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Nuclear transfer in cats and its application

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Abstract

Nuclear transfer (NT) technology is typically used for generating identical individuals, but it is also a powerful resource for understanding the cellular and molecular aspects of nuclear reprogramming. Most recently, the procedure has been used in humans for producing patient-specific embryonic stem cells. The successful application of NT in cats was demonstrated by the birth of domestic and non-domestic cloned kittens at a similar level of efficiency to that reported for other mammalian species. In cats, it has been demonstrated that either in vivo or in vitro matured oocytes can be used as donor cytoplasts. The length of in vitro oocyte maturation affects in vitro development of reconstructed embryos, and oocytes matured in vitro for shorter periods of time are the preferred source of donor cytoplasts. For NT, cat somatic cells can be synchronized into the G0/G1 phase of the cell cycle by using different methods of cell synchronization without affecting the frequency of in vitro development of cloned embryos. Also, embryo development to the blastocyst stage in vitro is not influenced by cell type, but the effect of cell type on the percentage of normal offspring produced requires evaluation. Inter-species NT has potential application for preserving endangered felids, as live offspring of male and female African wildcats (AWC, *Felis silvestris lybica*) have been born and pregnancies have been produced after transferring black-footed cat (*Felis nigripes*) cloned embryos into domestic cat (*Felis silvestris catus*) recipients. Also, successful in vitro embryo development to the blastocyst stage has been achieved after inter-generic NT of somatic cells of non-domestic felids into domestic cat oocytes, but no viable progeny have been obtained. Thus, while cat cytoplasm induces early nuclear remodeling of cell nuclei from a different genus, the high incidence of early embryo developmental arrest may be caused by abnormal nuclear reprogramming. Fetal resorption and abortions were frequently observed at various stages of pregnancy after transfer of AWC cloned embryos into domestic cat recipients. Abnormalities, such as abdominal organ exteriorization and respiratory failure and septicemia were the main causes of death in neonatal cloned kittens. Nonetheless, several live domestic and AWC cloned kittens have been born that are seemingly normal and healthy. It is important to continue evaluating these animals throughout their lives and to examine their capability for natural reproduction.

Keywords: Nuclear transfer; Inter-species embryo transfer; Cats; Oocyte maturation

1. Introduction

Since the birth of the first cloned animal using adult somatic cells as the donor nucleus [1], nuclear transfer has progressed from being a novel technology to (1) a widely used technique for generating identical individuals, (2) a model for understanding the cellular and molecular aspects of nuclear reprogramming, and most recently, (3) a means to produce embryonic stem cells for their potential use in cell-based therapies. Normal pre- and postnatal development to maturity of cloned animals is the definitive measure of successful nuclear transfer and the ultimate confirmation of the value of this technology. The application of nuclear transfer in domestic cats (*Felis silvestris catus*) was demonstrated

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by the birth of a domestic cloned kitten after transfer of embryos reconstructed by inserting cumulus cells into enucleated oocytes [2]. Since then, an increased interest in the use of this technology for commercial purposes has been observed, with a recent focus on cloning domestic cats as pets. Although there are some ethical and social implications, the reality is that the technique is available and births of cloned cats for commercial purposes have been reported in the popular press.

Nuclear transfer in cats not only provides the opportunity to genetically duplicate a deceased pet, but also presents the prospect of preserving endangered felids. Clearly, natural breeding should be the preferred method for propagation and genetic management within a population. On the other hand, when populations or sub-populations are at risk of extinction, nuclear transfer may be a valuable approach for species restoration. Most wild felids are threatened, and the feasibility of using this technology to preserve endangered felids was demonstrated with the birth of African wildcat (AWC, *Felis silvestris lybica*) cloned kittens [3]. Even though live cloned animals have been produced in several mammalian species, there are numerous technical and biological factors affecting the success of nuclear transfer, some of which are associated with abnormal nuclear and epigenetic reprogramming that can lead, subsequently, to implantation failure, fetal abnormalities and poor postnatal health [4]. Although the implantation rates of cloned embryos from various mammalian species are low [5], the overall level of success obtained after transferring domestic or AWC cloned embryos into domestic cat recipients is similar to that reported for other mammalian species.

In this review, we summarize recent progress and factors affecting the success of nuclear transfer in cats, and we also discuss some problems associated with in vitro embryo production and abnormalities observed in the resultant fetuses and kittens.

2. Source of cytoplasts and length of in vitro maturation

2.1. In vitro versus in vivo maturation

The quality and source of oocytes is a key factor in determining the proportion of oocytes developing to the blastocyst stage and the efficiency at which live offspring are produced [6–8]. In the cat, both in vivo- and in vitro-matured oocytes have been used as recipient cytoplasts for production of cloned embryos. We compared in vitro development of AWC cloned embryos reconstructed with both sources of cytoplasts. When in vivo matured oocytes were used as recipient cytoplasts, a higher percentage of fusion was observed (97%) as compared to in vitro matured oocytes (90%). In contrast, cleavage frequency after reconstruction using in vitro matured oocytes (85%) was higher than that after reconstruction with in vivo matured oocytes (79%) [9]. Nonetheless, no significant differences were observed in frequency of blastocyst development between embryos reconstructed with in vivo or in vitro matured cytoplasts (27% versus 23%, respectively) [10]. Both types of cytoplasts have been used for cloning domestic and non-domestic cats. In fact, the first domestic cloned kitten was produced after transfer of embryos derived by reconstitution of cumulus cells with enucleated oocytes matured in vitro for 26–30 h [2]; whereas, AWC cloned kittens were produced after transfer of cloned embryos derived from cytoplasts matured in vivo [3]. These data clearly indicate that both types of cytoplasts can be used for cloning cats; however, a comparison of which type of cytoplast supports higher rates of development to term remains to be done.

2.2. Length of in vitro maturation

While it may be logically assumed that in vivo matured oocytes are a better source of cytoplasts for production of cloned cat embryos, their production and collection is much more difficult and expensive. Therefore, the use of in vitro matured oocytes as donor cytoplasts is a reliable alternative, since cat ovaries are easily accessible from local veterinary clinics and are available throughout the year. A high percentage of domestic cat oocytes undergo maturation in vitro (40–70%) and complete maturation to metaphase II during the first 24 h of culture [11]. Additional oocytes also mature between 24 and 48 h [12,13], although the highest proportion of mature oocytes is reached between 42 and 45 h of culture [14]. The length of in vitro oocyte maturation affects in vitro development of reconstructed cat embryos [15]. In the latter study, they observed that a prolonged maturation period of 43 h affects in vitro development, as indicated by lower fusion rates (58%), lower development of embryos to the morula stage (28%) and no blastocyst development. In contrast, when oocyte cytoplasts matured in vitro for 24 or 35 h were used, fusion rates (71% and 72%, respectively) and the percentage of cloned embryos developing to morula (56% and 34%, respectively) and blastocyst stages (8% and 9%, respectively) were significantly higher than that of embryos reconstructed