

Québec/2004 Canada



23^e Congrès mondial de buiatrie • Québec, Canada, 11-16 juillet 2004
23 Congreso Mundial de Buiatria • Québec, Canada, 11-16 de Julio 2004

23rd World Buiatrics Congress • Québec, Canada, July 11-16, 2004
23. Welt-Kongress für Buiatrik • Québec, Canada, 11.-16. Juli 2004

Pregnancy Loss Associated with Embryo Technologies in Cattle

Peter W. Farin^{1*}, Jeremy R. Miles¹ and Charlotte E. Farin²

¹Department of Population Health and Pathobiology, ²Department of Animal Science,
North Carolina State University, Raleigh, NC 27606 USA

Introduction

Although normal calves can result from the transfer of embryos produced in vitro (IVP) or by somatic cell nuclear transfer (cloned), these embryo technologies are more often associated with increased rates of abortion and abnormal development of the fetus and placenta, particularly pregnancies from cloned embryos (1,7,15). Neonatal health problems and calf mortality represent further limitations to the wide-spread use of in vitro embryo production and cloning in cattle. The purpose of this paper is to address pregnancy loss and development of bovine fetuses and placentas derived from IVP and cloned embryos.

Patterns of Pregnancy Loss

It is well established that the incidence of pregnancy loss in the normal cattle population is greater during the embryonic period (conception to the end of organogenesis of the fetus, Day 1 to Day 42) than during either the fetal period (completion of organ differentiation to parturition, Day 42 to Day 280) or the neonatal period (parturition to Day 28 of extra-uterine life; for review see 7). Early embryonic death associated with a failure of maternal recognition of pregnancy before Day 18 of gestation appears to account for approximately 40% of all pregnancy loss in cattle (7). The incidence of abortion decreases to less than 10% after 2 months of gestation in recipients that received in vivo-produced embryos from superovulated cows (14). There is a paucity of data on the mechanisms of pregnancy loss during the fetal period in cattle.

A variety of methods have been used to make IVP (2,10,16) and cloned (3,17) embryos in cattle. Several laboratories have demonstrated that these embryos have distinct differences in morphology, developmental competence and more recently gene expression, compared to embryos produced in vivo (7-9,19). Embryos produced in vitro have markedly higher rates of early embryonic death and abortions than that seen with either artificial insemination or the transfer of in vivo-produced embryos (8,10,15). Pregnancy loss following transfer of IVP embryos occurs most frequently prior to Day 21 of gestation (7), or within about 2 weeks of embryo transfer. Approximately 19% of conceptuses resulting from the transfer of grade 1 blastocysts produced in vitro were found to be degenerated by Day 17 of gestation (7). In contrast, no degenerated conceptuses were recovered from the in vivo controls. This resulted in a greater proportion of pregnant recipients with a viable conceptus after having received in

vivo-produced embryos compared to IVP embryos (65% *versus* 42%). These data are consistent with the hypothesis that IVP embryos may be compromised in their ability to survive and initiate mechanisms essential for maternal recognition of pregnancy.

Based on large field studies, pregnancy rates following transfer of IVP embryos have ranged from about 45% to 65% (2,8,10,15,16). Factors that have been shown to influence the maintenance of pregnancy following transfer of IVP embryos include embryo culture system, embryo quality, embryo evaluator, number of embryos transferred per recipient, synchrony of embryo development with the recipients day of estrous cycle, transfer technician, fresh *versus* frozen embryos and heat stress on the embryo or recipient (2,7-10). Pregnancy failure during mid- to late-gestation occurs more frequently in recipient cattle carrying fetuses derived from IVP embryos than in vivo-produced embryos (7% to 20% or greater; 7). Fetal loss during this period of gestation has been attributed to insufficient placental development and/or function.

Loss of pregnancies resulting from the transfer of cloned embryos was increased compared to pregnancies from IVP embryos; resulting in only about 10% of recipients carrying a cloned fetus to term (3,11,12,17). Early loss of cloned pregnancies was common during the first two to three months of gestation, and again during the late fetal and perinatal periods (11,12). Hill and coworkers (12) found that pregnancy rates at Day 30 of gestation were similar for recipient cows carrying cloned and control embryos (45% *versus* 58%). However, from Days 30 to 90 of gestation 82% of cloned fetuses died, whereas all of the fetuses in the control group survived. These authors attributed poor viability of cloned fetuses during Days 35 to 60 to inadequate development of the chorioallantois (12). Loss of pregnancies with cloned embryos has been linked to severe decreases in placental vascularization, the occurrence of complete avascular chorioallantoic membranes, decreases in placentome number and increases in placentome size (3,17). Other factors that may be responsible in part for early pregnancy loss of cloned fetuses include modifications of genomic imprinting and immunologic rejection of the conceptus. For example, genomic imprinting was recently shown to be disrupted at the XIST locus in placentas of cloned fetuses at Day 40 of gestation (5). In addition, early loss of pregnancies containing cloned fetuses were related to expression of major histocompatibility complex class I antigens by trophoblast cells (13). Expression of these trophoblast antigens was found in all cloned placentas, but not in the placentas of controls. Loss of cloned pregnancies by Day 100 of gestation was found to be associated with elevated concentrations of pregnancy serum protein 60 at Day 50 (11). During late gestation, abnormalities associated with placental function including an excessive volume of allantoic fluid (hydrallantois) and prolonged gestation account for significant loss in pregnancies with cloned fetuses (3,11,17).

Development of Fetuses and Placentas

Developmental abnormalities associated with pregnancies derived from either IVP or cloned embryos include increased calf birth weight, altered organ development, musculoskeletal deformities, altered energy metabolism, increased perinatal mortality, increased placental weight, hydrallantois, and alterations in placentome number and placental membrane structure (1,7,15). Collectively, these characteristics are described as 'large offspring syndrome' and are now recognized to occur with high frequency in pregnancies from cloned bovine embryos and with lower frequency in pregnancies from IVP embryos.

Alterations in the morphology and function of placentas have been reported for pregnancies from IVP and cloned embryos. For example, placentas from IVP embryos had decreased surface area of placentomes resulting in a reduced area for fetal-maternal contact (18). Fetal villous volume density was also reduced in these placentas. In contrast, the placentome blood vessel density-to-surface area ratio was increased during late gestation compared to controls (18). These perturbations in placental development may correspond to a disruption in the normal fetal-maternal fluid, gas and nutrient exchange mechanisms necessary for survival of the fetus. Changes in placental tissue morphology are often accompanied by altered expression of imprinted and non-imprinted genes. There is increasing evidence that disruption of components of the insulin-like growth factor (IGF) system may be responsible for placental overgrowth and altered placental development in IVP and cloned pregnancies (1,6,20). For example, the expression of mRNA for insulin-like growth factor type 2 receptor (IGF-2R) was altered in conceptuses from IVP embryos at Day 17 of gestation compared to controls (6). In pregnancies from cloned embryos, Ravelich et. al. (20) reported that the expression of IGF binding protein (IGFBP)-1 was increased in the allantoic fluid of cloned placentas compared to controls. IGFBP-2 and IGFBP-3 were also altered in the placentomes of these pregnancies.

The bodyweights of bovine fetuses or calves resulting from either IVP or cloned embryos often show a rightward shift in their weight distributions (7,15). These observations imply that animals within the normal weight range may exhibit deviations in development or physiological measurements compared to in vivo controls. For example, Chavatte-Palmer et al. (3) reported that a segment of cloned calves demonstrated obvious overgrowth abnormalities associated with large offspring syndrome. This is consistent with the hypothesis that altered expression of imprinted genes may be driving this syndrome. However, they also observed a number of cloned calves that appeared in the normal range for birth weight but still demonstrated physiological perturbations. These abnormalities included altered body temperature, increased abdominal fat, elevated leptin concentrations, increased plasma IGF-II at birth, lower plasma thyroxine levels, elevated erythrocyte cell volume and an increased neutrophil:lymphocyte ratio at birth. It is unclear if these physiological changes noted in normal-weight cloned calves are associated with alterations in imprinted gene expression. Similarly, changes in myostatin and glyceraldehyde-3-phosphate dehydrogenase gene expression associated with an increased ratio of secondary-to-primary muscle fiber types in IVP-derived fetuses (4) may not be directly associated with aberrant expression of imprinted genes. Therefore, some of the observed abnormalities of fetal and placental development seen with large offspring syndrome are not consistent with the hypothesis that this problem is solely caused by disturbed genomic imprinting. These changes may be physiological consequences that occur secondarily to changes in imprinted gene expression during gestation or they may represent physiological mechanisms that are perturbed as a result of in vitro manipulations apart from changes in imprinting status. The subtle alterations in the phenotypes of cloned animals that do not fall within the classically defined large offspring syndrome may, however, still represent forms of epigenetic dysregulation. Studies are needed to partition and attribute the variation observed in placentas, fetuses and calves derived from IVP and cloned embryos.

Résumé

Des taux d'avortements et des taux de développement anormaux du placenta, du foetus et des veaux seront souvent remarqué suite au transfert d'embryons produits in vitro ou par clonage

issu de cellules somatiques. Ce papier présente une analyse des données récentes sur les pertes de gestations et du développement anormal des foetus et placentas bovins issus de transferts d'embryons produits in vitro ou par clonage.

References

1. Bertolini M, Anderson GB. The placenta as a contributor to production of large calves. *Theriogenology* 2002; 57:181-187.
2. Bousquet D, Twagiramungu H, Morin N, Brisson C, Carboneau G, Durocher J. In vitro embryo production in the cow: an effective alternative to the conventional embryo production approach. *Theriogenology* 1999; 51:59-70.
3. Chavatte-Palmer P, Heyman Y, Richard C, Monget P, LeBourhis D, Kann G, Chilliard Y, Vignon X, Renard JP. Clinical, hormonal, and hematologic characteristics of bovine calves derived from nuclei from somatic cells. *Biol Reprod* 2002; 66:1596-1603.
4. Crosier AE, Farin CE, Rodriguez KF, Blondin P, Alexander JE, Farin PW. Development of skeletal muscle and expression of candidate genes in bovine fetuses from embryos produced in vivo or in vitro. *Biol Reprod* 2002; 67:401-408.
5. Dindot SV, Farin P, Farin C, Alexander J, Crosier E, Walker S, Long C, Piedrahita JA. Analysis of epigenetic modifications and genomic imprinting in nuclear transfer derived *Bos Gaurus* x *B. Taurus* concepti. *Reprod Fertil Dev* 2004; 16:140 abstr.
6. Farin CE, Alexander JE, Rodriguez KF, Farin PW. Expression of insulin-like growth factor (IGF) mRNA in bovine conceptuses from embryos produced in vivo or in vitro. *Reprod Fertil Dev* 2004; 16:237 abstr.
7. Farin PW, Crosier AE, Farin CE. Influence of in vitro systems on embryo survival and fetal development in cattle. *Theriogenology* 2001; 55:151-170.
8. Hansen PJ, Block J. Towards an embryocentric world: the current and potential uses of embryo technologies in dairy production. *Reprod Fertil Dev* 2004; 16:1-14.
9. Hasler JF. The current status of oocyte recovery, in vitro embryo production, and embryo transfer in domestic animals, with an emphasis on the bovine. *J Anim Sci* 1998; 76 (Suppl 3): 52-74.
10. Hasler JF. In vitro culture of bovine embryos in Menezes's B2 medium with or without coculture and serum: the normalcy of pregnancies and calves resulting from transferred embryos. *Anim Reprod Sci* 2000; 60-61:81-91.
11. Heyman Y, Chavatte-Palmer P, LeBourhis D, Camous S, Vignon X, Renard JP. Frequency and occurrence of late-gestation losses from cattle cloned embryos. *Biol Reprod* 2002; 66:6-13.
12. Hill JR, Burghardt RC, Jones K, Long CR, Looney CR, Shin T, Spencer TE, Thompson JA, Winger QA, Westhusin ME. Evidence for placental abnormality as the major cause of mortality in first-trimester somatic cell cloned bovine fetuses. *Biol Reprod* 2000; 63: 1787-1794.
13. Hill JR, Schlafer DH, Fisher PJ, Davies CJ. Abnormal expression of trophoblast major histocompatibility complex class I antigens in cloned bovine pregnancies is associated with a pronounced endometrial lymphocytic response. *Biol Reprod* 2002; 67:55-63.
14. King KK, Seidel Jr GE, Elsdon RP. Bovine embryo transfer pregnancies. I. Abortion rates and characteristics of calves. *J Anim Sci* 1985; 61:747-757.

15. Kruip TAM, den Daas JHG. In vitro produced and cloned embryos: Effects on pregnancy, parturition and offspring. *Theriogenology* 1997; 47:43-52.
16. Lane M, Gardner DK, Hasler MJ, Hasler JF. Use of G1.2/G2.2 media for commercial bovine embryo culture: equivalent development and pregnancy rates compared to co-culture. *Theriogenology* 2003; 60:407-419.
17. Lee RS, Peterson AJ, Donnison MJ, Ravelich S, Ledgard AM, Li N, Oliver JE, Miller AL, Tucker FC, Breier B, Wells DN. Cloned cattle fetuses with the same nuclear genetics are more variable than contemporary half-siblings resulting from artificial insemination and exhibit fetal and placental growth deregulation even in the first trimester. *Biol Reprod.* 2004; 70:1-11.
18. Miles JR, Farin CE, Rodriguez KF, Alexander JE, Farin PW. Vascular morphometry of bovine placentomes in late gestation from embryos produced in vivo or in vitro. *Reprod Fertil Dev* 2004; 16:259 abstr.
19. Niemann H, Wrenzycki C, Lucas-Hahn A, Brambrink T, Kues WA, Carnwath JW. Gene expression patterns in bovine in vitro-produced and nuclear transfer-derived embryos and their implications for early development. *Cloning Stem Cells* 2002; 4:29-38.
20. Ravelich SR, Breier BH, Reddy S, Keelan JA, Wells DN, Peterson AJ, Lee RS. Insulin-like growth factor-I and binding proteins 1, 2, and 3 in bovine nuclear transfer pregnancies. *Biol Reprod* 2004; 70:430-438.