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Short communication

Aberrant expression of retinol-binding protein, osteopontin and fibroblast growth factor 7 in the porcine uterine endometrium of pregnant recipients carrying embryos produced by somatic cell nuclear transfer

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Abstract

The technique of somatic cell nuclear transfer (NT) is a useful tool to produce cloned animals for various purposes, but the efficiency to generate cloned animals using this technique is still very low. To improve the low efficiency in production of cloned pigs it is critical to understand the reprogramming process during development of cloned embryos, but it is also essential to understand the uterine function interacting with the transferred cloned embryos during implantation and placentation. Thus, to understand the uterine responsiveness to NT cloned embryos during pregnancy, we investigated expression of retinol-binding protein (RBP), osteopontin (OPN) and fibroblast growth factor 7 (FGF7), which play important roles in implantation and/or maintenance of pregnancy as a transport protein, an extracellular matrix protein and a growth factor, respectively, in the uterine endometrium in pigs. The uterine tissue samples were obtained by C-section from pigs with NT cloned normal (NT-normal) embryos and NT cloned abnormal (NT-abnormal) embryos and pigs with non-NT (Non-NT) embryos at term. Immunoblot analysis showed that expression of RBP and FGF7 decreased in the uterine endometrium of recipient gilts carrying NT embryos than in the endometrium of gilts carrying Non-NT embryos. Levels of OPN protein of 70 and 45 kDa were not different in between the uterine endometrium of gilts carrying Non-NT and NT-normal embryos, but in the uterine endometrium of gilts carrying NT-abnormal embryos 70 and 45 kDa OPN proteins increased compared to

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those in the endometrium of gilts carrying Non-NT embryos. Immunohistochemistry results showed that RBP expression was lower in the endometrial glandular epithelial cells, while OPN expression was higher in the endometrial luminal epithelial cells of the uterus of gilts carrying NT embryos than in the uterus of gilts carrying Non-NT embryos. Results of this study showed that maternal uterine genes were aberrantly expressed in the uterine endometrium of gilts carrying NT cloned embryos in varying degrees depending on the normality of the developing embryos. These results indicate that abnormal maternal–fetal interactions of the uterus carrying the developing NT cloned embryos may cause problems in development of cloned embryos.

Keywords: Pig; Uterus; Somatic cell nuclear transfer; Retinol-binding protein; Osteopontin; Fibroblast growth factor 7

1. Introduction

Cloning by somatic cell nuclear transfer (NT) in livestock animal species including pigs is a valuable tool for basic research and agricultural and biomedical applications. Since the birth of Dolly, the first cloned sheep produced by using the technique of NT, production of many animal species by NT cloning has been successful (Campbell et al., 2005). However, the efficiency of animal cloning by NT is very low due to high embryonic mortality during pregnancy (Yanagimachi, 2002; Jouneau et al., 2006) and many other problems such as large offspring syndrome (Young et al., 1998), increased placental weight (Wakayama et al., 1998; Ono et al., 2001), respiratory problems (Loi et al., 2006) and aberrant phenotypes (Park et al., 2002). Much effort has been made to understand the cause of high embryonic mortality and the defects of cloned embryos. Currently, it is believed that inadequate nuclear reprogramming of cloned embryos and their subsequent inadequate development result in high rates of embryonic death in NT embryos. In addition, it has been shown that defects of extra-embryonic tissue formation occur during development of NT embryo (Kim et al., 2005; Chae et al., 2006; Jouneau et al., 2006) and that changes in DNA methylation patterns are higher in the trophectoderm than in the inner cell mass of the NT embryos (Kang et al., 2002). These results indicate that placental development in NT embryos is abnormal, causing problems in proper communications with the maternal uterus.

To understand problems in cloning by using NT, it is required to determine how the uterus interacts with the NT cloned embryos during pregnancy, because the uterus provides an environment for implantation, placentation and fetal development during pregnancy. In pigs, which form a non-invasive, diffuse type of true epitheliochorial placentation, the uterus plays a critical role in the support of fetal and placental development by secretion of many regulatory products and exhibits considerable control over the ability of a conceptus to develop (Roberts and Bazer, 1988). Uterine secretions in pigs include transport proteins (Roberts and Bazer, 1988), extracellular matrix proteins (Burghardt et al., 2002) and growth factors (Jaeger et al., 2001). Thus, to understand the uterine responsiveness to the NT cloned embryos during pregnancy, this study determined expression of uterine secretory proteins, retinol-binding protein (RBP), osteopontin (OPN) and fibroblast growth factor 7 (FGF7) as markers of uterine activity and uterine–placental interactions. Among many uterine secretory molecules, these are known to be produced by the endometrium from early to late gestation and relatively well studied in pigs. This study provides insights into the uterine function in development of the NT cloned embryos during pregnancy.