Advances in interspecific pregnancy

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Abstract **Interspecific pregnancy in which the conceptus and female carrying the pregnancy are of different species is a key step to interspecific cloning. Cloning endangered animals by interspecific pregnancy is such a highlight catching people's eyes nowadays. In this article, the history of interspecific pregnancy, the methods for establishment of interspecific pregnancy, the corresponding theories, barriers and applied prospects are reviewed.**

Keywords: interspecific pregnancy, cloning, reproduction.

The phenomenon of intraspecific pregnancy and breeding is common to society. While the fact that hybrid mule can be born by mating of an equine and an ass has inspired new ideas: Can interspecific pregnancy be realized in other species by artificial means?

1 The history of interspecific pregnancy study

The early studies of interspecific pregnancy were mostly focused on interspecific nuclear transfer and interspecific chimeras, which played an important role in the studies of embryonic survival in xenogeniec uterus and embryonic cell differentation and migration, genetic interactions between two species. As for successful interspecific pregnancy, a few interspecific embryo transfers have been established. The most common examples were between *Bos taurus* and *B. indicus* (cattle). Other interspecific transfers involved *Bos gaurus* and *B. Taurus* (cattle); *Ovis musimon* and *O. aries* (sheep); *Equus asinus* and *E. caballus* (horse). Meanwhile, there are several examples of intergeneric embryo transfers such as between goat and sheep and between mouse and rat in which embryos have been implanted but did not develop into term[1].

The earliest interspecific chimeras were interspecific transplantations of embryonic gonads of phasianidea conducted by French scientists Akram et al. in 1967[2]. The earliest interspecific nuclear transfer was that between *Carassius euratus* and *Rhodeus sinensis* conducted by Chinese scientists Tong et al. in 1973[3]. The studies in interspecific pregnancy have been conducted since.

Chimera between *Mus musculus* and *M. caroli* came out in 1980 and was the first mammal interspecific chimera by artificial means[4]. Fehilly and Meinecke[5,6] reported that the interspecific chimeras of sheep and goat can overcome the barrier of interspecific pregnancy respectively at nearly the same time in 1984. Chinese scientist Chen et al.[7] reported that cow embryos can be impregnated and developed to term in yak successfully by embryo transfer in 1995.

The facts of obtaining sheep, mouse and calf from somatic cells not only render common people to trust intraspecific cloning technology, but also inspire scientists to bring forward more challenging and daring ideas: interspecific cloning. Chen et al.[8] transferred somatic cells of giant panda passage cultured *in vitro* as a donor nucleus into rabbit's oocyte by removing the nucleus to make reconstructed embryo in 1999. They obtained hatched blastula by culturing these reconstructed embryos *in vitro*. American scientists Neal L. First et al.[9] transferred dermal fibroblasts of bovine, sheep, swine, monkey and rat donor cells into bovine oocytes by removing the nucleus, respectively in the same year. All the interspecific reconstructed embryos can develop forward. Except those of the rat reconstructed embryo used in embryo transfer at two-cell stage, other interspecific reconstructed embryos developed to the blastula stage by culturing *in vitro*. However, none of reconstructed embryos could be impregnated in recipients. This indicates that interspecific pregnancy is one of the most urgent problems involved in mammal interspecific cloning.

The understanding of the interspecific pregnancy tendency in theory and in practice can be helpful to breaking through the second difficult step of mammal interspecific cloning: implantation and pregnancy. Therefore, several aspects of interspecific pregnancy are reviewed here.

2 The methods of interspecific pregnancy establishment

(ⅰ) Produce interspecific hybrids by interspecific male and female animals by natural mating or by artificial insemination and then establish an interspecific pregnancy by natural pregnancy or by embryo transfer[10].

(ⅱ) Rossant et al.[4] injected the inner cell masses (ICMs) of *Mus musculus* into the blastocyst of *Mus caroli*, then produced live chimeras between these species of mice. The chimeras were entirely similar to *M. musculus* in equilibrium with *M. musculus* chimeras in their somatic tissue organization[4]. Viable *M. caroli* offspring were produced by reconstitution using trophoblast of *M. musculus* genotype and inner-cell mass of *M. caroli* in 1983[11].

(ⅲ) Interspecific chimeras between *Mus musculus* and *Mus caroli* were made by aggregation of eight-cell embryos[12]. Chimeras produced by methods 2(ⅱ) and

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2(iii) were transferred to *M. musculus* recipients. The former did not survive to term, but viable chimeras were produced following embryo aggregation.

(iv) Two demi-embryos came from *Bos. taurus* and *B. indicus* were placed in a single zone pellucida and developed to early blastocyst stage. Then, the interspecific chimeras offspring were produced by embryo transfer[13].

(v) It is possible to achieve pregnancies after transfer of ibex embryos into domestic goats, but this requires a great change of the PAG profiles, which increase significantly. Live ibex kids can be produced when embryos from both species share the uterus[14].

(vi) Interspecific reconstructed embryo could be made by injecting mammal somatic cell cultured *in vitro* into other specific oocyte without genetic material and by cell fusion (such as somatic cells of giant panda and rabbit's oocytes). Interspecific pregnancy could be established by embryo transfer after culturing these reconstructed embryo to blastocysts or hatched blastocysts *in vitro*[8,9].

3 Related theories to the study of interspecific pregnancy

(i) Interaction of interspecific cytoplasm and nucleus. The cytoplasm and nucleus cooperate to carry out cellular physiological function and constitute an ambivalent and uniform together. A mass of data testified that embryonic cells' development is omnipotent before eight-cell stage which is related to cytoplasm equality. It is still necessary to study, before and after eight-cell stage, how the interaction of cytoplasm and nucleus support the reconstructed embryos made by interspecific nuclear transfer to blastula. For reconstructed embryos, cytoplasm and nucleus are from different species. All proteins and mRNAs in cytoplasm and those controlled by nucleus are not entirely congenetic. The difference will possibly cause genes in nucleus to express incompletely and affect the development and succedent implantation of the reconstructed embryos.

It may be an incompatibility between the maternal *Oncorhynchus masou* cytoplasm and paternal *O. mykiss* genome that contribute to mitotic abnormalities led by chromosome loss or deletion after fertilization to the blastula stage. This may depend on the interaction of cytoplasm and nucleus from different species in interspecific hybrid embryos. But such annormalities were seldom or never observed in the reciprocal hybrids[15].

(ii) Developmental differences of interspecific embryo and normal embryo. There are certainly great differences in development *in vivo* and *in vitro* and genic expression between interspecific hybrid embryo and normal embryo. But the related reports are still very limited and it needs further development.

Development of interspecific hybrid embryo is relatively slow compared to that of normal embryo once it becomes dependent on embryo-encoded gene products. Gene activation in hybrid embryos is stage-specific, rather than age-specific. Both the paternal and maternal alleles were equally expressed in hybrid embryos and that the paternally derived allele was not activated before the maternally derived allele[16].

Gene imprinting is considered to be one of the barriers for pregnancy of interspecific hybrids. Gene imprinting can cause some cytokines not to express successfully. For example, the expression of mouse H_{19} gene (histocompatibility gene) is controlled by parental gene imprinting which is located at downstream of *IGF Ⅱ* (insulin-like growth factor type Ⅱ) and affects its expression. Therefore, the development of embryo would be affected when the expression of *IGF Ⅱ* is deficient. Mouse *Impact* is an evolutionarily conserved imprinting gene that is expressed in oocytes as well as in early embryos. But overexpression of *Impact* results in gastrulation defects. It is documented that *Impact* is expressed biallelically in the whole embryonic stage, suggesting the need for tight control of its dosage to better allow for the interspecific hybrid embryonic early development[17]. But there is still disputation focus in the effect of gene imprinting on interspecific pregnancy.

Moreover, for most of interspecific hybrids, inhomogeneous nucleus cells will come into being because of asynapsis or disorder of homologous chromosomes in mitosis in the early embryonic development, even if sperm and ovum from different species can identify and fertilize. Interspecific pregnancy would be terminated by the barrier of anaphase embryonic development even if these interspecific hybrid embryos can develop to blastula stage and be pregnant. But in this facet, the phenomenon of hybrid mule root in equine and ass perhaps is an exception. Nevertheless, the hybrid offspring mules of equine and ass have a distinct shortcoming: infertility.

(iii) Interrelation of interspecific nu□lear and mitochondria. Mammalian mtDNA codes for 13 enzymes used in the mitochondrial energy-generating pathway, oxidative phosphorylation, 22 tRNAs and two rRNAs. Although all transcripts of mtDNA and their translational products remain in the mitochondria, most proteins used in mitochondria are from nuclear DNA and are imported after synthesis on cytoplasmic ribosomes. Spermatozoa introduce a small number of mitochondria into the cytoplasm of the egg at fertilization, which appear to be digested soon after penetration. Although the paternal contribution of mtDNA to the offspring is not believed to occur in mammals, some interspecific crosses have suggested that it does occur. Experiments with animals derived from reconstituted embryos, using nuclear or cytoplasmic transplantations, suggest that nuclear-mitochon-

drial interactions are important but not essential in the survival and replication of exogenous mitochondria introduced into the egg.

As the levels of heteroplasmy varied in several tissues of animals derived from reconstituted embryos, it is suggested that differential partitioning the mitochondria occurs during embryogenesis. Mitochondrial morphology changes substantially during oogenesis and throughout the early cleavage stage. In pig oocytes and embryos, mitochondria aggregate and are closely associated with endoplasmic reticulum, lipid granules and large vesicles. Although the direct correlation of mitochondrial genes with reproductive traits is still unclear, some human degenerative diseases and performance traits in cattle can be related directly to specific mtDNA polymorphisms. Information on the transmission of mtDNA and its effects on performance will have many implications for the increased productivity of animals. There are also potential ramifications to the animal cloning industry[18].

Electron microscopic observations indicate that foreign mitochondria transfered into mouse fertilized ova can be kept alive in blastula stage embryos[19]. DNA sequencing of interspecific reconstructed embryo between giant panda and rabbit indicates that the nucleus of reconstructed embryo come from giant panda and cytoplasm contains giant panda's mitochondria[8]. It is still worthy to study the new birth of paternal mitochondria, the fate of maternal mitochondria and interrelation of them in reconstructed embryos.

4 The barriers of interspecific pregnancy

It is an interesting fact that interspecific hybrids can be produced between donkey ($2n$=62) and horse ($2n$ = 64) despite their chromosome difference, which inspires us for further investigation. More surprisingly, intergeneric embryo transfer between zebra ($2n$=44) and horse ($2n$=64) can also be successful. However, their offspring are usually infertile. About 70% were aborted at the gestation of 85 d when donkey embryos were transferred into horse uterus. *M. musculus* embryos can survive in uteri of *M. caroli* females. While 69 *M. caroli* embryos were transferred into *M. musculus* uterus, only an embryo survived to term and it would die at once postnatally. But the reverse transfer was a failure[20]. It shows that the successful rate of interspecific pregnancy is very low and is associated with the orientation of embryo transfer. So are bovine and sheep.

Compared with high success rate of intraspecific pregnancies, interspecific pregnancies obviously have intrinsic barriers. Immunological rejection is presumably regarded as the foremost barrier but its mechanism is unknown. Commonly, the immunological relationship between the maternal-fetal interface in intraspecific pregnancy is similar to that of transplant and receptor in allogeneic transplantation. Presumably so is between interspecific pregnancy and between xenogeneic transplantation. If the similarity is reasonable, the complement-mediated hyperacute immunological rejection will be predominant. Once the rejection is controlled, the following difficulty is similar to that of intraspecfic pregnancy. Immune response in intraspecfic pregnancy is mainly the involvement of T lymphocyte pathway, but natural killer (NK) cell is reported to play a crucial role in this process recently. It is well known that the immunological rejection in intraspecfic pregnancy can be exempted. If the hyperacute immunological response is removed, it is possible that the immunological barrier of interspecific pregnancy will be overcome.

The incompatibility of maternal-fetal genotype is another reason of the failure in interspecific pregnancy. The genotypes of both maternal recipient and fetus affect the development and function of recipient uterine endometrum. The genotype of recipient makes a great influence on the fetal growth. MacLarcen et al.[10] reported that the maternal-fetal abnormal interaction led to the failure of goat embryos in sheep or their chimera uterus. Fetal abortion rate was regarded to be relative to the genotype of embryos. The incompatibility of maternal-fetal genotype may cause their asynchronous development with a result of their incomplete talk or even the failure of talk. Maybe it induces immunological rejection. Up to now the actual mechanism has not been clear. The factors that determine the incompatibility of embryo and uterus in interspecific and intergeneric pregnancy and the difference that donor and receptor telorate the factors are also unknown.

Besides maternal-fetal immunological rejection and genic incompatibilty, a lot of other factors affect interspecific pregnancy. Successful interspecific pregnancy includes three key processes: Pregnancy recognition, establishment and maintenance. Pregnancy recognition signals vary with species. For example, trophoblast protein-1 (TP-1) of sheep, goat and bovine conceptuses acts as recognition signal which can initiate the maintenance of corpus luteum, while horse conceptus can secrete not TP-1 but an unknown factor which can initiate the maintenance of corpus luteum. If the signals secreted by xenogenic embryos cannot be recognized by recipient uterus when xenogenic embryos are transferred into recipient uterus, corpus luteum will not be maintained and pregnancy will not be established. In pregnancy establishment, not only corptus luteum but also the synchronous development of xenogenic embryo and uterus is indispensable to embryo implantation. Culture condition *in vitro* and the difference of embryo implantation time bring about asynchronous development thus embryos could not implant. Furthermore, the varying implantation types with species also have an influence on the embryo implantation rate of interspecific pregnancy. Most rodents belong to the type of partial implantation but primates to the type of complete

implantation. Placenta, as the immunological barrier and tache of maternal-fetal nutrition, plays a crucial role in pregnancy maintenance. Different animals have different types of placentas. For example, placentas of horse and swine are of epitheliochorion but those of bovine and sheep are of connection tissue chorion. If the types of placentas in interspecific pregnancy are different, maternal uterus will not provide sufficient nutrition for fetus and it is more important that the immunological barriers would be formed, which results in the failure of interspecific pregnancy. Furthermore, the different duration of gestation exerts an effect on the fetal growth so that the fetus cannot develop to term.

Although the failure of interspecific pregnancy among all kinds of animals is mainly attributed to the same barriers, the barriers are partially different with different species of animals.

(ⅰ) Barriers of interspecific pregnancy among equine species. The placentas of equidae have special structures called endometrial cups, which can secrete equine chorionic gonadotropin (eCG). A function ascribed to eCG is luteinization of secondary follicles that are important in supporting pregnancy until the placenta takes over progesterone production. The donkey chorionic girdle develops so poorly in horse uterus that the endometrial cups are smaller or even do not form and eCG remains absent, which cause low quantity of progesterone. So the fetus cannot absorb nutrition from the surrogate mares and becomes increasingly stressed until it finally dies. It is aborted by 80—100 d of gestation and a strong cytotoxic response occurs in 30 d before the abortion.

Later, it is discovered that mule embryos in horses are more often aborted than those in donkeys and the placentas of the former have narrower and thinner chorionic girdles than those of the latter. It shows that chorionic girdles are the keys to the success or failure of equine interspecific pregnancy. Earlier studies revealed that it is due to genome imprinting. The maternal genome contributes preferentially to the makeup of the embryo and the paternal genome to the placenta. Regardless of the uterine environment, the horse genome, as the paternal genome, is certain to give rise to a broad girdle, while the donkey genome stimulates the development of a narrow girdle[21]. However, someone opposed it through an experiment. In the experiment, a mule morula was bisected and one of the resulting two demi-embryos was transferred surgically to a mare while the other was transferred to an unmated female donkey. As a result, in the surrogate mare carrying one demi-mule embryo, the endometrial cups were smaller and narrower than those in the donkey carrying the other demi-mule embryo. It can be concluded that the fate of the girdle is determined not by genic imprinting, but by the uterine environment[22].

Allen et al.[23] postulated a mechanism to explain the dramatic influence of the donkey uterus on the development of the mule chorionic girdle. Perhaps a growth factor secreted by the endometrium under maternal genetic control plays a leading role in the development of the chorionic girdle. It may be able to bind only weakly to its receptor and result in a much smaller and narrower chorionic girdle when a mule conceptus is in a horse uterus. But when some mule conceptus is placed in a donkey uterus, the growth factor produced by the donkey endometrium can bind avidly to the placental receptor and so stimulate the makeup of a larger girdle. This hypothesis still awaits experimental testing.

Glycosylation patterns were similar between the placental tissues of the horse and donkey, but the glycosylation patterns of the horse and donkey placenta were strikingly different from those of the camel. The glycodiversity is viewed as one of the factors preventing implantation and subsequent placental development in interspecific pregnancy between horse or donkey and camel. Glycodiversity may induce the weak contact between plactental trophoblast and uterine endometrium and immunological rejection. When interspecific pregnancy succeeds, the mechanisms that normally suppress the expression of MHC class Ⅰ molecules by the epithelial trophoblast layer of the equine placenta can only function if the apical surface of the cells is in a close and stable contact with other tissues such as the endometrial epithelium. Therefore the abnormal pregnant termination can be prevented[24]. When equine interspecific pregnancy fails, MHC class Ⅰ strongly expresses in conceptus allantochorion and a lot of lymphocytes appear nearby the endometrium matrix.

(ⅱ) The barriers of interspecific pregnancy among murine species. The success rate of interspecific pregnancy among murine species is related with the direction of embryo transfer. *M. muculus* embryos can survive in *M. caroli* uterus, but in the reverse condition, most *M. caroli* embryos cannot develop. One explanation is that presensitization of *M. musculus* against *M. caroli* antigens exists, but presensitization of *M. caroli* against *M. musculus* antigens does not exist. Clearly, immunological rejection are closely associated with the genotype of murine interspecies[25]. The trophoblast plays an important role in immune response. When all or parts of trophblast are of the same specific genotype with uterus, immunological tolerance can be obtained. The trophblast cells can down regulate the proliferation of decidua lymphocytes and suppress the activity of CD56+ natural killer (NK) cells. Loss of trophoblast cell function rather than lymphocyte-mediated destruction of trophoblast appear to underlie the death of *M. caroli* embryos in the *M. musculus* uterus[26]. However, someone refuted that failure of *M. caroli* embryos in the *M. musculus* uterus does not involve response by classical cytotoxic T lymphocyte or

cytotoxic T lymphocyte or NK cell pathways, while the immune system does participate in the resorption process[27].

The development of hybrids from *M. musculus* and *M. caroli* was retarded in comparison with that either parent, suggesting intrinsic problems of genomic incompatibility. Contrary to the phenomena in intraspecific pregnancy, mouse fetal weights in interspecific pregnancy are not related to the placental weights. And glycogen cells are able to negatively modulate fetal growth by an as yet unidentified mechanism[28].

(iii) The barriers of interspecific pregnancy of sheep and goat. In the uteri of sheep-goat chimeras, sheep conceptus can develop but the hybrid cannot survive. No differences in serum progesterone, oestrone, prostaglandin F-2 alpha metabolites, cortisol concentrations and cotyledon numbers could be detected during pregnancy between recipients of the normal sheep and interspecific sheep. Anatomical inspection of interspecific dead fetus indicated that all fetuses were premature and had various degrees of hydranencephaly. The transfer of Dall's sheep embryos to domestic ewes results in the establishment but subsequent loss of pregnancy and that these losses occur throughout gestation. Complement-mediated lymphocytotoxic and hemolytic assays were used to monitor onset and titer of antibodies. The data did not support the hypothesis that the failure of caprine pregnancy in ewes or chimeras is due to a species-specific, maternal antibody response. In contrast, a maternal cytotoxic antibody response to species-specific antigens may contribute to the failure of hybrid or ovine pregnancy in does[29]. The humoral immune responses by ewes and does pregnant with a blastomere-aggregation sheep-goat conceptus includes an allogeneic response that appears to involve recognition of parentally inherited, polymorphic antigens and a xenogeneic response that appears to involve species-specific, monomorphic antigen. In addition, fetal trophoblastic or serum antigen in the xenogeneic response[30]. Can the mechanism be used to explain the failure of other interspecific pregnancy? It needs further investigation.

5 Remove the barriers of interspecific pregnancy

Removing the barriers of interspecific pregnancy is a key to the success of interspecific cloning. Although the barriers of interspecific pregnancy in different species are different, the main barrier is basically the same. According to the technology at present, the methods of overcoming the barriers of interspecific pregnancy are as follows.

(i) Remove the maternal-fetal immunological rejection. The maternal-fetal immunological rejection is the main barrier to interspecific pregnancy. According to immunity, the following methods can be used: i) Immunise recipients by active immunization or passive immunization. In the experiment of donkey-in-horse, the recipient horse obtained active immunization by infusing into recipient horse with donkey peripheral blood and passive immunization by infusing into recipient horse with serum recovered from mares carrying normal intraspecies horse pregnancies at equivalent stages of gestation. The two types of immunological therapy seemed to result in a marked improvement in fetal viability. ii) Treat the recipient with immunosuppressor. Immunosuppressors such as Dexameth or hydrocortone are injected into recipients before transfer. Its aim is to inhibit phago function of the macrophage and decrease the function of reticuloendothelial system killing granules or cells and lead to the lysis of lymphocytes. iii) Destroy the complement of the recipient. Snake poison can directly prevent the complement-mediated hyperacute rejection. iv) Non-immunogenicity embryos or recipients can be obtained by transgene or gene knock-out.

(ii) Remove the maternal-fetal genomic incompatibility. The same MHC genotype of the donor and the recipient can decrease the rate of immunological rejection in organic transplantation. Chimeras produced by injection of *M. caroli* ICMs into *M. musculus* blastocysts are viable, whereas *M. caroli* blastocysts cannot survive in the *M. musculus* uterus. These results indicate that the same genotype of trophoblast cells and maternal uterine allows the cells of both *M. caroli* and *M. musculus* to be in equilibrium in chimeras so that the chimeras can survive in the *M. musculus* uterus. It is suggested that *M. musculus* trophoblast components may protect the *M. caroli* embryonic cells from maternal immune rejection. Otherwise, if the key genes such as *HOXa-10* and *MHC* gene can be found and their expression is regulated properly, it will provide a new avenue to overcome the maternal-fetal genomic incompatibility.

(iii) Promote maternal recognition, establishment and maintenance of pregnancy. Among the factors that restrict interspecific pregnancy, the type of embryo implantation, the type of placenta and the duration of gestation are inalterable. So long as the species with the same or similar inalterable factors are selected as the donor and recipient, it will be helpful for xenogeneic embryo implantation. In order to improve the rate of embryo implantation, some factors may be modulated through the following ways.

(1) Induce the expression of integrin and matix metalloproteinase (MMP) in pre-implantation embryo. Integrin can prepare for embryo adhesion and implantation and contribute to the transition of endometrium from non-adhesive state to an adhesive state. Furthermore, integrin α Vβ 3 affected the process of embryo implantation by route of mediating both the attachment and outgrowth processes of blastocyst on uterine epithelial cells. Therefore, integrin has been regarded as an epithelial marker of

the opening of the window of implantation[31]. Matrix metalloproteinase (MMPs) is a kind of proteinase which degrades extracellular matix (ECM) components and has been widely viewed as a marker of trophoblast invasiveness in embryo implantation[32]. The abnormal maternal-fetal talk in interspecific pregnancy is attributed to the asynchronous spatio-temporal expression of integrin and MMPs. It results in the asynchronous development of the invasiveness of blastocyst and the receptivity of uterine endometrium. If the expression of integrin and MMPs can properly be stimulated in pre-implantation embryo in order to improve the attachment and invasiveness and coordinate the maternal-fetal synchronization, it is possible to remove the barriers of interspecific embryo implantation. Certainly, excessive stimulation will cause to overinvasiveness of trophblast. Therefore, it is very important to select proper inducement.

(2) treat pre-implantation embryo with cytokines. A lot of proofs show that cytokines play an important role in intraspecific pregnancy by controlling endocrine and immune systems. It can affect the invasiveness of blastocyst and the receptivity of uterine endometrium, as well as coordinate the synchronization of the maternal and fetal development. For example, EGF, TGF α can increase the expression of integrin in uterin epithelial cell, which indicates that cytokines can modulate the receptivity of uterine endometrium and initiate embryo implantation by regulating the expression of adhesive molecules. The mouse embryo lack of LIF cannot implant. When cytokines produced by Th1 cells predominate over those produced by Th2 cells, the maternal-fetal immune equilibrium will be destroyed so that the pregnancy cannot be maintained. Th2 type cytokines, IL-4 and IL-6, induce the release of CG from trophoblasts and CG stimulates progesterone production. Progesterone stimulates the secretion of Th2 cytokines and reduces the secretion of Th1 cytokines, which contribute to pregnancy maintenance[33].

Interspecific pregnancy is often viewed as an extension of the process occurring in intraspecific pregnancy. If interspecific embryos before transfer are treated with several key cytokines, it is possible to establish interspecific pregnancy by controlling immunological rejection, promoting embryo implantation and development in concert. The hypothesis awaits experimental testing.

6 The application prospect of interspecific pregnancy

Rescuing endangered animals is one of the human being's great duties. As for the endangered animals, intraspecific cloning is not an ideal method, because , for example it is very difficult to obtain oocytes for nuclear transfer from only less than 1000 giant pandas presently alive. Carrying out interspecific cloning becomes a reasonable way. Interspecific cloning depends on construction of interspecific reconstructed embryo, establishment and maintenance of interspecific pregnancy. Fortunately, there are reports on successful construction of interspecific reconstructed embryo[8,9]. Because of the involvement of implantation regulation and immune repulsion, establishment and maintenance of interspecific pregnancy are much more difficult and still lack of systemic research. Further research on interspecific pregnancy will open a promising direction for reproductive biology and developmental biology, meanwhile, greatly contribute to rescuing rare and endangered animals, such as giant panda ——China's national treasure.

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