THE PLACENTA AS A CONTRIBUTOR TO PRODUCTION OF LARGE CALVES

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ABSTRACT

Unusually high birth weights frequently occur in calves born from cultured bovine embryos. The mechanism(s) through which in vitro manipulations during early cleavage are translated to enhanced fetal growth is (are) incompletely understood. Accelerated growth is primarily prenatal, and the placenta of an in vitro-derived conceptus could account for abnormal fetal growth. Results from a systematic comparison of placental morphology and function in bovine concepti produced in vitro versus in vivo are discussed.

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INTRODUCTION

More than a decade ago, rumors circulated that some calves developing from embryos produced by nuclear transfer procedures, using blastomeres as nuclear donors, were excessively large at birth. Speakers at a 1992 satellite symposium of the International Embryo Transfer Society (IETS) confirmed these rumors (17). Several years later, our laboratory reported that calves with excessively high birth weights had developed from embryos cultured in vitro from fertilization to the blastocyst stage (2), an observation reported earlier for cultured sheep embryos (22). Abnormally high growth was also reported in 7-mo fetuses developing from oocytes having undergone IVM, IVF, and IVC (7). Despite early skepticism by some researchers that in vitro embryo manipulations were responsible for development of large calves, skepticism fed by the circumstance that some laboratories using some culture systems had not observed large calves from cultured embryos, in vitro manipulation of bovine cleavage-stage embryos is now widely accepted to produce the "large offspring syndrome" in some calves. Ironically, as described by Barnes (1), the first nuclear transfer calves with abnormally high birth weights were produced using donor cells from in vivo-derived embryos, recipient cytoplasm from in vivo-matured oocytes, and in vivo culture to the blastocyst stage in a sheep oviduct.

The observation that in vitro manipulation of cleavage-stage bovine embryos can lead to aberrant fetal growth is of both practical and biological significance. "Large calves" frequently present delivery and/or survival problems that raise questions of animal welfare. Embryo manipulation procedures that produce large calves will find only limited practical field use until the large calf problems are solved. Large calves also raise fascinating questions of fundamental biology. For example, why is it that bovine embryos can be split into halves, frozen in liquid nitrogen, or subjected to numerous other insults and still develop to term normally, but in vitro attempts to mimic in vivo conditions for early development can result in abnormal post-embryo transfer development to term?
The large offspring syndrome has been the subject of numerous recent reviews (9), including a symposium paper at the 2001 IETS meeting (11) and a satellite symposium at the 2000 IETS meeting (6). During 2001 the popular press even announced, based on a scientific publication with more restrained conclusions (26), that the gene producing large offspring had been identified. What new is there to report?

Molecular analysis of tissues from large fetuses resulting from nuclear transfer and from in vitro embryo production has led to the suggestion that imprinted genes are involved in the large offspring syndrome. Blondin et al. (4) reported that in vitro embryo production increases IGF-2 mRNA expression in Day 70 fetal liver and decreases expression in skeletal muscle. Young et al. (26) found similar steady-state transcript levels of IGF-2 mRNA in liver, kidney, and muscle but reduced methylation and expression of the IGF-2 receptor gene of control and “large” in vitro-produced (IVP) Day 125 sheep fetuses; however, reduced methylation was not seen consistently across all large fetuses. These authors proposed that cleavage-stage embryos are vulnerable to epigenetic alterations of imprinted genes and that unfavorable (i.e., unphysiological) IVC conditions lead to faulty gene expression.

The physiological link between individual gene expression and the large-calf phenotype is not always clear. Moreover, phenotypes and expression of the large calf syndrome are variable. For example, in addition to high birth weights, some IVP calves suffer perinatal health problems that may or may not be associated with birth weight. The degree to which these symptoms occur, similar to the range in birth weights, is variable and unpredictable.

What do we know about birth weights and other characteristics of IVP calves? Despite the attention paid to large calves, most IVP calves are normal. The distribution of birth weights is shifted to the right, meaning that both the mean birth weight and the upper end of the range are higher (15), an outcome also described for lambs born from cultured embryos (23). Importantly, the excessive growth appears to be a prenatal problem; once born, with a few exceptions (16), growth rates seem to normalize (24). The latter observation is particularly intriguing, because it implies that conditions for expression of the large-calf phenotype exist during the prenatal but not postnatal period. In seeking a physiological explanation for a problem that is primarily prenatal, altered placental function would provide a logical role. The remainder of this paper will focus on the potential role of the placenta in promoting excessive growth in IVP calves.

DISCUSSION

Evidence for Placental Abnormalities in IVP Calves

The earliest characterizations of IVP calves included mention of abnormal placental morphology or function. Hasler et al. (13) and van Wagendonk-de Leeuw et al. (20, 21) reported an increased incidence of hydrallantois in IVP calves, a problem typically associated with placental (versus fetal) abnormalities. Farin and Farin (7) reported that IVP embryos develop fewer placentomes than do normal, in vivo concepti, but, in another experiment conducted by Farin et al. (8), the number of placentomes in Day 70 IVP concepti was not different from in vivo concepti. The same laboratory...
(10) further studied Day 222 IVP concepti and reported heavier placentas in association with larger fetuses and reduced caruncular surface area and villous density.

From results of a study conducted on nuclear transfer calves during the perinatal period, Garry et al. (12) proposed that some observed conditions affecting calf survival and well-being could be due to placental dysfunction. Stice et al. (19) reported failure of cotyledonary development in nuclear-transfer concepti, and Hill et al. (14) later proposed that the primary cause of pregnancy losses in bovine nuclear transfer pregnancies is faulty placental development. These authors further speculated that an abnormal placenta could affect development of an otherwise normal IVP fetus. Most recently, DeSousa et al. (5) concluded that ovine nuclear transfer concepti suffer both fetal and placental deficiencies, including fetal liver enlargement, dermal hemorrhage, and lack of placentomal vascular development reflected in reduced or absent cotyledonary structures. The retarded development of cloned fetuses was strikingly associated with avascular or hypovascular placentas, similar to the observations of Hill et al. (14).

Evidence Contrary to Placental Involvement in Large Calves

Although aberrant placental development has been reported in IVP concepti, some researchers have not found an association between abnormal placentas and excessive growth. Sinclair et al. (18) reported that, in Day 125 ovine fetuses developing from cultured embryos, increased fetal weight was not the consequence of larger placentas. These and other data led Young et al. (25) to conclude, "Thus, the overgrowth appears to be driven by the fetus and not the placenta, although placental function, rather than size, may be affected."

New Evidence of Placental Dysfunction in IVP Bovine Embryos

We conducted a series of experiments aimed at measuring placental morphology and function in IVP versus typical embryo transfer calves. Oocytes were collected at slaughter from beef females for IVM, IVF, and IVC. Embryos were co-cultured to the blastocyst stage with oviductal cells in medium containing serum. In vivo-produced blastocysts were collected from superovulated, inseminated beef females of similar breeding to oocyte donors. In vivo and IVP Day 7 blastocysts were transferred nonsurgically to the uteri of synchronized recipients.

In one experiment, IVP and control embryos were allowed to develop in a physiologically synchronous uterine environment to Day 16, after which they were retrieved from the uterus for physical and molecular analysis. Distinct patterns were observed in Day 7 versus Day 16 IVP embryos; specifically, Day 7 IVP embryos compared with in vivo embryos displayed differences in gene expression that became less distinct by Day 16. Morphologically, Day 16 IVP concepti presented smaller embryonic discs and a trend toward shorter trophoblastic lengths than controls.

In another experiment, pregnancies for IVP and in vivo control embryos were diagnosed on Day 30 of gestation, and recipients were either slaughtered on Days 90 and 180 of gestation or allowed to carry the pregnancies to term. On Days 90 and 180, placental and fetal morphology and placental function (substrate permeability, placental hormones, and proteins) were studied. Sixty minutes before slaughter, a saline solution containing 10% D-xylose, a marker for substrates using the
glucose transporter system, was infused intravenously into each recipient. On Day 90 of gestation, fetal D-glucose concentrations were lower (P < 0.05) in IVP pregnancies, which presented fetuses with greater crown-rump lengths and organ weights (P < 0.05) and a tendency for greater total placentome gross surface area (P = 0.07). Physical traits such as fetal, liver, heart and placentome weights correlated with one another and were greater in IVP pregnancies on Day 180 (P < 0.05), except for a trend toward greater fetal crown-rump length (P = 0.06). Physical traits also correlated with total D-glucose and D-xylose accumulated in the allantoic and amniotic fluid and total fluids. These results suggest differences in substrate transport between IVP and control pregnancies; enhanced substrate transport to IVP fetuses might be expected to support greater fetal growth during late pregnancy.

For the group of recipients allowed to carry pregnancies to term, fetal and placental development during pregnancy and after delivery was examined. Ultrasound examinations were performed weekly with images from embryos/fetuses and placentomes (amniotic vesicle and crown-rump length, foreleg and humeral length, diameter and surface area of eye socket, heart rate, diameter and thickness of placentomes) recorded. After birth, calf birth weights and several physical traits were recorded. Our results revealed significant differences in embryonic, fetal, and placental development between IVP and control pregnancies at different stages of development. Following the same developmental pattern seen on Day 16, IVP embryos and early fetuses were smaller than in vivo controls, based on ultrasonographic examinations (Figure 1). Day 180 IVP pregnancies sustained larger concepti, which coincided with increased substrate transport from the maternal circulation to the fetus and associated fluids.

Birth weights of IVP calves were 33% higher (P < 0.05) than controls, but gestation lengths were similar. Linear measurements (e.g., crown-rump length, heart girth circumference, femoral length, height at shoulders) for each calf increased linearly relative to birth weight, i.e., calf body size was proportional to body mass in both IVP and in vivo-derived calves.

The most dramatic morphological observation related to the range in size of placentomes and term cotyledons in the IVP group. Differences existed in the distributions of placentomes and cotyledonary diameters, thicknesses, and surface areas in the fetal horn between IVP and control.

Figure 1. Sagittal views of in vivo- (left: 45.7 mm) and in vitro-derived fetuses (right: 35.0 mm) on Day 51 of gestation (scale bars = 10 mm).
pregnancies. Placentomes immediately surrounding the fetus, in the mid region of the fetal horn, were longer and thinner from mid to late first trimester of IVP pregnancies. Placentome size distribution, weight, and surface area in the fetal horn differed between groups on Days 90 and 180 of gestation. The presence of enlarged placentomes and cotyledons on the fetal membranes of IVP concepti was associated with a larger gross surface area in the fetal horn on Days 90 and 180 and at term. Fetal membranes from IVP calves contained 21% fewer cotyledons (P >0.05) than calves in the control group but had a larger cotyledonary surface area in the fetal horn, as a result of a different distribution of cotyledonary size (P <0.0001). The largest cotyledons measured 13 and 21 cm in diameter in the fetal horn for control and IVP calves, respectively. Approximately 13% of cotyledons in the fetal horn were larger than 13 cm in diameter in IVP calves. Cotyledonary surface area was greater in IVP than control calves, which likely was linked to the presence of giant cotyledons in IVP concepti (Figure 2).

Figure 2. Fetal membranes of in vivo- (left) and in vitro-derived (right) calves in the umbilical cord area (scale bars = 20 cm; numbers on the cotyledons are the individual diameters in cm) (3).

Concentrations of IGF-1 and IGF-2 and their binding proteins were also measured in maternal and fetal plasma and amniotic and allantoic fluids. Maternal plasma concentrations of IGF-1 in IVP pregnancies were elevated throughout gestation; the highest levels occurred in the first trimester of pregnancy, coinciding with the period of growth retardation in IVP concepti. Components of the IGF system were differentially regulated, according to gestation period (Day 90 or 180), fluid type (maternal or fetal plasma, amniotic or allantoic fluid), and origin of conceptus (IVP versus control embryos). Increases in fetal weight resulting from IVP embryos appeared to be associated with increases in concentrations of IGF-1 and IGF-2 in the maternal circulation and alterations in the IGF binding proteins within amniotic and allantoic fluids that are evident at or before Day 90 of gestation.

Significant embryonic and fetal losses (27/49) occurred between Days 30 and 65 of gestation in the IVP group. The majority of losses took place between Days 30 and 44 (17/27). The in vivo group presented lower losses (11/51), which occurred predominantly dispersed throughout the first trimester, between Days 30 and 90 of gestation. Although we have not systematically analyzed the physiological explanations for this high loss rate in IVP pregnancies, it is intriguingly suggestive to
speculate that faulty placental development may be linked to early losses and growth retardation, as reported by DeSouza et al. (5) and Hill et al. (14) for cloned sheep and cattle.

Placenta: Important or Not in Producing Large Calves?

Our results presented in the previous section strongly suggest that morphological and functional differences between IVP and in vivo-derived pregnancies could affect fetal growth and calf birth weight. How are these results reconciled with other reports of large IVP calves in the absence of placental defects? Certainly in some previous studies in which large calves were reported, including our own (2), placental membranes were neither collected nor examined. Other studies in which placental anomalies were not identified involved examination of placentas during pregnancy but not at term. Moreover, just as all IVP calves are not large, variations in phenotypes might be expected with regard to placental normalcy in IVP concepti. Placental and fetal development in IVP concepti might be viewed as a spectrum instead of in discreet categories. Finally, similarities and differences might be expected in IVP concepti resulting from IVM/IVF/IVC and those from nuclear transfer; it has yet to be determined whether large calves and associated anomalies resulting from IVC and those from nuclear transfer result from the same developmental perturbations or hold different positions along the same spectrum.

REFERENCES


