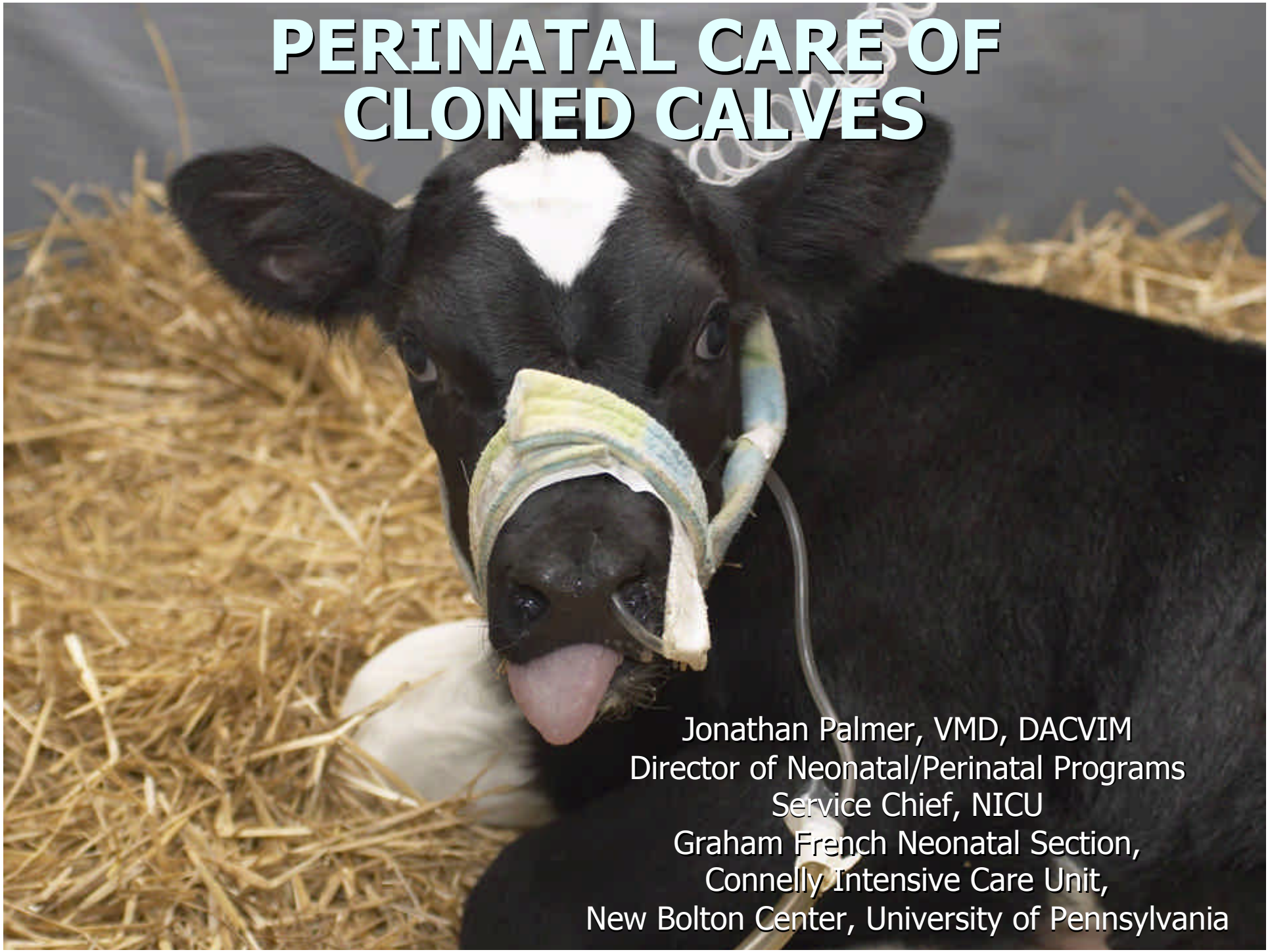
PERINATAL CARE OF CLONED CALVES



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470

Cloned Calf Births 2001-2003

- 20 different cell lines
  - 1-5 calves per cell line
- 43 calves
  - 3 stillborn
  - 3 euthanized for economic reasons
- 37 calves treated
  - 28 survived – 76%
  - 4 died
  - 5 euthanized
- Wt – mean 52.3 Kg (115 lb)
  - 23.6 – 77.7 kg (52-171 lb)
  - 11 < 45 kg (100 lbs)
  - 11 > 60 kg (130 lbs)

Delivery
Choosing the Day

- Gestational length
  - 270 – 288 days, mean 275
- Induction method
  - Dexamethasone
- C-section
  - Before Labor – 31 deliveries
  - During Stage II – 9 deliveries
  - Vaginal delivery – 3 deliveries

Preparation

- Resuscitation space
  - Recovery stall
- Available equipment
  - Airway, INO_2
  - Capnograph
  - ECG
  - BP
  - IV Pumps, catheter setup
  - Resuscitation drugs
  - Defibrillator
- Team – assigned roles
  - Neonatologist
  - Resident
  - Nurse
  - Students

Coordinated Resuscitation Team

Elements of Resuscitation

- *Initial Quick Overview*
- *Neonatal Assessment*
  - *Apgar score*
- *Establishing an Airway*
- *Tactile Stimulation*
- *Free Flow Oxygen*
- *Ventilation*
- *CPR*
- *Post resuscitation care*

Resuscitation Steps

- *Initial Quick Overview*
  - Assess viability
  - Monitor heart rate
  - Quick assessment for dysmorphisms
    - Malformations
  - Insure umbilical hemostasis
- *Neonatal assessment*
  - APGAR Score

APGAR Score

Current Researches in Anesthesia and Analgesia—July-August, 1953

A Proposal for a New Method of Evaluation of the Newborn Infant.*

Virginia Apgar, M.D., New York, N. Y.

Department of Anesthesiology, Columbia University, College of Physicians and Surgeons and the Anesthesia Service, The Presbyterian Hospital

RESUSCITATION OF INFANTS at birth has been the subject of many articles. Seldom have there been such imaginative ideas, such enthusiasms, and dislikes, and such unscientific observations and study about one clinical picture. There are outstanding exceptions to these statements, but the poor quality and lack of precise data of the majority of papers concerned with infant resuscitation are interesting.

There are several excellent review articles[1] [2] but the main emphasis in the past has been on treatment of the asphyxiated or apneic newborn infant. The purpose of this paper is the reestablishment of simple, clear classification or "grading" of newborn infants which can be used as a basis for discussion and comparison of the results of obstetric practices, types of maternal pain relief and the effects of resuscitation.

The principle of giving a "score" to a patient as a sum total of several objective findings is not new and has been used recently in judging the treatment of drug addiction.[3] The endpoints which have

Bovine APGAR Score

Score	*0*	*1*	*2*
Heart Rate	Absent	< 100 irregular	> 100 regular
Respiratory Rate	Absent	irregular	regular
Muscle Tone	Limp Lateral	Some Flexion	Active Sternal
Reflex ***Nasal Stimulation*** ***Ear Tickle***	No Response	Grimace Weak Ear Flick	Sneeze/Cough Ear Flick/Head Shake

Resuscitation Steps

- Tactile Stimulation
  - Secure airway
- Cardiovascular monitoring
  - Heart rate
  - ECG
  - BP
- ABG obtained
  - With 10 minutes of birth
  - As nasal O2 line placed
  - Second sample on INO2
- Place IV catheter
  - Jugular
  - Blood culture
- Blood samples
  - Hematology
  - Chemistry

Birth Resuscitation

- Respiration – 33 cloned calves
  - Immediate – 24
  - Require intubation – 1
  - 1 minute – 3
  - 2 minutes – 5
  - 4 minutes – 1
- APGAR – 40 cloned calves
  - 1 minute 6.2 (range 1-8)
  - 5 minute 7.3 (range 3-8)
  - 10 minute 7.7 (range 4-8)

Indicators of Fetal Distress

- Meconium passage
- Timing of Breathing
- Apgar score
- Hematology
  - WBC, Differential
  - Fibrinogen
- Chemistry
  - Lactate – 8.2 (3.4-20.4, N=32)
  - pH 7.262 (6.952 - 7.392, N=34)
  - SID
  - PCV (12-49%)
  - Dextrose (<8 - 451 mg/dl)
  - Ca++

Reassuring Signs

- Vocalization mean 16 min (3 to 51 min)
- Standing
  - 2 never stood
  - With or without assistance 11.8 hr (1-84 hr)
  - Unassisted 15.8 hr (2-84 hr)
- Suckle (22 calves) – 47 min (3-134 min)
- Nurse (23 calves) 10 hr (2.5-50 hr)
  - Many forgot to nurse

Initial Therapy

- INO2 – all cases
- Dextrose – 60%
- Cardiovascular support
  - Fluids
  - CRI
    - Dopamine
    - Doutamine
    - Vasopressin
    - Epinephrine
    - Norepinephrine

Birth Resuscitation Problems

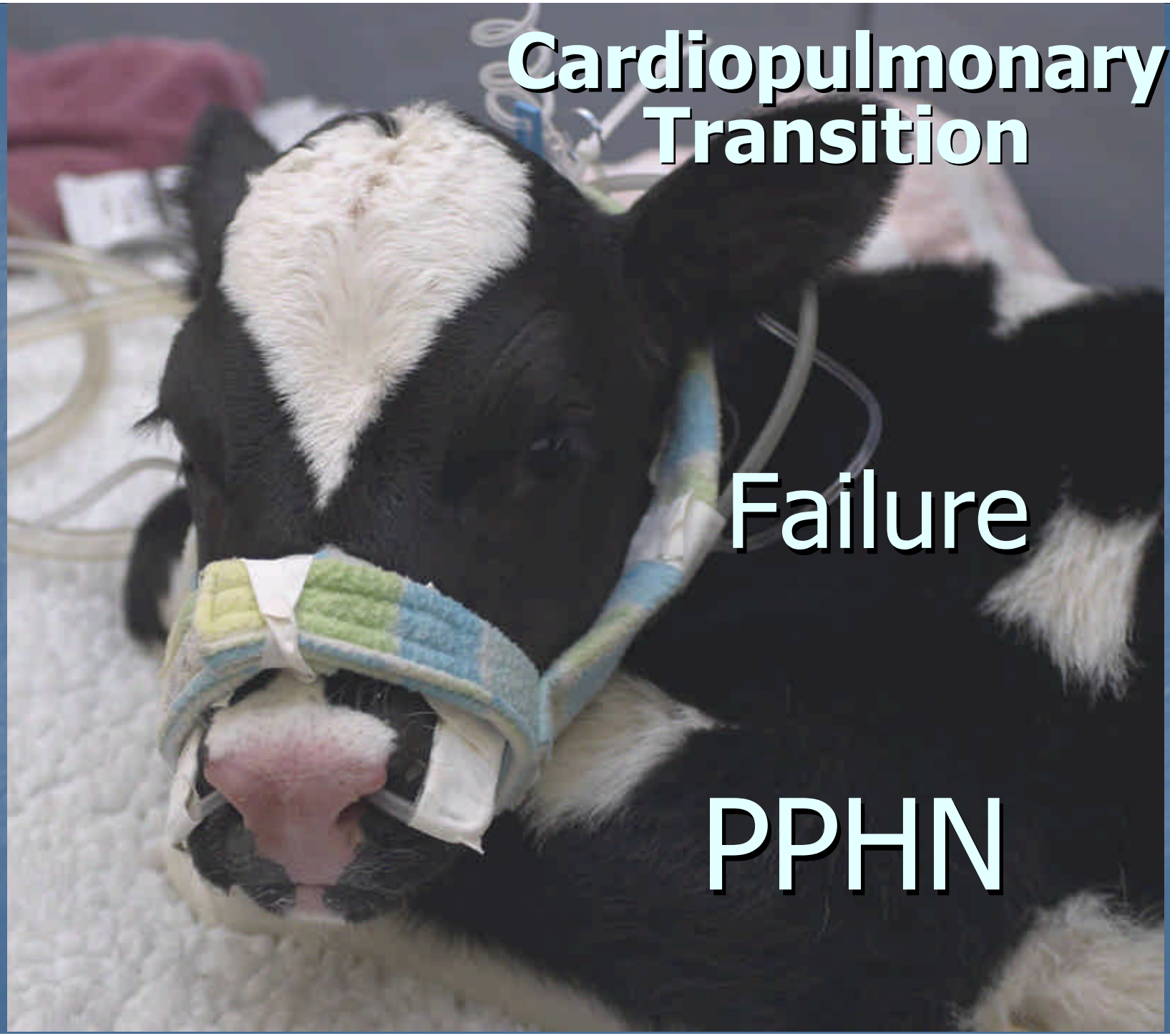
- Deformation dysmorphisms
- Cardiopulmonary transition
- Hypoglycemia/hyperglycemia
- Congenital anemia

Birth Resuscitation Deformation Dysmorphisms

- Severe musculoskeletal deformations – 14%
  - Stillborn – 3 calves
  - Born alive – 3 calves
- Musculoskeletal abnormalities
  - Carpal/Fetlock 90° contracture
  - Hyperextension of the hocks with 90° fetlock contracture
  - Limited motion hock/stifle
  - Chondrodysplasia
  - Rotational deformities
  - Head deformities

Birth Resuscitation Deformation Dysmorphisms

- Other abnormalities accompanying musculoskeletal deformations
  - Abdomen distension, large fluid filled abomasum
  - Cardiac
    - Tricuspid valvular dysplasia
    - Ventricular hypertrophy
    - Cardiomegaly
  - Hepatic abnormalities
  - Small, tubular nasopharynx
  - Skeletal muscle myocellular dysmaturation


Cardiopulmonary Transition
Failure
PPHN

Calf 0126-3

	10 min	38 min	2.5 hr	18 hr	21 hr
PH	7.226	7.329	7.349	7.288	7.367
$Paco_2$	42.6	44.0	53.8	56.2	56.0
Pao_2	49.8	60.8	75.9	90.2	244
HCO_3	23.8	23.3	29.8	27.1	32.3
BE	- 3.4	- 1.9	+ 4.2	+ 6.3	+ 6.8
Lac	9.2	9.1	5.2	2.7	2.1
O_2 Sat	78.1	86.2	92.7	94.2	99.5
O_2 Cont	10.5	11.0	11.0	11.4	12.2
INO_2	RA	10 lpm	10 lpm	10 lpm	10 lpm

Calf 0069-1

	30 min	90 min	8 hr	18 hr	24 hr
PH	7.156	7.080	7.320	7.336	7.262
$Paco_2$	47.4	50.3	63.2	61.1	78.4
Pao_2	41.3	39.5	67.7	39.1	46.2
HCO_3	16.9	15	29.9	32.8	35.6
BE	- 12.1	- 12.7	+ 4.6	+ 7.4	+ 8.4
Lac	20.4	19.8	4.4	1.9	2.6
O_2 Sat	55.6	66.9	88.7	61.2	59.6
O_2 Cont	-	4.5	5.2	5.0	5.4
INO_2	RA	4 lpm	10 lpm	10 lpm	10x10

Transition from Fetal to Neonatal Circulation

- Intermittent fetal breathing
  - To continuous neonatal breathing
- Initial drop in pulmonary vascular resistance
  - Not complete for 4 weeks
- Decrease in pulmonary artery pressure
- Increase pulmonary blood flow

Transition from Fetal to Neonatal Circulation

- Pulmonary vasodilation at birth
  - Distention of lungs with gas
  - Local changes in P_{O_2} and P_{CO_2}
  - Increased PG
  - Bradykinin
  - Nitric oxide
  - Reduction in vascular wall thickness
- Nitric oxide
  - Major mediator of oxygen induced vasodilation

Fetal Circulation
Ductus Arteriosus

- Patent Ductus Arteriosus
  - Prostaglandins keep ductus patent
  - Adenosine also keeps ductus patent
  - Nitric oxide dilation
- Closure of Ductus Arteriosus
  - Closure begins immediately after birth
    - First stage complete in 10-15 hours
    - Second stage takes 2 to 3 weeks
  - Sympathetic nervous system important

Closure of Ductus Arteriosus

- Increase oxygen tension
- Increased plasma catecholamine levels
- Suppression of PGI_2 production
- Switching off PGE receptors
- Synergistic role of $PG_{F2\alpha}$ and oxygen
- Fall in plasma adenosine level
- Production of endothelin 1

Disruption of Birth Transition

- Inflammatory mediators play central role
  - Thromboxanes
  - Leukotrienes
  - TNF
- Hypoxic ischemic disease
- Imbalance of vasodilators/constrictors
  - Nitric oxide – induces angiogenesis
    - Deficiency – lack of vascular development
  - Endothelin – smooth muscle mitogen
    - Muscular hypertrophy
- May never make the transition
- May revert the fetal circulation with stimulus

Failure to Reverse
Increased Pulmonary Vascular Resistance

- Perinatal stress
  - Anemia, hypoglycemia
  - Aspiration, hypoxia
- Result in failure of vessel dilation
- Result in ventricular dysfunction
- Increased production endothelin
  - Role?

Role of Endothelin 1 (ET1)?

- Wilkins et al in press
- 40 cloned calves
- Calves requiring supplemental oxygen
  - Based on low pulse ox reading
  - Based on clinical signs
- 13/40 calves Rx O_2
  - Maternal ET1 levels – higher in O_2 Rx group
  - Fetal fluid ET1 levels – very high in O_2 Rx group
  - Not fetal blood levels
- ET1 – marker
  - Distress
  - Abnormal placentation
  - Role in PPHN in cloned calves?

PPHN Therapy

- Free flow oxygen
- Ventilation
- NO
- Pulmonary vasodilators

Therapy Vasodilators Tolazoline

- Traditional treatment PPHN
  - No controlled studies
- Complications
  - Significant systemic hypotension
    - Increase Rt-to-Lt shunting
  - Pulmonary bleeding
- Dosage
  - Test doses – goal increase P_{ao_2} > 15 torr
  - Infusion – continuous drip

Therapy
NO

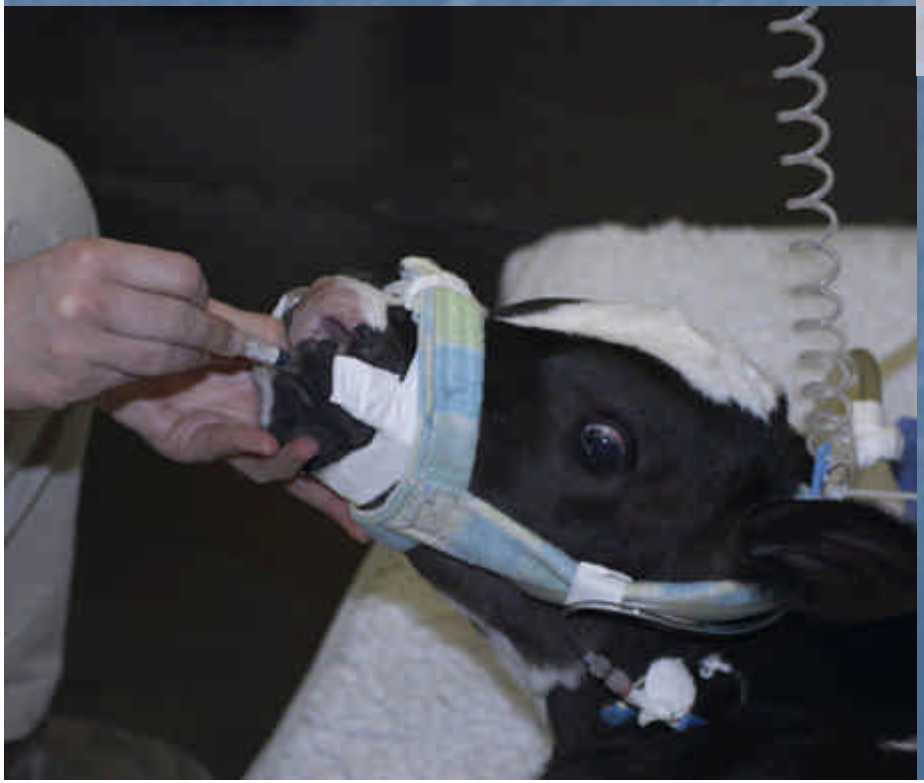
- Normal birth transition
  - NO plays a major role
  - Prostacyclin also important
  - Deficiency leads to PPH
- Replacement therapy
  - Prostacyclin infusion not help
  - NO appears to reverse PPH
    - At least reversible component
  - Experimental reversal PPH
    - Prenatal ductus closure
    - Hypoxia
    - Thromboxanes
    - Bacterial sepsis
- NO toxicity – problem?

Therapy
Sildenafil

- Type V phosphodiesterase
  - cGMP-selective
    - PDE in lung 1,2,3,5,9
- Phosphodiesterase Inhibitors
  - Augment vasodilating effects of inhaled NO
- Sildenafil
  - Lung specific at low doses
  - Others may be more lung specific

Therapy
Sildenafil

- Requires NO induced cGMP
  - Endogenous
  - Exogenous – inhaled NO
    - Continuous
    - Pulse dosing
- Dose ???
  - 0.5 – 2.5 mg/kg
  - Oral, PR?



Birth Resuscitation
Cardiopulmonary Transition

- Transition
  - Mean 5hr 47 min (10 min – 26 hr)
  - 4 calves never made a transition
    - All had at least 1 cardiac anomaly
    - 3 died, 1 euthanized
  - 4 calves revered to fetal circulation
    - All euthanized
    - 2 had cardiac anomalies
    - 1 meconium aspiration
    - 1 Abnormal pulmonary vasculature

Clone Calves
Fatal Outcome

- 8 - associated with PPHN
  - All had multiple other problems
  - One survived until day 18
- 3 - multiple musculoskeletal deformities
- 3 - stillborn
- 1 - Congenital muscular dystrophy induced hyperthermia

PERINATAL CARE OF CLONED CALVES

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BACKROUND

Between 2001 and 2003 I was fortunate enough to care for 41 cloned calves representing 19 cell lines (primarily Holstein but also other breeds e.g. Long Horns, Angus, Jersey and others) born at our hospital as part of our high risk pregnancy program. I was allowed to administer a high level of supportive care to these neonates. These calves were owner by a commercial cloning company and 2 private individuals. At their encouragement, these clinical cases received close scrutiny of clinical laboratory changes as well as intensive clinical monitoring and aggressive therapeutic interventions although mechanical ventilation was rarely allowed. The cloned calf recipients were admitted to our hospital approximately 2 weeks before their calculated due date and elective cesarean sections performed 36 hours after dexamethasone induction approximately 1 week before the calculated term gestation. Most cesarean sections were performed before the cow began labor. I have no knowledge of the origin of the cell lines and the various methods used in performing the cloning. Also, the cases I received were biased toward the more difficult since many of the recipients sent to our high risk pregnancy program were carrying clones from cell lines which the cloning company had not been successful in delivering a live calf or only a few calves from the line had survived to term. So the frequency, types and severity of the problems encountered in these calves may not represent what would be expected in other situations. The program committee has asked me to share the lesions from this experience in the following talks.

INTRODUCTION

In 1997, after 277 attempts, Dolly, the first cloned sheep, was born. Since then adult sheep, goats, cattle, mice, pigs, cats, rabbits and horses have been cloned using somatic cell nuclear transfer.[1] Since Dolly's birth, hundreds of cloned calves have reached weaning age but these represent less than 5% of all cloned embryos transferred into recipient cows.[2] Many of the losses have been blamed on abnormal placentation.[3] Abnormal placentation includes reduced placentome numbers and inadequate allantoic epithelium and vascularization.[4] Perinatal morbidity and mortality has frequently been reported. A number of congenital defects, failure to make a successful birth transition and characteristic phenotypic abnormalities such as the large offspring syndrome (LOS) make perinatal care of these patients a challenge.

PERINATAL CARE

Effective perinatal care is more successful when the problems faced can be anticipated. Of the 41 births, 73% survived till discharge. Of the ones that didn't, approximately 1/3 were euthanized at birth because of the combination of severe musculoskeletal defects and LOS phenotype. Other frequent problems that may need immediate attention at birth include delayed transition from fetal circulation, significant hypoglycemia, congenital anemia and significant umbilical hemorrhage.

Birth Resuscitation

The calves in this series were delivered by elective cesarean section before onset of labor after dexamethasone induction 24 to 36 hours previously approximately 1 week before the calculated due date. This scheme was requested by the clone calf owners to insure vaginal delivery was not attempted because of the possibility of dystocia and the added perinatal distress. None of the calves appeared premature although many had problems that could have been influenced by readiness for birth. Whether or not these problems would have been modified by waiting until labor before cesarean section or by vaginal delivery is a matter for speculation. The frequent occurrence of multiple deliveries early in our experience dictated a routine of being prepared to resuscitate twins.

Birth resuscitation includes initial assessment, immediate delivery of respiratory support, tactile stimulation, thermal management, calculation of serial Apgar scores, monitoring blood gases, blood glucose, heart rate, respiratory rate, blood pressure and special attention to umbilical care. The initial assessment should include a quick evaluation of phenotype so that resuscitation efforts do not go forward on calves with severe malformations as well as an initial survey of vital signs to determine if advanced life saving protocols should be immediately initiated. Respiratory monitoring should include stimulation of initial respiratory efforts, monitoring the success of those efforts, collecting an initial room air arterial blood gas (the median artery is a convenient source), initiation of free flow intranasal oxygen insufflation and repeating the arterial blood gas once on oxygen insufflation. The heart rate and respiratory rate should be monitored every 2 to 3 minutes. Apgar score should be recorded at 1 minute, 5 minute and 10 minute post delivery. If the Apgar score is ≤ 4, aggressive resuscitation should be initiated. If the Apgar score is dropping on subsequent evaluations, appropriate actions should be taken. If the Apgar score is not ≥ 7, further and more frequent evaluations may be indicated. The body temperature should be monitored at birth and at least every 5 minutes. It should be recalled that the calf will be born at the cows body temperature and decrease from there. With dystocia cases often the initial body temperature can be 106 – 107 F. The rapidity and magnitude of temperature drop should be closely followed since hypothermia/poor body temperature control is often a problem. Blood glucose should be followed closely as poor glucose response and glucose levels below the detectable level (< 12 mg/dl) are frequent problems. Within the 1st 10 minutes of life, samples for hematology and chemistry should be taken to serve as a base line and to establish the presence of fetal inflammation, fetal anemia, prenatal asphyxia leading to sick cell syndrome and other conditions.

One reflection of the abnormal placentation that seems to be prevalent in cloned calves is unusually large umbilical blood vessels which do not regress at birth. Although we tried a number of techniques aimed at achieving hemostasis, none was completely satisfactory. We generally settled for ligation or clamping of multiple large vessels which resulted in excessive manipulation of the

structures which may have predisposed the calf to infection with the umbilical remnant acting as an entry portal for sepsis. In some calves the remnant was surgically removed when the calf was 24 hours old, but this did not always prevent sepsis.

List of References: 1. Edwards JL, Schrick FN, McCracken MD, van Amstel SR, Hopkins FM, Welborn MG, Davies CJ. Cloning adult farm animals: a review of the possibilites and problems associated with somatic cell nuclear transfer. AJRI 50:113–123 (2003). 2. Oback B, Wells DN. Cloning Cattle. Cloning and Stem Cells 5(4):243-256, 2003. 3. Hill JR, Burghardt RC, Jones K, et. al. Evidence for Placental Abnormality as the Major Cause of Mortality in First-Trimester Somatic Cell Cloned Bovine Fetuses. Biology of Reproduction 63:1787–1794 (2000). 4. Hill JR, Edwards JF, Sawyer N, et. al. Placental Anomalies in a Viable Cloned Calf. Cloning 3(2):83-88, 2001.

Key words: Birth, resuscitation, placentation, Apgar score