Clone Calves: Medical Challenges

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The management of clone calves is different from that of other calves only because of their high value and because of their apparent tendency to develop certain conditions with a higher frequency than conventional calves. These conditions include placental abnormalities, persistent fetal circulation, flexural deformities, large umbilical vessels, and tachypnea. Many clone calves are at high risk of persistent fetal circulation and should be closely monitored by measuring arterial blood gasses; treatment should be early and aggressive. Tolazoline infusion and supplemental oxygen therapy resulted in a successful outcome in several sick clone and conventional calves.

It could be argued that the neonatal management of clone calves is really no different than the neonatal management of any other calf. Many times this is true. However, we now know that some of clone calves are very different from other calves and because the investment in, and potential value of, a clone calf is higher than for many conventional calves, we now handle neonatal clone calves quite differently than the way in which we handle conventional calves. I do not present myself as an expert in the management of clone calves, nor do I have extensive experience in treating a great number of clone calves. What I will do is related our experience with clone calves at Texas A&M University, good and bad. I will also review some of the techniques we use in clones and other high-value calves for diagnosis and therapy.

Our first "clone calves" were not clones in the contemporary sense, but were rather "split embryos." These calves were derived from dividing an 8 or 16 cell morula and placing the individual cells into separate eggs. Multiple identical siblings were produced. These calves were produced by a private company over 10 years ago. We were only involved with "problem" cattle and had little knowledge of the details of the process or of the percentage of normal to problem births. The most frequent reason for presentation to our clinic was prolonged gestation. Cows would be admitted days to weeks past the due date. The cattle were clinically normal except for an enlarged abdomen in some cases. We soon learned that some of the cows did not receive the signal to commence parturition. Hence the calves were extremely large when they were finally induced. The largest was approximately 75 kg at birth; one of its siblings was born at the expected time and weighed half as much. Another calf, delivered by caesarean section past the expected birth date, was diagnosed with diabetes mellitus. The producers of these calves were interested in protecting their proprietary rights and were not interested in releasing details of the successes or failures of their program to outsiders. They declined our offer to investigate the causes of the long gestations and oversized calves. The quality and accessibility of the records of these calves is limited, so what is presented is here is largely from memory, but these calves foretold the complications that were to come when true cloning began.

The next phase in clone-related clinical activity in our hospital came in 1998 when a series of transgenic Holstein calves cloned from fetal tissue were admitted to the hospital for assisted birthing. The details of these cases have been reported .[1] Our plan was to induce on day 284 with dexamethasone and dinoprost, allow parturition to begin, but perform a caesarean section if parturition was not rapid and easy. Two of 8 were delivered vaginally. The other 6 were delivered by caesarean section; of these 2 of the cows had hydrallantois. All but one of the caesarean sections was performed via a ventral midline incision.

The first calf was a learning experience. We decided to observe the calf as we would a routine caesarean–derived calf, intervening only in necessary. However, the caesarean section was not routine as the cow had severe hydrops and the calf was delivered a week before schedule. The cow died during surgery. At first the calf appeared relatively normal, maintaining himself in sternal recumbency moments after birth. An initial arterial blood gas revealed severe hypoxia, which I deemed incompatible with the clinical picture and attributed the values to a venous sample. After 3 hours the calf began to deteriorate and another arterial blood gas confirmed the results of the first. For the next 4 days, heroic measures including putting the calf on a ventilator were instituted, only to fail. A post-mortem examination revealed gross and histopathologic evidence of pulmonary hypertension and surfactant deficiency. Abnormal findings included atypical interstitial pneumonia, centrilobular atrophy of the liver, right ventricular hypertrophy, patent ductus arteriosus and severe pulmonary arterial enlargement. As a result of the seexperiences, we modified our approach to the peri-natal management of the other calves in this set.

The modified procedure for managing the new-born clone calves featured aggressive assessment and early intervention. Calves were intubated immediately after birth and 100% oxygen was administered by placing a small plastic tube approximately 30 cm down the endotracheal tube. The calves were vigorously rubbed and kept in sternal recumbency except when blood was collected for blood gas analysis. Suction was used to clear the endotracheal tube as necessary. Calves were extubated when they voluntarily assumed sternal recumbency and began to resist the tube. At that point, oxygen was administered by nasal insufflation or mask. Arterial blood was collected from the brachial artery within 30 minutes of birth and blood gases were measured. Administration of oxygen continued until clinical signs and blood gas results suggested that oxygen was no longer needed. Crude surfactant harvested from bovine lungs at a slaughterhouse was administered intratracheally to calves that appeared to be in respiratory distress or had unacceptable blood gas values. Colostrum was administered within an hour of birth.

I would like to digress for a moment for some important comments on arterial blood gas measurement. Measurement of arterial blood gas is not a routine procedure in most bovine practices, but it is essential for the assessment of respiratory distress in neonates. The site of collection we use is the brachial artery. The technique was described by Adams, *et al*.[3] and is easily accomplished if the recommended procedure is followed. The calf is placed in lateral recumbency. An assistant restrains the calf by holding the neck down with one hand and the up leg caudally with the other. The down leg is positioned forward at a 45 angle to the sternum and held there between the knees of the sampler. Clipping a small area at the site of sampling makes the procedure easier. The artery is located on the proximomedial aspect of the elbow by palpation of the pulse. The artery is stabilized by placing the index finger and second finger just proximal and distal to the puncture site. We used a heparinized tuberculin syringe and a 25 g needle to collect the sample. The needle is inserted perpendicular to the skin for about half a cm. After the blood is collected, apply digital pressure to the site for a minute to avoid hematoma formation. Several samples can be collected from the same artery over 24 hours. In Adams' study, they did not find a consistent effect of restraining the calves in lateral recumbency for 10 minutes. We attempted to collect the samples quickly and if there was a delay, we allowed them several minutes of sternal recumbency before attempting again.

Because there was a wide range in the rectal temperatures of the calves, we always corrected blood gas values for temperature. The temperature in the machine at which the measurement is made is calibrated to 37º C. If the measured P_aO_2, for example, is 53.9 mmHg, when temperature corrected to a temperature of 39.5º C, the value is 64 mmHg. This difference is great enough to change the level of concern substantially for us about a newborn calf breathing room air.

My experience with arterial blood gas measurement and neonatal respiratory distress syndrome prior to attending these calves was limited. Therefore, I was unaware of the difficulty in recognizing the seriousness of the syndrome and the degree of hypoxia using basic physical parameters like heart and respiratory rate, color and depression. Calves, especially very young calves, can look nearly normal and have horrendous hypoxia. Therefore it is imperative to measure arterial blood gasses. Another important lesson we learned concerned the manner and method we used to discontinue oxygen supplementation. Calves that appeared to be clinically normal with oxygen insufflations continued to look good for hours after the cessation of oxygen, only to rapidly decompensate. It appears that clinical signs lag the deterioration of the blood gas parameters. Therefore, it is now our practice to gradually wean calves off of oxygen over 24-48 hours and to monitor P_aO_2 during the process immediately prior to reducing the oxygen flow rate.

Returning to the story of the calves, the calf born to the second cow with hydrops was treated aggressively immediately after birth and did not develop respiratory distress. However, he became tachycardic at week 5 at the ranch and died during week 6. A field necropsy was performed, but findings were inconclusive. There was gross evidence of cardiomegaly and very thin ventricular walls.

The other 6 calves survived long-term. The perinatal complications associated with these calvings included: retained placenta, placental edema, respiratory distress requiring supplemental oxygen for 24 hours, flexural contraction or laxity, large umbilical vessels, tachypnea, and hyperthermia. The calves were not extremely large (range 44-58.6 kg), unlike those from the "split" embryos, but they were not allowed to go beyond day 285 in gestation.

The next clone adventure at Texas A&M was the birth of Second Chance, the first bovine clone derived from frozen cells of a mature animal. It was by chance that the cells were harvested. Dr John Hill, the researcher who ultimately cloned Second Chance, was passing through the clinic when a tumor was being removed from Chance. He and the owner began to talk, one thing led to another and a piece of skin was taken to the lab for cell culture and freezing. A single pregnancy was derived after a rather small number of cell manipulations and the pregnancy progressed without incident. The calf was born by caesarean section and was handled as described above. At 24 hours the respiratory rate increased and the blood gasses (P_aO_2) values deteriorated, a cardiology consultation was requested. A "bubble study" revealed right to left shunting through an open foramen ovale. This suggested persistent fetal circulation and pulmonary hypertension. A test dose of 50 mg of tolazaline was administered intravenously over several minutes. It resulted in an increase in P_aO_2 of about 50%. Subsequently, we began an intravenous infusion of 200mg of tolazoline in 1 liter of lactated Ringer's solution at the rate of 50 ml/hour. Nasal insufflation was maintained at 10 L/min for 24 hours. After the first liter was infused, a second liter with 100mg tolazoline/L was begun. Over 72 hours, the infusion of tolazaline was reduced gradually as was the flow rate of oxygen. Clinical parameters and arterial blood gasses were monitored to assess progress. With the respiratory crisis over and the calf back in the stall, a serum chemistry profile was performed on day 7 and hyperglycemia was present. Over the next 6 days, serum glucose remained elevated, reaching a high of 445mg/dl. During this period Second Chance was clinically normal. Insulin concentration in the face of hyperglycemia was 10µU/ml compared to 65 µU/ml in a control calf of the same age. A diagnosis of diabetes mellitus was made and after establishing an effective dose regimen, the calf was maintained on 5 U/day for several weeks. The insulin was then discontinued at 60 days of age when the glucose was consistently between 100 and 120 mg/dl. At 5 months of age, the serum glucose was 89mg/dl. At each yearly examination afterwards, the serum insulin has been within the reference range. At the age of 4 years, Second Chance is clinically normal.

Our last experience with cloned calves came with the birth of 86^2 or "Bruce" as the students called him. He was derived from frozen cells of a brucella-resistant bull. The amazing thing about 86^2 is that his cells were frozen in 1985, 12 years before the first cloned sheep, Dolly, was produced and 14 years before the first calf from a mature bovine animal was produced.[2] Dr Garry Adams, who owned the donor, 86, apparently believed in the saying, "Be prepared.". The birth and neonatal period of 86^2 was relatively uneventful except for a tendency to have periods of hyperthermia. Several of the other clone calves and later, the cloned goats and pigs at Texas A&M University displayed similar tendencies.

Résumé

La gestion de veaux clonés est différente de celle d'autres veaux, non seulement de par leur haute valeur monétaire, mais aussi de par leur tendance à développer certaines conditions pathologiques à une fréquence plus élevée que chez les veaux conventionnels. Ces conditions incluent les anomalies placentaires, la persistance de la circulation foetale, la contracture de membres, les vaisseaux ombilicaux anormalement développés, et la tachypnée. Chez les veaux à risque de persistance de circulation foetale, le suivi d'analyse des gaz sanguins artériels, combiné à un traitement agressif et précoce devraient être institués. L'utilisation de la

tolazoline en infusion et l'addition d'oxygène ont sauvé plusieurs cas de veaux clonés et conventionnels.

References

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