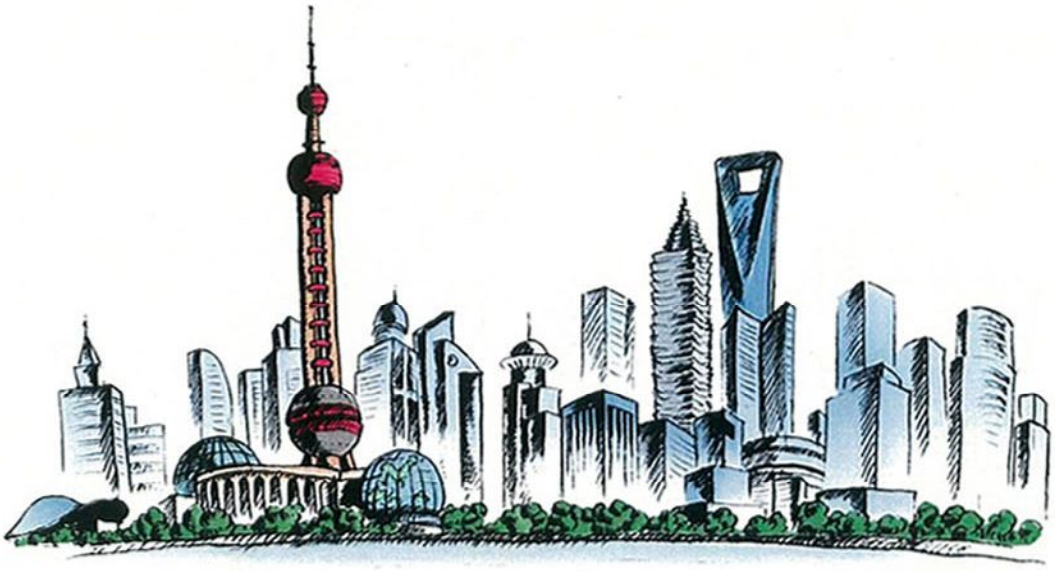
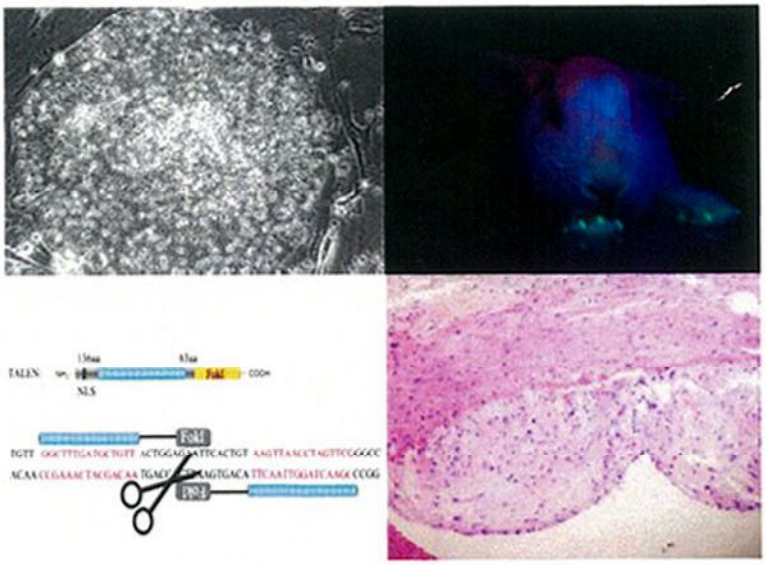


The 5th International meeting on rabbit biotechnology

Shanghai, China, 7-8 June 2013



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Program for 5th International Meeting on Rabbit Biotechnology

June 4th-6th, 2013

Speakers arrival (June 4-6)

Meeting of the organizing committee (June 6)

Preparatory committee of the second Japan-China Research and Exchange Meeting on Rabbit Biosciences (June 6)

Dinner (speakers and committee members) (June 6)

June 7th, 2013

8:00~8:45: Registration

8:45~9:15: Opening remarks

(Prof. Xuejin Chen, Chairman of the Organizing Committee)

School leaders

SALAS leaders

Session 1: New technologies in generating genetically modified rabbits

***(Chairpersons: Xuwen Peng and* Luca Fontanesi)**

9:15~9:50

Zsusana Bosze, Ph.D. Agricultural Biotechnology Center, Gödöllő, Hungary

Sleeping beauty transposon mediated transgenesis in rabbit

9:50~10:25

Liangxue Lai, Ph.D. GBHI, Guangzhou, China

Generation of gene disruption rabbits by embryo microinjection of TALENs

10:25~10:55

Tatiana Flisikowska, Ph.D. Chair of Livestock Biotechnology, Technische Universität München, Freising, Germany

Molecular scissors - new tools in transgenic technology

(Lecture times include 10 min Q&A session per speaker)

Coffee Break（10:55-11:05）

11:05~11:40

Shangang Li, Ph.D. Shanghai Jiaotong University, Shanghai, China

Transgenic rabbits by RNAi method

11:40~12:15

Y. Eugene Chen, MD, Ph.D. University of Michigan, USA

Novel Knockout, Knockdown, and Transgenic Pig and Rabbit Models for Cardiovascular Research

Taking photos and lunch (12:15-13:35)

Session 2: Genomics, genetics and ES cells

(Chairpersons: Eugene Chen and Pierre Savatier）

13:35-14:10

Pierre Savatier, Stem Cell and Brain Research Institute, INSERM, Bron, France

In search of naïve pluripotency in rabbit

14:10~14:45

Arata Honda, Ph.D. University of Miyazaki, Miyazaki, Japan

Naive-like conversion overcomes the limited differentiation capacity of pluripotent stem cells in rabbit

14:45~15:20

Jinsong Li, Ph.D. Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China

Generation of Mammalian Haploid Embryonic Stem Cells

Coffee Break (15:20-15:35)

Session 3: Novel rabbit models for human diseases

（Chairpersons: Tatiana Flisikowska and Zsusana Bosze）

15:35~16:10

Xuwen Peng, Ph.D. Pennsylvania State University College of Medicine, Hershey, PA, USA

A Novel Transgenic Rabbit Model for Studying Human Pathogens

16:10~16:45

Luca Fontanesi, Ph.D. University of Bologna, Bologna, Italy

Genomics of coat color in the rabbit: modeling and animal model for pigmentation related diseases in human

16:45~17:20

Enqi Liu, Ph.D. Xi'an Jiaotong University, Xi'an, China

Evaluation of new lipid-lowering drugs using cholesterol-fed rabbits

17:20

Closing remarks

Jianglin Fan, MD, Ph.D., President of the International Meeting on Rabbit Biotechnology

17:30~ Short reports and poster presentations（Chairpersons: Enqi Liu and Liangxue Lai）（14 posters submitted now）

18:30~ Dinner (All participants)

June 8th, 2013

1、 Getting-together Collaboration Forum

2、 A tour for visiting Department of Laboratory Animal Science, School of Medicine, SJTU

3、 City tour (optional for applicants only)

4、 Zhouzhuang tour (optional for applicants only)

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Sleeping beauty transposon mediated transgenesis in rabbit

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Since transgenic rabbits are widely used models of human diseases a novel and relatively simple method, which would increase the efficiency of additive transgenesis is long time awaited. In our earlier experiments the SIV lentivirus vector was applied in laboratory rabbit. Lentiviral transgenesis significantly increased the ratio of founder rabbits, albeit resulted exremly low germline transgenesis rate (Hiripi *et al.* 2010). Transposon mediated transgenesis, based on the hyperactive *Sleeping Beauty (SB)* transposon system SB100X which was applied –among others - in mice and rat reproducibly promoted generation of transgenic founders at frequencies of 50-64% and 14-72%, respectively (Katter *et al.* 2013). Because the SB system is applicable to a wide range of vertebrate species (Izsvak *et al.* 2000), we hypothesized that SB-mediated transgenesis may be extended to the valuable model species, laboratory rabbit.

The cocktail of a circular plasmid carrying the T2/Venus transgene on the transposon and mRNA coding for the transposase was microinjected into the male pronucleus of rabbit zygotes. 472 injected embryos were transferred into 25 pseudo-pregnant females. Altogether 46 rabbits were born from 10 does. Young hairless transgenic F0 pups had a mosaic pattern of *Venus* fluorescence expression. All four rabbit founders which reached sexual maturity were germline transgenes, 35 out of the 80 F1 rabbits (44%) inherited the transgene and all F1 transgenic rabbit expressed the *Venus* protein ubiquitously. A single copy transgene expressed from the transposon was capable of supporting significant gene expression in one of our transgenic rabbit lines. So far we bred four generations of the transgenic line carrying one integration site and epigenetic silencing of indicator protein expression was not found. Thus, SB transgenesis is applicable in rabbits and at a 15% transgenic founder rate with high germline transmission rate; it is a very efficient and widely applicable method.

This work was supported by grants OM-00118/2008 and OTKA NK 104397.

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Generation of RAG 1 and 2 deficient rabbits by embryo microinjection of TALENs

Jun Song[1], Juan Zhong[1], Xiaogang Guo[1], Yongqiang Chen[1,2] Qingjian Zou[1,2], Huaqiang Yang[1], Jiao Huang[1], Xiaoping Li[3], Quanjun Zhang[1], Zhiwu Jiang[1], Peng Li[1], Duanqing Pei[1] & Liangxue Lai[1,3]

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Abstract

The rabbit is an important laboratory animal. However, the gene-targeted rabbit has been hardly established because no germline-competent rabbit ES cells are available and the efficiency of rabbit somatic cell nuclear transfer is such low[1]. An only single gene knockout rabbits was achieved by zinc-finger nucleases technology, the off-targeting of which limits its broad practice[2]. Transcription activator-like effector nucleases (TALENs) is a new genome modifying technology with fewer off-target effects and lower toxicity[3]. Here, we describe the generation of recombination activation genes (Rag1 and 2) deficient rabbits based on TALEN mediated gene editing. We designed TALENs targeting both rabbit RAG1 and RAG2, and microinjected their mRNAs into 40 and 24 embryos respectively. The injected embryos were transferred into surrogate rabbits and gave birth to 18 and 4 bunnies respectively. Sequencing analyses of the targeted alleles revealed that most of the rabbits were mosaic animals with compound heterozygous mutations. Interestingly, we found that 11 RAG1talen KO and all 4 RAG2 Talen KO rabbits were biallelically modified, and two RAG1talenKO rabbits and one RAG2 talen KO rabbit carried the same mutation at both loci. We observed a dramatic decrease of lymphocytes in RAG1talen KO and RAG2talen KO rabbits than in wild type animals. The arrested lymphocytic development at an immature stage and the absence of V (D) J recombination suggest that the functions of RAG1 and 2 are lost in the double KO rabbits. Considering their easy availability and high efficiency, we believe that the application of TALENs would tremendously promote the specific genetic modification of rabbits for biomedicine and agriculture. Our results also demonstrate the feasibility to establish RAG-deficient rabbits without mature T and B cells for drug discovery and stem cell research.

Molecular scissors - new tools in transgenic technology

Tatiana Flisikowska

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Rabbits are important laboratory animals, widely used in many areas of biomedical research, including the production of antibodies and recombinant proteins. Rabbit models have contributed to the understanding of human diseases and the development of therapeutic compounds, devices and techniques. However until recently it has not been possible to engineer precise genetic alterations in rabbits because they have so far been refractory to the two key enabling technologies; (i) rabbit embryonic stem (ES) cells capable of contributing to the germ line have yet to be derived, and (ii) rabbits are particularly difficult to produce by somatic cell nuclear transfer [1].

The production of synthetic enzymes called zinc-finger nucleases (ZFNs) was a major breakthrough in transgenic technology. An appropriately designed ZFN can create a double-strand break at a single predetermined site in the genomic DNA of an organism. In eukaryotes, double-strand break repair pathways often create small insertions and deletions at the break site, a useful means of inactivating genes of interest [2]. ZFN cleavage can also stimulate homology-directed genetic exchange between an episomal donor construct and a chromosomal locus, as first demonstrated for a native locus in Drosophila [3] and for endogenous loci in human cells [4]–[6].

To enable the production of targeted rabbits and streamline the production of gene targeted animals in other large mammals we investigated ZFN-mediated targeted gene replacement directly in rabbit oocytes. The immunoglobulin M locus was chosen as a suitable target because inactivation of endogenous immunoglobulins is a necessary first step for the production of humanized polyclonal antibodies in rabbits. ZFN mRNA was co-injected into fertilised oocytes with linear targeting vector DNA and embryos transferred to foster mothers. Analysis of late stage rabbit fetuses showed that ZFN-mediated gene targeting by homologous recombination could be achieved with 17% efficiency [7]. Correct targeting was confirmed by PCR, DNA sequencing and Southern hybridization.

Our approach circumvents the need for ES cells or SCNT to carry out precise alterations in any gene, because ZFNs can be designed against any native locus of interest. However specific and effective ZFNs can be difficult to produce. Now, new tools are emerging that are considerably simpler to make, these are highly specific transcription-like effector nucleases (TALENs) [8] and Cas9 RNA-guided endonucleases [9]. Their usefulness has been already successfully shown in many species, including human, and now need to be investigated whether they can be extended also to rabbits.

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RNAi mediated Stable Silencing of HGPRT Expression in Rabbit Fibroblasts and SCNT Rabbit Production

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Abstract

The hypoxanthine-guanine phosphoribosyl transferase(HGPRT)gene mutation is responsible for gouty arthritis. Kidney stone, and Lcsch. Nyhan Syndrome. It has been reported that the expression of HGPRT is decreased or even absent in these diseases. Rabbits are an ideal model for studying the pathology of these diseases. Therefore, the development of all HGPRT-knockdown rabbit model will be highly beneficial in such studies. Stable HGPRT-knockdown transgenic fibroblast lines were generated by transfecting rabbit fibroblasts with RNA interference (RNAi) plasmids. Polymerase chain reaction (PCR) analyses indicated that the average positive rate was 83.3%. The mRNA and protein level of in the transgenic fibroblast lines were significantly lower than that in the control. Transgenic rabbit blastocysts were derived after performing nuclear transfer. When the HGPRT-RNAi embryos were transferred to recipients, ten clone rabbits were derived, but no one of them survived more than 5 days. So a tet-on system was used, and the shRNA were inserted under H1tet promoter. The new vector was transfected into fibroblast and single cell clones were derived. When the new transgenic cell line was used for SCNT, one live cloned rabbit were derived. The results show that RNAi can be used to stably knock down expression of the HGPRT in rabbit fibroblasts and further improvements in related technologies will facilitate the use of this method for the generation of gene knockdown rabbits.

Key words: RNA interference, rabbit fibroblast, HGPRT, nuclear transfer

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Novel Knockout, Knockdown, and Transgenic Pig and Rabbit Models for Cardiovascular Research

Y. Eugene Chen, MD, Ph.D.

Frederick Huetwell Professor of Cardiovascular Medicine, Vice-Chair for Basic and Translational Research, Department of Cardiac Surgery Director, Center for Advanced Models for Translational Sciences and Therapeutics, University of Michigan Medical Center

A major bottleneck in cardiovascular research and drug development is the lack of larger animal models that more accurately simulate human disease in the pre-clinical stages of drug discovery and in the analysis of disease mechanisms. Although the study of the cardiovascular system has benefited significantly from the use of gene-targeted and transgenic mouse models, small rodents do not accurately reflect human cardiovascular physiology. To generate more appropriate and useful animal models for better mimicking the human cardiovascular system, it would be beneficial to explore larger mammalian models for CVD. In contrast to mice and rats, the rabbits/pigs are good models for CVD research. The cardiovascular system of the rabbits/pigs is similar to that of humans. However, the use of large animal (including rabbits and pigs) models has been prohibited by the un-availability of embryonic stem cell lines, which are highly amenable for genetic manipulations to create various necessary research models. Because of the compelling need to address this bottleneck in cardiovascular research and drug development, we have built a leading research team with multiple approached and expertise in the world to produce gene-targeted (knockout) and transgenic rabbits/pigs as CVD model animals.

In search of naïve pluripotency in rabbit

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Pluripotent stem cells (PSCs) can exist in at least two morphologically, molecularly and functionally distinct pluripotent states, designated as the naïve and primed states; PSC exist in both states in rodents, whereas only the primed stated has been described in primates so far. Not much is known about the pluripotent state of PSCs in rabbits. To address this, we derived and characterized four types of rabbit PSCs from the same breed of New Zealand White rabbits: three lines of induced PSCs (rbiPSCs) that were obtained by reprogramming adult skin fibroblasts with FGF2, three lines of iPSCs stably expressing mouse Krüppel-like factor 2 and 4, four lines of embryonic stem cells (rbESCs) established in the presence of FGF2, and six lines of ESCs established in the presence of LIF. All of these lines were compared to rabbit Inner Cell Mass (ICM) cells for the expression of pluripotency markers (both naïve and primed) using Agilent microarrays, real-time PCR, and single cell PCR. The four types of PSCs showed marked differences in the expression of naïve markers, including mosaic expression of *Esrrb*, *Tbx3* and *Dazl*. They also exhibited marked differences in their capacity to colonize the ICM of the rabbit blastocyst after injection into early cleavage-stage embryos. These results will be discussed in light of the rodent data, which show that only naïve pluripotent stem cells have the capacity to colonize the blastocyst to generate germ line chimeras.

Naive-like conversion overcomes the limited differentiation capacity of pluripotent stem cells in rabbits

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Rabbits pose fewer demands as laboratory animals than mice or rats. Once we noticed the congeniality between rabbit and pluripotent stem cell research, this combination became a very attractive analysis tool. Rabbit embryonic stem (ES) cells and induced pluripotent stem (iPS) cells have been established and share important major characteristics with human pluripotent stem cells. In addition, rabbit iPS cells fulfill all the requirements for the acquisition of a fully reprogrammed state, thus showing strong similarity to their ES cell counterparts. However, although the global gene expression profile of rabbit iPS cells becomes closer to that of the rabbit ES cells as the number of cell passages increases, a slight but rigid difference between the two types of rabbit pluripotent stem cells remains. Recently, we have demonstrated that the *in vitro* neural differentiation capacities of rabbit iPS cell lines may vary with the donor cell type, passage number, and target cell type into which they are induced to differentiate. Although the limited early neural differentiation capacity observed in the iPS cell lines was improved by continuous passage, more mature types of neural cells, such as oligodendrocytes, were poorly generated, even from continuously passaged iPS cells. It is noteworthy, however, that such limited neural differentiation capacity could be ameliorated by converting the cells to a naïve-like state.

We expect that rabbit pluripotent stem cells will provide unique, easily accessible animal models for assessing the efficacy and safety of new pluripotent stem cell-based treatments. Moreover, this rabbit model should enable us to compare iPS cells and ES cells under the same standardized conditions when evaluating their ultimate feasibility in cell-based regenerative medicine.

Keywords: ES cells, iPS cells, rabbit

Generation of Mammalian Haploid Embryonic Stem Cells

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Abstract

Haploid cells are amenable for genetic analysis. Recent success in the derivation of mouse haploid embryonic stem cells (haESCs) via parthenogenesishas enabled genetic screening in mammalian cells. However, successful generation of live animals from these haESCs, which is needed to extend the genetic analysis to the organism level, has not been achieved. Here, we report the derivation of haESCs from androgenetic blastocysts. These cells, designated as AG-haESCs, partially maintain paternal imprints, express classical ESC pluripotency markers, and contribute to various tissues, including the germline, upon injection into diploid blastocysts. Strikingly, live mice can be obtained upon injection of AG-haESCs into MII oocytes, and these mice bear haESC-carried genetic traits and develop into fertile adults. Furthermore, gene targeting via homologous recombination is feasible in the AG-haESCs. Our results demonstrate that AG-haESCs can be used as a genetically tractable fertilization agent for the production of live animals via injection into oocytes.

A Novel Transgenic Rabbit Model for Studying Human Pathogens

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A novel humanized transgenic rabbit model expressing HLA-A2.1 gene, a well-characterized human major histocompatibility complex class I (MHC-I) gene, has been established in our labs. The HLA-A2.1 strongly expresses and co-localizes exclusively with rabbit MHC-I on the cell surface of peripheral blood lymphocytes and various organ tissues of all transgenic rabbits. This transgenic rabbit model has been successfully used to assess the protective efficacy of HLA-A2.1 restricted $CD8^+$ T cell epitope-based vaccines. The transgenic rabbits vaccinated with the human papillomavirus 16 (HPV16) E7 epitope (82-90) DNA vaccine showed an almost complete protection against infection with the modified cottontail rabbit papillomavirus (CRPV) DNA containing an embedded HPV16 E7/82-90 epitope. Similar results were also observed in an independent study from testing several HLA-A2.1 restricted epitopes from herpes simplex virus type 1 (HSV-1) against ocular HSV-1 infection in the transgenic rabbits. Data from these studies indicate that the HLA-A2.1 transgenic rabbit model have provided a powerful model to directly screen and evaluate HLA-A2.1-restricted epitopes of HPV and HSV-1 proteins for protective immunity in the context of a human MHC class I gene. Furthermore, this transgenic rabbit model may also have potential application in testing $CD8^+$ T-cell epitope-based vaccines against other human pathogens that are permissive or semi-permissive in rabbits, such as human T cell leukemia virus, adenovirus, EBV-like viruses, syphilis and tuberculosis.

Genomics of coat colour in the rabbit: modeling and animal model for pigmentation related diseases in human

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Coat colour genetics in rabbits has been the matter of pioneering studies at the beginning of the last century. From these early studies, a quite large number of loci affecting coat colour has been identified and comparative analysis has made it possible to establish putative homology across species. As in most species, coat colour loci in rabbits are also associated with genetic defects. Only recently, the identification and characterization of genes affecting coat colour phenotypes has opened new avenues to investigate or detect mutations causing associated genetic defects. We first characterized several mutations in the melanocortin 1 receptor (*MC1R*), agouti signaling protein (*ASIP*) and melanophilin (*MLPH*) that cause different alleles at the *Extension*, *Agouti* and *Dilute* loci, respectively. In particular, the identification of the causative mutation for the *Dilute* locus provides a natural animal model for human Griscelli syndrome type 3. According to the first coat colour studies in rabbits, the *English Spotted* locus is also associated with a megacolon defect. Two alleles have been inferred: allele *En*, that gives spotted phenotypes and megacolon; allele *en*, that gives a solid coat colour phenotype. We first created a reference population crossing Checkered Giant rabbits that were expected to be heterozygous at the *English Spotted* locus. We first selected a few candidate genes for this phenotype and identified several polymorphisms. A single nucleotide polymorphism (SNP) in the *KIT* gene was completely associated with the different coat colour phenotypes of the *English Spotted* locus. Quantitative gene expression analysis in colon and cecum specimens indicated that the expression level of the *KIT* gene in *En/En* rabbits was only 5-10% compared to the level of control *en/en* normal animals. Histological and transmission electron microscopy of cecum and colon specimens revealed a few alterations in the cells of Cajal. Other studies are under way to further characterize rabbits homozygous *En/En* at the *English spotted* locus as model for human congenital megacolon.

Evaluation of new lipid-lowering drugs using cholesterol-fed rabbits

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Abstract

Introduction: Probucol (PB) and cilostazol (CZ) both show anti-atherogenic effects. However, it is fully unclear for their combinatorial effects in rabbits. This study was designed: 1) to investigate their combinatorial (PB+CZ) anti-atherogenic effect in rabbits. 2) to elucidate whether addition of LPC (PB+CZ) would increase anti-atherogenic effects of atorvastatin (statin) in cholesterol-fed rabbits.

Materials and Methods: 1) The rabbits were fed with a cholesterol diet with PB or CZ alone or LPC for 16 weeks; 2) rabbits were treated with statin alone or LPC+statin for 12 weeks. Plasma total cholesterol, LDL-cholesterol, HDL-cholesterol, C-reactive protein, superoxide dismutase, malondialdehyde, and NO were measured. Aortic atherosclerosis lesions were grossly and microscopically evaluated.

Results and Conclusion: We found that 1) anti-atherogenic effect was the most prominent in PB +CZ. Histological analysis revealed that although PB or LPC significantly reduced the lesion size but only LPC significantly decreased macrophages accumulation and smooth muscle cells proliferation in the lesions. 2) LPC combined with statin treatment exhibits a combined effects on the inhibition of atherosclerosis in cholesterol fed rabbits. These effects are attributed to diverse mechanisms, including lipid-lowering, anti-oxidization, anti-inflammation, up-regulation of levels of NO and endothelial protein S-nitrosylation. These findings may provide us with a new clue for the development of new therapeutics for treatment of atherosclerosis.

Keywords: Probucol, cilostazol, statin, atherosclerosis, rabbits.

The rabbit pre-implantation embryo as a paradigm to explore naive embryonic stem cell derivation in non-rodent species

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Rabbit is a good model to improve embryonic stem cell (ESC) derivation in non-rodent species, as it can produce up to 30 embryos per super-ovulated females. All rabbit ESC line produced so far showed the characteristics of primed pluripotency. In this work, we investigated the conditions suitable for the generation of naïve ESCs in rabbits. A first experiment aimed to study the effect of the pharmacological inhibitor of MEK signaling (PD0325901) on ESC line derivation. ICMs were isolated by immunosurgery from 576 blastocysts and plated onto gelatin- or fibronectin-coated dishes in various media (DMEM/F12 + 20% KOSR, N2B27) supplemented with GSK3β inhibitor (CHIR99021), PD0325901, and LIF (2i/LIF), or not. 80% of the ICMs plated, but none could be expanded beyond passage 2. We concluded that inhibition of MEK signaling fails to prevent spontaneous differentiation of pluripotent stem cells in rabbit. A second experiment aimed to study the effect of LIF on ESC line derivation. We collected 262 ICMs, which were plated onto growth-inactivated mouse embryonic fibroblasts in DMEM/F12 supplemented with 20% KOSR (72), 20% KOSR + LIF (90), or 10% KOSR + 10% FCS + LIF (80). No outgrowth cultured in media lacking LIF could be expanded beyond passage 4. By contrast, 7 ESC lines were derived from outgrowths cultured and expanded in the presence of LIF, 2 in 20% KOSR + LIF, and 5 in 10% KOSR + 10% FCS + LIF. Two lines, designated rbES-LIF1 and rbES-LIF2, were expanded by gentle dissociation with collagenase until passage 40, and showed a normal karyotype. RbES-LIF1 and rbES-LIF2 displayed the cardinal features of pluripotent stem cells, *i.e.* expression of pluripotency markers, differentiation into derivatives of the 3 germ layers, and teratoma formation. However, they could not be cultured onto gelatin-coated dishes, they did not express markers (*Zfp42*, *Klf4*, *Pecam1*, *Dazl1*) associated with naïve pluripotency in rodents, and they did not survive in 2i/LIF medium. We concluded that LIF facilitates the derivation of ESCs but does not support naïve pluripotency in rabbits. When rbES-LIF1 and

rbES-LIF2 cells were propagated for 20 passages in 10% KOSR + 10% FCS + LIF, and were enzymatically dissociated with Acutase into single cell suspensions, they acquired chromosomal abnormalities (43XX; 45XY). The same observation was made with 5 freshly derived ESC lines in 10% KOSR + 10% FCS + LIF with Acutase, of which 3 displayed abnormal chromosome numbers. We concluded that LIF-dependent rabbit ESCs cannot be propagated under stringent conditions without chromosomal rearrangement-based adaptation.

Characterization of Multipotent Stem Cells from Rabbit Amniotic Fluid

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Abstract

A rabbit to serve as a good biological model for *in vivo* tissue engineering (embryonic, germinal or adult stem cells) experiments. Amniotic fluid-derived multipotent stem cells are newly described, excellent seed cells that have good differentiation capability and are convenient to obtain and characterize.

The aim of our preliminary study was describe a method of isolation of rabbit multipotent stem cells from the amniotic fluid and examine the phenotype of these cells using flow cytoemtry. New Zealand White rabbit amniotic fluid was recovered from uterus about 23 day of gravidity using sterile syringe. The composition of the culture medium for the amniotic fluid stem cells was as follows: EBM-2 basal medium (CC-3156, Lonza) supplemented with 20% fetal calf serum (FCS); recombinant growth factors: bFGF, EGF, R3-IGF-1; vitamin C (all are part of the medium kit); penicillin and streptomycin (15140122, Life Technologies). Rabbit amniotic fluid was mixed with the culture medium in proportion of 5:6, respectively. 5 ml of this mixture was then transferred into a T25 tissue culture flask. Approximately 5 days following plating, colonies af adherent cells start to be visible. At this point, a medium change is performed and from this point onwards, the medium is changed every day. On day 10, the adherent outgrowths are after washing with PBS harvested using 2ml of Accutase (A1110501, Invitrogen) for 5 min at 37°C and 5% CO2. The cells were counted and replated for the purpose of expanding the cell numbers. We found the optimum density of the cells to be $6\text{-}8\times10^4/cm^2$ of cell culture surface.

Our preliminary resutls show rapid proliferation of amniotic fluid-derived adherent cells. We found two predominant types of morphology in the cultures of rabbit amniotic fluid stem cells – "spindle-shaped" and "tile-shaped". We hypothesize that these cells have a broadly multipotent mesenchymal stem cell phenotype based on their morphology, the expression of CD44 (Slamečka and Chrenek, 2013). Furthermore, these cells can potentially serve as an excellent and amenable cell source for reprogramming experiments into naïve induced pluripotent stem cells.

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Sperm mediated gene transfer in rabbit

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Abstract

Fresh collected, extender washed rabbit sperm cells were co-cultured with linear 14.3 kb gene construct and subsequently they were used for artificial insemination. The gene construct used for sperm mediated gene transfer (SMGT) consisted of a 2.5 kb murine whey acidic protein promoter (mWAP), 7.2 kb cDNA of the human coagulation factor VIII (hFVIII) and 4.6 kb of 3´ flanking sequences of the mWAP gene. The plasmid pPolyII-D was digested with *Not I* to release the 14.3 kb insert and gel purified in a QIAEX. Transgene integration in rabbit genome was verified by PCR analysis. Litter size of does inseminated with DNA treated sperm was in normal range (9.1±1.5). In total 86 live born and 5 dead born animals were screened for transgene presence. Number of genetically modified youngs after SMGT varied from 12.5 % to 55.5 %. Results of PCR demonstrate transmission of transgene to next generation. This shows that sperm mediated gene transfer may be an effective method for production of genetically modified rabbits.

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The effect of different passages of donor cell on the efficiency of rabbit somatic nuclear transfer

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Abstract: **(objective)** the time and cultivate passage of donor cells in vitro culturing has a significant effect on the efficiency of somatic cloning. (methods) we carry on a research to 5-10 low generation group and 20-25 high generation group of rabbit fibroblasts by nuclear transfer, **(Results)**and found that the efficiency of transferred donor nuclei in two experimental groups are differences (Table1). Through comparison of the fusion rate, cleavage rate, blastocysts rate and in vivo development of the reconstructed embryos after donor cell nuclear transferring, relatively low generation was found more efficient, but high generation can also get normal cloned offspring (Table2). **(Conclusion)**This study verifies the influence of different passages cells of in vitro culturing on the efficiency of rabbit cloning, and provides basis for cloning gene targeting rabbits.

Passages of donor cell	NO. of reconstructed	NO. of fusing oocytes	NO. of cleavage	NO. of blastocyst
5-10	281	197(70)	147 (74)	85(43)*
20-25	118	103(87)**	86 (83)	33 (32)

Keyword: Rabbit; adult fibroblast; nuclear transfer; cell passage

Table 1 The number of fusion、cleavage and blastocyst of reconstructed complex after in vitro culturing
*Values with the superscripts in same column are significantly different ($P < 0.05$).

Table 2 *In vivo* development of clone embryo with different passages donor cell

Item	group 5-10	group 20-25
No. of embryos	145	214
recipients	5	11
Pregnancy recipients	3	5
Full term fetus (%)	7（5%）	4（2%）
Alive kit at wean（%）	2	1

The WHHLMI rabbit is a good BioResource for studies about hypercholesterolemia, atherosclerosis, and coronary heart disease.

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Abstract

[Purpose] One of the important roles of experimental animals is contribution to translational researches for human diseases. In hypercholesterolemia and atherosclerosis, the pathophysiologic features of mice and rats are largely different from those of humans. In contrast, pathophysiologic features of WHHL or WHHLMI rabbits resemble to those of human familial hypercholesterolemia. In Kobe University, WHHL or WHHLMI rabbits have been provided to many researchers in the world. In the present study, we would like to introduce features of WHHLMI rabbits and the activity as a bioresource center.

[Methods] After over 15 hours of fasting, serum lipid levels were measured at 2, 6, 12, 18, and 24 months old (mos). Plasma lipoproteins were fractionated with ultracentrifugation. Atherosclerotic lesions and myocardial lesions were evaluated histopathologically after the death. WHHLMI rabbits were bred using a selective breeding, because development of coronary plaques and myocardial infarction is controlled by poly genes in rabbits. Selective breeding was carried out using the offspring derived from rabbits showing myocardial infarction and/or severe coronary atherosclerosis. Examination of pathogenic organism was carried out on 9 species every year. Rabbits have been provided under agreement to our MTA and import permission issued by the Government of recipient country is necessary to export rabbits or the fertilized eggs from Japan.

[Results and Discussion] The serum cholesterol levels were 874±8 (mean ± SEM) mg/dl (2 mos), 1066±9 mg/dl (6 mos), 879±11 mg/dl (12 mos), 783±18 mg/dl (18 mos), and 728±23 mg/dl (24 mos). Coronary atherosclerosis was developed from 2 mos, and was detected in all rabbits from 4 mos. The most severe cross-sectional narrowing was about 80% at 12 mos and about 90% at 18 mos. The coronary plaques were characterized various features as similar to humans, such as xanthomatous lesion (fatty streak), fibrous lesion, fibroatheroma, thin-capped fibroatheroma with a large

lipid core (vulnerable plaque), and advanced lesion. The cumulated incidence of myocardial infarction was about 60% in 20 mos and more than 80% in 30 mos in WHHLMI rabbits. In addition, WHHLMI rabbits with hyperinsulinemia showed insulin resistance and accumulation of visceral fat. WHHL and WHHLMI rabbits have been used development of hypocholesterolemic and/or anti-atherosclerotic compounds, in addition to clarify the mechanism of suppression of coronary events. Recently, acute coronary syndromes were induced pharmacologically in WHHLMI rabbits. WHHLMI rabbits are free from infectious diseases. Until now, more than 4,000 WHHL or WHHLMI rabbits were provided from Kobe University and more than 640 research papers were published in international journals.

[Conclusion] WHHLMI rabbits are a useful bioresource for translational researches about hypercholesterolemia, atherosclerosis, and coronary heart disease.

Probucol stabilizes the atherosclerotic plaques in WHHL rabbits

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Probucol is a lipid-lowering drug with anti-oxidation effects and often prescribed for the treatment of familial hypercholesterolemia. However, it is not known whether probucol can stabilize the lesions of atherosclerosis. In the current study, we examined this hypothesis using WHHL rabbits, a model of human familial hypercholesterolemia. Male WHHL rabbits at 3-month-old were treated with either 0.3% probucol (n=6) or 0.02 atrovastatin (n=5) for 16 weeks and their plasma lipids and aortic lesions were compared with control group (n=5). We found that probucol treatment reduced plasma cholesterol levels but less remarkably than atorvastatin treatment. In spite of this, probucol treatment led to prominent reduction of aortic en face lesions by 39% ($P<0.01$) whereas atorvastatin reduced by 16% ($P>0.05$) compared with control. Histological examinations revealed that the aortic lesions of probucol-treated rabbits were characterized by reduced macrophage, increased smooth muscle cells, and increased fibrosis compared to both control and atorvastatin groups. Furthermore, probucol treatment reduced coronary artery stenosis and increased plaque stability. These results suggest that probucol treatment may be beneficial for the plaque stability of hypercholesterolemic patients.

The Oct4 promoter-EGFP transgenic rabbit: A new model for monitoring pluripotency of rabbit stem cells

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Abstract

Background: The rabbit has long been used as a laboratory animal model for developing reproductive and stem cell-related technologies, as well as for studying human disease. However, germ line-competent pluripotent rabbit stem cells have not yet been reported. The Oct4 transcription factor plays a crucial role in the maintenance and regulation of pluripotency in embryos and stem cells.

Methods: We constructed a reporter plasmid containing the gene encoding the enhanced green fluorescent protein (EGFP) under the control of the rabbit Oct4 promoter (prOG) and transfected it into E14 mouse stem cells. In addition, prOG transgenic fibroblasts were derived and prOG transgenic rabbits were produced by somatic cell nuclear transfer (SCNT).

Results: Ectopic EGFP was expressed at similar levels in E14 mouse stem cells whether under the control of the rabbit (prOG) or mouse Oct4 promoter (pmOG). Both prOG transgenic SCNT embryos and F1 prOG transgenic embryos derived from adult transgenic rabbits expressed green fluorescence at the morula and blastocyst stages. EGFP was also expressed in the testis of newborn prOG transgenic rabbits.

Conclusion: The prOG transgenic rabbit represents a new model for studying the derivation and maintenance of rabbit pluripotent cells, and for investigating rabbit embryo development.

Keywords: Oct4, rabbit, stem cell, EGFP, development

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Polymorphisms of *GnRHR*、*FSHβ* and *GDF9* Gene and Their Relationships with Reproductive Traits in Meat Rabbits

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Objective: Meat rabbit's reproductive traits' heritabilities are very low and conventional breeding techniques are difficult to improve them. In order to improve these traits, this study aimed at finding the SNPs in *GnRHR*, *FSHβ* and *GDF9* gene for molecular marker assisted selection.

Method: In this study, 227 meat rabbits including163 come from Kangda strain 2 and 64 from Kangda strain 7, were selected and cultivated by Qingdao Kangda Ltd and Shandong Agricultural University to detect genetic variants by Mix-sequencing and PCR-SSCP. SAS and R software were applied to analysis allelic frequency and relations between reproductive traits and different genotypes.

Results: For *GnRHR* gene, two mutations were found in exon1 and 3---T229A and TG insertion into 13430bp. For T229A mutation on the first parity TNB and later parity NAB in strain 2, the genotype BB was 1.23 and 1.56 more than AA as a significant effect ($P < 0.05$). The mutation inserted into the base of TG in 13430bp in strain 7 on 21 day's weight, weaning weight, weaning number, first parity TNB has a significant effect ($P < 0.05$). The genotype FF were higher than EF on 21 day's weight, weaning weight, weaning number, first parity TNB for 80.1g, 362 g, 3.61, 2.75 respectively.

For *FSH β* gene, two mutations were found in exon2---T695C and A919G. The T695C mutation has a significant effect ($P < 0.05$) in strain 2 on later parity TNB and NAB, as the genotype MM was higher than NN for 1.75 and 1.81. While in strain 7, the mutation has a significant effect ($P < 0.05$) on the weaning weight trait. The genotype MM was higher than NN for 161g. The A919G mutation has a significant effect ($P < 0.05$) in strain 2 on first parity TNB, later parity TNB and NAB. On born weight and first parity TNB, CT was higher than TT for 81.75g and1.17. On later parity TNB and NAB, CT was higher than CC for 1.17 and 1.34.

For *GDF9* gene, a gene mutation was found in exon2---T1650C. For the mutation on weaning weigh and number in strain7, GH was 328 g and 3.31 more than HH as a significant effect ($P < 0.05$).

Conclusion: The study of *GnRHR, FSHβ* and *GDF9* gene detected five mutations and analyzed their relationship with the meat rabbit's reproductive traits. The results show that these three genes could be or cooperate with major genes to

affect meat rabbit's reproductive traits. Under the certain conditions, these five mutations could be used in the meat rabbit selecting for high reproduction traits in molecular marker assisted selection programs to improve the level of prolificacy.

Key Words: Meat rabbit, *GnGHR*, *FSHβ*, *GDF9*, Reproductive traits, SNPs

Creation of macrophage-specific overexpression of human urotensin II transgenic rabbits

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Objective Urotensin II (U II) has been implicated in atherosclerosis. To define U II functions in vivo, we generated transgenic rabbits that expressed human U II gene under the control of a macrophage-specific promoter, the human scavenger receptor promoter.

Methods U II transgenic rabbits were created by microinjection method. We compared the follicle-stimulating hormone (FSH) administration protocol and the pregnant mare serum gonadotropin (PMSG) administration protocol in collection of more zygotes.

Results The results showed that the FSH administration protocol was better than the PMSG administration protocol in collection of more zygotes. We successfully established the U II transgenic rabbit model by microinjection method.

Conclusion We have successfully created U II transgenic rabbits by microinjection method and provided a powerful tool for studies on U II biological function.

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Comparison of heritability and repeatability of pre-weaning traits in four rabbit lines selecting as maternal lines in China

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A five-year breeding project involving HPLUS (GP and PS), Champagne Rabbit and Taishan White Rabbit was carried out to produce two maternal lines (KD Ⅰ and KD Ⅱ) and three paternal lines (KD Ⅲ, KD Ⅳ and KD Ⅴ) in Shandong Province, China. Another two pure rabbit breed Californian Rabbit (CAR) and New Zealand White Rabbit (NZW) were selected at same time as candidate lines.

A total of 4422, 13222, 2430 and 6267 litters from 818, 1761, 361 and 916 does from KD Ⅰ, KD Ⅱ, CAR and NZW, respectively, were used to evaluate the heritability and repeatability of pre-weaning traits in this study. Doe traits such as conception rate (CR) and milking capacity (MC); litter traits such as litter size and weight at birth (LSB and LWB), 21 d (LS21 and LW21) and weaning at 28 d (LSW and LWW); new born alive (NBA) and litter survival rate at 21 d and weaning (SR21 and SRW) were studied in each lines separately. Data were analyzed using a repeatability uni-trait animal model to estimate the genetic parameters of each line using the software ASReml.

Estimates of heritability for most of studied traits in each line were low ranging from 0.0003-0.0911 except CR in KD Ⅱ and LW21 and MC in CAR were higher ranging from 0.1475 to 0.1602. Taking all the pre-weaning traits into account the heritability of the four lines nearly equals in most of traits and KD Ⅱ has a less narrow range than others. The heritability of LS and NBA ranging from 0.0393 to 0.0887 and there are little difference between CAR and NZW which are lower than KD Ⅰ and higher than KD Ⅱ; LWB ranging from 0.0483 to 0.0592 and have little difference among four lines; LWW (0.0118-0.0508) and SR21 (0.0241-0.0578) have similar distribution with LS and NBA in four lines; LSW (0.0121-0.0348) and SRW (0.0003-0.0180) of the two new maternal lines are lower than CAR and higher than

NZW; LS21 ranging from 0.0014 to 0.0509 and the two new maternal lines are higher than both CAR and NZW. The traits tested at birth and 21 d have higher heritability than weaning and traits related to litter weight have higher heritability than litter size. The range of repeatability of LSB, NBA, LWB, LS21, LW21, SR21, LA, LSW, LWW and SRW are as follows: 0.0961-0.1823, 0.0980-0.1722, 0.0858-0.01838, 0.0044-0.0827, 0.1646-0.2340, 0.0488-0.1131, 0.1548-0.2017, 0.0249-0.1408, 0.0416-0.1568 and 0.0180-0.0679. NZW has the highest value of repeatability in all traits except LS21. The values of repeatability of the two new maternal lines are very close and slightly higher than CAR. The repeatability of traits tested at birth have lower value than 21 d and have higher value than weaning in all lines just as we have found in heritability.

Overall, good agreement was observed among the four lines selected as maternal lines. From the previous estimates of heritability and repeatability, it is conducted that the selection of CR, LSB, NBA, LWB, LW21 and SR21 could be efficient to improve these traits in all four lines. KD Ⅰ could have the greatest genetic improvement than CAR, NZW and KD Ⅱ which could have the least genetic improvement with the same selection intensity.

Keywords: rabbit; maternal lines; litter traits; heritability; repeatability

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The Establishment of Immunosuppressive Model for Fujian Rabbit

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Abstract: In order to develop an immunosuppression animal model with easy replication and good economic application which also applicable in the field of traditional Chinese medical research. At the same time, it provided a new kind of experimental basis for Fujian rabbit. The experiment took the hydrocortisone as injection, 6 experimental groups, 1 control group and five dosage groups (at 20, 25, 30, 35, 40 mg/kg daily respectively), CD_4, CD_8 cell content would be examined by flow cytometry and spleen was removed in order to calculate the spleen index. The results showed that, after injection, CD_4 and CD_8 cell in B, C dosage group (25, 30 mg/kg respectively) had gone down obviously which demonstrated that they were the most appropriate injection dosage. There were different degrees of reduction in weighted spleen of dosage groups when compared with the control group, which implied the immunity function had damaged. Then, B, C, D group showed a more significant level of decline, especially with group C. It confirmed that the C group was the most appropriate injection dosage in immunosuppression animal model from this research.

Key words: Fujian Rabbits; immunosuppression; T-lymphocytes; spleen index

Study on the Sensitivity of the Fujian Rabbits Response to Bacterial Endotoxin

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Abstract: To obtain the body temperature instance and sensitivity response to bacterial endotoxin of the Fujian rabbits, and to discuss whether the Fujian rabbits can be adapted to suit the use of pyrogen test. According to the standard pyrogen test from 'Chinese Codex', to choose Fujian rabbits with the eligible body temperature, which were administrated with three doses of endotoxin (5 Eu/mL, 10Eu/mL, 20Eu/mL) to analyze the sensitivity of these rabbits response to endotoxin. The 65.85% of the tested Fujian rabbits were up to the mustard and the body temperature of most rabbits was 38.5-39.5℃. The eligibility whereas under the Japanese White rabbit, WHBE and SPF New Zealand rabbits. The sensitivity of the Fujian rabbits response to bacterial endotoxin was positive correlativity to the dose of endotoxin, the big dose resulted to the high sensitivity. Through the test, it was known that most of the Fujian rabbits' body temperature was steady, which had some sensitivity to the bacterial endotoxin. If the Fujian rabbits were fed in a standard way of laboratory animal, the quality of rabbits would more stable, and these rabbits would fit for the pyrogen test, which could broaden out the Fujian rabbits' application field.

Key words: Fujian rabbits; bacterial endotoxin; sensitivity; rabbit pyrogen test

Equilibration temperature before vitrification effect the morphology, zona pellucida and developmental competence of human mature oocytes

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Abstract

Objective: To determine the changes of morphology , zona pellucida and developmental competence of human mature oocyte following vitrification at different equilibration temperatures.

Design: Randomized, comparative study.

Setting: University-affiliated hospital.

Patient(s): Thirty-seven women undergoing infertility treatment in reproduction center of Ruijin hospital, all of the mature oocytes at retrieval were vitrified on account of no spermatozoa.

Intervention(s): Human mature oocytes were vitrified and warmed at 37℃ or room temperature(RT), group A:vitrification 37℃/warming 37℃, group B:vitrification RT/warming RT. The oocyte morphological parameters were evaluated before and after oocyte vitrification using OCTAX EyeWare software. The time taken to dissolve the zona pellucida by enzyme-digestion was evaluated also.

Main Outcome Measure(s): Equilibration time, Oocyte morphological parameters, dissolution of zona pellucida, embryo development, and clinical pregnancy.

Result(s): A total of 480 oocytes were vitrified. During vitrification, the equilibration time of group A was significantly shorter then group B. Oocyte survival, fertilization and cleavage rate were similar in two groups, however, a statistically significantly higher embryo available rate, implantation rate and pregnancy rate were observed in group A compared with group B ($P < 0.05$). There was no significant difference in oocyte morphology obtained for each oocyte parameter and each type of vitrification ($P > 0.05$). The time required for the dissolution of the zona pellucida was statistically

significant different between the group A and B ($P < 0.001$).

Conclusion(s): The present study demonstrated that vitrification/warming at 37°C can cut down equilibration time, improve the embryo available rate, implantation rate and pregnancy rate, reduce the influence of the vitrification upon the dissolution time of the zona pellucida. At the same time, the vitrification has no effects on oocytes morphological parameters.

Key Words: Human mature oocytes, vitrification, Oocyte morphology, zona pellucida

Fatty Acid Analysis of Desaturase Transgenic Rabbits

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Objective: the purpose of the study is to analyze the expression of fatty acid desaturase transgenic rabbits produced by testicular injection.

Method: firstly using PCR to detect the integration of exogenous gene in transgenic rabbit of all the 425, and then detect the relative content of the fatty acids by gas chromatography for all PCR-positive transgenic rabbit (182/267). All the n-6 PUFAs and n-3 PUFAs ratio of F0，F1 and F2 generations were analyzed through SAS9.2 program between experimental and control group as well as the genetic expression of three generations, different ages and tissues.

Results: the study shows that there are no significant difference ($P>0.05$) of the n-6/n-3 in muscle and liver. The n-6/n-3 of liver, muscle, ear tissue, kidney and blood of the weaning are higher than the 120 days. Among these five of the weaning, ear tissue of n-6/n-3 is the lowest, and then muscle, blood, kidney and liver. The n-6/n-3 of the F0 experimental group between muscle and liver are significantly lower than the control group ($P<0.01$)，and dropped from 13.19 and 10.96 to 5.30 and 4.91. The n-6 PUFAs of F0 transgenic rabbit's muscle and liver, the C18:2n-6 of experimental group is significantly lower than the control group ($P<0.01$). The C18:3n-3 of experimental group is significantly higher than the control group ($P<0.05$). Besides, C20:5n-3, C22:5n-3 and C22:6n-3 of liver are significantly higher than the control group ($P<0.01$). What's more, C22:6n-3 of muscle is significantly higher than the control group ($P<0.05$).

The n-6/n-3 of F1 transgenic rabbit in the muscle and liver are significantly lower than the control group ($P<0.01$), dropped from 13.19 and 10.96 to 3.35 and 3.43. Additionally, the n-6/n-3 of F2 ear tissue is significantly lower than the control group ($P<0.01$), dropped from 2.87 to 1.06.

The comparison of n-6/n-3 of muscle and liver between F0 and F1 shows that there are no significantly difference ($P>0.05$). However, to liver, the n-6/n-3 of F1 are lower than F0 ($P<0.05$).

Conclusion: N-3PUFAs can be synthesized from n-6 PUFAs in transgenic rabbits catalyzed by exogenous gene Δ15 which is stably inherited to the next generation. It doesn't work in weaning period, and maybe it's caused by the age limitation. Besides, we found that individual transgenic effect can be represented by any tissue, and this study provides a theoretical foundation for the relationship between exogenous gene position of chromosome and its expression. We have obtained 67 transgenic rabbits rich in n-3 PUFAs, and it proved the efficiency and practicality of the testicular injection in producing transgenic rabbit.

Key words: Transgenic; Rabbits; Fatty Acid; Gas Chromatography

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Performance of rabbits fed diets containing genetically modified corn.

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Genetically modified (GM) crops offer a wide variety of benefits to producers including resistance to insects, diseases and herbicides. Slovakia ranks among other seven EU countries, which have practical experience in GM maize cultivation.
The objective of this work was to determine the effect of selected maize hybrids on live weight growth, feed conversion and health of rabbits after feeding the complete feed mixtures with 12 % proportion of MON 88017, MON 88017 x MON 89034, isogenic maize and reference maize (LG 3475). It was tested on 120 broiler rabbits (Hycola).
MON 88017 - contains two novel genes, *cry*3Bb1 for insect resistance, and *cp4 epsps*, which confers tolerance to glyphosate. MON88017xMON 89034 - Maize resistant to Lepidoptera and Coleoptera and tolerant to glyphosate herbicide (*cry1A.105*, modified *cry2Ab2*, modified *cp4 epsps*, modified *cry3Bb1*)
The testing period lasted from weaning at day 35 to day 77 of animals' life. The trial ended with slaughter (LW≈2500g). The average daily weight gains were 41.9g and 36.9g resp., with a daily feed consumption 129.6g and 130.6 g, which gives a feed conversion rate 3.43 and 3.49 resp. The results of chemical analysis MLD muscles at the age of 11 weeks showed the high content of total proteins (21.83-22.43 g. 100 g^{-1}) and very favourable content of intramuscular fat that did not exceed 0.93-1.8 g.100 g^{-1}. Energetic value does not overstep the value of 410.9-433.5 kJ.100 g^{-1} MLD. Data were analysed using one-way ANOVA. Differences between the groups were determined by t-test. Obtained results demonstrate minimal, statistically no significant differences in individual nutrient digestibility in tested mixtures and performance, physical and chemical characteristics of meat in MLD muscle substance, and caecal fermentation pattern of rabbits.
On the basis of our results we can summarize that genetically modified maize in rabbit nutrition did not have a negative influence on the health status of animals. It had no negative effect on growth performance of rabbits and influence of body weight as well as. Bt maize deteriorated neither the health in animals nor the production of animal proteins valuable for human nutrition compared with conventional maize.

Rescue death transgenic rabbit by Intracytoplasmic Sperm Injection and Electric Activation

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ABSTRACT

Objective: the aim of the present study was production of full-term development rabbit from the death GFP rabbit sperm and carried out to investigate the effect of electric stimulation before intracytoplasmic sperm on rabbit oocyte activation.

Methods: Mature New Zealand rabbits were superovulated by injection of HCG, and oocytes were collected at 14-15 hr. post–HCG injection and fertilized by microinjection of a single sperm from a death heterozygote GFP rabbit into the ooplasm of each oocyte. After ICSI, the zygotes were cultured in B_2 medium for 4 days or transferred into oviducts of recipient does at the 2-cell or 4-cell stage. Series electric pulse procedures were applied to activate the oocytes before or after ICSI operation.

Results: the embryos in group without electric treatment, showed the second polar body extrusion and pronuclear formation at 5-7 hours after ICSI, but the electric treatment group showed just at 3-4 hours. Evaluation on blastocyst rate, there is no difference between once shock and twice shock group before sperm injection (34% VS 37%), but the two groups are significantly higher than the other groups. In the two sham ICSI groups, twice shock before ICSI appeared higher parthenogenetic development (40 %VS 0%, respectively $P<0.05$). In addition, An average of 23% of the blastocysts express the GFP fluorescence in the once electric shock before ICSI group, which was significantly more than that of the twice electric shock (12%) and without treated group (10%)($p<0.05$). Once shock before and after group, and once shock after group showed poor embryo survived rate, the survive rate was less than 50%. Ninety three ICSI embryos from once shock before ICSI group were transferred into four recipients. One of the four recipients was pregnant and a full term fetus was delivered.

Conclusions: electric stimulation before Sperm injection was an effective method to activate the oocyte. The sperm from the death rabbit could support the full term development of ICSI embryos.