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# Ultrasonographic fetal parameters and neonatal survival in somatic cell nuclear transfer–derived beef calves

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ABSTRACT

The objectives of this study were to identify prognostic indicators of calf survival in SCNT-derived beef calves. Ultrasonographic parameters of fetal well-being and development, maternal clinical parameters, and neonatal parameters were evaluated as predictors of calf survival in cows carrying SCNT-derived beef fetuses (n = 38). Calf survival was 61.5% (88.2% female and 40.9% male calves; P = 0.0026). Cow respiratory rate and cow temperature were significantly greater in the nonsurviving (NS) group 1 week prepartum. In surviving (S) calves, fetal heart rate (FHR) decreased during the last 2 weeks of gestation (P < 0.01). However, this final deceleration was not observed in NS calves, resulting in higher FHRs in this group (P < 0.0001). Fetal movement and fluid scores did not differ with calf classification. Mean amniotic fluid depth was smaller in S (5.5 ± 0.7 cm) than NS (8.7 ± 1.4 cm) calves (P = 0.0398). However, mean allantoic fluid depth did not differ (P = 0.6120). There was a significant association between the body weight of calf and the diameter of the fetal aorta (P = 0.0115; $r^2 = 0.3762$). Surviving calves were lighter at birth (P = 0.0028) and were born later (P = 0.007) than NS calves. Calves born vaginally had a smaller fetal aorta (2.1 ± 0.1 cm vaginal and 2.4 ± 0.1 cm Cesarean) (P = 0.0487) and a lighter birth weight (41.4 ± 4.2 kg vaginal and 60.4 ± 2.1 kg Cesarean) (P = 0.0001) than calves born by Cesarean. Also, calves that underwent spontaneous labor (52.2% S and 0% NS; P = 0.0029) had a lighter birth weight (44.9 ± 3.8 kg) than calves that did not initiate labor (61.6 ± 2.2 kg) (P = 0.0004). Frequent ultrasonographic fetal monitoring allowed identification of differences between S and NS calves. Calves without a final decrease in FHR or with a large aortic diameter were more likely to require a Cesarean because of failure to initiate labor or fetomaternal disproportion. Parameters of fetal well-being and development during the last 3 weeks of gestation were first described in SCNT-derived beef calves.
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## 1. Introduction

Somatic cell nuclear transfer (SCNT) is associated with a high rate of conception failure or pregnancy and neonatal loss. Only 5% of transferred SCNT-derived embryos result in birth of a live calf. Although most pregnancies are lost during the embryonic period, fetal losses also occur in association with abnormal placental development [1,2]. Placental abnormalities vary from fewer large, irregular, and edematous placentomes to the presence of microplacentomes, edema of the fetal membranes, and enlarged umbilical cords [3–7]. Of the calves born, 30% reportedly die within 6 months of birth [8]. Fetal and neonatal problems include a high birth weight and abnormalities of the lungs, kidneys, and heart [5,7,9].

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Identification of pregnancy complications and fetal distress allows anticipation of neonatal problems and might provide an indication for pregnancy termination if the welfare of the dam is compromised. Ultrasonographic examinations during pregnancy allowed detection of hydroallantois and placental edema in cows carrying SCNT-derived calves [8]. Hyperechoic particles in fetal fluids, increased thickness of the amniotic membrane, decreased depth of fetal fluids, and fetal hyperactivity or inactivity were all associated with fetal or neonatal death [7,10].

SCNT-derived calves are typically delivered by elective Cesarean after inducing fetal maturation with dexamethasone and PGF2α. This prevents unassisted births and minimizes the risk for dystocia because of fetomaternal disproportion [2]. Prediction of birth weight based on ultrasonographic fetal measurements could allow identification of calves affected by Large Offspring Syndrome. Ultrasonographic measurement of metacarpal or metatarsal width was correlated with birth weight in cloned Holstein calves [11]. The diameter of the thoracic aorta was also correlated with birth weight in *in vivo* produced calves. However, this correlation was not present in SCNT-derived Holstein calves [10].

In spite of the advances in fetal monitoring, most information on SCNT-derived calves was generated from Holstein calves. There is no information available on fetal parameters in SCNT-derived calves of beef breeds. Moreover, in most reports examinations were performed weekly to biweekly, or information from only one examination was included. More intensive fetal monitoring may be more accurate for identification of variations in fetal parameters and compromised fetuses. The objectives of this study were to identify prognostic indicators of calf survival in SCNT-derived beef calves during the last month of gestation. Ultrasonographic parameters of fetal well-being and development, maternal clinical parameters, method of delivery, and initial neonatal parameters were proposed as predictors of calf survival.

## 2. Materials and methods

### 2.1. Animals

Pregnant mixed-breed recipient cows carrying SCNT-derived fetuses were included in the study (n = 38). The cows presented to the Veterinary Health Center of Kansas State University (KSU) for management of parturition between 16 days before and 3 days after their expected parturition date. The protocol for veterinary management of delivery limited access to the cows to the last 2 weeks before the expected birth date. Expected birth date was calculated as 283 days for Aberdeen Angus and 286 days for Continental European breeds or crosses. Cows were 2 to 8 years old (median 6 years). They were housed in individual stalls and fed brome and alfalfa hay, 2 kg/day of grain, and water *ad libitum*. The SCNT-derived calves were produced in a commercial embryo biotechnology facility from adult fibroblasts derived from skin biopsies. Calves were derived from 11 genetic donors and cows presented to the Veterinary Health Center of KSU in 11 groups of recipients. Cows within a group (1–6 cows) carried SCNT calves derived from the same genetic donor of Aberdeen Angus (n = 19) or Continental European (n = 20) breed or crosses. Of the 38 pregnancies evaluated, 15 cows were missing at least one examination or one parameter in an examination. Two cows delivered twin heifer calves, one of which was born dead at the farm before admission. Therefore, postpartum data were available from 39 calves. Prepartum data from the twin pregnancies were not included in the analysis of fetal parameters. Therefore, prepartum data from 484 ultrasonographic examinations were included from 36 singleton pregnancies. Seventeen (43.5%) of the calves were female, and 22 (56.4%) were male. Calves were born between 10 days before and 18 days after the expected parturition date.

### 2.2. Embryo production

Embryos were produced in a commercial biotechnology facility and the information not disclosed here was considered proprietary. Bovine ovaries were obtained from slaughterhouse specimens. Cumulus oocyte complexes were aspirated from ovarian follicles and were matured for 24 hours in tissue culture medium (TCM-199) with 10% (v:v) fetal bovine serum, LH, and estradiol. Incubation was done at 39 °C in a humidified atmosphere of 5% $CO_2$ in air. Donor adult fibroblasts were obtained from skin biopsies. Fibroblasts were cultured at a density of 10,000 cells/$cm^2$ for 2 to 4 days, and their cell cycle was not synchronized. Cells were then trypsinated and suspended in a holding medium before nuclear transfer. Cumulus was removed and the oocytes were enucleated. A fibroblast was injected underneath the zona pellucida of each oocyte. Injections were performed in the holding medium with 5 μg/mL of cytochalasin B. Injected oocytes were then transferred to a medium containing 0.28 M of manitol and magnesium chloride for electrofusion. Activation was performed in a medium with 5 μg/mL of cytochalasin B and 10 μg/mL of cycloheximide for 6 hours. Reconstituted embryos were cultured for 7 to 8 days at 39 °C in 5% $CO_2$. One blastocyst was then transferred into each recipient cow. Transfer was done into the uterine horn ipsilateral to the ovary containing the corpus luteum. Recipient cows were 7 to 8 days postovulation at the time of transfer.

### 2.3. Ultrasonographic fetal monitoring

Ultrasonographic fetal monitoring was performed at presentation and daily at 8 AM and 4 PM thereafter until parturition. More frequent monitoring, up to four times daily, was performed if the fetus was thought to be undergoing *in utero* stress. Fractious cows were occasionally examined once daily or not examined on some days. Non-sedated cows were restrained with a head catch within the stall. An ultrasound machine (Aloka SSD-2000; Hitachi Aloka Medical Ltd., Wallingford, CT, USA) equipped with a 3.5 MHz curvilinear transducer was used for B-mode real time and M-mode ultrasonography. Alcohol was applied to the skin to facilitate contact with the transducer. Ultrasonographic examination started cranial and lateral to the mammary gland. The transducer was then moved cranially