Physical and clinicopathologic findings in foals derived by use of somatic cell nuclear transfer: 14 cases (2004–2008)

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Objective—To describe the health status of foals derived by use of somatic cell nuclear transfer (NT) at a university laboratory.

Design—Retrospective case series.

Animals—14 live-born NT-derived foals.

Procedures—Medical records from 2004 through 2008 were evaluated to identify all pregnancies resulting in live-born NT-derived foals. Information obtained included gestation length, birth weight, foaling complications, gross abnormalities of the fetal membranes, appearance of the umbilicus, mentation of the foal, limb deformities, and any other abnormalities detected in the neonatal period. Clinicopathologic data were also evaluated when available. Records of 4 recipient mares during gestation were included.

Results—Six foals were clinically normal for all evaluated variables. The most common abnormalities detected in the remaining 8 foals included maladjustment, enlarged umbilical remnant, and angular deformity of the forelimbs. Two foals died within 7 days after parturition; in the remaining foals, these conditions all resolved with medical or surgical management. Large offspring syndrome and gross abnormalities of the fetal membranes were not detected. The 12 surviving foals remained healthy.

Conclusions and Clinical Relevance—Associated problems of calves resulting from use of NT have been reported, but there are few data on the outcome of foals resulting from adult somatic cell NT in horses. Although this population of foals had a lower perinatal mortality rate than has been reported for NT-derived calves, some NT-derived foals required aggressive supportive care. Birth of foals derived from NT should take place at a center equipped to handle critical care of neonates. (*J Am Vet Med Assoc* 2010;236:983–990)

The technique of somatic cell NT has yielded thousands of animals since it was first successfully used in 1996.[1] Despite major advances, the process of cloning remains inefficient, and it is estimated that across species, fewer than 5% of all transferred NT-derived embryos result in live births.[2–5]

Abnormalities of the placenta are a leading cause of pregnancy loss for bovine fetuses derived by use of NT, particularly between 30 and 90 days of gestation.[6,7] Small numbers of large, irregularly shaped placentomes, placental edema, and adventitious placentation are commonly observed in fetal membranes of aborted or stillborn calves.[6,8,9] Hydroallantois secondary to placental abnormalities develops in 33% to 50% of bovine pregnancies that result from the use of NT techniques.[8,10,11] In addition, the diameter of the umbilical cord is increased at most stages of gestation for NT-derived calves.[8] The umbilical vessels of NT-derived calves at birth are dramatically enlarged and often require ligation to control hemorrhage.[2,10,12,13] In 1 study,[12] 100% of the calves were affected.

Abbreviations

CTUP	Combined thickness of the uterine and placental tissues
LOS	Large offspring syndrome
NT	Nuclear transfer

Problems in cloned calves at birth include an increase in birth weight (ie, LOS). This syndrome develops in both NT- and in vitro fertilization–derived calves and is associated with dystocia and musculoskeletal problems in the neonatal period.[9,12,14] The incidence of LOS has decreased over time with modifications in the NT procedures, but it is still a major problem in NT-derived calves in many laboratories. Flexural and angular limb deformities are also common in NT-derived calves and range in severity from mild abnormalities that

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respond to application of splints to severe abnormalities that require that the animal be euthanatized.[2,9,12,13,15] Additional life-threatening conditions that are reported more often in NT-derived calves are persistent pulmonary hypertension,[2,10,13,15] neonatal hypoxia,[2,10] and paradoxical hyperthermia that is unresponsive to conventional treatment.[2,14] Abnormal hematologic variables in NT-derived calves include poor glucose regulation leading to hypoglycemia,[2,12,15] transient anemia,[2] azotemia 36 to 48 hours after birth,[2] and hyperfibrinogenemia.[2]

Problems associated with NT-derived animals continue into the postpartum period in many species, with mortality rates as high as 30% during the first 6 months after birth reported in cattle.[2,13] However, in NT-derived calves that survive through weaning, performance for growth,[16,17] milk production and composition,[17,18] meat products,[19] and reproductive efficiency[17] are similar to those of conventionally conceived calves.

Nuclear transfer in horses is in its infancy. The first report[20] of an equid resulting from the use of NT was that of a mule born in 2003, which resulted from the use of cells obtained from a fetus. The same month the mule birth was reported, researchers in Italy reported[21] the birth of a horse foal resulting from use of NT with adult somatic cells. Subsequently, birth of 2 additional cloned foals from adult somatic cells was reported[22] by the laboratory group in Italy, and birth of 14 cloned foals from adult somatic cells has been reported[23–25] by our laboratory group in Texas. A commercial cloning company has also announced the birth of cloned foals in the popular press,[26] but information on the production of these foals is not available.

The health and hematologic findings for cloned mules have been reported,[27] and these animals have been clinically normal from birth. However, these mules may not be representative of all equine clones because they were the result of tissues and methods not typically used for NT. For example, the donor cells used for NT were obtained from a fetus, rather than from an adult; and the oocytes used were collected ex vivo from mature preovulatory follicles (rather than from immature follicles, followed by oocyte maturation in vitro). In addition, immediately after NT, the oocytes were surgically transferred to the oviduct of recipient mares, rather than being cultured in vitro until they reached the blastocyst stage. All of these variations, especially use of fetal donor cells, increase the chance that a healthy offspring will result; these variations are not readily applicable to cloning as a clinical procedure.

Little information on the health of cloned foals resulting from the use of adult somatic donor cells and standard techniques has been reported.[21–25] The objective of the study reported here was to describe neonatal physical and clinicopathologic data of foals resulting from use of NT techniques at a university laboratory.

Materials and Methods

Case selection—Medical records for all pregnancies diagnosed at 11 to 15 days of gestational age (day 0 = day NT was performed) that resulted from transfer of NT-derived embryos at Texas A&M University from 2004 through 2007 were evaluated. This included records of foals born in 2005 through 2008 (4 foals were born at the Texas A&M University Veterinary Teaching Hospital, and 10 were born at an equine referral hospital).

Medical records review—Information obtained from the records included gestation length, birth weight, whether the birth was assisted, gross abnormalities of the fetal membranes, appearance of the umbilicus, mentation of the foal, limb deformities, and any other abnormalities detected during the neonatal period. Clinicopathologic data also were evaluated when available. Records analysis of 4 recipient mares included information on prepartum analysis of the CTUP via transrectal ultrasonography and progesterone concentrations prior to parturition.

Scoring of abnormalities—To standardize grading of the severity of the most commonly encountered abnormalities of flexural and valgus limb deformities, enlarged umbilicus, and degree of maladjustment, a scoring system for each was used. The forelimbs and hind limbs were scored on a scale from 0 to 4 as follows: 0 = limbs were straight, 1 = slight changes similar to those seen in clinically normal foals and that corrected without treatment within days to weeks after birth, 2 = moderate changes (valgus or tendon contracture) treated by application of bandages for < 1 week that corrected without additional treatment within weeks to months, 3 = marked changes that required prolonged application of bandages or casts, and 4 = severe contraction that was deemed to be not correctable. The umbilicus was scored on a scale of 0 to 4 as follows: 0 = normal, no enlargement, and separated without assistance; 1 = slightly enlarged but no intervention required; 2 = moderately enlarged and required application of ligatures at parturition or assistance to separate; 3 = markedly enlarged and required surgical removal of the umbilical remnant; and 4 = umbilical hernia. Mentation and degree of maladjustment were scored on a scale of 0 to 4 as follows: 0 = clinically normal, 1 = slightly weak with a prolonged time to stand and suckle but no other maladjustment, 2 = moderately maladjusted with a poor suckling effort and difficulty standing but that resolved within 48 hours with palliative treatment, 3 = markedly maladjusted with intensive care required but the foal survived, and 4 = severely maladjusted and the foal died.

Results

A total of 1,675 oocytes were subjected to NT from 2004 through 2007.[23–25] Of those, 1,360 (81%) cleaved after activation. Including all donor cell and activation treatments investigated, 67 embryos developed to the blastocyst stage when cultured in vitro; this represented 5% of cleaved embryos and 4% of oocytes subjected to NT. Because of a lack of available recipient mares, only 54 embryos were transferred (all embryos were transferred via a transcervical technique). Of these, 31 (57%) resulted in establishment of pregnancy, as determined by use of transrectal ultrasonography on days 11 to 15 after the day of NT.[24]

Outcome of pregnancies by horse cell line was determined (**Table 1**). Nine pregnancies were lost before 90

Table 1—Results for NT-derived equine embryos at a university laboratory.

Horse	Cell line	No. of blastocysts transferred	No. of pregnancies	Loss at < 90 days of gestation	Loss at 3 to 10 months of gestation	Fetus died at end of gestation	Foal died after birth	Viable foals
DN	A	11	4	0	2	0	0	2
TT	A	7	5	2	1	0	1	1
SM	A	13	9	0	1	2*	1	5
GN	A	6	2	0	1†	0	0	1
SG	A	10	5	5	0	0	0	0
	B	4	4	1	1	0	0	2
SS	A	3	2	1	0	0	0	1
Total		**54**	**31 (57%)**	**9**	**6**	**2**	**2**	**12**

*Fetuses died at days 305 and 341 of gestation. †Pregnancy lost when recipient mare was euthanatized at 4 months of gestation because of neurologic disease.

Table 2—Results for 14 live-born NT-derived foals.

Foal	Cell line	Duration of gestation (d)	Umbilicus score*	Limb score†	Valgus deformity detected	Contracted tendons	Maladjustment score‡
1 DN	DN	389	2	2	Yes	No	2
2 ER	DN	341	3	2	No	Yes	1
3 HD	TT	342	2	3	No	Yes	2
4 IC§	TT	352	2	3	No	Yes	2
5 AL	SM	326	3	3	Yes	Yes	0
6 BO	SM	327	0	0	No	No	0
7 CB	SM	334	3	3	Yes	Yes	1
8 DV	SM	340	0	0	No	No	0
9 EL	SM	341	0	0	No	No	0
10 FE‖	SM	337	3	3	Yes	Yes	4
11 GR	GN	343	0	0	No	No	0
12 TY	SG	312	0	0	No	No	0
13 RO	SG	318	0	1	Yes	Yes	0
14 SS	SS	341	0	0	No	No	0

*The umbilicus was scored on a scale of 0 to 4 as follows: 0 = normal, no enlargement, and broke without assistance; 1 = slightly enlarged but no intervention required; 2 = moderately enlarged and required application of ligatures at parturition; 3 = markedly enlarged and surgical removal required; and 4 = umbilical hernia. †The forelimbs and hind limbs were scored on a scale from 0 to 4 as follows: 0 = limbs were straight, 1 = slight changes similar to those seen in clinically normal foals and that corrected without treatment within days to weeks after birth, 2 = moderate changes (valgus or tendon contracture) treated by application of bandages for < 1 week that corrected without additional treatment within weeks to months, 3 = marked changes that required prolonged application of bandages or casts, and 4 = severe contraction that was deemed to be not correctable. ‡Mentation and degree of maladjustment were scored on a scale of 0 to 4 as follows: 0 = clinically normal, 1 = slightly weak with a prolonged time to stand and suckle but no other maladjustment, 2 = moderately maladjusted with a poor suckling effort and difficulty standing but that resolved within 48 hours with palliative treatment, 3 = markedly maladjusted with intensive care required but the foal survived, and 4 = severely maladjusted and the foal died. §Foal died 6 days after birth. ‖Foal died 2 days after birth.

days of gestation; 5 of these originated from a single cell line and represented all pregnancies that resulted from use of that cell line. No fetal tissue was recovered from pregnancies that were lost before 90 days of gestation.

Six pregnancies were lost between 3 and 9 months of gestation; fetuses were not recovered for 2 of these pregnancies (lost between 3 and 5 months of gestation). One recipient mare developed signs of severe neurologic disease at 4 months of gestation and was euthanatized; no diagnosis for the mare's condition was achieved during necropsy, and no abnormalities were detected in the fetus. Three fetuses were recovered after they were aborted between 5 and 9 months of gestation; all 3 were submitted for necropsy. One fetus had no abnormalities, and the second fetus had an umbilical hernia (omphalocoele) with no other abnormalities. The third fetus was found in the pasture and had been subject to scavenging with loss of the abdominal organs; however, no abnormalities were observed in the available tissues.

Two pregnancies continued past 300 days but resulted in dead foals. One foal was born in the pasture at 305 days of gestation but was dead when found. Results of necropsy suggested that this foal had breathed and the eponychiae had signs of wear, which indicated that the foal had at least attempted to stand. The second foal was carried to term (341 days of gestation), but birth resulted in a dystocia that required delivery via cesarean section; the foal was delivered dead, and it had severe contraction of the forelimbs and an omphalocoele.

Fourteen foals were born alive. The mean length of gestation for the 14 viable foals was 339 days (**Table 2**). Two mares pregnant with fetuses from the same cell line foaled at 312 and 318 days of gestation, respectively, which were considered abnormal for a typical equine gestation length (> 320 days). One foal was born on day 389 of gestation (ie, after a prolonged gestation), and a second foal from the same cell line was born on day 341 (ie, after a gestation of typical length). The birth weight of each of the foals was

within the expected range, except for the foal born at 389 days, which weighed only 27 kg (59.4 lb) at birth. The fetal membranes for this foal appeared grossly normal. The foal born on day 389 of gestation grew rapidly, and by 1 year of age, it was comparable in size to the other foal from this cell line that had a typical birth weight.

Medical records of 4 mares were available for review. These mares were examined monthly throughout gestation via ultrasonography per rectum and were assessed for fetal viability (movement or heart rate), CTUP, and appearance of allantoic and amnionic fluid. Approximately 1 month before their anticipated date of parturition, each of the mares was moved into stalls for monitoring and fitted with a foaling monitoring system.[a] Serum progesterone concentrations were obtained weekly starting 6 to 8 weeks before the mares foaled. As the anticipated date of parturition approached, analysis of calcium concentrations in mammary gland secretions was performed daily. All 4 mares had a typical prepartum increase in progesterone before foaling,[28,29] with a mean increase in progesterone concentration of 8 ng/mL between samples obtained 3 weeks and 1 week before parturition (the sample obtained 1 week before parturition was the last sample obtained before foaling). Calcium analysis of mammary gland secretions was not accurate for use in predicting the day of parturition. Two mares did not have an increase in calcium concentration prior to foaling, and the other 2 had a prolonged increase. In 1 mare, the calcium concentration was elevated 3 weeks prior to foaling and remained high until the foal was born. Transrectal ultrasonography revealed that CTUP was within anticipated limits in 2 of the 4 mares. In a third mare, the CTUP was slightly higher than expected starting at approximately 9 months of gestation. The fourth mare had a marked increase in CTUP (2.3 cm; reference range, < 1.2 cm) at 10 months of gestation, which was associated with a pattern of nonechogenic areas within the placenta that was interpreted as edema. All 4 mares had unremarkable parturitions, with no evidence of premature placental separation or placental inflammation at the time of birth. No treatment was instituted in any of the mares. All fetal membranes were grossly and histologically normal.

All 14 mares were treated as high-risk pregnancies, and live-born foals were treated as high-risk neonates at the time of birth. All parturitions were attended, and all mares had an uncomplicated foaling. Postnatal treatment included administration of oxygen via nasal insufflation, assistance in standing and suckling, and aggressive supportive care when required. All 14 foals received a minimum of 500 mL of supplemental colostrum (quality confirmed by use of a colostrum refractometer) via a nasogastric tube within 1 hour after birth to ensure adequate ingestion of high-quality colostrum. Twelve foals were administered 1 to 2 L of plasma, IV, between 18 and 24 hours after birth on the basis of a low (< 800 mg/dL) serum IgG concentration,[b] which indicated partial failure of passive transfer. The 10 foals born at the private referral hospital also received a concentrated equine IgG product[c] via nasogastric intubation within 12 hours after birth.

All foals were administered supplemental oxygen immediately after birth as a precaution until it was determined that they were able to maintain adequate oxygenation (Pa_{O_2} > 80 mm Hg) while breathing room air. Arterial blood gas measurements were obtained after the supplemental oxygen had been discontinued for at least 15 minutes. Most samples were collected with the foals restrained in lateral recumbency. The foals were maintained in a sternal position if they were unable to stand unassisted. Seven of 14 foals required administration of supplemental oxygen for > 12 hours after birth, as determined by a Pa_{O_2} < 80 mm Hg. Oxygen administration was discontinued in 6 of the 7 foals within 3 days after birth (mean Pa_{O_2}, 93 mm Hg), but 1 foal required oxygen administration for 15 days. The Pa_{O_2} for this foal was as low as 58 mm Hg (mean, 76.6 mm Hg) until day 15 after birth, when it increased to 94 mm Hg.

Antimicrobial treatment was instituted in all foals at the time of birth and was continued for at least 5 days or until the foals were clinically normal. Reasons for continuation of antimicrobials for > 5 days were omphalitis (n = 3 foals), omphalectomy (3), sepsis (1), and diarrhea (1). Twelve foals received ceftiofur and amikacin, 1 received only ceftiofur, and 1 received only amikacin. One foal was considered to be septic and received polymyxin B because of the endotoxin-binding effects of that product.

Scores for valgus limb deformities, contracture, umbilicus, and maladjustment for each foal were summarized (Table 2). Six of the 14 live-born foals had a score of 0 for all variables evaluated. Seven foals had some degree of umbilical abnormality, which included dilated umbilical vessels, edema, and failure of the umbilicus to rupture after birth **(Figure 1)**. Omphalectomy was performed in 4 foals.

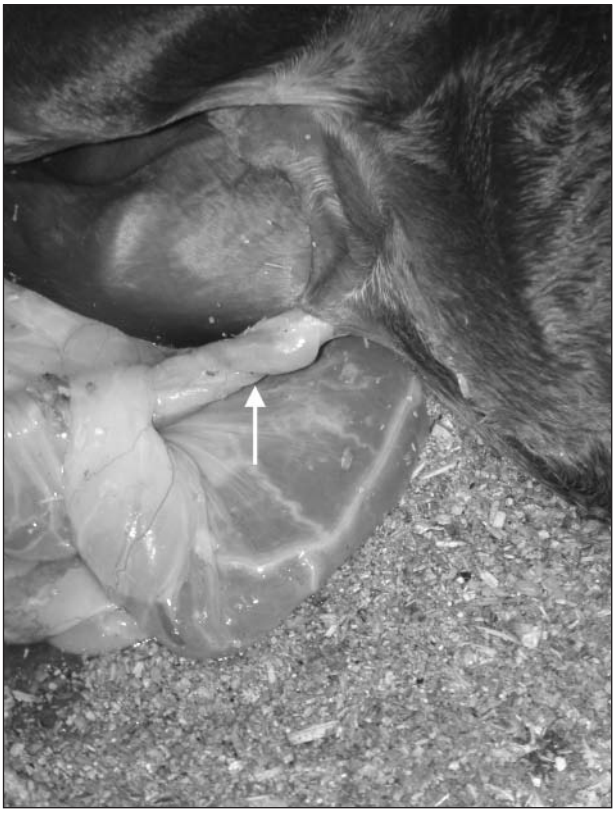

Figure 1—Photograph of the umbilicus of an NT-derived foal. Notice that it is thick and edematous (arrow) and lacks a natural rupture point.

Table 3—Abnormal results for available serum biochemical and hematologic variables during the first 3 days after birth for 14 live-born NT-derived foals.

Variable	No. of foals assessed	No. of foals with abnormal results	Mean (range) for affected foals	Reference range
Creatinine (mg/dL)	10	4	4.4 (2.2–7.1)	1.1–2.0
BUN (mg/dL)	10	1	31 (30–33)	7–28
Creatine kinase (U/L)	10	7	1,834 (462–8,430)	73–450
Chloride (mmol/L)	8	4	94 (90–97)	98–105
Sodium (low; mmol/L)	8	4	128 (125–130)	132–141
Sodium (high; mmol/L)	8	2	145 (142–151)	132–141
Potassium (mmol/L)	8	3	4.7 (4.3–5.7)	3.0–4.2
Phosphorous (mg/dL)	8	3	5.6 (4.6–7.2)	1.7–3.9
Calcium (low; mg/dL)	10	2	10.3 (10.2–10.4)	11.0–13.0
Calcium (high; mg/dL)	10	4	16.1 (13.9–18.6)	11.0–13.0
Lactate (mmol/L)	10	10	3.60 (0.99–22.00)	0.50–1.78
Fibrinogen (mg/dL)	10	2	500 (476–533)	100–400

Eight of 14 live-born foals had some degree of flexural or angular limb deformity (Table 2). Four foals required that bandages be applied to their limbs to assist with standing or tendon relaxation. One foal underwent surgical correction later in the postnatal period. All limb defects had corrected by the time the foals were 1 to 6 months old. Two foals had incomplete ossification of the cuboidal bones, as determined during radiographic examination of the carpus and tarsus; these foals were both from the same cell line. One of these foals died of anesthetic complications following a procedure performed before the cuboidal bones could mature, but the other was treated with supportive care and exercise restriction until 20 days after birth, when the cuboidal bones had fully ossified.

Two foals had signs of hypoxic ischemic encephalopathy at the time of birth. One of these foals initially had cortical blindness but regained vision after 2 days of supportive care. Both foals recovered completely. Three foals from 2 cell lines had some degree of brachygnathism (ie, parrot mouth).

Two live-born foals died after birth (1 at each of the foaling locations). One foal died of pneumonia 48 hours after birth. This foal was weak at birth and required extensive nursing care for hypoxia and respiratory distress until the time of death. The second foal, which was somewhat weak at birth but alert and able to stand and suckle, died after several days of intermittent seizures that began following a hypotensive event during induction of anesthesia. Blood was detected in the urine of this foal 2 days after birth, and ultrasonographic examination of the bladder revealed an apparent clot adjacent to the bladder wall. Because of an increasing BUN concentration, surgical exploration of a possible bladder tear and omphalectomy were scheduled 3 days after birth. The foal had a severe hypotensive event (arterial pressures of 20 to 25 mm Hg) during induction of anesthesia, and the surgery was cancelled. Seizures occurred during recovery and continued despite treatment. The foal never regained consciousness and died 3 days after the anesthetic episode. This foal was consistently hyponatremic before and during anesthesia, which may have contributed to the neurologic abnormalities. Necropsy of the foal revealed severe focally extensive cerebral edema with laminar cortical necrosis usually associated with prolonged hypoxia; there was no tear in the bladder. The pathological findings of this foal have been reported elsewhere.[30]

Rectal temperature, serum glucose concentrations, and results of CBCs and serum biochemical analyses in these foals were not clinically different from those expected in high-risk neonates that did not result from use of NT techniques. Complete hematologic values were not available for all foals.

Transient azotemia was detected in 4 foals at birth, as has been reported commonly in conventionally conceived foals[31]; however, IV administration of fluids resulted in resolution of azotemia by 3 days after birth in all foals, except for 1 (the foal with the suspected tear in the bladder). Serum lactate concentration was elevated at birth in all foals **(Table 3)** but returned to the reference interval within 4 days in most of the foals. The most common serum biochemical abnormalities detected at birth were elevated concentrations of creatinine (4/10 foals) and sodium (6/8 foals) and an increase in creatine kinase activity (7/10 foals). There was no evidence of abnormal thermoregulation or glucose regulation, which has been reported in calves. None of the foals required insulin treatment.

Foals received intensive care and monitoring for 3 (n = 2 foals) to 20 (1 foal) days. Six foals were considered stable after 6 days but remained under intensive observation as a precaution (n = 3 foals) or because of omphalectomy (3). Mean number of days of intensive care required for all foals was 8.5. All of the 12 foals that survived > 1 week after parturition continued to grow and develop normally. The only health concern in the foals after the neonatal period was for 1 colt at 7 months of age. This foal developed uroliths, which were removed surgically. The uroliths recurred and were again surgically removed when the colt was 9 months old. This foal had no further recorded problems after the second surgery.

Discussion

The efficiency for producing equine blastocysts by use of NT was low (4% of oocytes subjected to NT); however, the pregnancy rate after transfer of NT-derived blastocysts (31/54 [57%]) approached that for transfer of ex vivo recovered embryos (50% to 60%)[32,33] and for transfer of in vitro produced embryos (50%).[33] Although the rate of pregnancy loss after transfer of

NT-derived embryos was substantial (17/31 pregnancies failed to yield a live foal), the overall rate of live-born foals per embryo transferred (14/54 [26%]) compares favorably with that expected in cattle, in which only 5% to 10% of transferred NT-derived embryos are expected to yield live offspring.[2,3,34,35]

Five of the 9 pregnancies that were lost before 90 days of gestation originated from a single cell line. This cell line failed to yield a pregnancy that was maintained for ≥ 90 days. An additional tissue sample from the same donor animal was subsequently obtained, which yielded 2 live foals after transfer of 4 embryos; this indicated that, similar to results for other species, the quality of the originating cell line may be important to the success of the process.[25]

Survival of live-born NT-derived foals appears to be greater than that for NT-derived calves. Twelve of 14 live-born foals survived for 1 to 4 years, whereas in 1 report,[17] 50% of NT-derived calves alive at 1 day after birth died by 4 years of age. The problems observed in foals resulting from use of NT are similar to a subset of those observed in NT-derived calves. Limb abnormalities, including valgus deformities and contracted tendons, appear to be commonly observed abnormalities in both species. In calves, musculoskeletal abnormalities were associated with up to 10% of postnatal deaths in 1 study[36] in which investigators used blastomere NT and accounted for 24% of deaths in the postneonatal period in a second study.[17] The limb abnormalities in the foals of the present study ranged from a condition in a severely affected stillborn foal to those that were mild and corrected without treatment. Limb problems on all surviving foals were corrected by the time the foals were 1 to 6 months old, which suggested that in NT-derived foals born with limb and tendon abnormalities, standard treatments appropriate for the severity of those conditions are effective.

Placental abnormalities are a common finding in NT-derived calves. In calves, placental edema has been linked to congenital pulmonary hypertension that leads to respiratory distress in neonates.[10] In 2 of the 4 mares whose records were available, the CTUP was increased in the area of the cervical star. The cause of the increased CTUP was suspected to be placental edema. Parturition proceeded without complications, and the fetal membranes were grossly and histologically normal. Thus, placental edema may be associated with foals resulting from use of NT. Treatment may be unnecessary if there are no other clinical signs indicative of placentitis.

Seven foals required supplemental oxygen for > 12 hours after birth because of hypoxia (Pa_{O_2} < 80 mm Hg) detected via arterial blood gas analysis. This suggests that cloned foals may be susceptible to dysmaturity of the lungs at birth; however, the hypoxia was transient and resolved with treatment. Supplemental oxygen should be available during the birth of cloned foals. No signs of congenital pulmonary hypertension were observed.

Umbilical cord abnormalities were evident in 7 of the foals in this study. Abnormalities of the umbilicus are a potential site of infection that leads to generalized septicemia in neonates. The umbilical stump is a common point of entry for bacteria, which leads to hepatic abscesses and further hematogenous spread of bacterial contamination and is especially concerning if the umbilical vessels are enlarged at birth.[37] Surgical removal of the umbilical remnant is effective in eliminating the umbilicus as a nidus of infection; however, the loss of 1 foal after a hypotensive event during induction of anesthesia suggests that surgery should only be undertaken in these foals when definitively indicated.

Signs of LOS were not seen in these NT-derived foals. Some NT-derived calves with LOS weigh as much as 20% more than related non–NT-derived calves, and this increase in body weight is associated with an increase in dystocia and postpartum deaths.[2,9] However, the difference in initial birth weight is not maintained as the calves reach adulthood.[9] In the cloned foals in the study reported here, birth weights were as expected for the breed and sex of each foal, except for 1 foal with a low birth weight. This lack of LOS in horses may be attributable to the reported ability of mares to control the growth of a foal in utero.[38] Because of LOS in cattle, many cows pregnant with NT-derived calves are induced to calve or scheduled for cesarean section. This does not appear to be necessary in mares because 15 of 16 mares that carried a pregnancy to ≥ 300 days of gestation foaled without difficulty. It is interesting that of the 12 mares with a recorded time of parturition, 11 foaled during daylight hours (between 6 AM and 8 PM). Reportedly as many as 83% of all mares foal at night.[39] It is possible that the NT-derived foals somehow disrupted the natural tendency to give birth at night. However, these mares were monitored continuously, which may have also upset the natural time of parturition. More data are needed to determine foaling times of mares with NT-derived foals and other high-risk pregnant mares that are continuously monitored.

Hydroamnion or hydroallantois was not observed in any of the pregnancies in this study. This may have been related to differences in placentation between cattle and horses because naturally developing hydrops is much less common in horses than it is in cattle.[40] Hydroamnion is usually associated with placental pathological changes[6,8] and may be related to abnormal placentome formation[8] in pregnancies with NT-derived calves. Renal dysfunction has been reported in cloned calves; renal lesions were detected during necropsy in 4 of 15 NT-derived calves that died shortly after birth.[13] Grossly, the kidneys of affected calves appeared disproportionately enlarged.[14] Renal abnormalities were not observed in any of the live-born foals in our study, nor were they detected during necropsy in any of the aborted or stillborn fetuses. It has been hypothesized in cattle that renal lesions may be related to hydroallantois associated with pregnancies resulting from transfer of NT-derived embryos.[41] Lack of renal lesions in the foals in our study may thus have been related to a lack of hydroallantois in these equine pregnancies.

One interesting characteristic in 3 foals was the incidence of mild to moderate brachygnathism (ie, parrot mouth). This trait is widely accepted to be a congenital and heritable abnormality.[42] The horse whose cell line yielded 2 of the affected foals reportedly has conventionally conceived offspring that are affected with this condition. This trait appears to have incomplete pen-

etrance because other foals that resulted from use of the affected cell lines did not have brachygnathism.

The long-term health of foals resulting from use of NT is yet to be determined. The oldest NT-derived horse, a female resulting from use of standard NT in Italy, has reached the age of 5 years and is reported to be in good health; in addition, this NT-derived mare conceived and delivered a foal after a typical pregnancy.[43] Thus, once through the critical neonatal period, the health of NT-derived horses appears to be typical of that of conventionally conceived horses.

Twelve viable foals were the result of 31 pregnancies after transfer of 54 NT-derived embryos. Pregnancy loss rates appeared to vary by cell line. Nuclear transfer–derived foals are at an increased risk for umbilical abnormalities, contracted flexor tendons, and hypoxemia. Foals derived by use of NT should be born at a center equipped to handle critical care of neonates. Mares pregnant with NT-derived foals may give birth after a shortened gestation (in our study, 2 foals were born at 312 and 318 days of gestation, respectively, and 1 foal was found dead after unexpected birth at 305 days of gestation but may have survived with better monitoring and appropriate supportive care). Thus, to maximize survival of NT-derived foals, mares should be observed carefully for onset of parturition beginning at 300 days of gestation.

On the basis of the findings in this group of neonates, aggressive supportive care should be implemented beginning at the time of birth. Supplemental oxygen should be administered immediately after birth and continued until an arterial blood-gas analysis reveals an adequate Pa_{O_2} while the foal is breathing room air. Because a high rate of failure of passive transfer was observed in our foals despite the provision of colostrum of adequate quality (as measured with a colostrum refractometer), plasma should be available for IV infusion, and prophylactic administration should be considered to prevent sepsis. Intestinal absorption may be impaired in NT-derived foals, which makes it necessary to administer immunoglobulins IV to achieve adequate concentrations. All NT-derived foals should be treated as high-risk neonates and monitored continuously until they are able to stand and suckle and all physical examination and biochemical values are within anticipated limits. The umbilicus of NT-derived foals should be monitored closely and treated aggressively if there are signs of infection. Once past the critical first few days after birth, foals derived by use of NT appear to be healthy and can be expected to grow normally. All 12 foals that survived the first week after birth were healthy for at least 1 to 4 years.

a. FoAlert, FoAlert Inc, Acworth, Ga.
b. SNAP foal IgG, IDEXX Laboratories Inc, Westbrook, Me.
c. Seramune equine IgG, Sera Inc, Shawnee Mission, Kan.

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