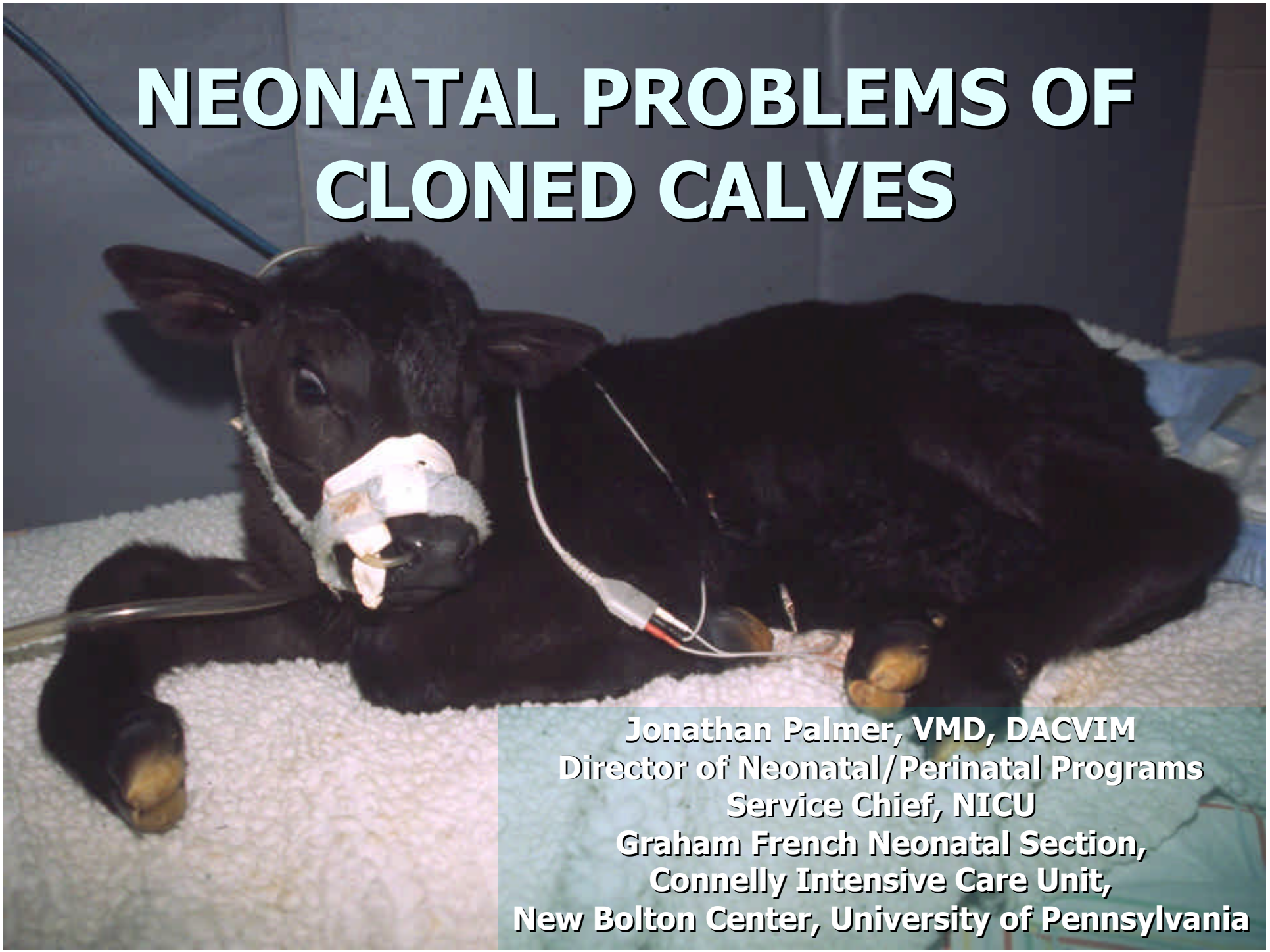
NEONATAL PROBLEMS OF CLONED CALVES



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Problems Recognized

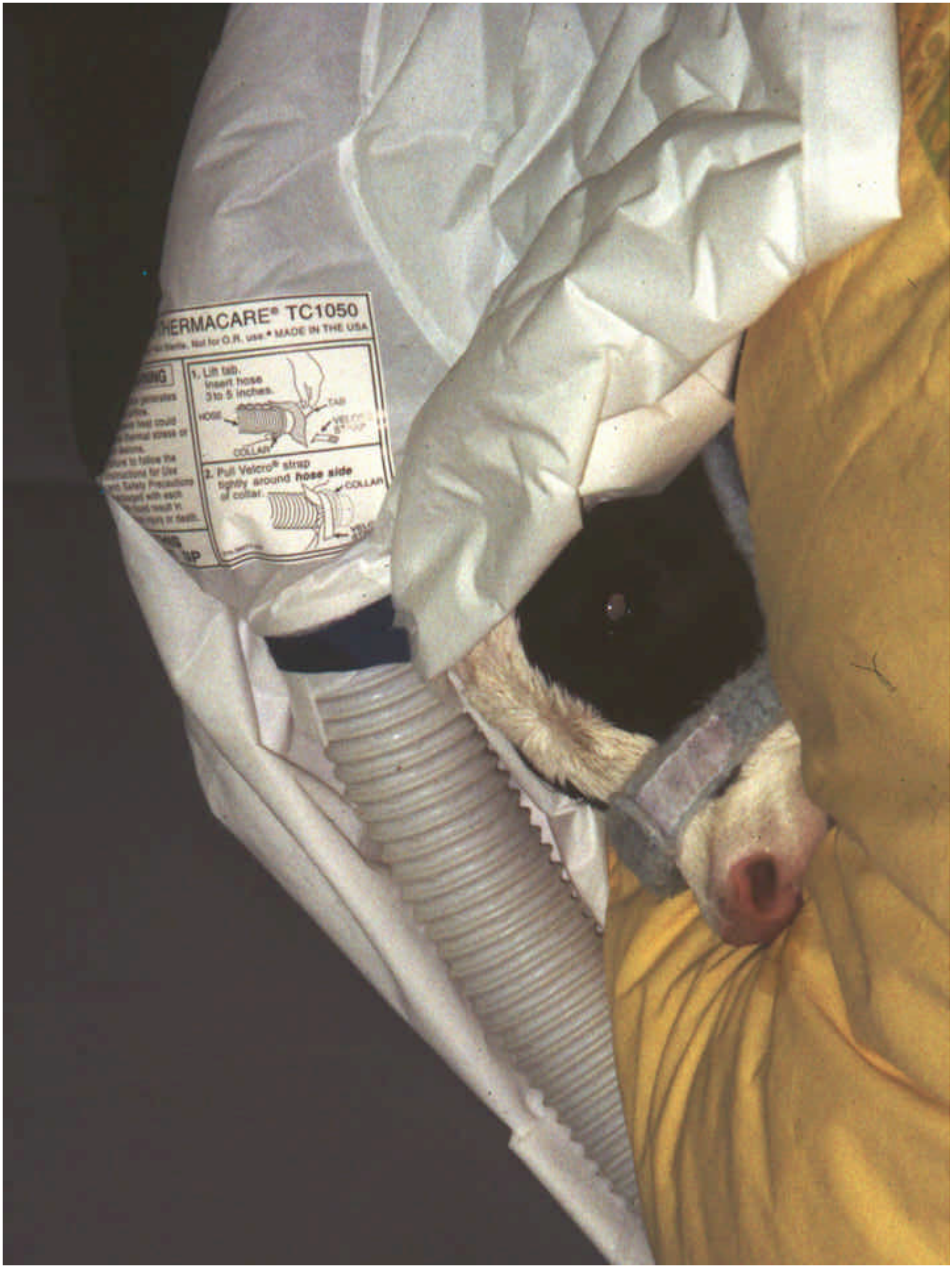
- Abdominal distension
- Alopechia
- Anemia
- Angular Limb Deformity
- Anuria
- Atelectasis/Pneumonia
- Aspiration pneumonia
- Bacteremia
- Birth bradycardia
- Birth Hyperlactatemia
- Birth Hypoglycemia
- Birth leukocytosis
- Birth polyuria
- Birth tachycardia
- Cardiac Arrhythmia
- Cardiomegaly
- Central hypercapnia
- Chondrodysplasia
- Colic
- Contracture limb, neck, torso
- Diarrhea

Problems Recognized

- Endocardiosis
- FPT
- Fever
- Fungemia
- Hematochezia
- Hepatomegaly
- Hyperglycemia
- Hypochloremia
- Hypothermia
- Hepatic cysts
- Large external umbilical remnant
- Lymphadenopathy
- Meconium Aspiration
- Muscular dystrophy
- Neonatal Encephalopathy
- Neonatal gastroenteropathy
- Neonatal Nephropathy

Problems Recognized

- nRBC
- Patent Urachus, Urachitis
- Periodic apnea
- Pigment nephropathy
- Porencephaly
- Portal fibrosis
- Retained Fetal Circulation
- Reversion to fetal circulation
- Rhinitis
- Rotational limb deformity
- Shock
- Sepsis
- SIRS
- Tachycardia
- Tachypnea
- Tracheal hypoplasia
- Tricuspid Dysplasia
- Tricuspid regurgitation
- Umbilical bleeding
- Umbilicomegaly
- Upper Airway Noise
- Valgus
- VSD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MADE IN THE USA
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Insert hose
3 to 5 inches.
HOSE
TAB
COLLAR
2. Pull Velcro® strap
tightly around hose side
of collar.
COLLAR

Sepsis

- 77% calves
  - Bacteremia – 9 calves – 24%
    - *Acinetobacter baumannii*
      - 3 isolates
    - *Pantoea agglomerans*
    - *Enterobacter cloacae*
    - *Enterococcus faecium*
    - *E coli*
    - Coag neg *Staph*
    - *Klebsiella* group 47
    - *Aspergillus niger*

Sepsis

- Infection usually not localized
- When localized
  - Umbilical infection
  - Pneumonia
  - Enteritis

Risk Factors

- Lower resistance
  - Secondary to other problems
- Aggressive nosocomial pathogens
- Abnormal immune function?
- Colostrum management
  - Use of colostrum substitutes
  - Lyophilized IgG products

Colostrum

Its not just about IgG

Colostrum
Neonatal Immune Development

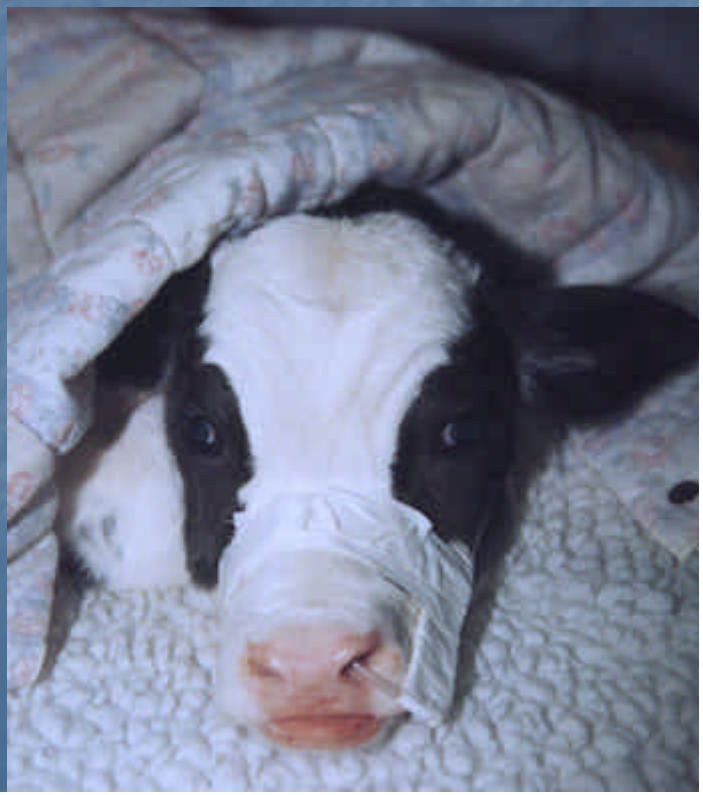
- Immature neonatal immune system
  - Provides replacement substances
    - Such as IgG
  - Provides substitutes
    - Augment functions poorly expressed
- Alters physiologic state of GIt
  - Transition from fetal to neonatal life
- Special quality of immune active substances
  - Protect without inflammation
  - Inhibit inflammatory response
  - Enhanced survival in GIt
- Bacterial growth factors
  - Augment proliferation of commensal enteric flora

Colostrum

- Development of a protective barrier
  - Targeting potential pathogens
    - before invasion
- Protecting fragile neonatal GIt
  - Promote maturation
  - Not disrupted by inflammatory damage

Colostrum
Bio-active Substances

- Antibacterial substances
- Anti-inflammatory substances
- Immune modulators
- Growth promoters



Colostrum
Antimicrobial Factors

- Immunoglobulins - local as well as systemic
- Lactoferin - targets bacterial outer membrane
- Lysozyme
- Complement – C3
- Mucins – inhibit binding of fimbriated bacteria
- Lactadhedrin – binds viruses
- Oligosaccharides
  - Receptor analogues, growth promoters
- Lipids – antiviral, antibacterial

Colostrum Antimicrobial Factors

- Leukocyte
  - Lymphocytes
    - Low cytotoxic activity
    - Produce cytokines
    - Memory cells appear to be transferred
  - Neutrophils
    - Reactivity/function modulated by colostrum
  - Macrophages
    - Reactivity/function modulated by colostrum
  - Cellular immunity transferred?

Colostrum
Anti-inflammatory Factors

- Proinflammatory cytokines in colostrum
  - Produce a dampened inflammatory response
- Anti-inflammatory Factors
  - Cytoprotective PGs
  - Epithelial growth promoters
    - Epidermal growth factor, lactoferrin
    - Cortisol, polyamines
  - Maturational factors – cortisol
  - Mediator binders/degraders
  - Leukocyte modulators
  - Antioxidants

Colostrum
Bioactive Factors

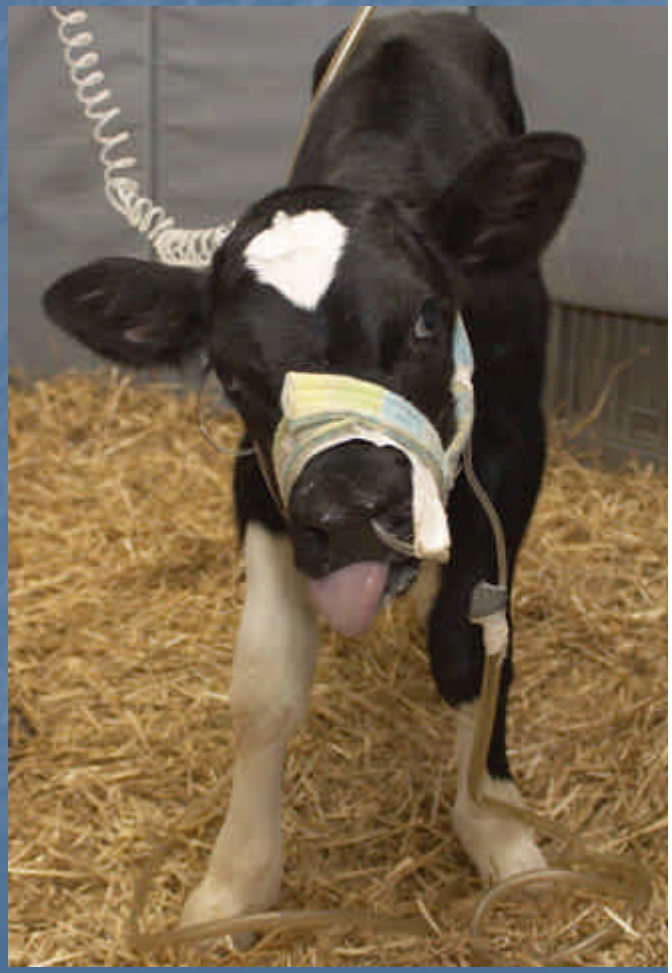
- Ery
  - F ells
  - U ess”
  - E optosis
  - F

Colostrum ≠ IgG

- Colostrum substituted by lyophilized IgG
  - Not a fare trade
- Obtaining a high IgG
  - Not the goal of colostrum feeding
  - Any more than obtaining a high GGT
- Finding plasma markers
  - Surrogate suggest immune barrier in place
  - Not the goal of colostrum feeding

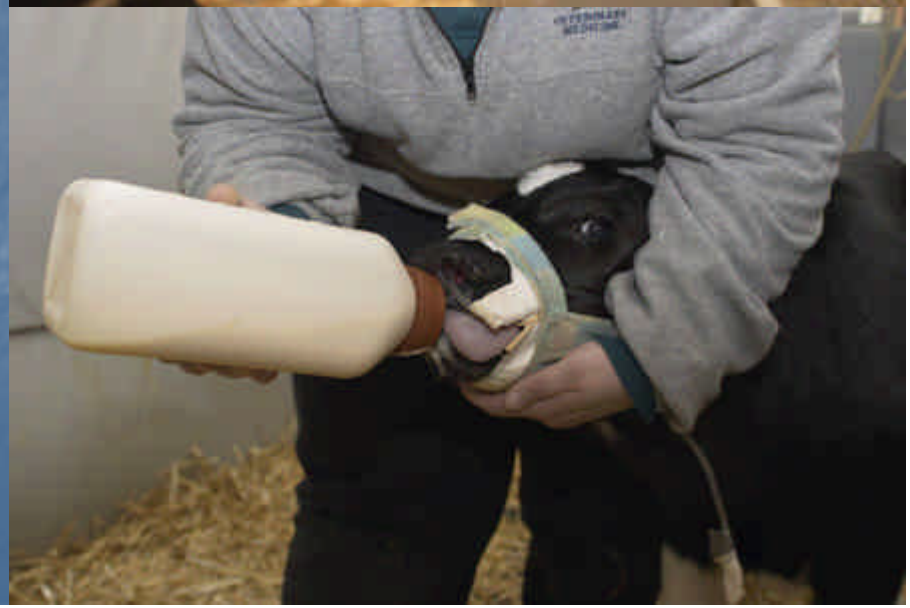
Neonatal Encephalopathy

- 15 calves
- Signs
  - Abnormal tongue position - 6
  - Abnormal suckle - 13
  - Loss of suckle – 8
  - Weak - 12
  - Somnolence – 5



Neonatal Encephalopathy

- Other signs
  - Abnormal search
  - Head tilt
  - Delayed vocalization
  - Novel head postures
  - Hyperresponsive
  - Hyperkenetic
  - Abnormal respiratory patterns
  - Hypercapnia
  - Strabismus
  - Not swallow saliva
  - Facial paresis

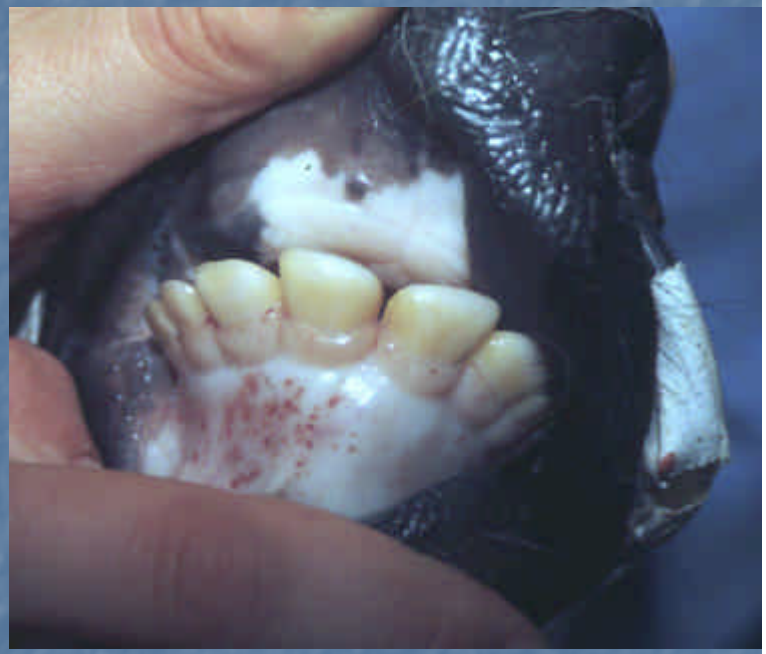


Umbilicus

- Large size
  - 50% of the cases
  - 4 – 3 cm diameter vessels, 9 cm diameter
- Infection
  - Frequent
  - Early resection – 24-48 hrs in 5 calves
- Patent Urachus – 5 calves
- Hemorrhage
  - 9 cases
  - 4 became anemic progressively anemic
    - 3 of these showed fetal anemia
  - 4 showed fetal anemia
    - 3 of these showed progressive anemia

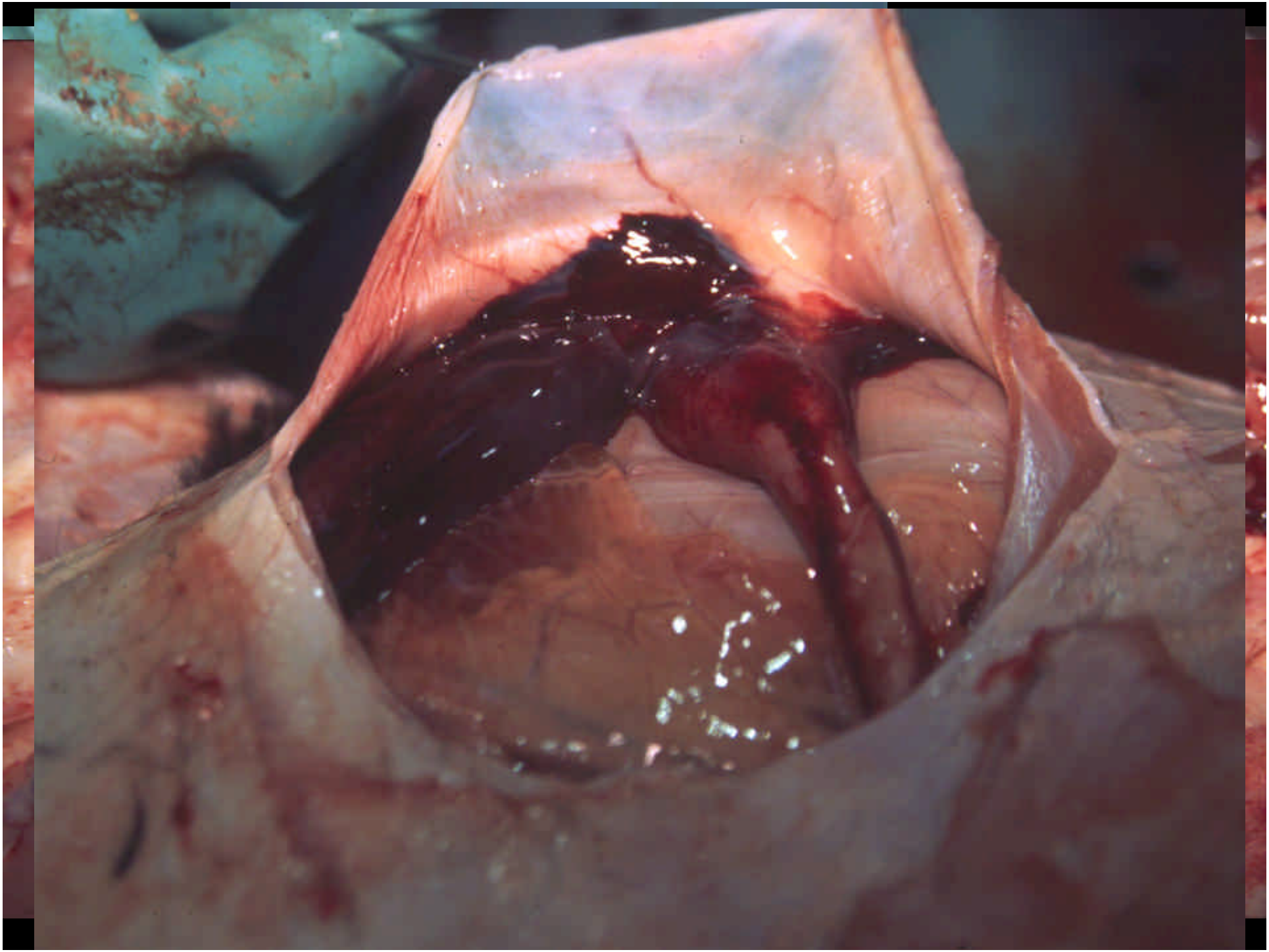
Anemia

- Fetal anemia
  - Calves PCV < 24 – 9 (23%)
  - Calves PCV < 20 – 3
  - Calves PCV < 15 – 2
- Acquired anemia - 8 calves
  - 4 with UA hemorrhage
  - 2 with GI hemorrhage
  - 2 with no source identified
- Fetal anemia followed by acquired
  - 3 calves - All with UA hemorrhage



Fetal Anemia

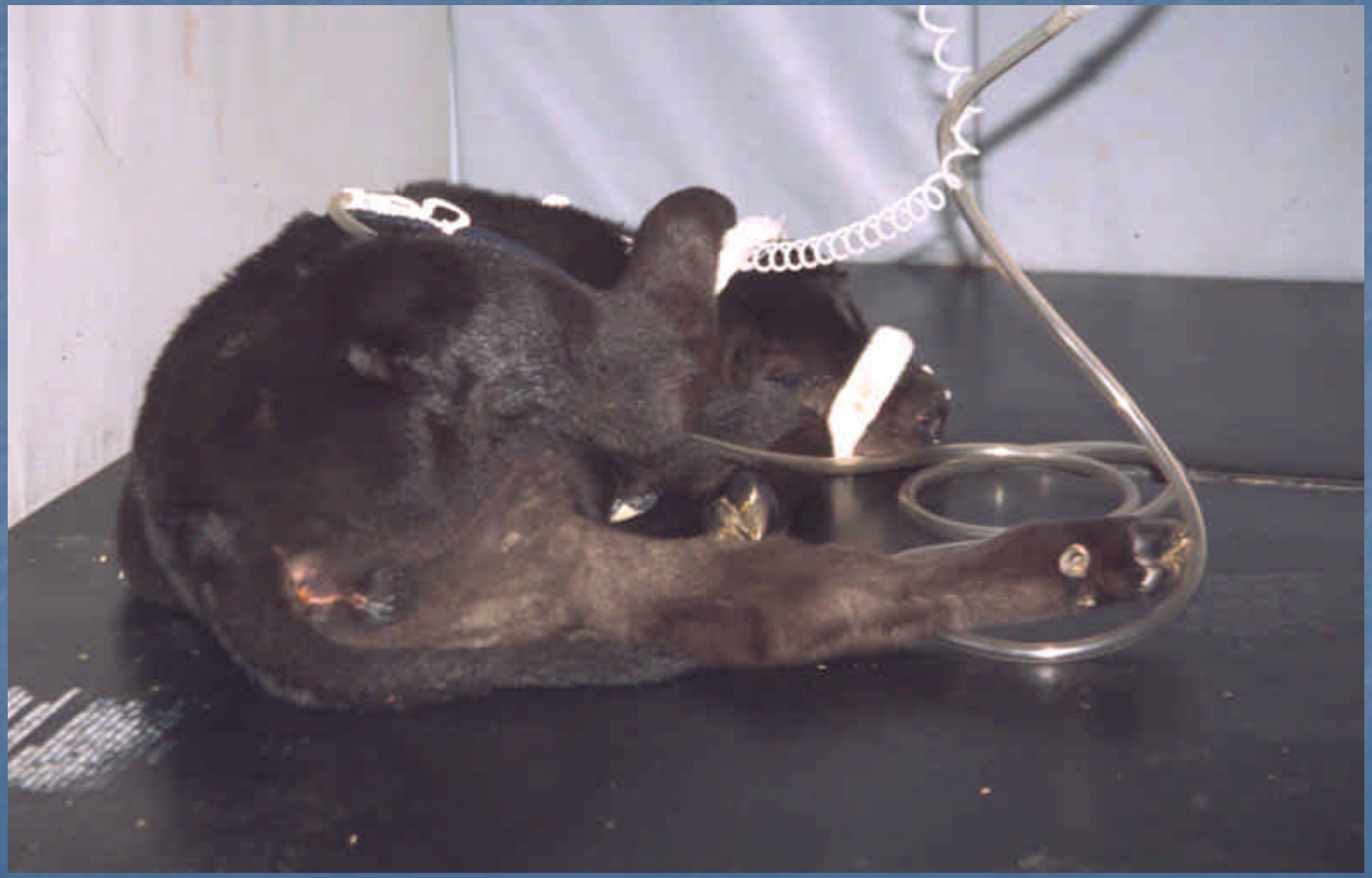
- Isoimmune hemolytic anemia
- Nonimmune hemolytic anemias
  - Erythrocyte structural defects
    - Structural protein defects
    - Membrane lipid defects
  - Somatic defects – G6PD
  - Defects in the synthesis of globulin chains
  - Hemoglobin variants
    - Hb E
    - Unstable hemoglobins
- Production anomalies
- Blood loss??



Musculoskeletal Abnormalities

- Contracture
  - Front legs – 9 calves
  - Hind legs – 3 calves
- Rotational and angular deformity – 4
- Laxity
- Adductor weakness
- Muscular dystrophy
- Spastic paresis

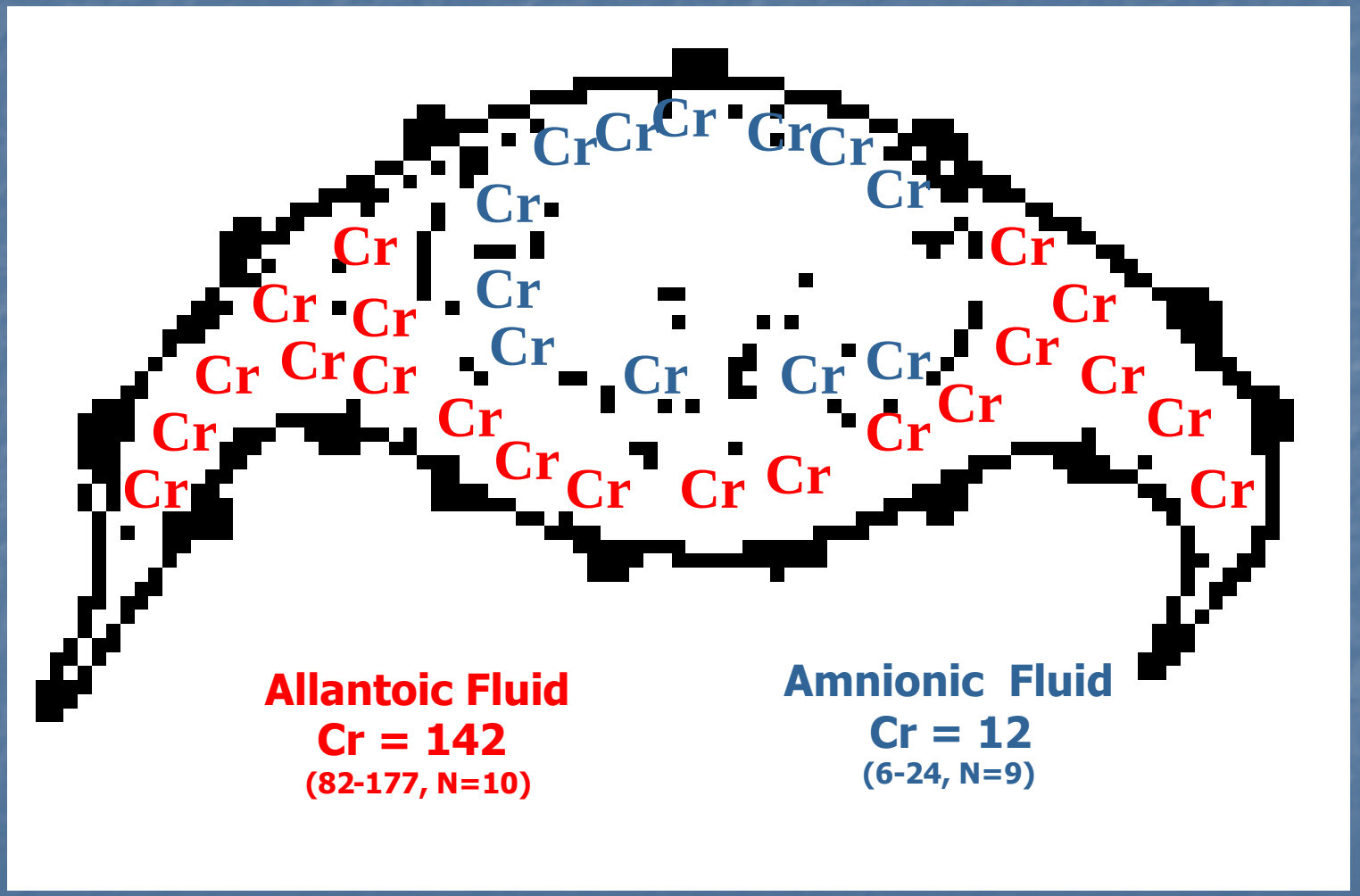
Neonatal Gastroenteropathy



Neonatal Nephropathy

- Neonatal Nephropathy – 8 calves
  - Birth Cr slow to drop
  - Cr rise after initial drop – 5 calves
  - Abnormal FxNa
  - High amikacin trough levels
- Abnormal urination patterns
  - Late 1st urination – 12 hrs or more
  - Decreased urine output
    - 1st urine 12 hrs, 2nd 33 hrs later, 3rd 12 hrs later
  - Anuria
  - Birth polyuria – 10 liters in 1st 12 hours
- Others
  - Polycystic kidneys
  - Pigment nephropathy

Fetal Calf



Birth Cr = 2.8 mg/dl (1.36 – 6.75, N = 37)

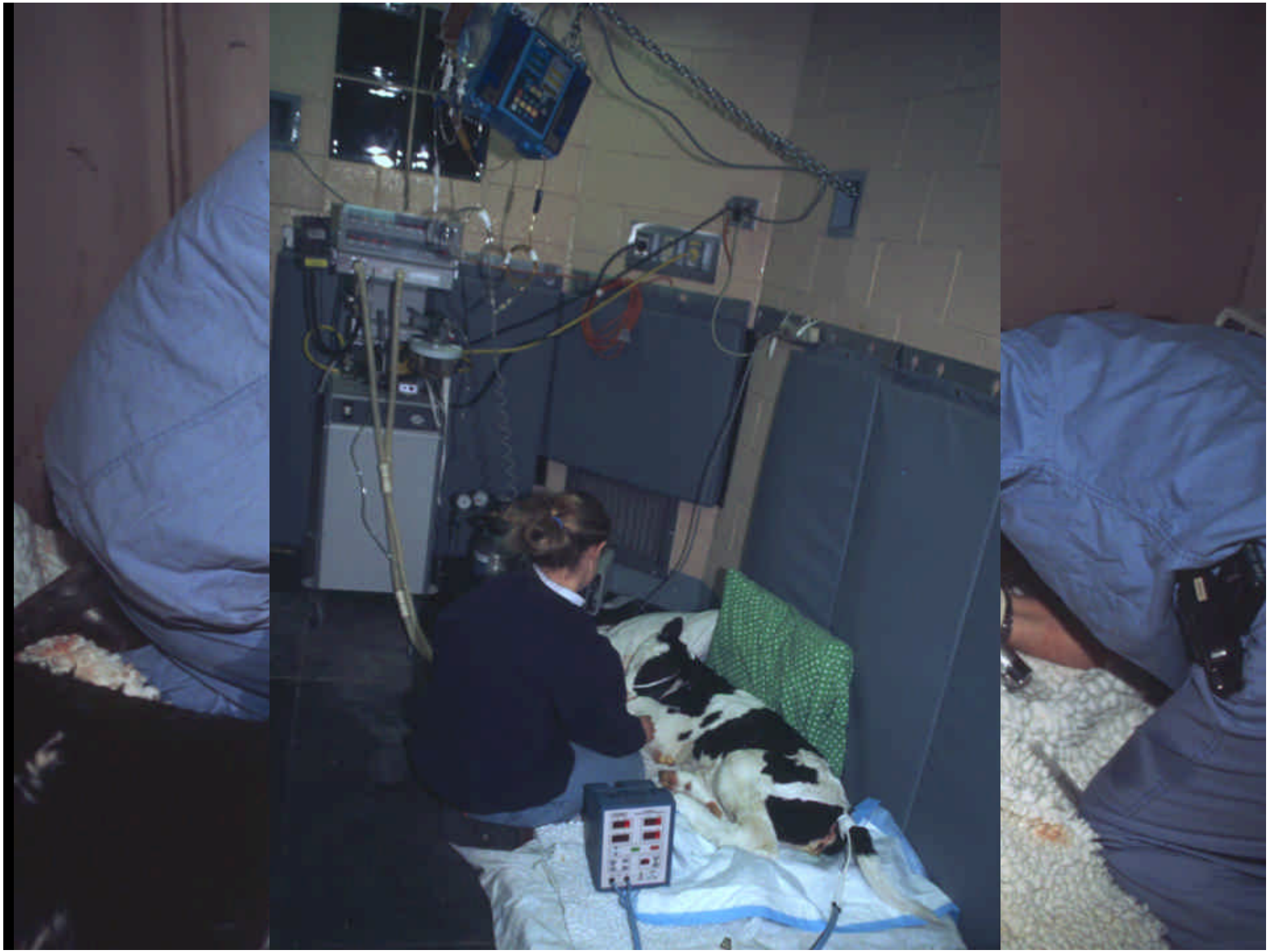
Sodium Balance

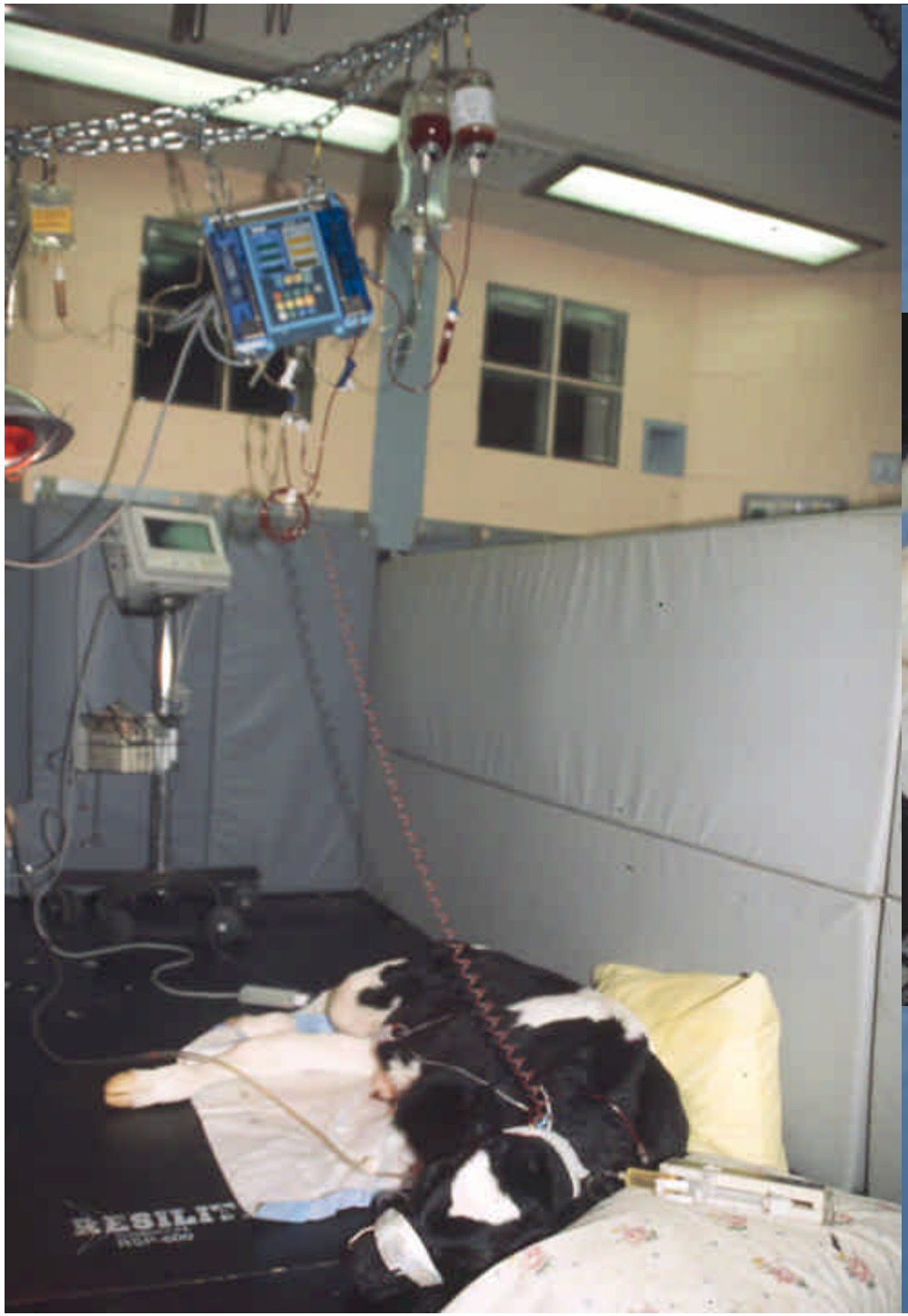
- Fresh milk is low in Na
- Neonate is preprogrammed to conserve Na
- Foals
  - Fxna = 0.3%, even when Na intake high
  - Requires a few days to adjust to high Na intake
- Cloned calves – adapted to milk replacer rapidly
  - < 24 hrs old
    - Fxna = 0.45% (0.05-0.82%, N=10)
  - On milk replacer
    - Fxna = 1.85% (0.89-2.68%, N=9)
- Fxna used to recognize Na wasting nephropathy
  - Requires age and diet matched normal values
- Cloned calves with Na wasting
  - Fxna = > 3%, up to 8.21% - 7 calves

- LOS
- hyperosmolarity
- hyperthermia
- lactic acidosis
- cardiac dysfunction, cardiac malformations
- hypochloremic alkalosis
- hypoglycemia/hyperglycemia
- Cystomegaly

Therapy

- Antimicrobials
- INO_2
- Dextrose
- Fluids
- Dobutamine
- Vasopressin
- Dopamine
- Norepinephrine
- Phenobarbital
- Plasma
- Whole blood
- PPN
- Caffeine
- Tolazoline
- NO
- Sildenafil
- Ventilation
- Umbilical resection





NEONATAL PROBLEMS OF CLONED CALVES

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INTRODUCTION

Between 2001 and 2003, 41 cloned calves were born at our hospital as part of our high risk pregnancy program. Based on experience with these calves, it is apparent that cloned calves have a wide variety of neonatal problems. Many of these problems are probably secondary to failure of placentation. Others may be a reflection of developmental abnormalities. Of the 41 cloned calves treated, 11 (27%) had fatal outcomes. Of those with fatal outcomes, 45% were associated with persistent pulmonary hypertension of the neonate (PPHN)/reversion to fetal circulation, 27% associated with severe musculoskeletal defects/LOS (large offspring syndrome) calves and 18% were associated with congenital heart defects. Beyond these problems, there was a wide variety of neonatal problems producing significant morbidity resulting in the need for intensive interventions. The frequent occurrence of multiple serious neonatal problems in the same calf raises the question whether the resulting cow or bull will be completely free of problems which may prevent them from reaching their full potential.

NEONATAL PROBLEMS

Persistent Pulmonary Hypertension of the Neonate (PPHN)

PPHN existing from birth or reversion to fetal circulation soon after birth is the most serious problem encountered in cloned calves with the highest mortality in our experience. Almost half of the cases succumbing during the neonatal period prominently display this problem, although most calves had multiple serious problems. The transition from fetal circulation is a commonly misunderstood process. Although this transition may occur within minutes of birth, it is not unusual for it to be delayed for anywhere from a few hours up until about 24 hours after birth. This slower than expected transition is not unique to cloned calves and occurs often enough in otherwise normal calves to be considered normal. A delay does not mean failure. In fact one of the cloned calves made the transition between 48 and 72 hours without signs of significant problems. This slow transition has been mistaken for surfactant failure, which is much less likely to occur. The cause of the slow transition or complete retention of fetal circulation is not known. It is no doubt a complex problem involving many factors. One interesting observation we have made in another group of cloned calves is that calves that require oxygen therapy to help with the transition have unusually high levels of endothelin in their fetal fluids, suggesting that this vasoactive substance may play an important part in that slow transition.

Sepsis

Sepsis, either supported by hard evidence such as positive blood cultures or cultures from localized foci (e.g. umbilical, pulmonary cultures) or supported by soft evidence such as hematological abnormalities, hyperfibrinogenemia, nutritional intake/weight gain patterns or pyrexia, seemed to be nearly universal in these cases. This observation does not necessarily imply immunoincompetence in these cases as they received more frequent invasive procedures increasing their sepsis risk, they were exposed to our nosocomial flora which tends to contain more aggressive pathogens and these calves were more closely scrutinized so mild infections may have been discovered which otherwise would have been missed.

All of the calves received a lyophilized IgG product as a colostrum substitute instead of fresh or frozen colostrum primarily because of concern about transferring specific antibodies which could confound future serology. Rather than following the traditional view that the primary role of colostrum is to transfer IgG, I feel that colostrum's primary function is the establishment of an immune barrier between the luminal bacteria and 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. 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 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.[1] Since the gastrointestinal tract is the most likely portal of entry of pathogens, colostrums action 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 (lyses bacterial cell wall by degrading peptidoglycans), MUCI (inhibits the binding of S-fimbriated *E coli* to epithelial cells), lactadherdrin (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 (disrupt envelope viruses, inactivate certain bacteria, defend against *Giardia*). Other important factors n colostrum include

PAFacetylhydrolase[2] (PAF-degrading enzyme; PAF is an important proinflammatory mediator in the GIt with high levels in the neonate; this enzyme protects mucosal cells from damage caused by PAF by degrading it), erythropoietin[3,4] which protects against programmed cell death in intestinal epithelium, epidermal growth factor[5,6] which has been shown to play an important role in mucosal barrier function in developing intestine, and down-regulates apoptosis of intestinal epithelium.[7]

Placing cloned neonates in a referral hospital environment, using invasive techniques and denying them the full benefit of the protection afforded by fresh colostrum may have resulted in the high occurrence of sepsis in these cases. Using IgG concentrates as a substitute for colostrum is a poor trade off. When we measure IgG plasma levels as a reflection of passive transfer, what we a doing in essence is making the only measurement we can. 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 colostral substances have had their stimulating effect on the neonate's immune function. 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 influenza virus is not helpful in protecting the neonate against bacterial pathogens. But since we have no method to measure the quality of IgG transfer, 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 to pathogens that aren't a threat to the neonate, but when the donor is stimulated to produce the 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.

Anemia

Anemia was a common problem. In these cases anemia can be classified as fetal anemia (congenital), physiologic anemia or blood loss anemia. Occasionally the blood loss is obvious as external bleeding from large umbilical vessels. Because of the size of the vessels and because of the lack of normal regression of the vessels after birth, without rapid hemostasis, the blood loss can be considerable. Not all the umbilical bleeding is external. When the normal bovine umbilical vessels rupture at birth, they often break internally, near the level of the bladder. In the usual course of events, they seal as they stretch and break. With the large, non-regressing umbilical arteries of cloned calves, the arteries may break internally but not seal resulting in a large quantity of internal hemorrhage. Likewise, if the vessels are bleeding externally and in an attempt to achieve hemostasis, the external umbilical remnant is occluded but the artery is not, bleeding will continue with the blood dissecting along the internal fascial plains of the umbilical arteries or up the urachus into the bladder. Frequently a large hematoma forms along the umbilical arteries. There may be repeat bleeding episodes over the first 24 to 36 hours. Although not palpable initially because of its liquid nature, these hematomas can easily be felt by abdominal palpation within 24 hours.

Congenital anemia not associated with blood loss can also be present. These interesting cases may have many underlying causes and were not completely characterized. They were occasionally combined with PPHN compounding the negative effect of that condition. Physiologically, because of neonatal fluid shifts, there is frequently a decrease in PCV during the first day of life. This should not be mistaken for blood loss or hemolytic anemia.

Anemic calves responded positively to whole blood transfusion. Calculations for the amount of blood necessary to achieve a given rise in PCV follow that of other species.

Umbilical Problems

As indicated above, the large size of the umbilical remnants and the lack of normal post natal regression lead to a number of problems. The most serious are infection and bleeding. The umbilical remnants are a frequent focus of chronic, low grade sepsis. Infection can ascend the arteries leading to abscess formation very near the aorta which makes surgical removal difficult.

Musculoskeletal Abnormalities

Sever contracture and musculoskeletal malformation was a frequent reason for euthanasia at birth, accounting for 27% of the neonatal losses. Less severe carpal and fetlock contracture, angular and rotational deformities were present which responded to conservative therapy.

Other Problems

A number of other problems were encountered. LOS with abomasal distension probably secondary to hydrops was occasionally present. Some of these cases responded well to supportive therapy while others did not. Other occasional problems included hyperosmolarity, hyperthermia secondary to muscular dystrophy, renal dysfunction, lactic acidosis, cardiac dysfunction, cardiac malformations, hypochloremic alkalosis, hypoglycemia/hyperglycemia, neonatal encephalopathy, patent urachus and others.

OUTCOME

The survival until discharge for this case series was 73%. Unfortunate, because of the nature of the cases, there was no ability to collect information of long term outcome.

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Key Words: persistent pulmonary hypertension, sepsis, colostrum, anemia, umbilicus