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Transfer of Inner Cell Mass Cells Derived from Bovine Nuclear Transfer Embryos into the Trophoblast of Bovine *In Vitro*–Produced Embryos

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ABSTRACT

Presence of placental tissues from more normal noncloned embryos could reduce the pregnancy failure of somatic cloning in cattle. In this study, inner cell mass (ICM) cells of *in vitro*–produced (IVP) embryos was replaced with those of nuclear transfer (NT) embryos to reconstruct bovine blastocysts with ICM and trophoblast cells from NT and IVP embryos, respectively. A total of 65 of these reconstructed embryos were nonsurgically transferred to 20 recipient beef females. Of those, two females were diagnosed pregnant by ultrasonography on day 30 of gestation. One pregnancy was lost at 60–90 days of gestation, and the other recipient cow remained pregnant at day 240 of gestation; however, this female died on day 252 of gestation. Gross pathology of the internal organs of the recipient female, a large fetus, and a large placental tissue mass suggested the massive size of the fetus and placental tissue were likely involved in terminating the life of the recipient female. Biopsy samples were harvested from the skin of the dead recipient cow, the fetus and from cotyledonary tissue. Microsatellite DNA analysis of these samples revealed that the genotype of the fetus was the same as that of the NT donor cells and different from that of the recipient cow. Correspondingly, neither the fetus nor recipient cow had the same genotype with that of the fetal cotyledonary tissue. These results present the first known documented case of a bovine somatic NT pregnancy with nonclone placental tissues after transfer of a blastocyst reconstructed by a microsurgical method to exchange of ICM cells and trophoblast tissue between NT and IVP blastocysts.

INTRODUCTION

AN UNUSUALLY HIGH proportion of fetal or neonatal losses has been consistently documented in bovine cloning by somatic cell nuclear transfer (NT), which is the major impediment towards widespread application of this methodology. Among the contributing factors, placental deficiency of the NT conceptus has been associated with lowered cloning efficiency (Stice et al., 1996; Hill et al., 2000; De Sousa et al., 2001, Bertolini and Anderson, 2002).

In cattle, both structural and epigenetic anomalies have been detected in later stage somatic NT embryos, such as decreased trophectoderm cell to total embryonic cell ratio and trophectoderm-localized methylation aberrancy (Kang et al., 2002; Koo et al., 2002; Han et al., 2003). Research indi-

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