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Placental abnormalities in equine pregnancies generated by SCNT from one donor horse

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ABSTRACT

Placental changes associated with SCNT have been described in several species, but little information is available in this area in the horse. We evaluated the ultrasonographic, gross, and histopathological characteristics of placentas from three successful and five unsuccessful equine SCNT pregnancies, established using cells from a single donor horse. Starting at approximately 6-month gestation, the pregnancies were monitored periodically using transrectal (TR) and transabdominal (TA) ultrasonography (US) to examine the placentas, fetal fluids, and fetuses. Of the five mares that aborted, one mare did so suddenly without any abnormal signs detected by US and four had enlarged umbilical vessels visible on TA-US before abortion. Placental edema (TR-US) and intravascular thrombi in the umbilical cords were seen (TA-US) in two of these four mares; one mare aborted shortly after acute placental separation was identified on TA-US. In three mares that delivered live foals, TA-US showed engorged allantoic vessels and enlarged umbilical vessels. Two of these mares had placental thickening visible on TR-US, interpreted as a sign of placentitis, that subsided after aggressive medical treatment. Seven of the eight placentas were submitted for gross and histopathological examinations after delivery. All placentas had some degree of edema, abnormally engorged allantoic vessels, and enlarged umbilical vessels. Placentitis, large allantoic vesicles, cystic pouches in the fetal part of the cord, and hemorrhages and thrombi in the umbilical vessels were detected only in placentas from mares that aborted. Equine pregnancies resulting from SCNT may be associated with placental pathologies that can be detected using ultrasonography. However, interpreting their severity is difficult. Although placental abnormalities have been observed in SCNT pregnancies in other species, to the best of our knowledge, placentitis has not been previously reported and may be an important complication of equine SCNT pregnancies, leading to pregnancy loss.

1. Introduction

Animal cloning by SCNT was introduced almost two decades ago [1], and since then more than 20 different animal species have been successfully cloned [2,3]. This technology has applications for the propagation of valuable genetics, the production of transgenic animals, the

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conservation of endangered species or rare breeds, and the replication of valuable individuals [2]. However, despite the continuous efforts of many research and commercial laboratories, the efficiency of this technology remains relatively low in comparison to other assisted reproduction techniques, such as artificial insemination, embryo transfer, or fertilization *in vitro* [4]. Across all the species of domestic livestock that have been cloned, approximately 5% to 15% of all embryos transferred to the recipients develop and result in viable offspring [2,3].

Commercial cloning is most commonly used in the cattle industry. Genetic copies of highly valued bulls or steers have been produced which have normal fertility, and have sired cows with expected physiological and reproductive parameters [2,5,6]. However, most bovine pregnancies that result from cloning are typically lost between Days 30 and 90 of gestation (GD) [7]. These losses are associated with various placental abnormalities, such as poor development of placentomes, reduced villous vascularization, hypoplasia of trophoblastic epithelial cells, and reduced numbers of binucleate cells [7–10]. Between Days 12–200 GD, 25% of bovine cloned pregnancies develop hydrallantois [11], and many develop severe placental edema and enlargement of the umbilical cord [9]. Owing to these abnormalities, approximately 20% to 75% of SCNT bovine fetuses are lost from 90 GD onward [9,11]. The small proportion of pregnancies that survive to term seem to compensate for poor placental development and function by placental overgrowth, which leads to fetal overgrowth known as large offspring syndrome [12,13]. These abnormally large fetuses can lead to dystocia and the need for delivery by cesarean section.

Bovine pregnancies that result from cloning are very valuable, and therefore, they are often monitored closely using ultrasonography [14]. Various abnormalities have been detected in bovine SCNT pregnancies using this technique, such as amniotic thickening and plaques, increased echodensity and/or amount of fetal fluids, abnormal placentomes, and increased diameter of the umbilical cord. Because hydrops conditions are life-threatening to the recipient cows carrying these fetuses, pregnancies showing signs of hydrops are often terminated on diagnosis. To prevent complications due to fetal overgrowth, parturition can be induced approximately a week before expected full term, after administration of exogenous corticosteroids to the recipient cow, which enhances fetal maturation [11,15,16].

Somatic cell nuclear transfer is also associated with placental abnormalities in other domestic and laboratory species, such as pigs, sheep, and mice [10,17,18]. Although the pathogenesis of this phenomenon is not fully understood, aberrant reprogramming of imprinted genes by the recipient cytoplast, resulting in improper epigenetic modification of key regulatory genes essential for placental development, is likely involved [10].

The use of SCNT to clone horses was first introduced in 2003 [19] and has resulted in the estimated production of over 200 viable foals to date [20]. Recently, live foal production from transferred embryos reached 35% in one laboratory [21]; however, 50% of these live born foals had neonatal maladjustment syndrome, an enlarged umbilical cord, and/or front leg contracture [22]. All newborn foals were given aggressive supportive therapy, such as supplemental oxygen, antibiotics, and infusion of exogenous plasma.

This report describes the results of the ultrasonographic, gross, and histopathologic examinations of the placentas obtained from eight equine pregnancies produced by SCNT from a single donor animal that showed a high rate of late gestational loss.

2. Materials and methods

2.1. General description

Somatic cell nuclear transfer was performed at Texas A&M University, using fibroblasts obtained from subcutaneous connective tissue from one donor horse, a 29-year-old Lipizzaner stallion. Sixteen blastocysts were transferred to recipient mares by standard transcervical embryo transfer technique (one embryo per mare) at three private equine embryo transfer centers. Eleven pregnancies were initially established, but three of them were lost before 90 GD. The eight remaining pregnant recipients (seven Quarter Horses, one Thoroughbred; aged 5–11 years) were monitored by local veterinarians for approximately 140 to 280 GDs; these mares are designated #1 through #8, according to the order of embryo transfer. Three mares were initially supplemented with altrenogest (Regumate, 0.044 mg/kg, given orally once a day), and five mares were given long-acting progesterone (Biorelease P4 LA 300 mg/mL; 5 mL, intramuscuscularly, every 7 days). The long-acting progesterone was replaced with altrenogest later in these pregnancies (Table 1).

Two mares were admitted to the University of Florida's Large Animal Hospital (UF LAH) after exhibiting signs of pending abortion (premature udder development and production of milk [#1; 280 GD]); increased combined thickness of uterus and placenta (CTUP) and placental separation (#8; 253 GD). Two mares (#2 and #6) aborted at 274 and 239 GD without premonitory signs. The remaining four mares (#3, #4, #5, and #7) were admitted to the UF LAH between 145 and 282 GD for pregnancy monitoring and foaling assistance. The fetal viability, fetal heart rates, and placental ultrasonographic morphology were evaluated using transrectal ultrasonography (TR-US) and transabdominal ultrasonography (TA-US) on a monthly basis through approximately 250 to 270 GD. The frequency of TR-US examinations increased to weekly for the remainder of gestation. Transabdominal US was performed weekly until approximately 300 GD, after which the frequency of this examination increased to at least twice a week. The concentrations of Ca^{++}, Na^{+}, and K^{+} in the mammary gland secretions were monitored in all hospitalized mares once or twice a week after 300 GD. All mares were observed closely for signs of parturition or abortion. University of Florida's Large Animal Hospital veterinarians assisted all deliveries of live foals.

Five recipients (# 1, #4, #5, #7, and #8) received antibiotics and nonsteroidal anti-inflammatory drugs because of one or more of the following findings: premature udder development, increased CTUP, edema of fetal membranes,