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Placentation in cloned cattle: Structure and microvascular architecture

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Abstract

To elucidate the morphological differences between placentas from normal and cloned cattle pregnancies reaching term, the umbilical cord, placentomes and interplacentomal region of the fetal membranes were examined macroscopically as well as by light and scanning electron microscopy. In pregnancies established by somatic nucleus transfer (NT), the umbilical cord and fetal membranes were edematous. Placentomal fusion was common, resulting in increased size and a decreased number of placentomes. Extensive areas of the chorioallantoic membrane were devoid of placentomes. An increased number of functional or accessory microcotyledons (<1 cm) were present at the maternally oriented surface of fetal membranes. Extensive areas of extravasated maternal blood were present within the placentomes and in the interplacentomal region. The crypts on the caruncular surface were dilated and accommodated complexes of more than one primary villus, as opposed to a single villus in non-cloned placentae. Scanning electron microscopy of blood vessel casts revealed that there was also more than one stem artery per villous tree and that the ramification of the vessels failed to form dense complexes of capillary loops and sinusoidal dilations as in normal pregnancies. At the materno-fetal interface, however, the trophoblast and uterine epithelium had normal histology. In conclusion, the NT placentas had a range of pathomorphological changes; this was likely associated with the poor clinical outcome of NT pregnancies.

Keywords: Cloning; Fetal membranes; Microvasculature; Placentation; Nucleus transfer

1. Introduction

Animal cloning is one of the most important biotechnical advances of recent years, and the results of somatic nucleus transfer (NT) in ruminants have served to clarify the genetic and epigenetic aspects of cloning [1]. However, NT can be an inefficient process, with a limited number of pregnancies reaching term [2],

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compared to pregnancies established by IVF [3]. In cattle studies, only 6% [4] or 8% [5] of the embryos transferred to recipient cows resulted in healthy, long-term viable calves. Large offspring syndrome is another feature of NT in cattle and sheep [6]. Moreover fetal loss sometimes involves death of the surrogate mother [7]. Finally, cloning by NT is a bio-medical tool with great potential for agricultural, economical and social impact but lacks regulatory and consumer acceptance [8,9].

As the main organ of materno-fetal interaction, the placenta plays a critical role in maintaining pregnancy [10–12]. Indications of placental failure in NT pregnancies include anomalies such as large offspring syndrome, altered placental and fetal membrane proteins, increased placental weight, and placentome enlargement and edema in cattle [3,6,13–18]; placentomegaly in the mouse [19]; and placental insufficiency in the sheep [20].

The objectives of the present study were to elucidate the macroscopic and microscopic morphology of the placenta in NT bovine pregnancies that went to full term. The study includes the umbilical cord, the number and structure of the placentomes and the interplacentomal areas of the fetal membranes. Within the placentomes, fetal villi interdigitate with the maternal caruncular crypts; their ramifications were described from vascular casts studied by scanning electron microscopy.

2. Materials and methods

Authorizations for this research were provided by the Veterinary Bioethics Committee of the School of Veterinary Medicine, University of Sao Paulo; UNESP, Jaboticabal; FZEA, Pirassununga; and EMPRAPA, Brasília.

2.1. Enucleation, nucleus transfer and culture

Cumulus oocyte complexes (COC) of cows (*Bos taurus* and *Bos indicus*) were aspirated from ovaries collected at an abatoir and matured *in vitro* in TCM 199 medium. After a maturation period of 18 h, cumulus cells were totally removed and oocytes with the first polar body were selected for enucleation. This procedure was conducted after aspirating the first polar body and surrounding cytoplasm with a pipette needle. Fibroblasts from adult male and female animals were collected from ear skin, cultured *in vitro* in Dulbecco's modified Eagles's medium (DMEM), and used as nucleus donors after synchronization for 5 days by serum starvation. Thirty minutes before nucleus transfer, skin cells were individualized by treatment with trypsin. A single fibroblast nucleus was then introduced into the perivitelline space of the enucleated oocyte followed by a single DC pulse of 1.5 kV/cm for 65 μs. Reconstructed zygotes were kept in synthetic oviductal fluid (SOF) medium [21] for 2 h before chemical activation with 5 mM ionomycin for 5 min and 6-dimethylaminopurine (6-DMAP) for 3 h. Activated zygotes were co-cultured with a layer of granulosa cells in 100 μL drops of SOF medium supplemented with 10% fetal calf serum (Gibco^{TM}, Invitrogen, Carlsbad, CA, USA) overlaid with mineral oil for 7 days. On day 7 after fusion, embryo quality was evaluated and grades 1 and 2 blastocysts were transferred to recipients. For further details of the protocols, see Mello et al. [22] and Yamazaki et al. [23].

2.2. Collection of material

Material was collected from 19 pregnancies (*Bos taurus* three, *Bos indicus* 12 and two cross breed) established by NT. Details are given in Table 1. In 15 cases, the calf was delivered by Cesarian section. During this procedure, one to four placentomes with adjacing interplacentomal tissue were removed from the uterus (eight cases) using clamps and a knife for histological examination (see below). In a subset of seven pregnancies, the cow was slaughtered following delivery of the calf by cesarian section and then the uterine horns were opened, injected with fixative, and the fetal membranes inspected. Nude or cotyledon-free areas of chorioallantoic membrane were recorded. Then the uterus was totally everted to register the size, shape and distribution of the placentomes. As controls, pregnant uteri were obtained from 12 animals at the slaughterhouse. Only pregnancies judged to be near term (>210 days, according to crown-rump-length) were included as controls [24].

2.3. Light and scanning electron microscopy

For light microscopy, placentomes were perfused through chorioallantoic blood vessels with 4% buffered paraformaldehyde, processed by standard procedures and embedded in Paraplast. Sections were cut at 5 μm and stained with hematoxylin and eosin, Van Gieson, and picrosirius [25]. The PAS reaction was applied as a histochemical method for detection of glycosaminoglycans. For the study of semithin sections prepared by routine methods, fragments of placentomes were fixed in 2.5% glutaraldehyde, 0.1 M PBS, pH 7.4 [26]. Selected tissue samples were washed in 0.1 M PBS