Development of porcine embryos reconstituted with somatic cells and enucleated metaphase I and II oocytes matured in a protein-free medium

ABSTRACT

Porcine M I oocytes have a potential to develop into blastocysts after nuclear transfer of somatic cells.

INTRODUCTION

Background Many cloned animals have been created using M II oocytes as the recipient cytoplasm (sheep, cattle, mouse, goat and pig). Maturation/meiosis/mitosis-promoting factor (MPF) activity of recipient M II oocytes appears to be important for the reprogramming of nuclei of reconstructed embryos. Two distinct and different protocols emerge for embryo reconstruction by nuclear transfer when using M II oocytes as recipients. The first is the transfer of nuclei in G1, S or G2 phase into the preactivated recipients after reducing MPF activity and the second is the transfer of nuclei in GO or G1 phase directly into M II oocytes having high MPF activity. Recently, it has been shown that when mouse embryonic stem cells and bovine somatic cells in the M phase were transferred into non-treated recipients, the chromosome constitution of reconstructed embryos was normal in that the second polar body was excluded after parthenogenetic activation and the embryos developed to offspring. The first protocol is effective for production of blastomeres derived cloned embryos because cell cycle synchronization of embryonic nuclei is difficult and the majority of blastomeres are in the S phase at any given time. In the preactivated oocyte, the nuclear membrane of the donor cells remains intact due to the low activity of MPF and DNA synthesis occurs according to the original cell cycle stage at the time of nuclear transfer and nuclear reprogramming occurs during the expansion of the donor nucleus. Additionally, nuclei of cells of established embryonic cell lines are reprogrammed in preactivated recipient oocytes, porcine embryos reconstituted with blastocyst-derived cells and preactivated recipients have the ability to develop to blastocysts. However, there is only one report in which cloned animals have been produced from embryos reconstructed by transferring differentiated cells into preactivated recipient oocytes and the ability of preactivated recipient oocytes to reprogram differentiated cells is now in debate. When bovine somatic cells were transferred into preactivated recipient oocytes, the resulting reconstructed embryo development was limited, since all embryos arrested at the 8-cell stage regardless of the cell cycle of the donor cells. Because embryonic genome activation of bovine embryos occurs between the 8- to 16-cell stages, preactivated recipients may not reprogram somatic nuclei. In contrast, when nuclei in G0 or G1 phase are transferred into non-treated recipients, reconstructed embryos can develop to offspring in many species. In the M II oocyte, the membrane of the donor nucleus is broken down and the chromosomes are prematurely condensed due to the high activity of MPF. After parthenogenetic activation, the membrane reforms and DNA synthesis begins. MPF activity during oocyte maturation is maximal at both M I and M II. Because many studies suggest that exposure to high activity of MPF is effective for reprogramming a donor nucleus from a differentiated cell, M I oocytes may reprogram somatic cell nuclei as well. In the amphibian, the greatest yield and most advanced tadpoles came from differentiated somatic cell nuclei injected into M I oocytes compared to M II oocytes, although adults could not be generated from these reconstructed embryos. However, there are no reports that experimentally tested this hypothesis in mammals. In the present study, we examined the ability of

porcine M I oocytes to reprogram somatic cell nuclei.

CONCLUSION

Conclusions We have shown that porcine M I oocytes have a potential to develop into blastocysts after nuclear transfer of somatic cells. However, the process of nuclear reprogramming may be different between M I and M II oocytes because developmental abilities of reconstructed embryos vary between the maturation stages of recipient oocytes. Comparison of reprogramming events between M I and M II oocytes would bring about important information to understand the mechanisms of nuclear reprogramming in reconstructed embryos. The use of defined IVM medium will be useful for these studies. Taken together, the use of a defined medium and optimal reprogramming conditions such as the type of donor nuclei and recipient oocyte will lead to improvements in porcine cloning outcomes.