# Diagnosis of Colostrum Deprivation in Calves

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Colostrum is the primary source of passive natural protective antibody for the neonatal ruminant. Early ingestion of this immunoglobulin (Ig) rich milk is critical for neonatal calf survival; colostrum deprivation results in neonatal failure of passive transfer of antibody (FPTA).[1,2] Maternal serum $IgG_1$ and $IgG_2$ are concentrated in the udder as colostrum during the month prior to calving; $IgG_1$ is the major antibody constituent of colostrum.[1,3] Two hundred to three hundred grams of total colostral Ig mass must be delivered to the calf's alimentary tract within 4-12 hours of birth to achieve absorption of maximum protective levels of Ig.[4] Administration of two liters (quarts) of colostrum to the neonate historically has been presumed to supply this Ig mass. However, selection for excessive milk production (most extreme in the Holstein breed) is hypothesized to have resulted in dilution of Ig concentrations of colostrum to such an extent that even four liters (1 gallon) of colostrum may contain inadequate antibody mass for optimal protection of the neonate.[5,6] Recent demonstrations of low and variable Ig concentration in Holstein colostrum explains some of the higher incidence of FPTA experienced by dairy calves as opposed to beef calves.[6,7]

Colostrum contains additional factors inhibitory for pathogenic bacteria, including complement, transferrin and lactoferrin. Complement facilitates neutrophilic phagocytosis through bacterial opsonization. Transferrin and lactoferrin bind iron rendering it unavailable for bacterial growth.

FPTA is still a major cause of death and debilitating diseases in the neonatal calf. Partial or complete FPTA has been reported to occur in 25 to 65% of naturally suckled calves, especially those destined for intensive rearing units.[1] Ninety percent of calves dying in the first week of life had not absorbed adequate amounts of Ig.[2] Of those dying in the second and third week of life, 80% had inadequate Ig concentrations.[2]

When FPTA is coupled with exposure to an invasive *Escherichia coli* serotype, acute coliform septicemia can occur. Calves with FPTA have up to ten times greater risk of septicemia than calves with normal Ig levels. The resulting neonatal coliform endotoxemia usually leads to cardiovascular collapse and death. Partial failure of passive transfer antibody may not result in the immediate death of the calf, but may be characterized by omphalitis, omphalophlebitis, urachitis, or septic arthritis. Bull calves are disproportionately affected with FPTA on dairies where good calf management is exercised for potential replacement heifers only.

### Clinical Signs of Colisepticemia

Colisepticemia usually occurs in the first two to five days of life. Septicemic shock may occur peracutely and a calf may be found in complete collapse a few hours after being apparently normal. An early indication of septicemia is anorexia, fever, and scleral vessel injection. As the calf becomes more severely affected it becomes cold, limp, and moribund with a profound tachycardia (140 to 200/beats per minute). If terminal meningoencephalitis occurs, the calf displays neurologic signs including paddling of the limbs, seizures, nystagmus, anisocoria, hyperesthesia, hypopion (uveitis), vocalization, etc. Frequently the hydration status of septicemic calves is normal since coliform strains capable of causing septicemia differ from those causing diarrhea. Diffuse petechial and ecchymotic hemorrhages will be detected in the carcass of the calf dying from colisepticemia. Increased quantities of watery, turbid joint fluid containing fibrin plaques is a hallmark of the colisepticemia. Organ cultures or blood aspirates from the intact heart collected prior to transection of the gastrointestinal tract can be submitted for bacterial isolation. An excellent description of technique for complete necropsy of the neonatal calf is available.[8]

### Specific Techniques for Detection Ig Absorption

Several methods of Ig assessment can be used to detect FPTA. A total solids refractometer that measures serum

plasma refractive index can identify hypoglobulinemia. Serum protein refractive index correlates more directly with Ig absorption than plasma protein refractive index, because soluble fibrinogen is not present to increase the measured refractive value. A serum refractive index less than 5.0 g/dl (or 5.5 g/dl for plasma) is cause for concern; levels greater than this may be partially or completely protective in the normally hydrated calf that is only a few days old. Preferred values are greater than 6.0 g/dl for serum and 6.5 g/dl for plasma.[4 9] Dehydration of the calf results in falsely elevated values; the hypoglobulinemic calf may appear normal while the normoglobulinemic calf may have a serum refractive index greater than 7.0 g/dl. Immunoglobulin levels decline after the first week of life, so lower refractive indexes in older calves are not as significant as in neonates.[3 10] Serum refractive index is a rapid test that lends itself easily to practice situations and is a good screening test when large numbers of animals are involved, although interpretation should always be tempered by clinical assessment and evaluation of hydration.

The gluteraldehyde coagulation test is an effective and rapid means of screening large numbers of calves in a field situation. 50 ul of 10% gluteraldehyde is added to a 0.5 ml aliquot of serum (a 1:10 dilution of reagent to serum) and is agitated thoroughly. The time required for complete coagulation of the mixture is inversely proportional to Ig concentration of the sample. Samples from neonatal calves with high serum Ig concentration coagulate within five to fifteen minutes. This rapid test requires only test tubes, a volume measuring device, a mechanical mixer and a centrifuge for serum separation.[11] Dehydration will result in false elevations of Ig concentration. The gluteraldehyde coagulation test may soon be utilized in the neonatal equine. It has been demonstrated to be very sensitive and less expensive than commercially available kits for evaluation of Ig absorption in the foal.[12]

The zinc sulfate turbidity (ZST) test can be performed as a general screening test or as a means of accurate Ig quantitation. Zinc sulfate solution selectively precipitates immunoglobulins. $ZnSO_4$ $7H_2O$ is mixed with boiled water to form a solution of 208 mg/l. One hundred microliters of calf serum is then added to six mls of this solution, and the mixture is incubated at room temperature for one hour. The turbidity noted is directly proportional to the serum Ig levels. The ZST test cannot be performed on plasma, since fibrinogen will also be precipitated. Carbon dioxide absorption alters the accuracy of the test, therefore the zinc sulfate reagent must be made from water that has been boiled to remove $CO_2$; it must be freshly mixed or have been stored in an airtight vessel containing soda lime straws. Immunoglobulin levels can be estimated subjectively on the basis of total precipitate formed. Accurate quantitation of serum Ig concentration is possible with this technique, but require special electronic instrumentation not available to practitioners.[4 5] Dehydration will also result in false elevation of Ig measurements.

A commercially available Ig precipitation test kit (Bova-S[a]) is a semi-quantitative means of identifying calves with FPTA. Each kit contains enough reagent to test 18 calves at a cost of about $5 each. Insertion of an accurately measured quantity of serum into a pre-measured bottle of sodium sulfite reagent results in formation of a flocculent precipitation in the calf with adequate serum Ig levels.[13] Standards for comparison are not provided in the kit, but a photograph of results is included to help in test interpretation. The kit contains everything necessary to conduct this test with the exception of a centrifuge. Errors in interpretation arise when calves with intermediate levels of Ig absorption are tested. Partial but visible precipitation may occur in calves which later develop joint or navel infections.[10]

Electrophoresis is a laboratory technique by which serum proteins can be fractionated and serum albumin and individual gamma globulin classes can be quantified. Gamma globulin levels below 1 g/dl indicate partial failure of clostral Ig absorption, while values less than 500 mg/dl are associated with severe Ig deficiency.[14] This test must be submitted to a laboratory, takes 8-12 hours for the actual test to be performed, requires expensive equipment, and usually costs less than $15.

Single radial immunodiffusion (SRID) is the most expensive and time consuming test for FPTA, but it is the most specific diagnostic tool for IgM and IgG quantitation.[3] It overcomes a problem inherent in each of the previously mentioned tests which are incapable of differentiating between absorbed IgG and IgM. In the calf, IgM is the Ig principally responsible for protection against coliseptic-emia.[15] It is possible for a calf to have adequate IgG absorption simultaneously with inadequate maternal IgM absorption. Such a calf could develop septicemia despite satisfactory results achieved on one of the previously mentioned tests. When measured by SRID, values less than 800 mg/dl of $IgG_1$ are considered marginal Ig absorption, and less than 500 mg/dl would be considered complete FPTA.[9 14] Serum $IgG_1$ values greater than 1000 mg/dl are considered ideal, and 1500 mg/dl or more of total IgG is considered indicative of excellent colostral antibody absorption. Less than 80 mg/dl of IgM and less than 22 mg/dl of IgA is also indicative of FPTA.[4] IgM and IgA have short half lives and rapidly decline in colostrum-fed calves.

Although presently considered the "gold standard" for Ig quantitation, there are disadvantages to SRID. Serum must be submitted to an appropriately equipped diagnostic laboratory, serum may be stored for several days so "batching" of samples is possible, but the test requires 24-48 hours to conduct. Therefore, the test is not useful when used as a prognostic test for an individual calf. To have all immunoglobulins quantitated ($IgG_1$, $IgG_2$, IgA and IgM) is expensive (greater than $50). Despite the fact that SRID offers the most accurate means of quantitifying $IgG_1$, $IgG_2$,

[a] *V.M.R.D., Inc., P. O. Box 502 Pullman, WA 99163*

IgM and IgA, comparison of absolute values between laboratories is difficult.

The present explosion in application of biotechnology may result in production of other methods for assessment of Ig absorption. A bovine Latex Agglutination test has been developed in Great Britain that satisfactorily identifies calves with FPTA. A calf-side ELISA test for $IgG_1$ may also become available in the near future.

### Non-specific Tests for FPTA

Neonatal intestinal absorption of macromolecules from colostrum is non-specific. Polysaccharides and non-immunoglobulin proteins (such as beta-lactoglobulin and serum albumin) exist in the colostrum and may be absorbed by the neonatal calf.[1][16] The neonatal kidney excretes these low molecular weight proteins for several days after absorption occurs. A transient proteinuria is evident within 24 hours of colostrum ingestion.[17,18] This proteinuria can be detected by simply shaking a urine sample and checking for short-term formation of a stable foam, or by addition of protein precipitating substance (such as 5% solution of sulfosalicylic acid) which will cause the urine sample to become flocculent.[19,20] Formation of a precipitate confirms that the calf absorbed lactoglobulin; this implies that gammaglobulin must also have been absorbed simultaneously.

The maternal enzyme gamma-glutamyl transpeptidase (GGT), is concentrated in bovine colostrum, up to 1000 time higher than in maternal serum.[21,22,23] Maternal colostral GGT has a lower molecular weight than globulins and is probably similarly absorbed by the neonate. Maximal GGT peaks in neonatal plasma are obtained within 24 hours of colostrum absorption. These levels show much individual variation but can be up to 2000 times greater than pre-colostral values. GGT levels do not increase in calves deprived of colostrum. Although this exogenous GGT declines rapidly over the next six days, significant levels are still detectable on the 20th day following colostrum ingestion. A significant correlation exists between neonatal log plasma GGT and plasma globulin (beta plus gamma) concentration.[23] If specific immunoglobulin tests are unavailable, this may be one means of confirming that colostral ingestion did occur.

Serum alkaline phosphatase (SAP) levels also increase dramatically within 24 hours of colostrum ingestion in the calf capable of macromolecule absorption. But in the lamb, SAP has been concluded to be of intestinal origin rather than of colostral origin. Therefore, SAP could be utilized as a non-specific indicator of colostral ingestion in the bovine animal but should not be used for this purpose in the neonatal lamb.[24]

Serum alkaline phosphatase and plasma GGT are unlikely to be widely utilized for confirming bovine colostral ingestion because of the easy, more specific tests for Ig absorption. However, a practitioner without access to the specific diagnostic techniques previously mentioned could use plasma GGT or SAP levels as a non-specific indicator of colostral Ig absorption. Experimental application of SAP and GGT levels in the neonatal puppy are presently being investigated, appear to show trends similar to that of the calf, and may prove to be a useful diagnostic tool in the dog.

Assurance of adequate colostral intake is necessary to facilitate survival of neonatal calves. Detection of inadequate colostral intake is an integral component of veterinary involvement in calf health programs, and available techniques have been summarized. It is advisable to use the more specific tests of Ig absorption (total solids refractometry, sodium sulfite precipitation, ZST, gluteraldehyde coagulation, electrophoresis, SRID) for valuable calves or in practices where monitoring of FPTA is frequently required. The non-specific tests (precipitation of urine proteins, and maternal enzyme absorption) are available when the practitioner has no immediate access to one of the other tests listed above.

Based upon total cost per test, expense is minimal for the ZST, gluteraldehyde coagulation and urine precipitation tests but practitioners wishing to use these tests must obtain and mix the reagent themselves. Total solids refractometry requires an initial expense for the refractometer and micro-hematocrit centrifuge, but this is standard equipment in many practices. The sodium sulfite precipitation and maternal enzyme absorption tests are moderately expensive ($4 to $10 apiece). The electrophoresis and SRID are too expensive to justify frequent use on calves of moderate value, and should not be selected as a screening tool for large numbers of calves.

When rapidity of diagnosis is essential or large numbers of calves are screened, the best tests to utilize are total solids refractometry, sodium sulfite precipitation, ZST, or gluteraldehyde coagulation. When time and expense are not a factor but accuracy is important, the SRID is the best test to select. The calf must be 24 hours or older before *any* of the tests discussed will accurately measure absorbed Ig. Therefore, by the time FPTA has been diagnosed by any of these methods, it is too late to supplement the calf orally with colostrum, and other therapeutic techniques are required. These techniques will be discussed in the second paper in this series.

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