

Failure of Passive Transfer

Jon Palmer, VMD, New Bolton Center, University of Pennsylvania

Colostrum and Passive Transfer

The primary function of colostrum is the establishment of a healthy immune barrier between the luminal bacteria and the foal at the GI mucosa. Although colostrum is an important source of IgG, it contains many other biologically active proteins, immune modulators and pro- and anti-inflammatory substances. All of these substances are important in insuring the development of an effective protective barrier targeting potential pathogens before their invasion and insuring that the fragile development of the gastrointestinal tract is not disrupted by inflammatory damage.

It was Paul Ehrlich in 1891 who first recognized the importance of colostral transfer of protective factors. Colostrum is tailored for the neonate who has yet to develop a complete compliment of immune functions. Certain agents in colostrum initiate or augment functions which are otherwise poorly expressed in the neonates. In fact, without some agents in colostrum, immune development will be delayed. Equine neutrophils only become mature killing cells after exposure to substances in colostrum. Certain immune functions that are initially absent in neonates are replaced by factors in colostrum. In addition, defense agents in colostrum have enhanced survival in the gastrointestinal tract of the recipient compared to their plasma derived counterpart. Also, defense factors in colostrum protect without provoking inflammation and some agents inhibit inflammation both allowing targeting of pathogens without allowing the inflammatory reaction to disrupt the development of the neonate's gastrointestinal tract. There are also agents in colostrum that alter the physiologic and biochemical state of the gastrointestinal state from one suited to fetal life to one appropriate to extrauterine life. Finally and perhaps most importantly, growth factors in colostrum augment the proliferation of the commensal enteric bacteria. Since the gastrointestinal tract is the most likely portal of entry of pathogens, the action of colostrum in preventing luminal establishment, proliferation and invasion of pathogens is vital in protecting the neonate from sepsis.

Antimicrobial factors in colostrum include proteins such as lactoferrin (bacteriostasis by Fe chelation), lactoferricin (causing bacterial killing), lysozymes (bacteriolysis by degrading peptidoglycans), MUCI (inhibits the binding of S-fimbriated *E coli* to epithelial cells), lactadherin (binds viruses so prevents

epithelial attachment), oligosaccharides and glycoconjugates (receptor analogues which inhibit binding of enteric pathogens and toxins to epithelial cells) and monoglycerides and fatty acids (disrupts enveloped viruses, inactivate certain bacteria, defend against Giardia). Other important factors in colostrum include PAF acetylhydrolase (PAF-degrading enzyme; PAF is an important proinflammatory mediator in the GI with high levels in the neonate; this enzyme protects mucosal cells from damage caused by PAF by degrading it), erythropoietin which protects against apoptosis of intestinal epithelium, epidermal growth factor which has been shown to play an important role in mucosal barrier function in developing intestine, and down-regulates apoptosis of intestinal epithelium.

Measuring Passive Transfer

When we measure IgG plasma levels as a reflection of passive transfer, what we are doing in essence is making the only measurement of the establishment of this immune barrier and transfer of immune competence available to us. There is no way to test to see if the enteric protective barrier has been established, to insure that protective and modulating substances are present and in place at the mucosal level resulting in an effective immune barrier. There are no simple techniques to see if the colostrum substances have had their stimulating effect on the neonate's immune function or have stimulated the healthy maturation of the neonate's mucosal barrier. So we use the measurement of plasma IgG levels as a surrogate for these things. Transfer of a quantity of IgG is important but not the most important part of passive transfer. It's not the quantity but the quality of IgG that's important. Having a large quantity of IgG targeted against encephalitis virus is not helpful in protecting the neonate against bacterial pathogens. But since we have no method to measure the quality of IgG transferred, we rely on quantity as a surrogate. It is unfortunate that we have largely lost sight of this and frequently teach that the surrogate, IgG quantity, is the aim of passive transfer. In fact a whole industry has grown out of this misconception and IgG concentrates are frequently marketed as colostrum substitutes. Even when hyperimmune plasma transfusion is used as a colostrum substitute, a significant quantity of IgG transferred will be directed against pathogens that aren't a threat to the neonate. But when the donor is stimulated to produce this unhelpful IgG, other, more useful antibodies will also be produced as well as immune modulating substances which may be important in the neonate who has not benefit from colostrum.

Classically failure of passive transfer (FPT) is defined as failure to absorb adequate

colostral antibodies. The failure is classified by degrees: complete if IgG < 400 mg/dl; partial if IgG 400 - 800 mg/dl; normal transfer > 800 mg/dl. Normal foals often have IgG > 3000 mg/dl. Septic foals, even if they absorbed adequate amounts of IgG, often have very low levels since they catabolize all proteins, including IgG causing levels to drop rapidly. Some of the IgG may be used specifically in the foal's response to the infection, but most is nonspecifically catabolized. It should be replaced by plasma transfusions (which will also provide other important proteins helpful in fighting the infection).

The prevalence of neonatal infections in the foal population is important in determining the value of the IgG level in predicting likelihood of infection. If the prevalence of infection is very low (as on well managed breeding farms), foals who do not receive antibodies are unlikely to be challenged by pathogens and it will appear that measurement of IgG levels has little importance in predicting disease. On the other hand, if the risk of infection is high (such as in hospitalized neonates with diseases which lower their innate resistance and who are in a high density hospital situation where aggressively virulent nosocomial pathogens are common) the IgG level may not predict protection. Poor understanding of the role of disease prevalence in different populations and its effect on diagnostic tests has lead to confusion and conflicting study conclusions not only in FPT in foals but a number of other disease problems in veterinary medicine.

The difficulty in measuring the quality of the IgG transferred has lead to the development of a quantitative guideline which is commonly used to diagnose FPT and guide therapy. Since the relative risk of infection for the neonate is difficult to predict, except on large farms with carefully recorded medical histories, guidelines developed from experiences at referral centers are often universally applied despite the fact that based on disease prevalence, they may be too rigorous.

Factors associated with FPT

1. Premature lactation - dripping milk before parturition.
2. Poor colostrum quality - low levels of IgG and other factors.
3. Failure of foal to ingest adequate amounts because of musculoskeletal disease, weakness, abnormal behavior, etc.
4. Older mares.
5. Foals born early in spring (Jan-March).
6. Breed differences - on average Arabians have the highest colostrum IgG

levels (6.1 mg/dl) and Standardbreds have the lowest (4 gm/dl).

Prevention

At the time of parturition, the colostrum quality can be assessed. Good quality colostrum is thick, sticky (from high protein content) and somewhat yellow. A quantitative assessment can be made by measuring the specific gravity. A specially designed colostrometer makes this process easy. Colostrum of acceptable quality usually has a specific gravity >1.060. Excellent quality colostrum has a specific gravity >1.080. Quality can also be measured with a Brix refractometer (> 23% is good colostrum). Although a high specific gravity or Brix correlates well with successful passive transfer, the converse is not true. Consumption of colostrum with a low specific gravity can result in successful passive transfer, perhaps because of the quantity ingested.

If there is doubt about quality or quantity of ingested colostrum, the foal can be supplemented (bottle feed or NGT) with frozen colostrum during the first 18 hours of life. Oral colostrum substitutes (e.g. Seramune®) are marketed and may help with luminal immunity but usually aren't absorbed so will not increase blood IgG levels. If a mare develops premature lactation, another source of colostrum such as a colostrum bank should be identified to provide colostrum for the foal at birth.

Diagnosis

The foal's IgG level should be measured between 8 and 24 hrs of age. Some clinicians measure the value early so that if it is lower than expected, supplemental colostrum can be given (rather than waiting until later when plasma will be needed). Other clinicians wait until 24 hrs, since level obtained at 8-12 hrs may continue to rise. If a low IgG level is found at 24 hrs, it is too late for colostrum absorption and supplemental IgG must be given parenterally. I generally run levels 6 to 8 hours after the foal first nurses. Most foals will have IgG levels > 800 mg/dl that early although levels often increased to > 2000 mg/dl by 24 hours. I prefer to supplement with bank colostrum when the IgG is low.

Diagnostic tests:

1. Snap® Foal IgG test (ELISA)

This is a rapid test which can be run on whole blood or plasma. The value returned is <400, 400-800 or >800 mg/dl based on a color comparison. This is currently the most accurate rapid test and commonly used.

2. DVM Rapid Test™ (anti-horse antibody binding assay)

This is a rapid test which uses anti-horse antibodies to quantitate through turbidimetry. This seems to be a very accurate and rapid method which reports a quantity of IgG instead of a breakpoint range. This is the technique currently used at NBC.

3. ZnSO₄ turbidity test

This is a rapid, potentially stall side test performed on serum. It has 3 problems making it less than ideal. First the reagents lack stability resulting in unreliability. Second, even slight hemolysis will cause false positive results. Third, it is difficult to quantitate (often the mare's serum is used as the positive control).

4. Glutaraldehyde coagulation test

This is a relatively rapid (1 hr) test requiring serum. It suffers from the same draw backs as the ZnSO₄ turbidity test, especially false positives with hemolysis.

5. SRID (Single radial immunodiffusion) test

This test requires 24 hrs. It is the traditional "gold standard", but its accuracy has been questioned recently. It is a quantitative test (the other tests are semiquantitative).

Treatment

Oral therapy (1 gm/kg IgG): The window of time when oral colostrum is absorbed varies depending on circumstances. The gut will not "close" sooner in foals that ingest milk (e.g. poor quality colostrum) than those that don't ingest anything. Foals with Neonatal Gastroenteropathy resulting in dysmotility may have less effective absorption. In most circumstances the

gut should not be relied on to absorb significant amounts of IgG past 18 hours (although the IgG may rise after this time). Even when it is too late to take advantage of absorption, colostrum is beneficial. It not only provides local immunity, it is an effective laxative and contributes to the health of the epithelial cells.

1. Frozen equine colostrum: It should be no more than 18 months old. The IgG levels will drop with time even if it is kept frozen. It should be kept in a "deep freeze," not a freezer built into a refrigerator (so that it is colder) and not in a "frost free" freezer since these go through thaw cycles. It should be thawed in lukewarm water to preserve the IgG. Ideally 1 liter of >1.060 colostrum should be given, but if the quality is high, half that amount may be sufficient.
2. Colostrum substitutes (lyophilized IgG - Foal-Aid[®], Seramune[®]): These products are poorly absorbed and do not significantly result in an increase in foal plasma IgG levels after administration. They also lack important factors other than IgG. They are a solution when equine colostrum is not available but a poor substitute.
3. Bovine colostrum: When equine colostrum is not available, bovine can be used. At least 4 liters should be fed. The IgG will drop more rapidly and may not be as effective as equine IgG.

Intravenous therapy

1. Fresh plasma: Unless the donor is hyperimmunized, the IgG in the plasma may be very variable and often is lower than expected. As a result frequently 2-3 liters are required to raise the foal's IgG level. Unless the donor has been tested recently, anti-equine antibodies may be present and result in a significant transfusion reaction. It is safest to run a crossmatch, but this will significantly delay the time to transfusion. Also if the plasma must be harvested, this will also take significant time.

2. Frozen hyperimmune plasma (number of commercial sources): This plasma is harvested from hyperimmunized donors shown not to have anti-equine antibodies and have IgG levels above 2400 mg/dl. This is the most convenient source of plasma for most practitioners. It should be stored in a non-frost free deep freeze and thawed in lukewarm water to preserve as much IgG as possible (no matter how it is thawed, there is a significant loss which needs to be minimized) and the companies will ship it overnight so it is not difficult to get when needed. Usually 1 liter is all that is needed except for septic foals.

3. IgG concentrates (Endoserum[®], Promune E[®], Seramune[®]): These products are less expensive and more easily stored, however they are inferior to whole plasma since they lack some of the immunogenic factors other than IgG.