous plasma to uterine secretions of susceptible mares increased phagocytosis and chemotaxis by uterine-derived PMNs [Troedsson et al., 1993a], indicating that autologous plasma is capable of acting as both an opsonin and a chemoattractant.

In both resistant and susceptible mares, the concentration of IgG and C3 in uterine secretions decreases during the first 24 h following uterine infection, and in susceptible mares this decrease continues beyond 36 h [Troedsson et al., 1993b]. Thus, treating mares with autologous plasma 12–36 h after breeding appears to be an appropriate time for intrauterine infusion.

In summary, treatment of mares with antibiotics and autologous plasma after breeding significantly improved per-cycle pregnancy rates in lactating mares and barren mares to levels similar to those of normal maiden mares. The suggested mechanisms for this are that 1) plasma provides a bolus dose of fresh opsonins, which enhance chemotaxis and phagocytosis by PMNs, 2) the extra fluid volume dilutes uterine secretions containing toxic byproducts, and 3) the addition of antibiotics kills remaining bacteria.

ACKNOWLEDGMENTS

The author is pleased to acknowledge Dr. J. Gibson for reading the endometrial biopsies, T. Tran for statistical analysis, and the "Strange Angels" data entry service.

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