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A Preliminary Study of Uterine Derived Polymorphonuclear Cell Function in Mares with Chronic Uterine Infections

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Troedsson, M., C. Concha, S. Einarsson and O. Holmberg: A preliminary study of uterine derived polymorphonuclear cell function in mares with chronic uterine infections. Acta vet. scand. 1990, 31, 187–192. – From 6 mares with chronic uterine infection, polymorphonuclear neutrophils (PMNs) were obtained from the uterus. In order to recover an adequate number of viable PMNs, 0.1 % oyster glycogen was infused into the uterus as a mild irritant 12 h prior to the uterine flushing. Phagocytosis and chemotaxis of the uterine derived PMNs were determined. The supernatant from the uterine flushing was compared with autologous serum for its capacity as an opsonin and chemoatractant. There was a significant increase of both phagocytosis and chemotaxis when autologous serum was used compared with the supernatant from the uterine flushing.
This study indicates that autologous serum has a greater opsonizing capacity than uterine secretion from mares with chronic uterine infection. Since all mares conceived following this study, the use of oyster glycogen was not considered to have deleterious effect on the uterine mucosa.

horse; uterus; phagocytosis; chemotaxis.

Introduction

Chronic uterine infections is a common cause of subfertility in the mare. It is diagnosed on the basis of history, clinical examination, uterine culture, cytology and biopsy. Even if these mares are treated with appropriate antibiotics until negative on bacterial culture, the inflammation will not heal completely and the mares remain susceptible to uterine infection. A defect in the local uterine defense mechanism is thought to be responsible for this susceptibility (*Asbury et al.* 1984, *Cheung et al.* 1985, *Liu et al.* 1985).

Several studies of mares susceptible to chronic uterine infections, have shown a dysfunction of uterine polymorphonuclear neutrophils (PMNs) to migrate, phagocytize and kill ingested material, when compared to resistant mares (*Asbury et al.* 1982, *Cheung et al.* 1985, *Liu et al.* 1985). This functional defect could either be due to a dysfunction in the PMNs themselves or to an insufficient opsonisation in the uterus. Some encouraging results reported when susceptible mares were treated with local infusion of plasma in the uterus emphasized insufficient opsonisation as a possible cause of chronic uterine infections (*Asbury et al.* 1984). Though increased amount of immunoglobulin (IgG, IgA and IgM) has been found in uterine washings from mares susceptible to uterine infections when compared to resistant mares (*Asbury et al.* 1980, *Mitchell et*

al. 1982, *Williamson et al.* 1983), a lack of complement in uterine secretion was suggested to be responsible for the insufficient opsonisation in the uterus (*Asbury et al.* 1984). However, conflicting results have been reported (*Hansen & Asbury* 1987) and the exact mechanism behind the dysfunctional local uterine immune response in mares susceptible to chronic uterine infections remains unknown.

The purpose of this study was to develop a non-harmful method to derive cells associated with inflammatory reactions from the uterus in mares with chronic uterine infections. The opsonizing capacity and chemoattractant properties of the supernatant from the uterine flushing was compared with that of autologous serum.

Material and methods

This study included 6 mares (5 standardbred and 1 thoroughbred) with a history of infertility for at least the last 2 breeding seasons. The mares were sampled for uterine cytology, bacterial cultures and uterine biopsies. The uterine biopsies were divided into 3 categories as described by *Kenney* (1975) where grade 1 indicates normal uterine morphology and grade 3 indicates chronic inflammation and fibrosis. Blood samples were collected from the jugular vein, to determine the total and differential white blood cell counts.

Uterine washings

In order to stimulate PMN migration into the uterine lumen, 100 ml 0.1 % oyster glycogen, a mild irritant, was infused 12 h before the sampling. A total of 500 ml of a phosphate-buffered saline solution, pH 7.2 (PBS), containing 40 IU oxytocin, was flushed into the uterus using a modified Foley catheter. After a brief massage of the uterus through the rectum, the washings were allowed to flow into a sterile flask by gravity. The uterine washing was immediately transported to the laboratory and PMN function tests were performed within 2 h after each sampling.

The washings were centrifuged at 2000 rpm for 20 min and the cell pellets were resuspended in a smaller amount of PBS. Total number and cell viability was determined by the tryphan blue exclusion test (*Barta et al.* 1984). The total number of PMNs in uterine washings was determined by examining acridine orange stained cells in a fluorescence microscope (*Enright & Jeffers* 1984).

Chemiluminescence: An indirect measure of phagocytosis

The phagocytic capacity of the uterine derived PMNs was analyzed using chemiluminescence (*Washburn et al.* 1982). Zymosan (Sigma) was used as a phagozytizing substrate. The opsonic capacity of uterine larvage was compared with that of autologous serum. A solution containing 0.6 ml autologous serum and 0.2 ml PBS was added to 10 mg zymosan. In a parallel test 0.8 ml of the supernatant was added to 10 mg of zymosan. The resulting solutions were incubated in a 37°C water bath for 30 min. Opsonized zymosan particles were washed twice and resuspended to the original volume in PBS. One hundred µl of 2×10^6 PMNs/ml PBS, 500 µl PBS, 200 µl opsonized zymosan, in either serum or the supernatant from the uterine washing, and 200 µl (10^{-4}M) luminol was added to a disposable sample cuvette. Chemiluminescence generated by the PMN was measured in a Luminometer® (Model 1251, LKB, Sweden).

Chemotaxis

Chemotaxis was measured using a multiwell filter chemotactic chamber model described by *Axelsson et al.* (1981). Plastic microcen-

Table 1. Results of endometrial biopsies, cultures, cytology and the number of viable polymorphonuclear neutrophils (PMNs) obtained from uterine washings of 6 mares with uterine infections.

Mare no.	Biopsy grade*)	Bacterial culture	Cytology	Total no. of cells/ % viable PMNs
1	2	*Escherichia coli*	–	274×10^6 90 %
2	2	*Streptococcus zooepidemicus*	+	40×10^6 70 %
3	2	*Klebsiella species*	–	46×10^6 80 %
4	2	0	+	100×10^6 80 %
5	2	*Streptococcus zooepidemicus*	–	55×10^6 90 %
6	2	*Actinomyces pyogenes*	+	200×10^6 65 %

*) according to *Kenney* (1975).

trifuge tubes were placed in the wells of the lower compartment of the chambers. A total amount of 400 µl autologous serum or supernatant from the uterine washing was used as chemoatractant. Unstimulated migration was measured by adding 400 µl of a saline solution instead of a chemoatractant. Micro filters were placed over the wells and the top of the chambers was put in a place. A total of 4×10^5 PMNs was added to the upper chamber compartment, above the filters. After incubation in 30°C for 60 min, the filters were stained, removed from the chemotactic chambers and fixed on slides. Migration was determined in triplicate using the method described by *Zigmond & Hirsch* (1973) and expressed as the mean distance migration in 5 microscopic fields per filter.

Results

The results of cytology, bacteriological culture and endometrial biopsies are shown in Table 1. PMNs were present in large amounts in the uterine smears from mares no. 2, 4 and 6 while the rest of the mares only showed epithelial cells in the smears. All the mares except no. 4 tested positive on bacteriologic cultures. The endometrial biopsies categorized all the mares as grade 2. The total and differentiated white blood cell count from venous blood samples were within the normal range in all of the mares.

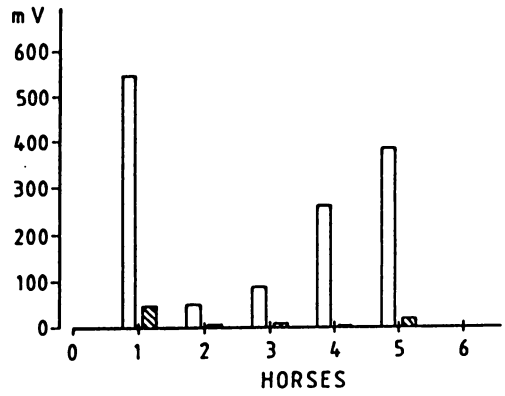

Figure 1. The phagocytic capacity, determined with chemiluminescence, of uterine derived polymorphonuclear neutrophils (PMNs) from mares with chronic uterine infections. Autologous serum (open bars) and the supernatant from uterine washings (striped bars) were used for opsonisation.

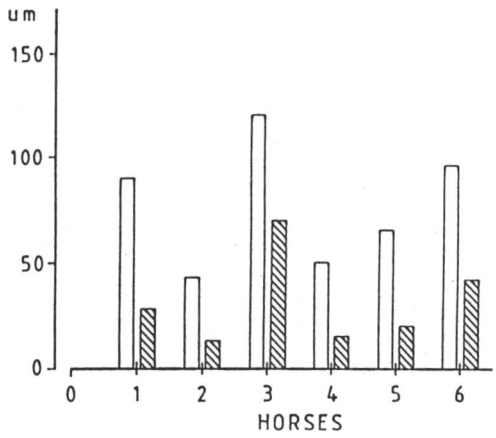

Figure 2. Chemotactic capacity of uterine derived polymorphonuclear neutrophils (PMNs) from mares with chronic uterine infections. Autologous serum (open bars) and the supernatant from uterine washings (striped bars) were used as chemoatractants.

The total number of cells derived from the uterine washings varied from 40×10^6 to 274×10^6 and 65–90 % of the cells were viable.

The capacity of the uterine derived PMNs to phagocytize zymosan after opsonisation with autologous serum and the supernatant from the uterine washing is shown in Fig. 1. There was a significant increase in phagocytosis when zymosan was opsonized in serum compared to the supernatant ($p < 0.05$, paired t-test). No results are available for the phagocytic capacity of mare no. 6, due to errors in processing the samples.

Chemotaxis is shown in Table 2. Chemotaxis of uterine derived PMNs increased in all the mares when serum was used as chemoatractant compared to the supernatant ($p < 0.01$, paired t-test).

Discussion

The method that was used to derive an adequate amount of viable PMNs from the uterus in the mares and test methods used to analyze phagocytosis (using chemiluminescence) and chemotaxis (using migration-chemotactic chambers) worked satisfactory. All the mares were treated for their uterine infections and became pregnant during the same breeding season. The pregnancies progressed normally during the 9 weeks the mares were monitored in all except 1 of the mares that resorbed her embryo around day 40. Hence our conclusion is that the technique used to derive PMNs from the uterus is safe and does not cause any damage to the endometrium.

The bactericidal capacity of uterine derived PMNs increased significantly after opsonisation of zymosan particals with serum compared to supernatant from the uterine washing. The supernatant was used as a model for the uterine secretion. Since the uterine washings was diluted with PBS, the autologous serum was also diluted (0.2 ml PBS to 0.6 ml serum) before opsonisation. However the dilutions of uterine washings were most likely higher and varied between samples. This could have affected the results but could probably not completely explain the large differences between serum and uterine washings observed.

The chemotactic capacity of uterine derived PMNs did also show significant differences depending on if serum or supernatant was used as chemoatractant.

This study indicates that serum has a positive effect on the function of PMNs with regard to both chemotaxis and bactericidal activity. The exact mechanism behind this effect is not known. Nevertheless, the fact that autologous serum has this positive effect on the local immune response in the uterus, is of great importance for the understanding of different treatment regimes of mares with chronic uterine infections. The mechanism behind serum's positive influence on chemotaxis and phagocytosis should be further investigated.

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Sammanfattning

En preliminär studie av cellfunktionen hos neutrofila granulocyter från livmodern hos ston med kronisk livmoderinfektion.

Från 6 ston med kronisk livmoderinfektion samlades neutrofila granulocyter från livmodern. För att få ett tillräckligt antal levande neutrofila granulocyter infunderades 0.1 %-ig oyster glycogen i livmodern, som ett svagt irritationsmedel 12 timmar före livmodersköljningen. Fagocytos och kemotaxis bestämdes hos neutrofila granulocyter från livmodern. Supernatanten från livmodersköljningen jämfördes med autologt serum avseende opsoniserande och kemotaktiska förmåga. Det var en signifikant ökning av fagocytos och kemotaxis när autologt serum användes jämfört med supernatanten från livmodersköljningen. Denna studie indikerar att serum har en positiv effekt på fagocy-

tos och kemotaxis hos neutrofila granulocyter från livmodern i jämförelse med livmodersekret från ston med kronisk livmoderinfektion. Då alla ston blev dräktiga efter detta experiment ansågs användningen av oyster glykogen inte ha någon skadlig effekt på livmoderns slemhinna.

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