REVIEW ARTICLE

# Progress in interspecies cloning of mammals*

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**Abstract** Interspecies mammalian cloning can be achieved by application of two key techniques, i.e. the technique of interspecies nuclear transfer and the technique of interspecies pregnancy. The general principles, problems and possible solutions, as well as the recent advances of interspecies mammalian cloning have been summarized in this review.

**Keywords:** interspecies cloning, interspecies nuclear transfer, interspecies pregnancy, mammals.

Intraspecies animal cloning is the technique for the production of clonally derived mammals. The procedure involves the transfer of a nucleus from a donor cell into an enucleated oocyte of the same species. Live offsprings have been born after somatic nuclear transfer in sheep, bovine, mouse, goat, pig, rabbit and cat. As opposed to intraspecies animal cloning, interspecies animal cloning (IAC), the transfer of a cell nucleus of one species into an enucleated oocyte of another species, is the technique for cloning those animal species whose oocytes are difficult to obtain. IAC usually includes the technique of interspecies nuclear transfer and the technique of interspecies pregnancy. As IAC breaks through the restriction of "reproductive isolation" among species and is involved in the interaction of different species in molecular, cellular and physiological level, it is an invaluable tool for studying the nucleus-cytoplasmic interaction, gene expression and regulation, differentiation and evolution. The technique of IAC also provides possible applications in animal breeding and conservation; meanwhile, it could become a hopeful approach to reviving those extinct species.

## 1 Principles and recent advances of IAC

IAC differs from intraspecies animal cloning in two aspects. Firstly, it involves a technique of transferring a donor nucleus of one species into an enucleated oocyte of another species to form a nucleocytoplasmic hybrids; secondly, the interspecies pregnancy is essential for the success of IAC. The nucleocytoplasmic hybrid embryos constructed *in vitro* need to be transferred back into the uterus of a surrogate animal to complete its full term of development. As the nucleus and cytoplasm of the hybrid embryos are from different species, the hybrid embryos are heterogeneous in some degree to the surrogate animal regardless of the surrogate species.

### 1.1 Interspecies nuclear transfer in mammals

In 1938, Spemann proposed a "thought experiment". He wondered what would happen if a nucleus from a differentiated cell, even an adult cell, were transferred into an egg whose nucleus had been removed. Fourteen years past before his *gedanken* experiment could be carried out in Amphibia. Brun in 1973 first reported interspecies nuclear transfer in mammals. He transferred several mammalian somatic cell nuclei into the *Xenopus* oocytes[1]. In 1977, De Roeper[2] found chromatin dispersal and DNA synthesis after injecting the nuclei of Hela cells into the *Xenopus* eggs. The result suggested that factors to reactivate mammalian nuclei exist in *Xenopus* oocyte cytoplasm. Professor Tong Dizhou, a Chinese scientist, is the pioneer of nuclear transfer in fish. He is also the first scientist who conducted interspecies nuclear transfer in fish and obtained interspecies cloned fish. He successfully conducted the nuclear transfer in

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two subfamilies of goldfish (*Carassius euratus* and *Rhodeus sinensis*) with his self-made micromanipulator[3]. Thereafter, Professor Tong and his successors obtained higher blastocyst rates for interspecies nuclear transfer embryos between species of families and orders, such as transferring the nuclei of goldfish into the enucleated oocytes of *Paramisgurnus dabryanus*[4]. These results demonstrated that cytoplasmic factors for dedifferentiating and reprogramming of differentiated cell nuclei exist in the oocytes of some low class animals, and these factors may not be species-specific.

The success of mammalian somatic cell cloning shows that factors in mammalian oocytes can make somatic cell nuclei dedifferentiated and reprogrammed. Are these factors not species-specific as those in Amphibia? McGrath and Solter[5] exchanged pronuclei between rodents and found that the reconstructed embryos could divide *in vitro*. In 1992, Wolfe et al.[6] transferred the bovine (*Bos Taurus*) nuclei from blastomere of 16-32 stage embryos into enucleated oocytes of buffalo (*Bubalus bubalis*), sheep (*Ovis aries*) and hamster (*Mesocricetus auratus*); the reconstructed embryos of bovine-buffalo and bovine-sheep could develop to the blastocyst stage after *in vitro* culture; however, the reconstructed embryos of bovine-hamster did not divide in his experiment. In 1993, Mei et al.[7] transferred the mouse (*Mus musculus*) nuclei of blastomere from the 8-cell stage embryos into enucleated rabbit (*Oryctolagus cuniculus*) oocytes. Nuclear swollen and premature chromosome condensation (PCC) were observed in the mouse-rabbit cloned embryos, 5.4% of the reconstructed embryos developed into the blastocyst stage, and karyotype analysis of the blastocysts confirmed that the chromosomes were from the mouse, not from the rabbit. These early studies showed that some mammalian oocytes could accept and dedifferentiate the nuclei from other species.

In 1999, Professor Chen et al.[8] reported that they obtained panda (*Ailuropoda Melanoleuca*) embryos, a highly endangered animal, by using the technique of interspecies nuclear transfer. They transferred the panda somatic cell nuclei from uterus epithelia, skeleton muscle and mammary gland epithelia into enucleated rabbit oocytes, resulting in 9.9%, 6.8% and 11.7% of blastocyst rates from these reconstructed embryos, respectively. Analyses of karyotype, mtDNA and nDNA confirmed that these reconstructed embryos were embryos of giant panda. At the same time, Dominko et al.[9] found that bovine oocyte cytoplasm supported development of the embryos produced by nuclear transfer of somatic cell nuclei from various mammalian species. They used enucleated bovine oocytes as nucleus recipients, transferring somatic cell nuclei from sheep, pig (*Sus scrofa*), monkey (*Macaca fascicularis*) and rat (*Rattus rattus*) into the enucleated bovine oocytes. Those reconstructed embryos of sheep-bovine, pig-bovine and monkey-bovine could develop to the blastocyst stage after *in vitro* culture. Although no pregnancies had been carried to term after transfer of these interspecies cloned embryos into surrogate animals, two surrogate sheep receiving sheep-bovine embryos showed signs of early pregnancies; however, no heartbeats were detected by transvaginal ultrasonography after 32 days of embryo transfer; the surrogates hysterectomized displayed sacks of fluid and distinctly developed caruncles within uterine horns; no fetal membranes or fetal remnants were found. Another group of scientists in USA established pregnancy after the transfer of nuclear transfer embryos produced from the fusion of argali (*Ovis ammon*) nuclei into domestic sheep (*Ovis aries*) enucleated oocytes. By transferring the argali somatic cell nuclei into the enucleated sheep oocytes White et al.[10] obtained 1.56% of blastocyst rate for these reconstructed embryos. 28 interspecies cloned blastocysts were transferred into 6 surrogate sheep, and one surrogate was found pregnant with ultrasonography 49 days after embryo transfer. Unfortunately, the pregnancy was terminated 10 days after the ultrasonograph detection. Although no live offspring was obtained, these studies have demonstrated that some mammalian oocytes (i.e. bovine, sheep and rabbit) are able to accept, dedifferentiate and reprogram the nuclei from other species.

In January of 2001, Advanced Cell Technology (ACT) announced[11] that an ordinary cow named Bessie gave birth to a remarkable baby bull, Noah. The 80-pound newborn gaur, an endangered type of wild ox from India, Indochina and Southeast Asia, was the first cloned mammals obtained by using the technique of IAC. The gaur and the cow are classified as two different species, for the karotype of gaur is 58 ($2n=58$) and the cow (*Bos Taurus*) is 54 ($2n=54$). Noah was produced by introducing nucleus from the skin fibroblast of an adult male gaur into enucleated oocyte of the cow, and transferred back into the uterus of a surrogate cow after the reconstructed em-

bryo developed to the blastocyst stage *in vitro*. Unfortunately, Noah succumbed to common dysentery within 48 hours after his birth, but his short life indicated that the somatic nucleus of the gaur could be dedifferentiated and reprogrammed in the enucleated oocyte of the cow and support the full term development of the interspecies cloned embryo. Shortly after the birth of Noah, another endangered animal, mouflon (*Ovis orientalis musimon*) was also cloned using the technique of IAC. Loi et al.[12] used post-mortem somatic cells from a mouflon as donor cells and enucleated sheep oocytes as recipients to produce cross-species nuclear transfer embryos, a total of 23 reconstructed embryos were produced and 7 of them developed to the blastocyst stage. Transferring these 7 blastocysts into 4 surrogate sheep resulted in 2 pregnancies, one surrogate aborted 40 ~ 50 days after embryo transfer, the other surrogate carried pregnancy to term. The success of interspecies cloning of gaur and mouflon demonstrated that the technique of IAC is practical, and it is another milestone in animal cloning after Dolly the lamb.

Many other results have been obtained using the bovine oocytes as recipients since Dominko et al. demonstrated that enucleated bovine oocytes could support the development of interspecies cloned embryos from various mammalian species. Lanza et al.[13] cloned gaur embryos by electrofusion of gaur fibroblasts with enucleated oocytes from a domestic cow. Twelve percent of the reconstructed embryos developed to the blastocyst stage, and 18% of these embryos developed to the fetal stage when transferred to surrogate mothers. Three of the five pregnant surrogates were removed at days 46 to 54 of gestation for other studies, and one surrogate aborted on day 201 of gestation, the abortive fetus weighed 10.7 kg with the same karyotype and phenotype as the gaur, the other surrogate developed normally to term. Kitiyanant et al.[14] transferred fetal buffalo fibroblasts into enucleated bovine or buffalo oocytes, and no difference of the blastocyst rates for buffalo-buffalo and buffalo-bovine embryos was observed. In 2001, Meirelles et al.[15] electrofused the blastomere of early *Bos indicus* embryos with enucleated bovine oocytes and obtained *Bos indicus* interspecies cloned embryos, five bovine surrogates received blastocysts of the cloned embryos and two of them became pregnant, finally, one live cloned offspring of *Bos indicus* was born. In the same year, Hwang et al.[16] in South Korea reported that tiger-bovine embryos were produced by transferring fibroblasts from ear skin of a tiger (*panthra tigiris altaica*) into enucleated bovine oocytes, one tiger and 3 lion surrogate mothers received the tiger-bovine embryos, however, no pregnancy was reported.

In our laboratory, we have produced various interspecies cloned embryos using enucleated rabbit oocytes as nucleus recipients. We cloned panda embryos by transferring panda fibroblasts into enucleated rabbit oocytes, these panda-rabbit cloned embryos at 2 ~ 4-cell stage were transferred into the oviduct of 21 cat recipients, 19 recipients returned estrus and the other two recipients died of pneumonia. One of them was found pregnant with 6 early fetuses after autopsy. Microsatellite DNA analysis of these early fetuses confirmed that two of them were from giant panda-rabbit cloned embryos[17]. Cat-rabbit cloned embryos were also produced by transferring cat fibroblasts into enucleated rabbit oocytes, 14.5% of the reconstructed embryos developed to the blastocyst stage after *in vitro* culture. Comparing the developmental capacity and timing of embryogenesis of the cat-rabbit cloned embryos with that of cat-cat cloned embryos, no significant difference of developmental capacity was observed between cat-rabbit and cat-cat cloned embryos. The timing of the first three cleavages for the cat-rabbit embryos was similar to that of the rabbit-rabbit cloned embryos, but significantly faster than that of the cat-cat embryos, while the time to form blastocysts was similar to that of cat-cat embryos, but significantly slower than that of the rabbit-rabbit embryos. The result indicated that the timing of embryogenesis for interspecies cloned embryos is recipient oocyte specific in early cleavage divisions, and is nucleus specific after the blastocyst stage (our unpublished data). The monkey cloned embryos also obtained in our laboratory by transferring somatic monkey cell into the enucleated rabbit oocytes, these monkey-rabbit cloned embryos could develop into the blastocyst stage after *in vitro* culture, analysis of mtDNA found that the mtDNA from both monkey and rabbit coexist before the blastocyst stage[18].

### 1.2 Interspecies pregnancy in mammals

Pregnancy between different species of mammals, namely, interspecies pregnancy, is rarely successful in nature, and it is prevented by the mechanisms of reproductive isolation at different level, such as genetic, cellular and physiological isolations. However, the reproductive isolation in mammals is not

stringent, in some of the cases, interspecies pregnancies of hybrids from different species do occur in nature. Interspecies pregnancies can be classified as three categories: (1) the pregnancies of hybrids of different species; (2) the pregnancies of chimeras; and (3) the pregnancies of embryos from different species via embryo transfer. Three different models of interspecies pregnancies have been established, the murine model using *Mus musculus* and *M. caroli*; the equine model involving primarily the domestic horse and donkey, and the bovid model of domestic sheep and goat[19]. Studying these models revealed that two main reasons attributed to the failure of interspecies pregnancies: (1) the failure of tissue interaction between the trophectoderm of embryos and the uterus of the surrogate mother[20]; (2) the immunological rejection of the surrogate mother to the fetuses after embryo implantation, this rejection prevents the formation of placenta[19, 21].

Embryo attachment and implantation to the uterus, occurring after the blastocyst stage, symbolize the beginning of pregnancy. The processes of attachment and implantation between mother and embryos are complex and numerous among species. In most of the mammals, embryos are able to attach and implant to the uterus after the blastocyst stage, at the same time, the uterine wall of the mother develops to a certain state which allows the embryos to attach and implant, the period of this state, specifically, "implantation window", is transient. Only the completion of embryo implantation in the period of window can pregnancy be normally established. In the delayed implantation mammals, the time of embryo implantation is determined by the state of the mother. In most of these mammals, embryos arrest at the blastocyst stage until the "implantation window" occurs and the process of implantation is triggered. Interestingly, these arrested embryos do not degrade and apoptosis for days or months according to different species *in vivo*[22]. Establishing a biological structure such as placenta for interactions between mother and fetus is crucial for the success of mammalian pregnancy. Trophoblast cells play pivotal role in the establishment of interactions between mother and fetus[23, 24]. The placenta, mainly derived from trophoblast cells, has been shown to secrete hormones and growth factors, and is considered to be an immunological barrier between the mother and her immunogenic conceptus[25, 26].

That trophoblast alone can control the success or failure of pregnancy has been demonstrated by successful gestation of chimaeras, composed of xenogeneic embryonic cells protected by trophoblast, species-homologous to the embryo transfer recipient[23, 27, 28]. In the murine model of interspecies pregnancy, involving *Mus caroli* and *Mus musculus*, fully xenogeneic *Mus caroli* were viable in *Mus musculus* following transfer of chimeric blastocysts composed of a *Mus caroli* inner cell mass within a *Mus musculus* trophoblast vesicle. Non-manipulated *Mus caroli* embryos gestating in the *Mus musculus* uterus failed to develop to term and most of the embryos died and resorbed during the second half of pregnancy[23, 27].

The success or failure of establishing interspecies pregnancy involves various factors, such as the genetic relationship, gestation period, body temperature and physiological structure of the uterus between the species of the embryos and the surrogate mother. Establishment of interspecies pregnancy between two genetic closer species should increase the possibility of success; this speculation has been supported by the facts of the successful cloning of gaur and mouflon[11, 12] and the successful gestation of Indian desert cat (*Felis sylvestris ornate*) in uterus of the domestic cat[29]. Although it is unlikely to establish interspecies pregnancy between any two species of mammals, it is possible to achieve interspecies pregnancy between two carefully selected species with considering the differences of the genetic relationship, reproductive physiology and anatomic structure after the proper remolding the embryos.

## 2 Problems and possible solutions in IAC

The technique of somatic animal cloning has solved the problem of nucleus dedifferentiation and reprogramming. At the basis of somatic nuclear transfer, the technique of IAC needs further to solve the problems of nucleo-cytoplasmic compatibility and the problems of interspecies pregnancy between species.

### 2.1 Problems of nucleo-cytoplasmic compatibility in IAC

Nucleo-cytoplasmic compatibility includes several aspects, i.e. whether the nucleus and cytoplasm support each other structurally and functionally, whether the signal passage can be correctly established between nucleus and cytoplasm, ect. The problems of nucleo-cytoplasmic compatibility in IAC mainly in-

volve whether the cytoplasmic factors in the oocytes of one species can support the dedifferentiation and reprogramming of the nucleus of the other species and whether the nucleus and mitochondria can support each other structurally and functionally.

2.1.1 Nucleo-cytoplasmic compatibility and selection of recipient oocytes Early embryo development is controlled by maternally inherited RNA and proteins and little or no transcription is detectable from zygotic nucleus. At a particular stage of development, which is species-dependent, a switch to zygotic control occurs. This transition, namely, maternal to embryonic (or zygotic) transition (MET), is characterized by a large increase in detectable transcription. The zygotic nucleus must then control development in a spatial and temporal manner and result in formation of specific differentiated cell types[30]. For successful development of nuclear transfer embryos, the transferred nucleus must therefore first abolish transcription and then reestablish the temporal, spatial, and quantitative patterns of gene expression associated with normal development[30, 31]. This process of reestablishment in nuclear transfer embryos is the like of MET in normal embryos[32]. The correct MET of the interspecies nuclear transfer embryos is crucial for the embryo development. The development can be continued only when the MET of nuclear transfer embryos occurs before maternal RNA and proteins are used up, or the embryo development arrest. The abilities to accept nuclei from other species for oocytes are different among mammalian species. Interspecies nuclear transfer between mouse and rat found that embryos produced by transferring rat nuclei into enucleated mouse oocytes were arrested at the 1-cell or 2-cell stage, and embryos produced by transferring mouse nuclei into enucleated rat oocytes could only develop to the 5 ~ 8 cell stage[33]; however, embryos produced by transferring nuclei from various mammalian species into enucleated oocytes of bovine, sheep and rabbit can develop readily to the blastocyst stage[8 ~ 10, 12, 13, 17]. The MET of normal bovine embryos occurs at the 8-cell stage[34], that of sheep and rabbit embryos occurs at the 8 ~ 16-cell stage[35, 36], whereas the MET of mouse embryos occurs at 1 ~ 2-cell stage[37]. From facts of these, we may propose that the ability to accept nuclei from other species for mammalian oocytes is related to the time of MET occurring in the species.

Nucleo-cytoplasmic compatibility involves the interactions of nucleus and various cytoplasmic factors. It remains largely unknown to these cytoplasmic factors for their composition, characters and functions. To clarify these factors and to specify their roles in the nucleus dedifferentiation and reprogramming is helpful to understand the mechanisms of animal cloning.

2.1.2 The fate of mitochondria in IAC The fate of mitochondria is one of the most concerned issues in IAC. Mitochondria, the "power plants" and the energy supplier for mammalian cell activities, are organelles that occupy a substantial portion of cell cytoplasm. Mammalian mitochondrion consists of a double-stranded circular DNA of about 16.5 kb, the genetics of which differs from that of nuclear genome. The mitochondrial DNA (mtDNA) carries genes for 13 proteins, 22 tRNAs and two rRNAs which are essential components of oxidative phosphorylation and electron transfer. Up to 95% of proteins involved in biogenesis and functions of mitochondria are encoded by the nucleus[38, 39]. The number of mitochondria in a typical somatic cell is about $2\times10^3$, while the number of mitochondria in a single oocyte is about $2\times10^5$[40]. In normal fertilized embryos, the mitochondria from the oocytes are multiplied, and the mitochondria from the sperm are eliminated by an unknown mechanism during the embryo development. In the process of nuclear transfer, mitochondria of the donor cell, together with the nucleus, are transferred to the recipient oocyte. Thus, the cloned embryo should harbor mitochondria from both the donor cell and recipient oocyte. What are the fates of mitochondria from the donors and recipients in IAC? Three different patterns of mitochondria transformation in IAC have been observed: (1) mitochondria from the donor cell are gradually eliminated, while the mitochondria from the recipient oocyte multiply to dominate in the embryo during development; (2) mitochondria from the recipient oocyte are eliminated, while the mitochondria from the donor cell multiply to dominate in the embryo, and finally mitochondria from the donor cell substitute for those from the recipient oocyte completely; (3) mitochondria from both the donor cell and the recipient oocyte coexist all the time during embryo development.

If mitochondria multiply without any selective pressure, mitochondria from both the donor cell and the recipient oocyte will increase geometrically after the blastocyst stage. However, the number of mitochondria from the recipient oocyte will dominate, and finally substitute for that from the donor cell after

many rounds of multiplication, since the number of mitochondria from the recipient oocyte is more than 10 times of that from the donor cell in the reconstructed embryo. Lanza et al.[13] reported that the mitochondria in 11 tissues from 3 cloned gaur fetuses produced by transferring gaur somatic nuclei into the enucleated oocyte of the domestic cow were exclusively from the oocyte of the domestic cow, and mitochondria DNA of the donor gaur was undetectable in the cloned fetuses. Loi et al.[12] cloned mouflon with the oocytes of domestic sheep; mitochondrial DNA of the recipient oocyte could be detected in the cloned mouflon, while mitochondrial DNA of the donor mouflon was undetectable.

If the nucleus from the donor cell does not support the biogenesis of mitochondria from the recipient oocyte, the interspecies cloned embryo will selectively multiply mitochondria from the donor cell during embryo development. Although the number of mitochondria from recipient oocyte is 10 times of that from the donor cell in the early cloned embryo, mitochondria from the donor cell will dominate those from the recipient oocyte after the blastocyst stage. The results from our laboratory have showed that the mitochondria from the donor panda cells and those from the recipient rabbit oocytes co-exist in embryos before implantation; whereas the mitochondria from donor panda cells remain detectable, mitochondria from the recipient rabbit oocytes are eliminated after implantation[17].

If the nucleus from the donor cell supports the biogenesis of mitochondria from both the donor cell and the recipient oocyte, however, the nucleus has a preference for multiplying mitochondria from the donor cell, in this case, mitochondria in the cloned animals will be at various levels of heteroplasmy. Steinborn et al.[41] found that mitochondria in 4 of the 11 cloned *Bos indicus* calves are heteroplasmy. Recently, Takeda et al.[42] used PCR-mediated single-strand conformation polymorphism (PCR-SSCP) to analyze the mitochondria of the cloned calves, and found that 3 of the 9 cloned calves exhibited heteroplasmy with donor cell mtDNA populations ranging from 6% to 40%. They contended about a significant replicative advantage of donor mtDNAs to recipient mtDNAs during the course of embryogenesis in cloned calves. Hiendleder et al.[43] also observed varying degrees of mitochondrial DNA heteroplasmy in nuclear transfer embryos, fetuses, and offsprings; however, their results did not suggest a replicative advantage of somatic nuclear donor cell mtDNA, but a mechanism of neutral segregation of mitochondria from both the donor cells and recipient oocytes in cloned animals.

### 2.2 Possible techniques for mammalian interspecies pregnancy

Interspecies pregnancy, an inevitable process in IAC, is one of the key factors to confine the success of interspecies mammalian cloning. Although the study of interspecies mammalian pregnancy is limited so far, it would be possible to achieve interspecies pregnancy with the help of the following techniques:

(1) Interspecies pregnancy by the help of conspecific embryos to the surrogate. Co-transferring the conspecific embryos and interspecies cloned embryos into the surrogate mother would achieve interspecies pregnancy. In our laboratory, we co-transferred the panda-rabbit cloned embryos at the 2 ~ 4 cell stage with the cat-rabbit cloned embryos into the surrogate female cats. One pregnant surrogate cat carried 6 fetuses after 21 days of embryo transfer. Of the 6 fetuses, two were from the panda-rabbit cloned embryos as confirmed by the satellite DNA analysis[17]. The giant panda is a delayed implantation animal, whose gestation period varies from 80 ~ 150 days. We hypothesize that the giant panda embryos play an inactive role during implantation for their delayed implantation characteristics; the cat-rabbit embryos may act as implantation "inducers" or "helpers" for the panda-rabbit cloned embryos, which may help implantation of panda-rabbit embryos in cat uterus. During embryo implantation, the signals from embryos to recipient uterus for implantation are controlled by the nucleus and these signals may be species-specific. The cat-rabbit cloned embryos may give implantation signals to the recipient cats, and trigger the process of implantation for both the cat-rabbit and the panda-rabbit cloned embryos.

(2) Replacement of inner cell mass. The trophoblast alone can control the success or failure of the mammalian pregnancy[25]. Interspecies pregnancy might be established with the composing of interspecies chimeric blastocysts, which can be produced by transferring the inner cell mass of one species into the blastocysts, with the inner cell mass being removed, of the species to the surrogate mother. Viable offspring of *Mus caroli* pregnant in surrogate mother of *Mus musculus* have been obtained by transferring the inner cell mass of *Mus caroli* into trophoblast

vesicles (blastocysts with inner cell mass being removed) of *Mus musculus*[23].

(3) Diploid/tetraploid ($2n/4n$) chimeras. $2n/4n$ chimeras are the chimeras produced by aggregating the diploid embryos or ES cells with the tetraploid embryos. Mouse $2n/4n$ chimeras display nonrandom distributive characteristics, which tetraploid cells mainly contribute to the extraembryonic tissues and diploid cells form the conceptuses during embryo development[44~46]. Aggregating the inner cell mass or ES cells of chinchilla 129/Sv mouse with the tetraploid embryos of albino CD1 mouse, chimeras embryos were transferred into the surrogate mother; most of the newborns were derived from the inner cell mass or ES cells, and only a minor contribution ($<2\%$) from tetraploid cells could be detected in some of the newborns[44,45]. Making use of the characteristic of $2n/4n$ chimeras, we may compose interspecies $2n/4n$ chimeras, of which the tetraploid embryos are from the species of the surrogate mother, and the diploid embryos are from other species. Transferring these interspecies $2n/4n$ chimeras into the surrogate mother, tetraploid cells, which is the same species as the surrogate mother, will be distributed to the placenta, and the diploid cells form the fetuses. The method of $2n/4n$ chimeras is widely used in production of gene deficient mice and mouse cloning. Whether or not this method can be applied in the species other than the mouse remains to be studied.

## 3 Perspectives

At present, the study of IAC is just in the state of exploration, and the technique of IAC itself is far from perfection. The mechanisms of IAC, such as nuclear dedifferentiation, reprogramming, nucleo-cytoplasmic interaction between species and interspecies pregnancy remain largely unknown. A deeper understanding of the mechanisms and the refinement of these techniques of IAC should be further carried out. Developmental biology, genetic regulations, conservation and utilization of animal resources, and many other newly developed research areas will benefit from the refinement of the techniques and the success of IAC.

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