Immunity in Foals

A report for the Rural Industries Research and Development Corporation

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Foreword

Given the importance of adequate passive immunity in the neonatal period, the intravenous administration of plasma has been recommended for foals with inadequate colostral immunity. However, the efficacy of plasma treatment has been poorly evaluated. Previous studies on neonatal equine immunity have focussed almost exclusively on the antibody (IgG) status of foals and, to date, little work has been completed evaluating other aspects of neonatal equine immunity, such as leucocyte function. Differences between the response of clinically normal foals with suboptimal passive transfer and those that are septicaemic have not been critically evaluated. Current recommendations are for the prophylactic treatment of all foals with suboptimal IgG concentrations. However, the necessity for, or benefits of, such treatment has not been evaluated.

These two aspects of neonatal immunity are assessed in this research. Firstly, the effect of delaying colostrum ingestion on the efficiency of intestinal absorption of colostral IgG was assessed in healthy foals. This part of the study provides information on the mechanism of gastrointestinal closure to the absorption of macromolecules. The effect of colostrum ingestion on neutrophil function was assessed using recently developed techniques. The second part of the study assessed the effect of plasma transfusion on IgG status and peripheral blood neutrophil function in healthy and septic neonates. The project is part of RIRDC’s programme aimed at establishing more effective treatments for foal diseases.

This report, a new addition to RIRDC’s diverse range of over 600 research publications, forms part of our Equine R&D program, which aims to assist in developing the Australian horse industry and enhance its export potential.

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Peter Core
Managing Director
Rural Industries Research and Development Corporation
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Clinical Pathology staff at Murdoch University Veterinary Hospital performed most of the haematology and biochemistry tests required during this project. Staff also made equipment and reagents available for the author to perform such tests when required outside normal laboratory hours.

Staff and students at Murdoch University Veterinary Hospital assisted in the observation and case management of foals included in these studies. This help was very much appreciated. David Lines provided Claudia with his considerable expertise in all areas of laboratory work and was particularly helpful in running single radial immunodiffusion tests on stored sera. We have very much valued his contribution. Professor John Penhale and Dr John Bolton also provided us with the benefits of their research experience. Dr Ian Robinson supplied statistical advice.

Staff of the Department of Immunology at Princess Margaret Hospital, particularly Ms Roslyn Hackshaw and Dr Richard Loh, provided us with access to their flow cytometer and were very tolerant of the additional work load we brought to their lab.

Meliss Henry and Norm Devine assisted greatly by supplying pregnant mares for inclusion in Part 1 of these studies. We thank them for this and also for their assistance in finding homes for mares and foals at the completion of the project.

Dr Charlie Stewart, of Saddleback Equine Hospital, and Dr Mark Young, of Serpentine Veterinary Clinic, assisted by providing blood samples from foals presented to their hospitals for plasma transfusion. We were very appreciative of their efforts in this regard and valued the opportunity to collaborate with our peers in private practice.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>FPT</td>
<td>failure of passive transfer (serum IgG 4 - 8 g/L)</td>
</tr>
<tr>
<td>pFPT</td>
<td>partial failure of passive transfer (serum IgG &lt; 4 g/L)</td>
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<tr>
<td>IgG</td>
<td>immunoglobulin G</td>
</tr>
<tr>
<td>SRID</td>
<td>single radial immunodiffusion</td>
</tr>
<tr>
<td>GCT</td>
<td>glutaraldehyde coagulation test</td>
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<tr>
<td>MUVH</td>
<td>Murdoch University Veterinary Teaching Hospital</td>
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<tr>
<td>SEH</td>
<td>Saddleback Equine Hospital</td>
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<tr>
<td>SVC</td>
<td>Serpentine Veterinary Clinic</td>
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<tr>
<td>IV</td>
<td>intravenous</td>
</tr>
<tr>
<td>DCFH-DA</td>
<td>dichlorofluorescin diacetate</td>
</tr>
<tr>
<td>DCFH</td>
<td>dichlorofluorescin</td>
</tr>
<tr>
<td>FITC</td>
<td>fluorescein isothiocyanate isomer I</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>PMA</td>
<td>phorbol myristate acetate</td>
</tr>
<tr>
<td>PBML</td>
<td>peripheral blood mixed leucocyte</td>
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Executive Summary

The effect of delaying the ingestion of colostrum for 12 hours after birth on the duration of intestinal permeability to immunoglobulins in foals was assessed by measuring the efficiency of absorption of colostral IgG in two groups of foals. Group 1 foals were given a glucose-electrolyte solution for 12 hours following birth and Group 2 foals were given a commercial milk substitute during this time period. No significant difference was found between groups, indicating that macromolecules do not mediate closure of the equine neonatal intestine to immunoglobulin absorption. These results are consistent with previous studies in calves, but contrast studies in piglets and lambs, suggesting that the process of closure is different in different species.

The efficiency of absorption of colostral IgG ingested 12 to 18 hours after birth was slightly higher for both groups than previously determined for foals less than 3 hours old. This suggests that closure had not progressed during the initial 12 hours post-partum and implies that factor(s) present in colostrum may mediate closure. The efficiency of absorption of IgG was not correlated with body weight, plasma volume, volume of colostrum ingested or the amount of IgG ingested. Despite the high efficiency of absorption, three (of 4) foals in each group had circulating serum IgG concentrations of less than 8 g/L. Where possible, colostrum should, therefore, be provided to foals less than 12 hours of age.

Colostral intake did not have a beneficial effect on foal neutrophil phagocytosis or oxidative burst activity. However, increased phagocytosis by adult peripheral blood neutrophils was observed when assayed with serum from foals that had ingested colostrum. It is likely that colostrum supplied opsonins, such as IgG and complement, which mediated this increase. Other factors present in colostrum may retard the ontogeny of other aspects of the neonatal immune system, resulting in no net improvement of function when cells are assayed in vitro with autologous serum. Cell function was not correlated with IgG concentration indicating that there are other mediators of neutrophil activity.

When foals cannot be provided with colostrum within 12 hours of birth, delayed administration of colostrum may still be beneficial. Foals treated in this way can be expected to absorb approximately 50% of IgG administered. A volume of 3 litres of good quality colostrum should raise circulating IgG levels to 4 – 8 g/L, which may represent adequate passive immunity for healthy foals in well managed environments and prevent the need for intravenous immune supplementation.

Preliminary studies on cell function yielded information important for the correct handling of cells. Studies on adult cells demonstrated that neutrophil function was preserved for up to 48 hours at room temperature (22°C) and at 37°C. Refrigeration at 4°C had a detrimental effect on cell function. Comparison of foal neutrophil function with that of adult cells demonstrated reduced phagocytosis, apparent for cells collected from foals less than 14 days of age. Foal neutrophils demonstrated an equivalent response when incubated with autologous or adult serum, suggesting that impaired function was due to an innate cellular deficiency. The oxidative burst activity of foal neutrophils was not significantly different to that of adult cells. However, the incorporation of foal or adult plasma into the assay caused a marked reduction in autofluorescence and oxidative response to soluble stimuli by foal cells, suggesting an inhibitory effect in response to a factor(s) in plasma. Plasma transfusion raised circulating IgG levels for healthy and septic foals with failure of passive transfer of immunity. Septic foals had lower serum IgG concentrations prior to treatment and responded less well to transfusion than healthy foals. Serum IgG levels for healthy foals were, on average, > 8 g/L 28 days after treatment.

Transfusion had no significant effect on neutrophil phagocytosis or oxidative burst response for cells collected from healthy foals. A transient reduction in phagocytosis was evident 12 hours following treatment and oxidative burst response was reduced 24 hours post-treatment. These differences were not significant. Conversely, neutrophils from septic foals demonstrated enhanced phagocytosis for 12 hours following treatment. The oxidative burst response of neutrophils from these foals was not
improved by transfusion and tended to be less than that of cells from healthy foals at all sampling times. Comparison of cell function between healthy and septic foals with failure of passive transfer and healthy foals with serum IgG concentrations greater than 8 g/L demonstrated no significant differences, although neutrophils from septic foals had lower oxidative burst activity.

The findings of the present study do not support plasma transfusion of healthy foals with failure of passive transfer of immunity. The clinical benefits observed from plasma transfusion of septic foals are likely to be due to improved neutrophil function. Such treatment is, therefore, recommended for such foals. Multiple treatments may be required due to the relatively short duration of effect.
Introduction

Neonatal mortality and morbidity due to septic conditions represent a source of considerable economic wastage within the equine breeding industry. Costs accrue due to mortality and the expense of treating affected animals. Treatment is often prolonged, with relatively poor long term survival rates (Koterba, 1987a; Brewer and Koterba, 1990). Foals which respond favourably to treatment may be subsequently unsuitable for their intended purpose due to the long term effects of the disease process.

A Texas study of 2468 foals demonstrated a crude mortality rate of 5% in foals up to 180 days of age (Cohen, 1994). Sepsis (excluding diarrhoea) was responsible for 43.3% of these deaths and has been identified as the most common cause of morbidity and mortality in the equine neonate (Carter and Martens, 1986). The true incidence of sepsis in these foals is likely to be higher than these studies suggest - diarrhoea was excluded because it is often of minimal clinical significance to the foal and the incidence usually does not correlate with passive immune status (Baldwin, et al., 1991; Raidal, 1996). However, diarrhoea may be the most common localising sign in septicemic foals (Koterba, 1987b). Other common manifestations of septic disease in foals include septicemia, pneumonia, haematogenous osteoarthritis (joint ill) and omphalophlebitis. Treatment of such conditions consists of prolonged antibiotic administration, supplementation of passive immunity and surgical drainage in some instances (especially septic arthritis). Treatment is expensive: the average daily cost for treating septic foals in a neonatal intensive care centre was US$310 (Koterba, 1987a), the average stay was greater than 7 days and ongoing treatment is usually required following discharge. Treatment failure may occur due to the rapidly progressive nature of most septic processes in foals and due to neutropaenia and impaired immune function in young horses (Furr, 1990). Retrospective studies have shown that only 45% of septic (blood-culture positive foals) are discharged from hospital (Brewer and Koterba, 1990). For foals with less than 4 g/L circulating IgG, survival is further reduced to less than 25%. Up to 45% of discharged foals may be subsequently euthanased for reasons related to the original illness (McGuire, et al., 1977). In an Australian study, only 37% of surviving foals treated for haematogenous osteomyelitis/septic arthritis subsequently raced, compared to over 60% of normal foals (Steel, et al., 1999).

Brewer and Koterba (1985) found that most neonatal infections are due to opportunistic organisms which inhabit the genital tract of the mare, the skin of normal horses and the environment. Their presence as pathogens suggests an immune deficiency in the affected animal. Although foals are capable of mounting a competent humoral (antibody) response at birth, the epitheliochorial placenta of the mare protects the foal in utero from contact with most infectious agents and antigens. The equine neonate is, therefore, incapable of the rapid anamnestic response characteristic of primed lymphocytes exposed to an antigen for a second time (Naylor, 1979). The weak primary response elicited on first exposure to most infectious agents is not sufficient to provide the foal with protection against disease. Inadequate passive transfer of immunity has been suggested as the most common predisposing cause of sepsis in foals from birth to 4 weeks of age (Rossdale and Leadon, 1975, Koterba, et al., 1984; Morris, et al., 1985; Barragry, 1991).

Although the association between low passive immune status and neonatal sepsis has been recognised in horses for some time (Crawford, et al., 1977; McGuire, et al., 1977), many terms and concepts integral to an understanding of passive immunity have been poorly defined or are contentious. For example, the volume of colostrum required to provide protective serum levels of IgG is not known (Clabough, 1988).

The efficiency of macromolecular absorption has been determined only once (Jeffcott, 1974a), in a study using PVP.60, a molecule analogous to IgG. The concentration of IgG which confers protection is also contentious. Perryman (1981) concluded that approximately 75% of foals with IgG concentrations of less than 2 g/L and 25% of foals with IgG concentrations between 2 - 4 g/L will require treatment for some infectious process. More recent studies (Baldwin, et al., 1989; Raidal, 1997) have suggested that IgG concentrations of less than 8 g/L correlate with reduced survival or an
increased incidence of septic disease. Most authors currently accept the following numerical classification of serum IgG concentrations to define the post-suckle passive immune status of the foal:

\[
\begin{align*}
< 4 \text{ g/L} & = \text{ failure of passive transfer (FPT)} \\
4 - 8 \text{ g/L} & = \text{ partial failure of passive transfer (pFPT)} \\
\geq 8 \text{ g/L} & = \text{ adequate passive transfer.}
\end{align*}
\]

The provision of an adequate volume of good quality colostrum within a short period after birth has been advocated for reducing the incidence of neonatal sepsis in foals. Determination of adequate serum IgG levels has been advocated for all foals (Raidal, 1996) and is now currently required by most insurance companies prior to the provision of mortality insurance for foals. To reduce the incidence of septic disease, foals detected to have failure of passive transfer (circulating IgG concentrations of less than 8 g/L) after intestinal closure should receive parenteral passive immune supplementation to increase circulating IgG levels (Varner and Valla, 1986; Raidal, 1996).

Plasma transfusion is, therefore, part of the routine treatment of septic foals and is now widely advocated for the treatment of healthy foals with FPT and pFPT. Increased IgG concentrations have been demonstrated following plasma treatment of sick and healthy foals (LeBlanc, 1988; White, 1989; Wilkins and Dewan-Mix, 1994). There has been little evaluation of the efficacy of such treatment on other aspects of neonatal immunity or on foal survival. Such treatment is expensive, invasive and carries some risk to the foal. Solid evidence of the benefit of such treatment to the foal would also be desirable.

There is currently no information in the veterinary literature of the effect of passive immune status on the development of cellular immunity in the foal or on the contribution of aspects of neonatal immunity, other than IgG concentration, to the incidence of neonatal sepsis. Our current understanding of neonatal susceptibility to disease and recommendations for treatment are based entirely on studies linking hypogammaglobulinaemia with increased incidence of disease. Recent epidemiological studies have questioned the association between circulating IgG levels and foal disease (Kohn, et al., 1989; Baldwin, et al., 1991). There is a paucity of information on the functional activity of foal phagocytic cells (LeBlanc, 1991). Functional deficiencies in peripheral blood neutrophils and pulmonary alveolar macrophages from foals have been noted in comparison to results obtained using cells from adult horses (Coignoul, et al., 1984; Bernoco, et al., 1987; Liu, et al., 1987; Fogarty and Leadon, 1987; LeBlanc and Pritchard, 1988; Wichtel, et al., 1991). The age at which these differences are no longer apparent has been poorly defined and the influence of passive immunity on the functional development of the equine neonatal immune system has also received little attention. The current study was planned to evaluate two important aspects of neonatal immunity:

**Part 1** - *Studies on the intestinal absorption of immunoglobulins by foals and the effects of passive immune status on neutrophil function.*

This part of the study was designed to evaluate the effect of withholding macromolecules (as seen in milk substitutes) on the duration of intestinal permeability to immunoglobulins in newborn foals. The influence of passive immune status was assessed by flow cytometric evaluation of neutrophil function during the first 48 hours following parturition.

**Part 2** - *Studies on neonatal foal neutrophil function and the effects of plasma transfusion on neutrophil function in healthy and septic foals.*

The second part of the study used recently developed techniques for the assessment of foal neutrophil function and was designed to evaluate and compare the response to plasma transfusion seen in healthy and septic equine neonates and specifically to assess the effects of treatment on neutrophil function.

These studies were conducted concurrently during the 1997/98 foaling season. Results are presented separately due to the large amount of information generated.
Objectives

The objectives of the current project were:

1. To determine whether the absorption of macromolecules by the equine neonate mediates the process of closure of intestinal cells to immunoglobulin uptake.
2. By application of this information, to further understanding of the mechanism of intestinal absorption on macromolecules and possibly to increase the potential for the oral supplementation of adequate passive immunity to foals.
3. To increase understanding of the development of septic diseases in equine neonates by determining the effect of age and passive immune status on the functional development of peripheral blood leucocytes in foals.
4. To determine whether the intravenous administration of plasma to healthy and ill foals has a beneficial effect on cell function. From this to re-evaluate the justification for plasma supplementation of clinically healthy foals with suboptimal IgG status.
5. To compare the response of healthy foals and clinically ill foals to plasma transfusion.

These objectives were met by conducting experiments in two parts. In the first, pregnant mares were purchased, their parturition attended and their foals prevented from suckling for 12 hours following birth. Colostrum was collected from the mare during this time for IgG quantitation and subsequent administration to foals. Foals were maintained on a glucose and electrolyte mixture (Group 1) or a milk substitute (Group 2) for the first 12 hours post-partum. The efficiency of colostral IgG absorption was determined for each foal and compared between groups to determine the effect of withholding macromolecules on the duration of gastrointestinal permeability to IgG. The effect of passive immune status on neutrophil function was assessed by evaluation of cell function prior to and following the ingestion of colostrum. Comparison of foal and adult cell function was performed in early studies aimed, in part, at validating experimental techniques that had not been previously applied to the evaluation of foal neutrophils. These results are presented in Part 2 of this report.

In the second part of the experiment, cell function prior to and following plasma transfusion was assessed from foals divided into 3 groups: Group 1 foals were healthy foals who were presented for plasma transfusion due to suboptimal passive immune status (serum IgG concentrations < 8 g/L), determined by IgG quantitation prior to or at the time of presentation. Included in this group were foals from the first part of the study which failed to achieve serum IgG concentrations greater than 8 g/L. Group 2 foals were septic foals presented to Murdoch University veterinary Hospital (MUVH), or other participating practices, for management of neonatal septic conditions. Results were compared with cell function of healthy foals with adequate colostral IgG (Group 3), presented to MUVH for treatment of non-septic conditions.

The satisfactory pursuit of these objectives was limited by a number of factors:

1. A satisfactory number of foals for inclusion of three treatment groups in Part 1 of the study, as originally planned, could not be obtained. For this reason, a “positive control group” (given colostrum from 0 to 12 hours post-partum) was not included in these studies.
2. Peripheral blood leucocytes could not be obtained from foals older than 2 – 4 weeks due to coagulation during the lysis procedure.
3. Foals presented to MUVH and participating practices were lost to follow up on discharge from hospital.

These factors are discussed in greater detail in the relevant sections of this report.
Part 1: Effect of withholding macromolecules on the duration of intestinal permeability to colostral IgG.

Introduction

Colostrum represents the best source of passive immunity for the neonatal foal because immunoglobulins are highly concentrated (Naylor, 1979; Higgins, et al., 1987; Koterba, et al., 1987) and because it contains substances which exert a local protective effect on the intestine and factors other than immunoglobulins which may enhance systemic immunity (Head and Beer, 1977; Sheldrake, 1989; LeBlanc, 1991). Proteins and macromolecules, such as immunoglobulins, are absorbed from colostrum without significant digestion and retain their biological activity (Jeffcott, 1974b). Uptake occurs uniformly throughout the entire small intestine (Jeffcott, 1973) via a complex mechanism involving specialised absorptive cells of the villous epithelium (Jeffcott, 1972). The intestinal absorption of colostral immunoglobulins occurs during a finite period after birth. Maximum absorption occurs soon after birth and progressively declines during the first hours of life until, by 24 hours, macromolecular absorption no longer occurs (Jeffcott, 1972). Many different estimates of the time of maximal absorption have been made. Kruse-Elliot and Wagner (1984) have suggested that maximal absorption occurs during the first 8 hours post-partum. Jeffcott (1974a) found that less than 1% of IgG administered at 20 hours reaches the systemic circulation. This progressive decline from maximal absorption soon after birth to complete cessation within 24 hours is termed closure. The linear decline in absorption efficiency from maximum recorded levels after birth to 20 hours of life suggests that cessation of intestinal permeability is a gradual process and not a sudden shutdown mechanism (Jeffcott, 1973).

It has been widely recommended that colostrum should be administered to foals within 12 hours of birth (Cowles, et al., 1983). As absorption may be dramatically reduced by 6 hours (Koterba, 1987b; Kruse-Elliot and Wagner, 1984; Clabough, 1988; White, 1989), it has been suggested that colostrum feeding should begin within 2 hours of birth for optimal uptake (LeBlanc, 1991). Good quality colostrum is not always available for administration within this time. If the administration of colostrum is delayed, immune supplementation by the intravenous administration of plasma or other sources of IgG is recommended. Such treatment is less desirable than the administration of colostrum because it is invasive, costly and has been associated with adverse reactions.

In piglets the absorption of immunoglobulins continues for much longer than expected if the ingestion of large molecules is not permitted. Absorptive capacity was retained for up to 106 hours after birth in piglets not fed milk products (Payne and Marsh, 1962), although the efficiency of absorption decreased with time. Vellenga, et al. (1988) found that the administration of a 5% glucose and electrolyte solution to neonatal piglets resulted in the retention of the ability to absorb macromolecules at 24 - 36 hours. The ability to absorb colostral proteins has also been preserved for 48 - 54 hours in starved lambs (Lecce and Morgan, 1962). Conversely, in calves, the period of permeability cannot be prolonged by withholding large molecules (McCoy, et al., 1970). This variation between species suggests that closure may be mediated by different processes in different species. The findings in cattle, for example, suggest that the absorptive process may be self-limiting, associated with a finite capacity for macromolecular uptake (Clarke and Hardy, 1969). In other species, closure may be mediated by other factors, such as a change in cell type from that lining the foetal gastrointestinal tract to that found in the adult (Naylor, 1979) or destruction of immunoglobulins due to increased digestion of protein in the stomach and small intestine as gut function improves after birth (Hill, 1956; Klaus, et al., 1969; Boyd and Boyd, 1987). Corticosteroids have been investigated as mediators of closure (Moog, 1953; Gillette and Filkins, 1966; Husband, et al., 1973; Logan and Irwin, 1977). However, Carrick, et al. (1987) were unable to demonstrate reduced absorption of colostral immunoglobulin in response to increased cortisol levels in neonatal foals. Similarly, Johnson and Stewart (1986) found that macromolecular absorption was actually enhanced in calves born prematurely to cows induced...
with corticosteroids when compared to calves born prematurely by caesarian section.

The possibility that the ingestion of large molecules may contribute to intestinal closure to immune protein absorption has not been investigated in the horse. If the absorption of macromolecules intrinsically mediates closure once the finite ability of the intestinal cells for absorption has been exceeded, closure could be delayed by withholding macromolecules. This then would prolong the period during which the intestinal absorption of colostral immunoglobulins could be achieved, resulting in more effective administration of colostrum to foals. The number of instances where plasma administration is subsequently required or where failure of passive transfer results due to inadequate absorption of colostral immunoglobulins would thus be reduced.

However, colostrum probably exerts a major controlling effect on the type of bacteria that establish in the digestive tract of the neonatal foal (Naylor, 1979). Delayed closure of the neonatal intestine may render the foal more susceptible to infection if the non-selective absorptive process permitted passage of bacteria across the intestine or facilitated the establishment of pathogenic bacteria within the neonatal intestine. Madigan (1997) has suggested that bacteria are able to pass from the neonatal intestine into the blood stream because of the non-selective absorptive process. He further contends that this problem is less likely to occur in foals that do not have failure of passive transfer because the finite capacity of intestinal cells to ingest large molecules is rapidly exceeded, thus rendering such foals less likely to absorb potential pathogens. If this hypothesis were true, rather than enhancing neonatal immunity, delayed ingestion of macromolecules would place the foal at increased risk for the development of septic disease. Absorption of non-immune macromolecules should be just as effective in achieving closure of the intestine and preventing the establishment of infection. Hence increased understanding of the mechanism of intestinal closure to the absorption of macromolecules is an important basis for decisions on foal management in the neonatal period.

**Methodology**

**Experimental Animals**

A total of 12 pregnant mares were purchased for inclusion in this part of the study. Two mares foaled unsupervised in the paddock and their foals could not be included in the study. One mare had a stillborn foal and one mare developed a ruptured pre-pubic tendon approximately 6 weeks prior to her expected foaling date. She subsequently delivered a live, small for gestational age foal which was considered unsuitable for inclusion in the study. Hence a total of eight foals were available for study. These foals were randomly assigned into two treatment groups, each of four foals:

**Group 1:** foals were prevented from suckling the mare for 12 hours and were maintained on commercial glucose and electrolyte replacer (Lectade\textsuperscript{1}) in this time; colostrum was milked from the mare and fed to the foal between 12 and 18 hours post-partum, at which time the foal was allowed to suckle the mare at will.

**Group 2:** foals were prevented from suckling the mare as for Group 1 foals, but were maintained on commercial milk replacer (DiVetelact\textsuperscript{2}) in this time; colostrum was stored and supplied to the foals as described for Group 1 foals between 12 and 18 hours post-partum.

A third group of experimental foals, fed colostrum from immediately after birth, was not included, due to the small number of foals available for inclusion in the study.

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1 Lectade\textsuperscript{1}, Beecham Veterinary Products, Dandenong, Victoria.

2 DiVetelact\textsuperscript{2}, Sharpe Laboratories, Artarmon, NSW.
Parturition was attended for all foals. Mares and foals were allowed to bond in the immediate post-partum period. Foals were observed for normal neonatal behaviour and, when able to stand, were given a physical examination and weighed. Colostrum was milked from the mare every 30 to 60 minutes during the first 18 hours post-partum. A volume of approximately 3 litres was stored at 4°C for administration to each foal from 12 hours post-partum. Foals were offered glucose-electrolyte solution or milk replacer, according to the group assigned, from within 30 to 60 minutes after birth and thereafter on an \textit{ad lib} basis until 12 hours post-partum. Each solution was made up according to the manufacturer’s instructions and stored at 4°C, if not consumed immediately. Stored fluid was heated to approximately 37°C in a microwave oven and offered to foals after careful mixing to ensure even heating.

After 12 hours, foals were offered stored colostrum by bottle on an \textit{ad lib} basis and the volume of colostrum consumed was recorded. Foals that failed to suckle colostrum offered were stomach tubed, to ensure an appropriate volume (approximately 3 litres) of colostrum was consumed within 18 hours of parturition. When repeated tubing was required a foal feeding tube was sutured in place to minimise the stress of repeated tube feeding. Colostrum from the foal’s dam was fed to each foal, that collected first (and presumably having the highest IgG concentration) was fed first. When greater than 3.5 litres of colostrum was collected from any mare, additional colostrum was stored frozen to supplement the volume of colostrum available to foals from mares producing less than 3 litres of colostrum. Stored colostrum was heated once to 37°C on low power in a microwave oven and mixed carefully prior to administration to ensure even heating of contents. Handling in this manner has been shown not to be detrimental to IgG concentration in bovine colostrum (Taylor and Hines, 1988) and has been employed in other studies on efficiency of colostral immune protein transfer (Boyd and Boyd, 1987). Colostrum not consumed immediately was stored briefly (less than 30 minutes) in a 37°C water bath. The volume of colostrum ingested by each foal was recorded. Aliquots of colostrum were stored frozen at -20°C for determination of IgG content by single radial immunodiffusion (SRID).

Foals were monitored by physical examination and observation. None experienced disease complications in the experimental period. Venous blood samples were collected at 0, 12, 24, 36 and 48 hours post-partum for haematology, IgG determination and cell function studies.

\textit{Blood Collection}

Blood samples were collected via jugular venipuncture into sodium EDTA vacutainers for haematology, plain (serum) vacutainers for IgG determination and lithium heparin vacutainers for cell function studies. Serum samples were split and utilised immediately (for cell function studies and determination of IgG concentration in 36 hours samples by glutaraldehyde coagulation test) or stored frozen at -20°C (for later determination of IgG concentration by single radial immunodiffusion and cell function studies using adult cells).

\textit{IgG Quantitation}

Serum samples collected at 0 and 12 hours (prior to the ingestion of colostrum) and 36 hours post-partum (18 hours after ingestion of colostrum) were stored at -20°C for determination of IgG status by SRID. Samples were stored frozen until the completion of the experiment so that all samples could be processed together. SRID was performed using a 1% solution of agarose in Barbital-EDTA pH 8.6. The agarose was melted in a water bath at 100°C and allowed to cool to that temperature at which the agarose did not set and the antiserum was not inactivated (usually 45 - 50 °C). The agarose gel containing antiserum was mixed rapidly and poured into a commercial slide. The agarose was allowed to set and wells were cut. The wells were then loaded with 1 µL aliquots of serum for the tests and the appropriate standards. The slides were placed in a humid chamber and left for 24 hours at room temperature. A calibrated magnifying eyepiece was used to measure the diameter of each ring of precipitation. A standard curve was prepared test results determined from this.
Aliquots of frozen colostrum were retrieved and thawed at room temperature. SRID was performed as described above for serum samples. Colostrum samples were diluted to permit accurate determination of their greater IgG concentration.

The passive immune status of each foal was also determined at 36 hours post-partum by glutaraldehyde coagulation test (GCT). GCT provides a semi-quantitative assessment of passive immune status and was used to identify foals with FPT and therefore at increased risk of developing septic disease. GCT was performed according to the method of Beetson, et al. (1985). Briefly, 0.5 mL aliquots of serum were transferred in 3DT disposable test tubes and mixed with 50 µL of glutaraldehyde solution (10% or 20%). The serum and glutaraldehyde was mixed immediately and each tube examined at intervals for up to 1 hour for evidence of coagulation. A positive reaction was judged to have occurred when a firm button developed in the bottom of the tube. A negative reaction was indicated when there was little or no change in the consistency of the serum following the addition of the glutaraldehyde reagent. If coagulation occurred within 10 minutes in the tube containing 10% glutaraldehyde, the IgG concentration was considered to be $\geq 8$ g/L; after 20 minutes the IgG concentration was considered to be $> 6.5$ g/L but $< 8$ g/L. If no change had occurred until 60 minutes, the IgG concentration was determined to be $> 4$ g/L (but $< 6.5$ g/L). If the 20% solution had not coagulated the IgG concentration was judged to be $< 2.5$ g/L. Foals which were determined to have suboptimal passive immunity (IgG $< 8$ g/L by GCT) were treated by intravenous plasma administration (Part 2 of current studies) to reduce their risk of septic disease.

**Efficiency of IgG Absorption**

The percentage of maternal IgG absorbed was calculated by determining the amount of IgG ingested by each foal (ie. the volume and IgG concentration of ingested colostrum) and dividing by plasma volume (calculated as 95 mL/kg, based on Splensly, et al., 1987) to give an expected IgG concentration. A ratio of 1:1 has been determined for equilibration of IgG between the intra- and extra-vascular spaces in the foal (Reilly and MacDougal, 1973). The expected plasma IgG concentration was then halved to allow for equilibration with the extra-vascular space. This result was compared to the measured IgG concentration to determine efficiency of absorption.

**Cell Preparation and Flow Cytometry**

Peripheral blood mixed leucocyte cell suspensions (PBML) were prepared as previously described (Raidal, et al, 1998a). Briefly, red cells were lysed by the addition of 10 mL of lysis solution (0.8% w/v ammonium chloride, 0.08% w/v sodium carbonate and 0.08% w/v potassium EDTA) at room temperature (22°C) to 5 mL of blood. After 5 minutes, 10 mL of phosphate buffered saline (PBS) was added, cells were centrifuged at 300g for 5 minutes, the supernatant discarded and the cell pellet resuspended in 5 mL of lysis solution. PBS (10 mL) was added immediately and cells were washed twice in PBS and resuspended in balanced salt solution (BSS$^2-$) (PBS with glucose 5 mM, gelatin 0.1%, calcium chloride 0.3 mM and magnesium chloride 1.0 mM) to a final volume of 4 mL.

Flow cytometric evaluation was performed using a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems) interfaced with a Macintosh computer. Data from 5000 events were collected in list mode and analysed using Cell Quest software (Becton Dickinson Immunocytometry Systems). Neutrophil populations were discriminated on the basis of forward scatter/side scatter cytograms of PBML cell populations. For evaluation of cell populations a threshold of 100 - 200 volts was set on FSC to remove small particles, such as free bacteria and cell debris. Fluorescence data were collected on log scale.
Phagocytosis Assays

Phagocytosis (the ability of peripheral blood neutrophils to associate with and to ingest bacteria) was determined using techniques described previously (Raidal, et al., 1998a). Briefly, paired 1000 µL aliquots of PBML cell suspensions were incubated at 37EC in an agitating water bath with 200 µL of autologous or donor serum and 200 µL of FITC labelled Staphylococcus aureus. 200 µL aliquots were removed, added to 200 µL PBS-EDTA on ice (to arrest phagocytosis) and evaluated by flow cytometry after 0, 10, 20 and 30 minutes of incubation. Data were collected from 5000 events within the neutrophil gate, established by passing cells and serum (negative control) through the flow cytometer prior to analysis. Cells able to associate with bacteria were recognised by their increased fluorescence and the proportion of positive cells was determined at each assay time. Cells able to internalise bacteria were discriminated by quenching the fluorescence of externalised bacteria with trypan blue and repeating the flow cytometric analysis of cells. Neutrophils collected every 12 hours to 48 hours post-partum from five foals were available for evaluation. Sera collected from all 8 foals was stored frozen and used as the source of opsonins in assays using cells collected from a donor (adult) horse to differentiate humoral effects from intrinsic cellular changes.

Oxidative Burst Assays

The oxidative burst capacity of peripheral blood neutrophils was evaluated by methods described previously (Raidal, et al., 1998b). Essentially, PBML cell suspensions with azide (100 µM) were preloaded with 7.5 µM dichlorofluorescin diacetate (DCFH-DA) for 15 minutes at 37EC in an agitating water bath. After incubation, one 200 µL aliquot of DCFH-DA loaded cells was withdrawn and placed on ice. Diluted phorbol myristate acetate (PMA) was added to the remaining suspension (final concentration PMA 5 ng/mL) and a 200 µL aliquot immediately withdrawn (0 minutes) and placed on ice prior to flow cytometric analysis, always within 15 minutes of collection. Subsequent 200 µL aliquots were withdrawn 5, 10, 20 and 30 minutes following the addition of PMA. A 200 µL aliquot of cells only was analysed as a negative control. Maximum oxidative burst response was determined in neutrophils collected at 0, 12, 24, 36 and 48 hours post-partum from 6 foals.

Statistics

The effect of withholding colostrum on efficiency of IgG absorption was determined by comparing results from Group 1 foals with those of Group 2 foals by unpaired t-test. Results were considered significant for P < 0.05. The possible effect of foal body weight, plasma volume and amount of IgG ingested on the efficiency of absorption were evaluated by simple linear regression analysis. The results of cell function studies from foals in Group 1 and Group 2 were pooled. The effect of time of collection was assessed by one way analysis of variance using a general linear model. Multiple comparisons were performed by Tukey test when a significant effect was determined. All analyses were performed using SigmaStat software).
Results

One foal (Group 1) developed acute septic arthritis of the tarsocural joint at 40 hours post-partum, but responded well to plasma transfusion, systemic antibiotics and lavage of the affected joint. All other foals remained bright and well during the experimental period.

Group 1 foals consumed, on average, 4.3 L of glucose-electrolyte solution during the first 12 hours post-partum. Group 2 foals consumed a slightly smaller volume of milk replacer (average 3.3 L). The volume of colostrum ingested by each foal is presented in Table 1. There was no significant difference in the volume of colostrum consumed by each group (Group 1, 2.9 L; Group 2, 2.9 L, P = 0.83). This volume was consumed within 7 hours of being offered (ie. 12 to 19 hours post-partum). No foal consumed this entire volume voluntarily - all were fed some part of the volume given by stomach tube. Repeat stomach tubing or indwelling nasogastric tubes were well tolerated.

The administration of colostrum at 12 to 18 hours post-partum was associated with a significant (P < 0.001) increase in serum IgG concentration (Figure 1). The amount of colostral IgG ingested by each foal is shown in Table 1. Despite the relatively uniform volume of colostrum ingested, colostral quality and hence IgG content varied dramatically, resulting in a very variable dose of maternal IgG received by each foal. Despite this individual variation, there was no significant difference between groups in the amount of IgG ingested (P= 0.91).

Most foals absorbed approximately 57% of ingested IgG into the intravascular fluid compartment, although again there was considerable individual variation. There was no significant difference between the two groups in the efficiency of absorption (P = 0.92); foals 3 and 8 appeared to absorb less than the other members of each group.

There was no correlation between efficiency of absorption and body weight (r² = 4.0%), plasma volume (r² = 4.1%), volume of colostrum ingested (r²=1.8%) or amount of IgG ingested (r²=11%).

Figure 1: Mean serum IgG concentrations for all foals (Groups 1 and 2 pooled). Time of collection had a significant effect on serum IgG concentration (P < 0.001, determined by one way analysis of variance). Significant differences, determined by pairwise multiple comparison (Tukey test) with 12 hour results (prior to ingestion of colostrum) are shown (**, P < 0.001).
Table 1 - Foals utilised in Part 1 of current studies. Plasma volume was calculated as 95 mL/kg. The amount of IgG ingested was calculated from the volume and IgG concentration of colostrum consumed. Expected IgG concentrated was determined by dividing the amount of IgG ingested by the foals plasma volume, assuming 100% absorption of ingested IgG. Equilibration of 1:1 with the extra-vascular space was assumed. The efficiency of absorption was determined by comparing the measured IgG concentration with the expected plasma IgG concentration. P values are the result of comparisons between groups, determined by unpaired t-test.

<table>
<thead>
<tr>
<th>foal</th>
<th>Birth weight (kg)</th>
<th>Plasma volume (L)</th>
<th>Volume of colostrum ingested (L)</th>
<th>Amount of IgG ingested (g)</th>
<th>Expected plasma concentration (g/L)</th>
<th>Measured IgG concentration (g/L)</th>
<th>Efficiency of absorption (%)</th>
<th>P</th>
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<td>Group 1 - Foals maintained on glucose-electrolyte solution for 12 hours post-partum.</td>
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<td>2.4</td>
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<td>Group 2 - Foals maintained on milk substitute for 12 hours post-partum.</td>
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<tr>
<td>Mean ± sem</td>
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<td>0.77</td>
<td>0.83</td>
<td>0.91</td>
<td>0.84</td>
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Results of cell function studies were pooled for Groups 1 and 2 because there was no difference in the efficiency of IgG absorption or in the serum IgG concentration achieved. When foal cells were assayed with autologous serum (n = 4), time of collection had no significant effect on the percent of neutrophils able to attach (P = 0.27) or ingest (P = 0.52) bacteria (Figure 2). The oxidative burst activity of neutrophils (n = 6) also demonstrated no significant effect due to time of collection (P = 0.57), although mean response was slightly lower at 24 and 36 hours post-partum (Figure 3).

**Figure 2:** Phagocytosis results for foal neutrophils assayed with autologous sera. Time of collection had no significant effect on the percentage of cells able to attach (P = 0.27) or ingest (P = 0.52) bacteria. Ingestion results at 24 hours post-partum appear higher because results from two foals were not available for inclusion.

**Figure 3:** Oxidative burst results from foal neutrophils. Time of collection had no significant effect (P = 0.57).
Time of collection had a highly significant effect on the phagocytic activity of adult neutrophils when foal sera was used as the source of opsonins in phagocytosis assays (P < 0.001 for attachment; P < 0.001 for ingestion; Figure 4). There was no correlation between serum IgG concentration and foal neutrophil phagocytosis ($r^2 = 6.4\%$ for attachment; $r^2 = 26.6\%$ for ingestion) or oxidative burst ($r^2 = 7.5\%$). There was also no correlation between serum IgG concentration and adult neutrophil phagocytosis ($r^2 = 19.7\%$ for attachment and $r^2 = 4.8\%$ for ingestion).

**Figure 4:** Phagocytosis by adult neutrophils using foal sera in assays. Time of collection had a significant effect on the percentage of cells able to attach (P < 0.001) and internalise (P < 0.001) bacteria. Significant differences between individual sampling times after the ingestion of colostrum and 12 hour results, determined by pairwise multiple comparison procedures, are shown (**, P < 0.001; *, P < 0.05).

**Discussion**

There was no difference in the efficiency of IgG absorption evident between the two treatment groups in the current study, suggesting that the administration of macromolecules (contained in milk replacer, but not in glucose-electrolyte solutions) did not influence the closure process. This finding is consistent with similar observations in calves (McCoy, *et al.*, 1970), but contrasts the situation in lambs (Lecce and Morgan, 1962) and piglets (Payne and Marsh, 1962; Vellenga, *et al.*, 1988). These discrepancies suggest that the process of closure may be mediated differently in these species.

The process of closure is complex and incompletely understood. Jeffcott (1972) has suggested that factors that influence closure may include changes in the absorptive cells lining the neonatal intestine, endocrine mechanisms and stress, prematurity or starvation, factors in colostrum, gastric development and the ingestion of solid food. There has been debate over the role of corticosteroids as mediators of closure. The absorptive capacity of puppies (Gillette and Filkins, 1966), calves (Husband, *et al.*, 1973) and lambs (Logan and Irwin, 1977) is prematurely terminated by the administration of corticosteroids or ACTH to the neonate or dam. However, Carrick, *et al.* (1987) were unable to demonstrate reduced absorption of colostral immunoglobulin in response to increased cortisol levels in neonatal foals.
Evaluation of the role of such endocrine changes was beyond the scope of the present study. Our results, however, do not support a role for stress hormones in premature closure of the absorptive process because, despite the additional handling imposed on foals, a premature reduction in the efficiency of absorption was not detected; the efficiency of IgG absorption was at least as high as that previously reported for foals (Jeffcott, 1974a) and calves (Boyd and Boyd, 1987). For this reason, factors present in colostrum should be considered likely to mediate closure.

The nature of such factors has not been investigated. There has, however, been some investigation of the possible role of colostral components in enhancing intestinal absorption of immune factors. Sansholm and Hankanen-Buzalski (1979) reported high antitrypsin activity in mare colostrum. Although the structure of the equine trypsin inhibitor is not known, its likely function is to protect immunoglobulin molecules from degradation within the gastrointestinal tract (Burton, et al., 1981). A peptide, designated colostrokinin, has been identified in bovine colostrum and may have a role in the absorption of maternal immunoglobulins because of its ability to increase capillary permeability (Schlagheck and Stott, 1983). Other compounds that are believed to accelerate absorption include lactate, pyruvate and salts of certain volatile fatty acids (particularly potassium isobutyrate, Hardy 1969), an inorganic phosphate and glucose-6-phosphate (Schlagheck and Stott, 1983). Further investigation of the possible benefits of the therapeutic administration of such substances to enhance immune protein absorption may be warranted.

Development of the intestinal enzyme system may also have a role in closure (Klaus, et al., 1969). Hill (1956) suggested that, for those species that receive antibodies from colostrum, gastric protein digestion is delayed by retarded development of the gastric glands; whereas in those species in which the major antibody transference occurs in utero, active gastric secretion occurs at or before birth. For example, pepsin activation, which occurs over the first 24 - 36 hours in the lamb, may result in increased protein digestion, with less protein then available for absorption.

The current study demonstrated a mean efficiency of absorption of approximately 57%, which is higher than the earlier findings of Jeffcott (1974a), who assessed the absorption of PVP 60, a macromolecule analogous to IgG as being approximately 22% three hours post-partum in colostrum fed foals. This study did not allow for equilibration with the extra-vascular space; hence the calculated absorption of PVP 60 from the small intestine was approximately 44%. The efficiency of absorption of macromolecules by equine neonates has not been assessed by other investigators. Boyd and Boyd (1987) demonstrated similar findings in calves fed a single dose of colostrum at 5 hours post-partum (46% for IgG1 and 49% for IgG2).

The dose of IgG administered to each foal in the current study was consumed over a 7 hour period between 12 and 19 hours after birth. The results generated hence represent a mean value for this period, whereas Jeffcott (1974a) reported relative efficiency of absorption at various sampling times. The value obtained in the current study was much higher than that obtained by Jeffcott at 12 hours post-partum (20%), suggesting that closure did not proceed as rapidly in colostrum deprived foals in the current study. The presence of hormones and trophic factors in colostrum may have a role in mediating mucosal proliferation and maturation of the neonatal gastrointestinal tract (Weaver, 1986), in which case withholding colostrum would be expected to delay closure. Jeffcott (1973) reported that the absorption of PVP K 60 was better in colostrum-fed foals than in colostrum-deprived foals, and suggested that factors in colostrum enhance macromolecular absorption. Such factors have been evaluated in other species (Balfour and Comline, 1962; Klaus, et al., 1969; Campbell, et al., 1977). Alternatively, differences may be due to experimental methodology, such as fundamental differences in the absorption of PVP and IgG.

The individual variation in efficiency of absorption, and in colostral IgG concentration, observed in the current study collaborates the clinical observations of erratic colostral transfer of immunoglobulins to foals (Madigan 1987) and supports routine testing of 24 hour serum IgG levels to document satisfactory passive immune status (Raidal, 1996). Studies in other species have demonstrated marked variation in efficiency of absorption between individual animals (Klaus, et al., 1969; Baird, et al.,
proven negative correlation between the efficiency of absorption and the mass of IgG1 and IgM fed to newborn calves, which, they claimed, suggested a physiological limitation to the mass of immunoglobulin that can be absorbed into serum from a given volume of colostrum. However, such findings were not evident in the current study, possibly due to differences in the mechanisms of absorption or closure employed by calves and foals. Neither the volume of colostrum consumed nor the amount or concentration of IgG administered could be correlated with the subsequent efficiency of absorption.

The measured IgG concentration obtained by foals in both groups was inadequate (less than 8 g/L) for 3 foals in each group. This suggests that, ideally, colostrum should be routinely administered to foals less than 12 hours old. However, if colostrum administration is delayed for 12 to 18 hours, the results of the current study suggest that approximately half the administered IgG would be absorbed. In the current study, delayed administration of colostrum resulted in serum IgG concentrations between 4 - 8 g/L (mean for both groups was 6.7 g/L), which may be adequate for healthy foals in well managed environments. The handling of foals and colostrum in the manner described did not appear to be detrimental to the efficiency of absorption because treated foals in both groups demonstrated an efficiency of IgG absorption higher than previously reported. However, the possible effect of handling procedures could be more accurately differentiated from the effect of time by assessing the efficiency of absorption in foals given collected colostrum by bottle or tube from birth.

One foal in the current study (foal number 1, Group 1) developed septic arthritis shortly after the completion of the experimental protocol. *Actinobacillus equuli* was isolated from the affected tarsocrural joint. This foal responded rapidly to appropriate treatment, which included intravenous plasma to boost suboptimal IgG (5.8 g/L), intravenous antibiotics and lavage of the affected joint. *A. equuli* is a common pathogen in equine neonatal septic disease and may be acquired in utero or soon after birth (Kowalski, 1998). Because these organisms cannot survive in the environment for long periods, the mare is the usual source of infection. The possible contribution of delayed intestinal closure to the development of neonatal septic disease, as suggested by Madigan (1997), cannot be commented on in this instance or for other foals in the present study. Based on the development of sepsis in Foal 1 and on the high incidence of fatal septic disease reported in colostrum deprived foals in other studies (Robinson, et al., 1993), routine plasma treatment of all foals was instituted at the completion of the current experimental period to minimise foal losses. Further evaluation of Madigan’s contention would require histologic demonstration of bacteria being absorbed by small intestinal cells. There is a large size difference between bacteria and macromolecules such as immunoglobulins. Whilst it is agreed that the absorptive process is non-selective, bacteria may fail to attach appropriately to small intestinal cells or may be too large to be internalised by pinocytosis or discharged by absorptive cells into regional lymphatics, as is the case for macromolecules.

Evaluation of phagocytosis and oxidative burst by foal neutrophils collected during the first 48 hours following birth demonstrated no effect which could be attributed to the ingestion of colostrum. Oxidative burst activity was transiently depressed coincident with the administration of colostrum, but the observed effect was not significant. Other studies have demonstrated improved neutrophil function in foals (Fogarty and Leadon, 1987) and calves (LaMotte and Eberhart, 1976) following the ingestion of colostrum. When adult neutrophils were assayed with foal sera a highly significant beneficial effect was evident for samples collected after the ingestion of colostrum. The percentage of adult neutrophils able to attach and ingest bacteria was not highly correlated with serum IgG concentration, suggesting that other factors transferred in colostrum and hence present in sera may be important mediators of the observed functional improvement. Such mediators may include chemotactic and opsonic factors such as complement (Jonas and Broad, 1972; Morgan, 1973) and other soluble components which mediate differences observed between colostrum fed and colostrum deprived foals in tumour necrosis factor synthesis or clearance (Green, et al., 1991; Allen, et al., 1993), interleukin production (Robinson, et al., 1993) and haemolytic complement activity (Bernoco, et al., 1994). Alternatively, differences observed in the neonatal period may be associated with physiological changes rather than the ingestion
of colostrum (Fogarty and Leadon, 1987). For example, plasma cortisol concentrations are raised in the serum of newborn foals and remain elevated during the first 24 hours post-partum (Malinowski, et al., 1990). Hence changes observed may be due to declining cortisol levels in sera used, rather than due to factors transferred in colostrum. This possibility could be assessed by evaluation of cell function in colostrum deprived foals.
Part 2: Studies on the effects of plasma transfusion on neutrophil function in healthy and septic foals.

Introduction

While inadequate passive immunity is undoubtedly the greatest risk factor for the development of septic disease in foals, our understanding of neonatal immunity and susceptibility to disease is poor. As discussed in Part 1, the level of IgG which confers protection to the neonate is contentious, with opinion currently divided between > 4g/L or > 8g/L. Routine screening of all foals for FPT has been recommended (Raidal, 1996) due to the serious consequences of inadequate passive immunity and due to the difficulty of predicting which foals are at risk to the development of FPT. Foals detected as having partial or complete FPT can then be given intravenous immune supplementation by plasma transfusion. As previously stated, most insurance companies will not insure foals against mortality due to septic disease unless their serum IgG concentration has been documented as being greater than 8 g/L. One result of such measures is that an increasing number of otherwise healthy foals are presented to veterinary hospitals for plasma treatment to boost circulating IgG levels.

When intravenous immune supplementation is required, plasma is preferable to other sources of passive immunity such as purified IgG or gammaglobulin preparations. Ideally, plasma should be collected from a properly immunised gelding or primiparous mare resident on the same property to ensure high antibody titres for pathogens likely to be encountered by the foal (Rumbaugh, et al., 1978). Donors should be screened for anti-red cell antibodies and should be Qa and Aa negative, as these are the red blood cell antigens most commonly associated with anti-red cell antibodies (Morris, 1986). Whole blood is collected from donors and plasma harvested by plasmapharesis, or following centrifugation or sedimentation of red cells.

As the response to treatment may be variable, the optimal amount and rate of plasma delivery has not been determined. Koterba (1987c) recommends 1 litre over 45 - 60 minutes. Fogarty (1988) recommended a slower rate of 500 mL/hour (or 5 - 8 mL/minute) depending on the circulatory, hydration and renal status of the patient. The volume required to raise circulating IgG levels may be large, up to 2 - 6 L (LeBlanc, 1987). This volume of fluid can be difficult to administer to neonates. Thomas and Pemberton (1980) demonstrated that thawing equine plasma concentrates its components so that the first fractions are very rich in all plasma constituents assayed. This represents a convenient way of reducing he volume of plasma to be administered to the foal.

Uncommon reactions associated with the intravenous administration of plasma to foals include hyperventilation, transient paresis, trembling and abdominal straining (Kruse-Elliot and Wagner, 1984). These abnormalities are rapidly corrected by stopping the transfusion for several minutes and resuming at a slower rate (Rumbaugh, et al., 1979). In the seriously ill foal, however, volume overload can occur, especially if cardiovascular and/or renal function is impaired. The effect of plasma administered to filly foals on the incidence of haemolytic disease in their offspring is uncertain. Although the ability of such treatment to raise circulating IgG concentrations for a short time has been demonstrated (White, 1989; Wilkins and Dewan-Mix, 1994), reductions in morbidity or mortality due to septic disease processes have not been demonstrated following plasma transfusion. The possible effects of such treatments on immune cell function have not been evaluated. Almost exclusively, studies on neonatal immunity have focussed on the antibody (IgG) status of foals and there is a paucity of information on the functional activity of foal phagocytic cells (LeBlanc, 1991). Functional deficiencies in peripheral blood neutrophils and pulmonary alveolar macrophages from foals have been noted in comparison to results obtained using cells from adult horses (Coignoul, et al., 1984; Bernoco, et al., 1987; Liu, et al., 1987; Fogarty and Leadon, 1987; LeBlanc and Pritchard, 1988; Wichtel, et al., 1991). The influence of passive immune status on observed cell function has received little attention.
Plasma transfusion is part of routine treatment septic foals. IgG levels are frequently low in such cases, either due to inadequate absorption of colostral IgG, or due to increased catabolism of circulating IgG. The response of sick foals to plasma transfusion is often disappointing. Clinically ill foals with failure of passive transfer exhibit a significantly lower rise in serum IgG when treated with plasma than do clinically healthy foals (LeBlanc and Tran, 1987; Wilkins and Dewan-Mix, 1994). This may be due to the exudation of serum proteins into infected tissues and body cavities or due to opsonisation of bacteria and thus depletion during phagocytosis (Raidal, 1992).

Neutrophils are the predominant phagocytic cell of the blood (Naylor, 1979). Neutrophil phagocytosis can be facilitated by complexing antigen with immunoglobulin or by the presence of complement. Evaluation of the functional integrity of foal neutrophils may, therefore, provide an effective method of assessing the response to plasma transfusion, permitting differences to be identified prior to and following treatment and for the response to treatment to be compared between healthy and septic foals.

Flow cytometric techniques have been developed for the evaluation of phagocytic and oxidative burst activity of equine neutrophils (Raidal, et al., 1998a, 1998b). Effective neutrophil function is dependent on chemotaxis (the movement of neutrophils from the blood towards areas of inflammation), phagocytosis (the ingestion potential pathogens) and the intracellular killing of ingested organisms. Oxidative burst activity is important for the killing of ingested micro-organisms and hence represents an important aspect of phagocytic capability. Hence, flow cytometry permits evaluation of these two important aspects of neutrophil function. Using flow cytometry, cells are aspirated into the cytometer from a sample reservoir under pressure and carried into faster flowing cell-free sheath fluid in a flow chamber. Cells are hydrodynamically oriented and confined to the centre of the stream of sheath fluid and carried past an argon laser generated light beam positioned at 90° to the direction of the cell stream. As the focussed light hits each cell, light is scattered, absorbed or emitted as fluorescence and detected by an array of photomultiplier tubes which transform the light signals to electrical pulses which are amplified, processed and displayed. This technology permits the rapid evaluation of large numbers of cells on a cell by cell basis, providing greater accuracy than conventional methods which rely on cell counting or the mean response of a group of cells. Subpopulations of cells can be identified and evaluated, negating the need for prior cell separation, which is time consuming and may alter cell function (Fearon and Collins, 1983; Hed, et al., 1987). The application of these techniques to the evaluation of foal cells has not previously been reported.

Methodology

Preliminary Studies

Comparison of foal neutrophil function with that of adult cells. The results obtained from phagocytosis and oxidative burst assays on cells from 5 clinically normal foals (# 14 days of age) were compared with the results of assays performed on cells from 12 healthy adult horses. Phagocytosis by foal cells was further assessed using autologous and donor (adult) sera with foal cells.

Effect of autologous or donor serum or plasma on neutrophil oxidative burst. The effect of the incorporation of 200 µL of autologous or donor serum in the oxidative burst assay was evaluated for foal and adult cells. Results were also evaluated following the addition of 200µL plasma to the assay. Serum or plasma was added to the assay at the same time as DCFH-DA, 15 minutes prior to the addition of PMA. Donor serum and plasma was collected from one horse and used in all subsequent assays.

Effect of storage at various temperatures on neutrophil oxidative burst. The effect of storage for up to 48 hours on neutrophil oxidative burst activity was assessed. Samples were collected for immediate
analysis (0 hours) and for evaluation after 24 or 48 hours storage at room temperature (22°C), 4°C, and 37°C. During the storage period cells remained suspended in their own plasma (ie. red cells were not lysed prior to storage).

Experimental Animals

Preliminary studies used to establish assay techniques and for the comparison of foal and adult cell function were conducted using samples from healthy foals resident on a nearby stud and foals born at MUVH. In all cases, foals were less than 14 days old when sampled. Samples from adult horses were collected from healthy horses resident at MUVH.

Foals presented to Murdoch University Veterinary Hospital (MUVH) between August and December 1997 were assigned to one of three treatment groups, based on the reason for presentation. Other equine practices in the greater Perth metropolitan area were approached to provide foals for inclusion in the study. Two other practices participated: Saddleback Equine Hospital (SEH) and Serpentine Veterinary Clinic (SVC). Foals from these practices were similarly classified. Plasma collection and administration procedures were similar at each practice. Foals available for inclusion in the current study are summarised in Table 2.

Group 1 - Healthy foals receiving plasma transfusion for FPT or partial FPT.
Eleven healthy foals were presented to MUVH (n = 8) or a neighbouring practice (SEH, n = 3) for plasma transfusion due to FPT or pFPT. All foals were less than three days of age at the time of transfusion. The most common reasons for FPT were premature lactation or delayed ingestion of colostrum. This group included foals from Part 1 of current studies.

Group 2 - Septic foals receiving plasma transfusion as part of treatment.
Eight foals received plasma transfusions as part of treatment of septic disease at MUVH (n = 2) or neighbouring practices (SEH, n = 5; SVC, n = 1). These foals all had FPT or pFPT at the time of admission. Septic disease was confirmed in all foals by positive bacterial culture and/or by a sepsis score of 11 or greater (Brewer and Koterba, 1988).

Group 3 - Healthy foals with adequate passive immune status (IgG ≥ 8 g/L).
Samples were obtained at 24 hours after birth from two foals born at MUVH. Five other foals less than 7 days of age were presented to MUVH for correction of angular limb deformities (ALD). A single blood sample was obtained from these foals at the time of admission, prior to undergoing any form of treatment. These foals all had adequate passive immunity (IgG ≥ 8 g/L) and were also classified as normal.

Table 2: Foals available for inclusion in current studies.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>description</th>
<th>number (n)</th>
<th>sampling times</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>healthy foals with FPT or pFPT</td>
<td>11 (MUVH, n = 8) (SEH, n = 3)</td>
<td>pre Tx: 0, 12, 24, 48 hours post Tx: 3, 5, 7, 14, 21, 28 days</td>
</tr>
<tr>
<td>2</td>
<td>septic foals with FPT or pFPT</td>
<td>8 (MUVH, n = 2) (SEH, n = 5) (SVC, n = 1)</td>
<td>pre Tx: 0, 12, 24, 48 hours post Tx: 3, 5, 7 days</td>
</tr>
<tr>
<td>3</td>
<td>healthy foals (not septic, IgG ≥ 8g/L)</td>
<td>7 (MUVH, n = 7)</td>
<td>on admission (&lt; 7 days post-partum) (24 hours post-partum for 2 foals born at MUVH)</td>
</tr>
</tbody>
</table>
**Plasma Collection**

Blood was collected from universal donor mares (primiparous mares which were Aa and Qa negative and had been screened for anti-red cell antibodies) resident on the Murdoch University farm. Up to nine litres was collected following aseptic jugular venipuncture into commercial 3L plasma collection bags and mixed with sterile acid citrate dextrose. Red cells were allowed to sediment, leaving plasma in the top half of the bag. This was syphoned into a smaller 2L satellite bag connected to the collection bag. Plasma was stored frozen at -20°C until needed. Collection techniques at SEH and SVC were similar to that described at MUVH. SVC utilised a closed collection technique essentially as described. SEH collected blood into open bottles following jugular venipuncture using a large bore bleeding trochar. Red cells were allowed to settle by gravity sedimentation before being syphoned into bags for storage. Neither outside practice had access to blood typed universal donors.

**Plasma Transfusion**

Foals with serum IgG concentrations < 8 g/L received IV plasma between 36 and 96 hours post partum. In all cases foals were restrained or sedated and prepared for aseptic venipuncture by clipping and surgical preparation (betadine or chlorhexadine scrub and alcohol). An intravenous catheter was introduced into the jugular or cephalic vein. Where possible the catheter was left in place for up to 72 hours to facilitate the collection of post-transfusion blood samples.

Stored plasma was thawed at room temperature or in warm water prior to administration. In some cases the plasma was concentrated by collecting the first litre (50%) to thaw (freeze-thaw concentration as described by Thomas and Pemberton, 1980). The volume of plasma administered was determined by consideration of the pre-transfusion status of the foal, the size of the foal and the IgG concentration of donor plasma (thawed whole vs concentrated plasma). In all cases a volume of 1 - 3 L of plasma or concentrated plasma was administered to each foal. Plasma was administered by gravity feed at a rate of approximately 10 to 20 mL/minute. Each foal was monitored during the transfusion and the rate of administration varied accordingly.

**Blood Collection**

Blood samples were collected via jugular venipuncture, or an indwelling cephalic catheter if there was one present. Blood was collected into lithium heparin vacutainers for cell function studies, sodium EDTA vacutainers for haematology and plain (serum) vacutainers for determination of IgG concentration. Serum samples were split and utilised for immediate determination of IgG concentration by glutaraldehyde coagulation (as described in Part 1). An aliquot of serum was stored at -20°C for subsequent determination of IgG concentration by SRID (as described in Part 1). Unless otherwise indicated, samples were kept at ambient temperature prior to processing for haematology or cell function studies, always within 3 hours of collection.

**Phagocytosis and Oxidative Burst Assays**

Peripheral blood mixed leucocyte cell suspensions (PBML) were prepared and flow cytometry performed as described in Part 1 of current studies. Phagocytosis and oxidative burst assays were also performed as described in Part 1. Unless otherwise stated, autologous serum (serum collected from the same individual as cells used in assay) was used as the source of opsonins in each phagocytosis assay and comparison between individuals was based on results obtained after 30 minutes incubation. For oxidative burst assays, unless otherwise stated, comparison between individuals was based on maximum results, usually obtained after 20 or 30 minutes incubation.
Statistical Methods

All analyses were performed using SigmaStat\textsuperscript{1} statistical software. The phagocytic and oxidative burst capacity of foal and adult neutrophils were compared by unpaired \( t \)-test. For Groups 1 and 2, the effect of plasma transfusion of foal cell function and serum IgG concentration was assessed in a number of ways. Time of collection was assessed within each group by one way analysis of variance using a general linear model. Results between groups were compared by two way repeated measures analysis of variance using a general linear model and examining the treatment versus time of collection interaction. When a significant effect was determined by analysis of variance, multiple comparisons were performed by Tukey test. When analysis of variance failed to demonstrate significance, but data suggested a difference between pre and post-treatment samples or between groups, results at individual sampling times were compared by paired (within group) or unpaired (between groups) \( t \)-test. When data did not demonstrate a normal distribution, comparisons between individual sampling times were performed using the Mann Whitney rank sum test. Results were considered to be significant when \( P < 0.05 \).

Results

Preliminary Studies

Comparison of foal neutrophil function with that of adult cells. Foal neutrophils demonstrated significantly less phagocytic capacity than adult cells when autologous serum was used in each assay (Figure 5). Differences in the ability of foal cells to associate with or ingest bacteria could not be discriminated when donor (adult) serum was compared with autologous serum (Figure 6). There was no significant difference in maximum oxidative burst response demonstrated by foal neutrophils when compared to that obtained from adult cells (Figure 7).

![Figure 5: Phagocytosis by foal neutrophils compared with that of adult cells. Autologous serum was used as the source of opsonins in each assay.](image)

\textsuperscript{1}SigmaStat\textsuperscript{®} 2.0 for Windows, SPSS Inc, Chicago.
Figure 6: Results of phagocytosis assays using foal neutrophils with autologous serum and donor (adult) serum. Results are typical of assays performed using cells from all five foals.

Figure 7: Oxidative burst results from foal and adult horse neutrophils.
The incorporation of plasma or serum into oxidative burst assays. The addition of both autologous (adult) and donor (foal) plasma to oxidative burst assays using adult PBML cells showed a large increase in neutrophil auto-oxidation, above that demonstrated by loaded cells without plasma. The oxidative burst stimulated by PMA peaked earlier but was of similar intensity to assays in which plasma was not included (Figure 8). Similar results were observed when serum was added to the assay. Conversely, the oxidative burst response of foal cells was dramatically inhibited by incorporation of autologous (foal) or donor (adult) plasma in the assay, when compared to that obtained from cells without plasma (Figure 9). The increased auto-oxidation (mean fluorescence intensity of loaded cells) observed in adult neutrophils following the addition of autologous or donor plasma was not observed in foal neutrophils. Results obtained following the addition of serum were similar.

**Figure 8:** Oxidative burst response of adult horse neutrophils with and with the addition of adult horse of foal plasma. Auto-oxidation (the fluorescence of DCFH-loaded cells prior to stimulation with PMA in markedly increased when compared to the response of cells incubated without plasma. Peak fluorescence following PMA stimulation is evident sooner than is the case for cells without plasma.

**Figure 9:** Oxidative burst response of foal neutrophils with and without the addition of foal and adult plasma. Auto-oxidation is not increased, as was demonstrated by adult cells. The cellular response to PMA stimulation is also dramatically inhibited. Results are typical of assays performed in triplicate using serum and plasma.
Effect of storage at various temperatures on neutrophil oxidative burst. The oxidative burst response of adult horse neutrophils stored at room temperature (22°C) for 24 or 48 hours was comparable to that obtained from cells assayed within three hours of collection (Figure 10). Storage of cells at 4°C appeared to cause impaired oxidative burst activity, while storage at 37°C yielded results that were similar to or slightly greater than those obtained from cells stored at room temperature.

![Figure 10](image)

Figure 10: Oxidative burst response of adult horse neutrophils stored for 24 or 48 hours at various temperatures. Results are typical of assays performed in triplicate.

Response to Plasma Transfusion

Transfusion was well tolerated by all foals. Minor transfusion reactions observed during administration included tremors and abdominal straining. More severe transfusion reactions were not observed at MUVH and were not reported at SEH or SVC. The rate of administration was slowed or temporarily discontinued when such reactions were observed. Total white cell counts for samples obtained at 0 hours post transfusion at SEH were markedly reduced, due to a profound neutropaenia. For most foals white cell counts had returned to pre-treatment values by 12 hours post-treatment. This response was observed in septic and healthy foals treated at SEH, but was not observed in foals in either group treated at MUVH or SVC.

Long-term follow-up of treated foals in all groups proved difficult for a number of reasons. Client foals presented to MUVH, SEH and SVC for transfusions were, in most cases, lost to follow-up on discharge. Samples were obtained for a longer period following treatment from nine foals that were accommodated at Murdoch University farm. Cells from five of these foals demonstrated marked haemagglutination when NH₄Cl lysis was attempted. In each case cellular aggregation first became apparent 1 to 4 weeks following transfusion and remained evident at all subsequent sampling times. A similar response was observed when hypotonic saline lysis was attempted and for cells collected into alternate anticoagulants. Light microscopic evaluation of aggregated cells demonstrated neutrophils “caught” in strands of platelets. The cellular clumps could be resuspended, but could not be passed through the flow cytometer. A similar response was not seen foals of similar age not accommodated at Murdoch University farm, but was observed in foals kept at MUVH and not transfused.
Group 1 - Healthy foals receiving plasma transfusion for FPT or partial FPT.

Plasma transfusion had a highly significant effect on serum IgG concentration (P < 0.001). Significantly higher serum IgG concentrations were evident 12 hours to 3 days following treatment than were evident prior to transfusion (Figure 11). Circulating IgG concentrations in samples obtained 12, 24 and 72 hours following treatment were significantly increased. Beyond 3 days after treatment mean values steadily declined.

Neutrophil phagocytosis was not significantly affected by plasma transfusion. There was a trend for decreased phagocytosis (attachment and ingestion) at 12 hours post transfusion (Figure 12), but analysis of variance demonstrated no significant time of collection effect within the group (P = 0.46 for attachment and P = 0.22 for ingestion). Comparison of pre-treatment and 12 hour post-treatment samples by paired t-test also demonstrated no significant difference (P = 0.54 for attachment, P = 0.15 for ingestion). Neutrophil oxidative burst activity at 24 hours post transfusion was reduced slightly relative to pre-treatment values (Figure 13). Observed differences were not significant (P = 0.85 by analysis of variance; P = 0.29 by comparison of pre-treatment and 24 hours post-treatment samples using paired t-test).

Figure 11: Serum IgG responses to plasma transfusion in healthy (Group 1) and septic foals (Group 2) with failure or partial failure of passive transfer of immunity. Time of collection had a significant effect on IgG concentration for Group 1 foals (P <= 0.001), but not for Group 2 foals (P = 0.18). Significant differences within Group 1, determined by Tukey test, are shown (Group 1: *, P < 0.05; ** P < 0.01). Comparison of individual sampling times for Group 2 foals by paired t-test demonstrated differences between pre-treatment and post-treatment results ( #, P < 0.05).
Figure 12: Neutrophil phagocytosis following plasma treatment of Group 1 foals (healthy foals with failure or partial failure of passive transfer). Time of collection had no significant effect ($P = 0.46$ for attachment and $P = 0.22$ for ingestion).

Figure 13: Neutrophil oxidative burst for Group 1 foals. Time of collection had no significant effect on response.
Group 2 - Septic foals receiving plasma transfusion as part of treatment.

Although plasma treatment was associated with increased serum IgG concentration in septic foals (Figure 11), a significant effect was not evident following analysis of variance (P = 0.18). Comparison of post-treatment samples with those obtained prior to treatment yielded a significant increase at 0 and 12 hours post-treatment (P = 0.014 and P = 0.023, respectively). Although mean serum IgG concentration following treatment was lower than that evident for Group 1 foals, there was no significant difference between the results obtained from Group 1 and Group 2 foals (P = 0.14). The difference evident between groups at 3 days post-treatment approached significance (P = 0.094).

Plasma transfusion had no significant effect on the percentage of neutrophils able to attach (P = 0.69) or ingest (P = 0.68) bacteria at any time following treatment (Figure 14). However, there was a trend towards improved phagocytic activity in the first 24 hours following transfusion. There was no significant difference in the phagocytic function of neutrophils from Group 1 or Group 2 foals prior to treatment (P = 0.89 for attachment and P = 0.87 for ingestion). Comparison of the response to treatment by the two groups suggested that Group 2 showed a positive response to treatment when compared to that demonstrated by Group 1 horses (Figure 15). There was no significant difference between the response to treatment between the two groups when compared by analysis of variance (P = 0.50 for attachment and P = 1.00 for ingestion). However, significant differences between the percentage of neutrophils able to ingest bacteria were evident between Group 1 and Group 2 at 0 and 12 hours post-treatment (P = 0.027 and P = 0.005, respectively. The difference between groups approached significance (P = 0.054) at 12 hours post treatment for percentage of neutrophils able to attach bacteria. Differences evident at other times were not significant.

The oxidative burst response of septic foals (Group 2) was not significantly affected by plasma transfusion (P = 0.15). The oxidative burst response of neutrophils from Group 2 foals was less than that of healthy foals at all sampling times (except at 24 hours post-treatment). However, there was no significant difference between the oxidative burst response of septic foals when compared to that of healthy foals (Group 1) by analysis of variance (P = 0.47, Figure 16) and no significant difference was evident when individual sampling times were compared by t-test.
Figure 14: Neutrophil phagocytosis following plasma treatment of Group 2 foals (septic foals). Time of collection had no significant effect (P = 0.69 for attachment and P = 0.68 for ingestion).

Figure 15: Comparison of phagocytosis by neutrophils from Group 1 and Group 2 foals. There was no significant difference between groups for attachment (left, P = 0.89) or ingestion (right, P = 0.87). Significant differences in the percent of neutrophils ingesting bacteria were apparent between groups when compared by unpaired t-test at 0 and 12 hours following treatment. Difference in the percent of neutrophils attaching approached significance at 12 hours post treatment (P = 0.054).
**Figure 16**: Oxidative burst response of neutrophils from Group 2 foals following plasma transfusion. Treatment had no significant effect on neutrophil oxidative burst ($P = 0.15$). Despite the trend for the oxidative burst of Group 2 foal neutrophils to be less than that of Group 1 foals, no significant difference was evident between groups following analysis of variance ($P = 0.47$) or comparison of individual sampling times.

**Group 3 - Healthy foals with adequate passive immune status (IgG ≥ 8 g/L).** The phagocytic and oxidative burst response of neutrophils from Group 3 foals was not significantly different to that of Group 1 or Group 2 foals prior to treatment (Figure 17). However, the oxidative burst response of septic foals tended to be less than that of healthy foals, with or without failure of passive transfer of immunity.

**Figure 17**: Comparison of neutrophil function of foals with failure or partial failure of passive transfer of immunity, with and without sepsis (Groups 1 and 2) with that of healthy foals with circulating IgG concentrations ≥ 8 g/L (Group 3). Results of the comparison of each group with Group 3 by unpaired $t$-test are shown.
Discussion

Phagocytosis assays using foal cells appeared to proceed in the same manner as described previously for adult cells (Raidal, et al., 1998a). Consistent with previous studies (Coignoul, et al., 1984; Bernoco, et al., 1987; Fogarty and Leadon, 1987), the phagocytosis of bacteria by foal neutrophils was less than that demonstrated by adult cells when autologous serum was used as the source of opsonins in each assay. There was no difference in phagocytosis by foal neutrophils when assays were performed using adult serum, compared to results obtained using foal serum. This suggests that the reduced phagocytosis by foal neutrophils, relative adult cells, is due to an innate cellular deficiency, rather than serum factors such as opsonins. This proposition is contrary to the findings of Bernoco, et al. (1987), who found that foal neutrophils demonstrated impaired phagocytosis when tested with autologous plasma but responded normally when tested with plasma from adult horses.

The oxidative burst response of neonatal neutrophils was not significantly different to the oxidative burst response of adult neutrophils. Such studies have not been performed previously using foal cells. A similar study of neonatal bovine neutrophils demonstrated reduced oxidative burst activity in response to PMA stimulation when compared to results obtained using adult cells (Dore, et al., 1991).

The addition of foal or adult plasma was associated with an increased auto-oxidation (fluorescence of DCFH-DA loaded, unstimulated cells) in oxidative burst assays using adult cells. Stimulation with PMA in such assays caused an accelerated response when compared with identical assays where plasma was omitted. The magnitude of cellular response to PMA stimulation was not compromised as the maximum response was similar with or without plasma. A similar effect was observed using serum, suggesting that it is not mediated by fibrinogen. As the reaction occurred with both autologous and donor serum, cellular activation is not in response to “non-self” factors in serum, such as Ig recognition of foreign cell surface antigens. Other factors present in serum or plasma may moderate neutrophil function. This would include various hormonal or neuroendocrine factors, such as cortisol, adrenalin or -endorphins. The effect of differing levels of such substances on equine cell function has been poorly characterised. Hydrocortisone has been shown to increase the chemiluminescence response of human neutrophils (Horan, et al., 1982). Although plasma cortisol concentrations are not affected by repeated sampling in horses familiarised to regular venipuncture (Larsson, et al., 1979), the horses used in the current study were relatively unaccustomed to repeated sampling. Earlier studies have shown that the approach of strangers and the procedure of venipuncture may cause a rapid and profound increase in the number of circulating leucocytes (Stewart, et al., 1970; Archer, 1974). Such changes are likely to be mediated by glucocorticoids or catecholamines. It is possible that observed changes were mediated by such factors, however further elucidation of the mechanism by which this occurred is beyond the scope of the current study.

The response of foal neutrophils to the incorporation of adult or foal (donor or autologous) serum and plasma was the reverse of that observed in adult neutrophils. Auto-oxidation was dramatically decreased and there was an apparent inhibitory effect throughout the entire assay. This in direct contrast to the adult cells, which demonstrated a stimulated response following the addition of autologous or donor plasma to the assay. As donor plasma (collected from an adult horse) had an identical influence to foal (autologous) plasma, the response must be due to a functional difference(s) between foal and adult cells. Reduced responsiveness to some factor present in plasma would appear to be likely, given that there was no difference between the oxidative burst response of foal cells when compared to that of adult cells assayed in the absence of plasma. Physiologic characteristics of the surface of neutrophils may be important in determining their functional capacity. Lichtman and Weed (1972) demonstrated that immature human granulocytes are characterised by a high negative surface charge, combined with low adhesiveness to glass and plastic, low predilection to aggregate, a slow rate of cell spreading, decreased pseudopodia extension and motility and a very low rate of phagocytosis. Bernoco, et al. (1987) demonstrated reduced chemotaxis in peripheral blood neutrophils from neonatal foals, consistent with the observations of Lichtman and Weed (1972). Reduced responsiveness to mediators of the oxidative burst response may render foals at increased risk to sepsis, despite being able to mount an oxidative response that is equivalent to that of adult cells.
Storage of whole blood at 4°C for 24 and 48 hours was found to have a detrimental effect on neutrophil oxidative burst activity, as has been observed in other studies on neutrophil function (Glasser, 1977; Steigbigel, et al., 1978). Conversely, storage at room temperature (22°C) and at 37°C did not adversely affect function. The observations reported are consistent with neutrophil physiology and function in that neutrophils are metabolically relatively inactive in circulation (they possess neither a rough endoplasmic reticulum nor a golgi apparatus, and contain small number of mitochondria). Neutrophil life in circulation has been estimated at up to 8 hours in circulation and up to 5 days in tissues (Tizard, 1984; Guyton, 1991). Neutrophil function primarily centres on the phagocytosis of foreign material, a process which, once begun, leads rapidly to cell death and disintegration. Evaluation of the effects of storage on phagocytic activity (results not shown) demonstrated a lesser effect at cold temperatures than that seen for oxidative burst. This was also consistent with the observations of Glasser (1977) that phagocytic capacity was better maintained than chemotaxis or microbiocidal activity.

Consistent with the findings of other studies and clinical observations, healthy foals with failure or partial failure of passive immunity (Group 1) demonstrated a significant increase in circulating IgG concentration. This response was relatively transient, with concentrations in samples collected greater than 3 days following treatment not significantly different to pre-treatment values. Mean concentrations remained greater than 8 g/L at 28 days post-treatment, suggesting that treatment was effective at maintaining the circulating IgG concentration of most foals above this level for a considerable period.

The importance of IgG status alone as a predictor of septic disease and mortality in foals has been questioned (Kohn, et al., 1989; Baldwin, et al., 1991; Madigan, 1997). Aside from increased circulating IgG concentration, possible benefits of the administration of exogenous plasma to healthy foals with suboptimal passive immune status have not been demonstrated (Brewer and Mair, 1988; Clabough, et al., 1991). Plasma transfusion is aimed at rendering treated foals less susceptible to septic disease by increasing the opsonic capacity of foal serum. However, the efficacy of such treatment in preventing septicaemia in foals with FPT is unknown (LeBlanc, 1991). Clabough, et al. (1991) found that the administration of plasma to healthy foals did not reduce the likelihood of illness requiring medical therapy within the first 3 months of life. Despite the observed increase in serum IgG levels, the current study found no beneficial effect of plasma transfusion on bacterial phagocytosis or oxidative burst activity of neutrophils from healthy foals. These results suggest that the routine administration of plasma transfusions to healthy foals with failure or partial failure of passive immune transfer should be reassessed given the expense and invasiveness of the procedure. As the endogenous production of immunoglobulins and other aspects of neonatal immunity, such as haemolytic complement activity, are higher in colostrum deprived foals (Bernoco, et al., 1994), it is possible that the functional integrity of the neonatal immune system may develop rapidly in the absence of further passive supplementation. Healthy foals maintained in a low-stress, well-managed environment may therefore benefit little from the administration of exogenous plasma.

Plasma transfusion of septic foals (Group 2) resulted in a lesser increase in circulating IgG concentrations than was observed for healthy foals. Wilkins and Dewan-Mix (1994) reported similar findings. The poorer response may be due to catabolism of administered IgG due to the septic process. Pre-treatment IgG concentrations were lower for Group 2 foals than was apparent for Group 1 foals. Although not significant, this difference may also be due to consumption of available IgG. Alternatively, it may be that lower IgG concentrations rendered these foals more susceptible to sepsis.

Despite the lower magnitude of IgG response to treatment in Group 2 foals, plasma transfusion appeared to have a short term beneficial effect on neutrophil phagocytosis. These results support the clinical administration of plasma as part of the routine treatment of septic foals. Although significant differences were not apparent in the current study, the oxidative burst response of neutrophils from septic foals tended to be less than that of healthy foals. This may be a consequence of the septic process or may have existed in these foals prior to the development of disease, thus rendering these
foals at increased risk to the development of sepsis. Phagocytosis was not different in neutrophils of septic foals prior to treatment, when compared to cells from healthy foals, suggesting that deficiencies in the attachment and ingestion of bacteria are not responsible for the development of disease and do not develop as a consequence of infection. The positive effect of treatment appeared to be due to enhanced phagocytosis above that evident in healthy foals and that evident in septic foals prior to treatment.
Implications

♦ The type of fluid administered to foals in the first 12 hours post-partum did not affect the efficiency of absorption of colostral IgG, indicating that macromolecules do not mediate closure of the equine intestine to immunoglobulin absorption.

♦ The time during which colostrum may be administered to foals cannot be prolonged by the administration of a glucose-electrolyte solution.

♦ The efficiency of absorption of IgG reported in the current study at 12 hours was slightly higher than that previously reported for absorption of a macromolecule analogous to IgG and much higher than reported for absorption at 12 hours post-partum. It is therefore likely that substances in colostrum have a role as mediators of closure.

♦ Despite a high efficiency of absorption, foals in the current study demonstrated IgG levels lower than is currently considered optimal. For this reason, colostrum should ideally be administered to foals within 12 hours of birth.

♦ The delayed administration of colostrum was, however, associated with IgG levels that may have been adequate for healthy foals in well managed environments. Furthermore, improved neutrophil phagocytosis was evident when adult cells were assayed with foal serum. Although this improvement may have been mediated by physiological changes, these observations suggest that even delayed ingestion of colostrum is likely to be beneficial for foals.

♦ Body weight, plasma volume, volume of colostrum ingested or the amount of IgG ingested are not useful as predictors of efficiency of absorption.

♦ Storage of adult neutrophils for up to 48 hours at room temperature (22°C) or 37°C had no effect on oxidative burst activity. Such delays may, therefore, be considered acceptable prior to performing functional assays. Refrigeration of samples intended for the evaluation of cell function is not appropriate.

♦ Neutrophils from foals demonstrated reduced phagocytosis when compared with adult cells. Autofluorescence and oxidative burst responses were inhibited for foal cells when autologous or adult plasma was incorporated in the assay. The activity of adult cells was increased by the addition of plasma. The age at which these functional differences are no longer apparent has not been determined.

♦ Plasma treatment of healthy foals in well managed environments may be of limited value as there was no demonstrable effect of plasma transfusion on cell function, despite increased circulating IgG concentrations. The value of such treatment for the prevention of septic disease may be limited.

♦ Conversely, plasma treatment of septic foals was associated with enhanced phagocytosis. The IgG response following treatment was less than that observed for healthy foals and the duration of improved cellular response was short-lived (12 hours).

♦ Improved neutrophil function was observed in septic foals despite the more modest increase in IgG levels, suggesting that provision of this component of the immune response alone may be a simplistic explanation for the beneficial effects of treatment.

♦ Neutrophils from septic foals tended to have lower oxidative burst activity than was observed in neutrophils from healthy foals with or without FPT or pFPT. Impaired intracellular killing may be an important predisposing factor for the development of sepsis, or may develop as a consequence of disease.

♦ The observation that the addition of plasma to oxidative burst assays had a dramatic and opposite effect on the activity of foal and adult cells suggests that humoral factor(s) may have an important role in regulating the oxidative response of neutrophils.
Recommendations

Industry/Management Recommendations

♦ Colostrum should be administered to foals within 12 hours of birth. When this is not possible, the administration of colostrum to foals between 12 – 18 hours post-partum should provide increased circulating IgG and may have a beneficial effect on cell function.

♦ The delayed administration of colostrum may be preferable to plasma transfusion of healthy foals.

♦ Circulating IgG concentration should be determined following the ingestion of colostrum due to marked individual variation in the efficiency of absorption of colostral IgG.

♦ Healthy foals with FPT or pFPT in low risk environments should not be routinely subjected to plasma transfusion. Close observation for the development of septic disease and prompt, appropriate and aggressive treatment should sepsis develop is likely to be effective.

♦ Plasma transfusion should be routinely used in the treatment of septic foals. Multiple transfusions at intervals of approximately 24 hours may be required due to the short duration of effect observed.

Avenues of Further Investigation

♦ More detailed studies on the process of closure are required. Evaluation of the possible role of colostral components as mediators of closure is indicated. Such studies may also yield information on the possibility that the non-specific nature of absorption permits entry of bacteria across the intestinal epithelium.

♦ Further studies are warranted to determine whether changes observed in cell function after 12 hours post-partum were due to the ingestion of colostrum or physiological changes. This could be achieved by studies on cell function in foals receiving colostrum at birth and in foals deprived of colostrum.

♦ The soundness of recommendations of withholding plasma treatment from healthy foals with FPT/pFPT and ensuring such treatment for septic foals should be evaluated by prospective studies aimed at identifying the effects of such treatment on the incidence of foal disease and long term outcome.

♦ Oxidative burst activity should be evaluated as a predictor of septic disease or as a prognostic indicator for sick foals.
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