2012 INTERNATIONAL SYMPOSIUM ON

ANIMAL BIOTECHNOLOGY, SHINSHU UNIVERSITY

Special Lecture:
Ms. Sarah Marie Cummings (Masuichi-Ichimura Sake Brewery)

Plenary Lectures:
Professor Dr. Robert Kneller (The University of Tokyo)
Professor Dr. Shosei Yoshida (National Institute for Basic Biology)
Professor Dr. Jianzhi Pan (Zhejiang Academy of Science)
Associate Professor Dr. Katsuhiko Hayashi (Kyoto University)
Professor Dr. Koh-ichi Hamano (Shinshu University)
Senior Researcher Dr. Takahiro Tagami (National Institute of Livestock and Grassland Science)
Researcher Dr. Tamas Somfai (National Institute of Livestock and Grassland Science)
Professor Dr. Kohzy Hiramatsu (Shinshu University)
Assistant Professor Dr. Shinichi Yonekura (Shinshu University)
Researcher Dr. Yoshiaki Nakamura (National Institute for Basic Biology)
Professor Dr. Hiroshi Kagami (Shinshu University)

International Symposium on Animal Biotechnology, Shinshu University

Date: January 31, 2012
Venue: Faculty of Agriculture, Shinshu University
Host: Shinshu University

Advisory Board:

Dean & Professor Shoichiro Nakamura, Faculty of Agriculture Shinshu Univ
Director & Dr. Takashi Nagai, National Institute of Livestock and Grassland Science
Professor Shun Nakamura, Tokyo Univ of Agriculture and Technology

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Member: Assistant Professor Shinichi Yonekura, Faculty of Agriculture Shinshu Univ
Member: Assistant Professor Takeshi Kawahara, Faculty of Agriculture Shinshu Univ
Member: Assistant Professor Masaki Ihara,Faculty of Agriculture Shinshu Univ
Member: Assistant Professor Takafumi Watanabe, Faculty of Agriculture Shinshu Univ

Cooperated Participants:

The University of Tokyo, Kyoto University, National Institute for Basic Biology,
National Institute of Livestock and Grassland Science,
Zhejiang Academy of Agricultural Sciences, Masuichi-Ichimura Sake Brewery

Cooperated Academic Societies & Organizations:

American Society of Animal Science, Japan Science and Technology Agency (JST),
The Molecular Biology Society of Japan, The Society for Bio Technology Japan,
Japanese Society of Developmental Biologist, Society for Reproduction and Development,
Japanese Association for Animal Cell Technology, The Japanese Society of Veterinary Science,
Japanese Society of Animal Science, Japan Society for Reproductive Medicine,
Japan Livestock Technology Association

Governments' Support:

The Ministry of Agriculture, Forestry and Fisheries of Japan (MAFF),
Ina City, Minamiminowa Village

Warm Welcome

On behalf of the Organizing Committee and Shinshu University, I would like to welcome cordially all delegates and accompanying persons to the International Symposium on Anaimal Biotechnology.

We are very pleased that the delegates from USA, Canada, China, Hungary, Bangladesh and others including Japan attend this symposium. During this symposium, we will have the special lecture, plenary lectures and poster session. It is also worth worthy of mentioning that the presented research data included one of the most important academic findings of which some parts had been published in international top journals; *Cell, Nature Reviews , Science, Molecular and Cellular Biology, Development, Biology of Reproduction* and so on.

In addition the lectures on the animal biotechnology, leading scientist will discuss in university-industry cooperation, the role of start-ups in innovation, the discovery and commercialization of biomedical technologies on conflicts of interests associated with academic entrepreneurship. In recent years, the issue is one of the most important on research activities both in academia, research institutions and industries. Another feature of the international symposium is issue of revitalization of the local area by industries based on biotechnology. Special lecture will be presented by a speaker who has been restructured and revitalized traditional Japanese society based on international point of view by means of Sake and brewing that is the art of biotechnology.

We believe their presence and lectures on a relevant topic would make this international symposium very successful. Many young scientists will present their latest research achievements in the poster session. The interaction of the established scientists and young promising ones will activate the academic activity.

I wish this international symposium will be the beginning of epoch for researchers from all over the world. Thank you very much for coming to this International Symposium on Animal Biotechnology.

Yours sincerely,

Hiroshi Kagami, Ph. D.
Chairman of the International Symposium on Animal Biotechnology
Chairman & Professor of Interdisciplinary Graduate School of Science & Technology for Ph. D. Course, Shinshu University
Professor of Faculty of Agriculture, Shinshu University

Scientific Program

Opening Address

Chairman: Professor Hiroshi Kagami

9:10-9:30

Dean & Professor Shoichiro Nakamura

Plenary Session 1

Chairman: Associate Professor Yuji Takagi

9:30-10:00

Perspectives for Animal Biotechnology

…**Professor Hiroshi Kagami** (Faculty of Agriculture, Shinshu University)

10:00-10:30

Sex Preselection in Bovine

…**Professor Koh-ichi Hamano** (Faculty of Agriculture, Shinshu University)

10:30-10:40 Tea Break

Plenary Session 2

Chairman Assistant Professor Takeshi Kawahara

10:40-11:10

Animal Biotechnology in China

…**Professor Jianzhi Pan** (IAHV, Zhejiang Academy of Science, China)

11:10-11:40

Behavior of the Mouse Spermatogenic Stem Cells in the Testis

…**Professor Shosei Yoshida** (National Institute for Basic Biology)

11:40-13:00 Lunch

Plenary Session 3

Chairman Assistant Professor Shinichi Yonekura

13:00-13:30

Reconstitution of the Mouse Germ Cell Specification by Pluripotent Stem Cells

… **Associate Professor Katsuhiko Hayashi** (Graduate School of Medicine, Kyoto University)

13:30-14:00

Japanese and American Models for Commercializing University Discovery

…**Professor Robert Kneller** (RCAST, The University of Tokyo)

14:00-14:30

Improvement of Germline Transmission in Chimeric Chickens

…**Senior Researcher Dr. Takahiro Tagami** (National Institute of Livestock and Grassland Science)

14:30-14:40 Tea Break

Plenary Session 4

Chairman: Professor Tamamo Ono

14:40-15:10

Neuroendocrine Control in Animal Production

…**Professor Kohzy Hiramatsu** (Faculty of Agriculture, Shinshu University)

15:10-15:40

Molecular Mechanisms of Neuronal Connectivity

…**Assistant Professor Shinichi Yonekura** (Faculty of Agriculture, Shinshu University)

15:40-16:10

Cryopreservation of Porcine Oocytes and Zygotes

…**Researcher Dr. Tamas Somfai** (National Institute of Livestock and Grassland Science)

16:10-16:20 Tea Break

Oral Session

Chairman: Assistant Professor Takafumi Watanabe

16:20-16:50

Genetic Conservation at the Cellar Level in Chicken

…**Researcher Dr. Yoshiaki Nakamura** (National Institute for Basic Biology)

Special Lecture

Chairman: Assistant Professor Takafumi Watanabe

16:50-17:20 Special Lecture

…**Representative Director Ms. Sarah Marie Cummings**

(Masuichi-Ichimura Sake Brewery)

Closing Address

17:20-17:30

…**Professor Hiroshi Kagami**

Commemorative Get Together Photo

17:30-17:40

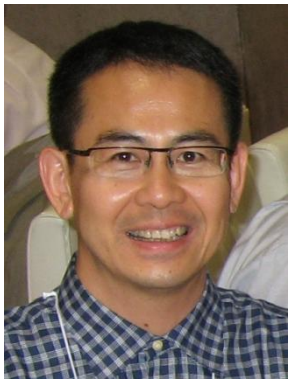
Poster Session

18:00-19:20

Dinner Party

19:30-21:00

Plenary Session



Hiroshi Kagami, Ph. D.

Chairman of The Department & Professor, Interdisciplinary Graduate School of Science & Technology for Ph. D. course, Shinshu University

Professor, Laboratory of Animal Developmental Genetics, Faculty of Agriculture, Shinshu University

Academic Major Interest: Avian Biotechnology by Use of the Stem Cells

Academic Background:

1987. 3. B. Sc. from Faculty of Animal Husbandry, Obihiro University, Majoring Animal Breeding &Genetics

1989. 3. M.Sc. from Graduate School of Agriculture, Nagoya University, Majoring Animal Breeding & Genetics

1992. 3. Ph. D. from Graduate School of Agriculture, Nagoya University, Majoring Animal Breeding & Genetics

Academic and Research Positions:

1992.6. Postdoctoral Fellow at University of Guelph, Canada, Majoring Avian Stem Cell Technology

1994. 6. Postdoctoral Fellow at National Institute of Animal Industry, Majoring Avian Stem Cell Technology

1998.4. Assistant Professor, Lab of Animal Genetics, Faculty of Veterinary Medicine, Azabu University

2000.10. Associate Professor, Lab of Animal Developmental Genetics, Faculty of Agriculture, Shinshu University

2005.6. Visiting Scientist, National Institute of Neuroscience

2006.10. Professor, Faculty of Agriculture, Lab of Animal Developmental Genetics, Shinshu University

2010.1. Invited Scholar, Graduate School of Agricultural and Life Sciences, Department of Veterinary Sciences, The University of Tokyo

2011. 4. Chairman of the Department & Professor, Ph. D. Course of Interdisciplinary Graduate School of Science & Technology, Shinshu University

2012.1. Chairman of the International Symposium on Animal Biotechnology

Research Awards and Gratitude:

1988.9. Certificate of Gratitude, XVIIIth World's Poultry Congress (awarded by President of the World's Poultry Congress)

1997.7. Goto Research Prize (awarded by Goto Hatchery Foundation)

2001.4. Research Funding Award for Excellent Research (awarded by Shinshu Agricultural Promotion Association)

2000.3. Best Poster Presentation Award (awarded by Japanese Society of Animal Science)

2001.10. Promising Research Award (awarded by Japan Poultry Science Association)

2003.11. Japan Prize in Agricultural Sciences (awarded by The Foundation of Agricultural Society of Japan)

2009.9. Outstanding Paper Award (awarded by Japan Poultry Science Association)

2010.9. Outstanding Paper Award (awarded by Japan Poultry Science Association)

Perspectives for Animal Biotechnology

Hiroshi Kagami

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Biotechnology could bring important changes in livestock production and veterinary medicine. In recent years, application of biotechnology in animal production has advanced very quickly. Many of all biotechnology research and development could expenditure to human health. At the experimental stage, a large number of drugs, diagnostic probes, vaccines etc. are frequently applied in livestock production prior to becoming available for use by humans. Therefore, pharmaceutical industry have had much interests for animal production since the biotechnology in this area are also applicable to human. One of the most promising application of the stem cell technologies should be regenerative medicine. The success could open up new frontier for the medical treatment of new century. An animal breeding, elucidation of the function and regulation of the economic genes of interests could increase the performance. Transgenic technologies would lead the production of human substrate in animal body, milk, muscle, egg and so on. To achieve these academic goals, novel strategies should be essential. Biotechnology could be applied for manufacture existing medicines relatively easily and cheaply. Human medicines designed to treat human diseases. In 1978, novel synthetic humanized insulin was produced in bacteria. The results contained big impact, since insulin have been widely used for the treatment of diabetes,. The insulin was previously extracted from the pancreas of animals such as dog, cat, monkey, cattle, pigs etc. Genetically modified bacterium enabled the production of much quantities of synthetic human insulin comparatively low cost as compared before.

In animal breeding and reproduction, biotechnology such as embryo cloning, stem cell regeneration, gene transfers, in vitro fertilization and sex alteration (Etches and Kagami, 1997) have been developed in livestock; cattle, swine, goat, chicken etc. The technological innovations will be very rapid for the next decades. Especially, stem cell technologies could be one of the most promising subjects in animal biotechnology. Martin and Evans (1975) isolated embryonic stem cells (ES cells) from ICM of mouse blastocysts. When the cells were injected into recipient embryos, somatic and germline chimeras were generated. It was suggested that the cells had pluriotency to differentiate into many cells types including germ cells, unlimited growth ability in culture and self-renewal capacity. These technology and finding largely contributed for the development of transgenic mice. Gene targeting and knockout led the detailed analysis of functional genes in higher animals. Mechanisms regulated functional genes alone cannot clarify how cellular traits are proliferated. Recent advances of the epigenetics have enabled us to reveal a molecular basis for how genetic information can affect gene function.

Embryonic stem cells (ES cells) were derived from human blastocysts. When the ES cells were microinjected into the recipient, somatic and germline chmimers could be obtained. Thus, it was considered that the ES cells could be one of the most useful cells for organ regeneration. More recently, it was found that retrovirus-mediated transfection with four transcription factors; Oct-3/4, Sox 2, KLF 4 and c-Myc, into mouse fibroblasts has generated induced pluripotent stem (iPS) cells (Takahashi and Yamanaka, 2006). The stem cells could open up new frontier for frontier medicine. The stem cells contained self-renewal ability and maintained pluripotentcy both in vivo and in vitro.The stem cells have been considered as one of the most useful tools for production of transgenic animals, generation of pharmaceutical substrates, sexual manipulation of offspring and conservation and propagation of endangered animals. Technical innovations of stem cells should lead the sexual manipulations and effective propagation of the animals. In poultry breeding, one of the most important subjects is

sexual alteration. Mixed-sex chimers were generated by injecting male donor cells into female recipient or vice versa. Gametes of the opposite sex could be produced in these chimeric gonads. The W- or Z sperm could be generated from female derived stem cells. Z ova could be generated from male derived stem cells. Chicken offspring was generated with sex-reversed Z-ova derived from male and with Z-sperm derived from female. Generation efficiency of the functional gametes of opposite sex largely depend on strain preference of the donor and recipient.

Avian stem cells holding puluripotency could be isolated from area pellucida of stage X embryos and could be cultured in vitro. Many type of tissues and organs could be regenerated using the stem cells. Inter-specific chimeras between mice and chickens could be generated mixed mice derived ES cells with chicken recipient embryos. Regeneration of tissues and organs, cloning, and transgenesis (van deLavoir et al., 2006; Lyall et al., 2011) should be one of the most important subjects for the future. Cloned mice could be generated by using frozen sperm. Genetic conservation of endangered animals should be another important issue on animal biotechnology. Many business persons have been applicable techniques for whole body regeneration in mammalian species. Bioethics should be important for correct use of these advanced technologies. Accumulation of the scientific knowledge should open up new frontier for avian biotechnology (Kagami et al., 2006).

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Kagami, H., Mushika, T., Noguchi, T., Yamamoto, Y., Fujiwara, A., Yamakawa, N., Okuizumi, H. and Ono, T. (2006) Pluripotent cell culture engineering and the application for avian biotechnology. In: *Animal Cell Technology, Basic & Applied Aspects*, Iijima, S. and Nishijima, K. (eds.), pp. 135-141. Springer, Dordrecht.

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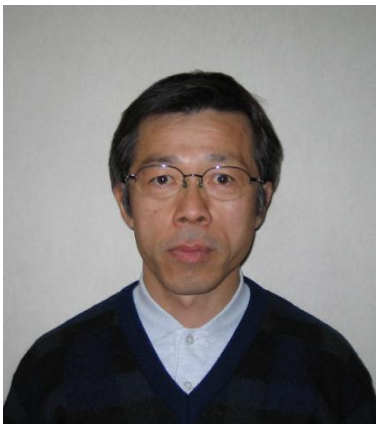
Martin, GR. and Evans, M.J. (1975) Differentiation of clonal lines of teratocarcinoma cells: formation of embryoid bodies in vitro. *Proceeding of the Natinal Academy of Science USA,* **72**, 1441–1445.

Takahashi, K. and Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell,* **126,** 663-673.



Koh-ichi　Hamano, Ph. D.
Professor, Faculty of Agriculture, Shinshu University, Japan

Academic Major Interest: Reproductive science by Use of mammalian sperm

Academic Background:
1980.3. Graduated from Iwate University, B. Sc.
1982.3. Graduated from Tohoku University, M. Sc.
1985.3. Graduated from Tohoku University, Ph. D.

Academic and Professional Positions:
1985.4. Researcher, Livestock Improvement Association of Japan
1998. 4. Associate Professor, Faculty of Agriculture, Shinshu University
2002. 4. Professor, Faculty of Agriculture, Shinshu University

Research Award
2006 Japanese Society of Animal Reproduction, Innovative Technology Award

Sex Preselection in Bovine

Koh-ichi Hamano

Faculty of Agriculture, Shinshu University, Japan

Introduction: Since the feed price soared and the infectious disease of livestock has broken out, the situation surround livestock production is very severe. The decline in the conception rate of the cow after artificial insemination (AI) caused the livestock productivity slowdown, and is a serious problem. Establishment of sex preselection technology which can improve and increase livestock efficiently has so far been desired. At present sex preselection technology is a stage of spread. Here, the flow cytometric sex preselection and the characteristic of separated sperm are outlined.

Sex preselection technology in bovine: Sex preselection technology in bovine has carried out with both embryo and sperm levels. If the sex of confirmed embryos is not desired, it is not economical. In the sperm level, sex predicted offspring are obtained with separated X, Y sperm by AI. Selected semen is applicable to AI like the usual ejaculated semen.

X, Y sperm separation in bovine: For separation of X, Y sperm, the differences between X and Y sperm was investigated and examined for size, density, antigen, surface charge and DNA contents. Now, the most confidential separation method is flow cytometric sorting based on the differences of relative DNA contents between X and Y sperm. X chromosome of bovine is larger than Y chromosome. The relative DNA content of bull X sperm is about 3.8 % higher than Y sperm. In 1989, Johnson et al. firstly succeeded in sex preselection in rabbit by surgical insemination of flow cytometric separated X, Y sperm. Now, X, Y sperm of many animals can be separated with high accuracy.

X, Y sperm separation for artificial insemination in bovine: X, Y sperm separation by flow cytometer is international patent technology acquired the United States Department of Agriculture. U.S. XY inc. has the exclusive royalty of the technology. In Japan, two or more semen production supply organizations sign a contract with XY inc., and are commercializing. Recently, the high-speed flow cytometers only for sperm sorting have been developed and the separating efficiency is improving. As a result, 10 million or more X, Y sperms can sort out per hour with high precision. The sex predicted calves were born by more than 90 % of accuracy using AI of flow sorted X, Y sperm. Selected X, Y semen for AI has been distributed from 2007.

Characteristics of separated sperm: Since the processing from semen collection to freezing preservation might require a long time, it worried about the decline of the viability, motility, and the conception rate after AI. In actual, since dead sperms are eliminated, only live sperms are collected during flow sorting. As a result flow sorted semen show as same motility as usual frozen-thawed semen. The conception rate after AI with flow sorting semen to undelivered cow is about 50 %, and to delivered cow is about 30～40 %. At present, flow sorting semen is used for AI and embryo production. In the field, in order to raise both conception rate and embryo production rate, improvement of the inseminating methods is advanced. Specifically, the method of delaying the timing of AI from

usual and the method of the deep-uterine AI are carried out.

Conclusions: Use of flow sorted semen enables increase of planned production of calf and a profit. From now on, sorted semen production efficiency will be further improved by the sperm processing method, sperm preserving solution and the performance of a flow cytometer. Furthermore, development of the wholesale X, Y sperm selecting technology which can be replaced with the flow cytometer is desired.

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Education:
2000: Science of Biological production, Gifu University of Japan (Ph. D.)
1992: Science of Environmental Biology, Zhejiang Agricultural University (M.S.)
1989: Husbandry and Veterinary Science, Zhejiang Agricultural University (B.A.)

Scientific Career and Employment:
2009-present: Professor, Zhejiang Academy of Agricultural Sciences, China
2007-2009: Senior Research Scientist, RIKEN Bioresource Center, Japan
2005-2007: Research Scientist, RIKEN Bioresource Center, Japan
2002-2005: Collaborative Researcher, RIKEN Bioresource Center, Japan
2000-2002: Postdoctoral Researcher, Dept. of Agricultural Science，Shizuoka University.
1989-1996: Assistant Researcher, Zhejiang Academy of Agricultural Sciences, China

Major Publications:
1. Pan, J, Nakade, K, Huang, Y-C, Zhu Z-W, Masuzaki, S, Hasegawa, H, Murata, T, Yoshiki, A, Yamaguchi, N, Lee, C-H, Yang, W-C, Tsai, E-M, Obata, Y, and Yokoyama, K; Suppression of cell cycle progression by Jun dimerization protein 2 (JDP2) involves down-regulation of cyclin A2. Oncogene 29:6245-6256, 2010.
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Animal Biotechnology in China: Research and Development of Genetically Engineered Farm Animals

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Current situation of animal biotechnology research in China

As in other countries, various biotechnologies are investigated and applied in farm animal breeding and reproduction in china, such as artificial insemination (AI), embryo transfer (ET), cryopreservation, sexing, in vitro fertilization (IVF), embryo bisection, nuclear transfer cloning (NT) and transgenic technology. Nowadays, transgenic technology has been one of the most hotspots in biology in China. Although there are many ethical issues surrounding this technology, its application prospects in agriculture, medicine and industry has attracted great attention.

There are several methodologies that can be used for the production of transgenic animals, including: (1) microinjection of DNA into pronuclei of fertilized zygotes; (2) genes transfer by viruses into zygotes or embryos, (3) stem cell (ES or EG)-mediated gene transfer, (4) sperm-mediated exogenous DNA transfer, and (5) somatic cell nuclear transfer (NT)-based gene transfer. The early method that has been employed widely to produce transgenic animals is microinjection, which injected the DNA fragments purified from any mammalian expression vectors, even large BAC clones, into the male pronuclei of the zygotes. Chinese investigators have already successfully generated numerous transgenic animals, such as mice, rats, rabbits, pigs, sheep and cows by this method. However, the transgenic efficiency by microinjection is very low for the farm animals, especially for swine, which includes dark organelles in their zygote blocking the view of pronuclei under microscope during microinjection. Recent major strategy of transgenesis for farm animals has shifted to somatic nuclear transfer (cloning). The advantage of the new procedures allow scientists to add gene(s) into in vitro cultured cellular genomes at random or at a specific locus (gene target), which result in the production of transgenic animal at 100% efficiency. By this method, a gene target farm animal could be produced for the first time. Transgenic and/or gene target farm animals have successfully generated in a number of laboratories in china including PPARγ and Myostatin gene knockout pigs. However, transgenesis procedure is still not efficient and economical, due to inefficiency of the animal cloning because of erroneous reprogramming of donor genome in the reconstructed embryos by somatic cell nuclear transfer (SCNT).

Support system for animal biology research in China

Since the 1980s, China government has formulated a series of programs for science and technology research and development, with the strategic aim of improving China's competitiveness in science and technology in the 21st century. The Key Technologies Research and Development Program, the "863 High-tech Plan", the "973" basic research program, and the National Natural Science Foundation of China (NSFC) form the main body of state programs for science and technology. Over the past dozen years, the NSFC has subsidized thousands of diverse research projects and about 60,000 scientists working in basic research. Animal Biotechnology is one of major and prioritized research areas in china, supported by above-mentioned national research programs, local government research funds, and also private investments. Recently, the government established the Project of Breeding of New Variety for Transgenic Biology as a major project under the National S&T Major Projects Program. This project will been implemented with a great amount of government investment in the next 15-20 years. The program aims to gain genes of significant application value and independent intellectual property, to develop high-quality, high-yield and disease-resistant genetically-modified new varieties, and to improve the overall level of genetically modified agricultural organism research and industrialisation. The government had also produced documents

to regulate and safe-guard the researches and applications of transgenic animals and plants. In May 2001, State Council of China announced a new rule for transgenesis: Regulation on Safety Administration of Agricultural GMOs. In the early 2002, the Ministry of Agriculture issued three detailed regulations on the bio safety management, trade and labeling of GM farm products. China's scientific research system is a cooperative one, comprising the Chinese Academy of Sciences (CAS), schools of higher learning and local scientific research institutes. The Beijing-based CAS is China's highest academic institute and comprehensive research center in natural sciences. It has more than 100 research institutes throughout China, and Its academic divisions including mathematics and physics, chemistry, geography, biology, technological sciences. However, National as well as local agricultural institutes and universities are important forces in scientific and technological research of genetically engineered farm animals for agricultural, medical and industrial application purpose in China.

Research and development of genetically engineered animals in China

There are only limited researches in China for producing transgenic farm animals for human consumption by increasing growth rate, feed utilization and meat quality. Transgenic pigs with bovine growth hormone gene were produced and reproduced 4th generation with foreign gene transmission. The growth rate of the GH-transgenic pig is increased by 20% in comparison to the non-transgenic control. Scientists in CAS have also developed lines of transgenic triploid carp with growth hormone gene. The transgenic fish had 42% higher growth rate and 18.5% feed conversion efficiency than their non-transgenic counterparts. But at the present, no transgenic animals have been commercially approved for human consumption. The transgenic bio safety studies of the GM animals are underway.

Animal pharming, the process of using transgenic animals to produce human therapeutic proteins, is staking its claim in a lucrative world market. The transgene inserted in genome enables an animal to make a certain pharmaceutical protein in its milk, urine, blood or eggs. Transgenic animals for producing high value functional proteins for use in medicine and food are widely carried out in China. Several universities, institutes and companies have made great progress in development of transgenic animals for producing human therapeutic proteins. The institutions, which invested a large amount of manpower and finances to this research, are China Agricultural University; Shanghai Jiao Tong University; Shandong Agricultural University; Institute of Genetics and Developmental biology and Institute of Zoology of CAS; Qingdao Samuels Industrial & Commercial Co., Ltd; Shanghai Genon Bio-Engineering Co, Ltd and others. The therapeutic proteins they are interested are: human lactoferrin, lysozyme, lactoalbumin, β-interferon, hepatitis B surface antigen (HBsAg), antithrombin III, erythropoietin and so on. They have produced transgenic cloned cows, goats, rabbits and pigs to express foreign proteins in their milk or blood. For example, China Agriculture University has developed transgenic cows expressing either a human lactoferrin or lysozyme gene. Transgenic goats have been developed by Shanghai Genon Bio-engineering Co. Ltd with those two human protein coding genes. Recently, scientists in China Agricultural University revealed their transgenic cloned cows, which express 3.4g/l of human lactoferrin in the milk, at the highest level in the world. Presently, those transgenic cows have been approved for an enlarged field trial, and the human-like milk from dairy cows has been approved for animal testing. If certified, the milk would become China's first transgenic animal product in commercial use within a few years.

Animal biotechnology research in the Zhejiang Academy of Agricultural Sciences

Zhejiang Academy of Agricultural Sciences is a comprehensive non-profit agricultural research institution. It was established in 1911, and now has a total of 16 institutes including the Institute of Animal Husbandry and Veterinary Science. Its research is mainly directed towards basic agricultural research and applications, high-tech agricultural innovation and service mechanism, covering almost all aspects of agricultural sciences. In our research division, several studies are been planning for promoting the agricultural and biopharmaceutical applications of animal transgenic breeding since 2009. The studies undertook are mainly as following: 1) Establishment of efficient

transgenic technology for pig by use of lentiviral vector, BAC clones, intracytoplasmic sperm injection (ICSI) and nuclear transfer cloning. 2) Establishment of transgenic methodology for chicken and duck with lentiviral vector. 3) Development of tissue-specific promoter resource for producing transgenic chicken oviduct bioreactor and other applications. 4) Marker-assisted breeding of High quality pig strain based on western and Chinese local swine breeds such as the Duroc pig and Jinhua pig. 5) Technical studies for isolation and culture method of stem cells of pig and chicken.

We had tried to generate transgenic pigs and chicken by injection of fluorescent protein-expression lentivirus into the perivitelline space of porcine zygote, or the subgerminal cavity of chichen embryo. Recently we have obtained several pups/chick, born or hatched from fluorescent-positive embryos. Presence of whole gene expression unit within genome DNA collected from the transgenic pig individuals were verified by PCR detection. Further analysis for integration and expression status of the transgene is undergoing. These studies were largely supported by Japanese scientists of RIKEN BioResource Center, Shinshu University and so on, who kindly provided genetic engineering tools and important technical guidance.

Poultry oviduct bioreactors have been one foci of transgenic research in various countries including China, because of the outstanding egg-laying capacity and other advantages of chicken as bioreactor. However, the strategy to achieve high expression level of foreign gene in an oviduct-specific manner was remained as the main technical bottleneck. We selected the chicken ovalbumin gene 5' upstream regulatory region as a promoter candidate for future development of chicken bioreactors. Primary study on this aspect revealed that a 2081 bp fragment contained the ovalbumin gene core promoter and upstream sequence possesses the strongest expression activity and better tissue-specificity among all fragments at length of 959, 1381, 3140, 5474 and 6293 bp. This assay also reveals some potential sequence regions of enhancers, repressors and sequence-specific regulatory elements within ovalbumin promoter region.

For analysis the promoter's activity and tissue specificity in vitro and in vitro, we development a novel lentiviral T vector which contain two report genes luciferase and green flurorescent protein (Venus) linked by IRES (internal ribosome entry site). By use of this reporter vector, we can easily evaluate the activity and tissue specificity of any PCR-amplified fragments of promoters, based on observation of green fluorescent under the fluorescence microscopy, or according to those luciferase assay result, in vitro and in vivo.

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Behavior of the mouse spermatogenic stem cells in the testis

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In mammalian testis, numerous sperms are produced persistently for a prolonged period, which plays an essential role for the continuity of life. It is generally considered that 'stem cells' that are both capable of self-renewal and differentiation supports the continuity of spermatogenesis. 'Stem cells' are also considered to be crucial not only for steady state spermatogenesis but also for regeneration after damage or transplantation. However, it is still largely to be elucidated what is the cellular identity and characters of the 'stem cells' and how they behave in the testis.

We have been challenging this issue by means of live imaging and pulse labeling, which allow investigating the cellular behavior in undisturbed testis that undergoes steady-state spermatogenesis. These have shown us, slowly but steadily, how the stem cell functions are achieved. It has been suggested that, rather than a very limited number of defined 'stem cells', extended population of undifferentiated cells with different degree of self-renewing and differentiating abilities compose the functional stem cell compartment. Interestingly, it is largely influenced by the tissue situation (steady-state, regenerating after damage, or post-transplantation) how much the cell population is recruited to the active population with the stem cell functionality. Live-imaging revealed dynamic migration of stem and differentiating progeny. We are also investigating the microenvironment for the spermatogenic stem cells and uncovering that it is related to the vasculature that surrounds the seminiferous tubules. These findings may expand our view regarding the definition of 'stem cells' in the mouse spermatogenesis.

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Reconstitution of the Mouse Germ Cell Specification by Pluripotent Stem Cells

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During embryogenesis, PGCs segregate from the somatic cell lineage at an early developmental stage (Hayashi et al., 2007). In mice, PGCs are derived from the post-implantation epiblast around embryonic day (E)6.25 in response to BMP4 secreted by the extraembryonic ectoderm, a tissue adjacent to the epiblast. Around PGC specification, about six cells of the posterior proximal epiblast at E6.25 start to express Blimp1/Prdm1, a zinc finger transcriptional repressor, and these Prdm1-positive cells are lineage-restricted to become PGCs (Ohinata et al., 2005). Soon after specification, PGCs undergo a unique program of gene expression that regulates epigenetic reprogramming. These successive steps during PGC specification have been thought to be important for establishment of toti-potency in germ cell lineage. However, study of PGC specification has been limited, mainly due to a small number of PGCs, for example less than 50 at E7.5. Therefore, an in vitro culture system reproducing PGC specification steps in vivo would be desired.

Embryonic stem cells (ESCs) are pluripotent stem cells that self-renew indefinitely while maintaining the capability of differentiating into various types of cells including germ cells. Therefore, ESCs are a suitable souse for developing the in vitro culture system reproducing PGC specification. There have been several attempts to produce PGCs, and gametes, from ESCs in vitro (Geijsen et al., 2004; Hubner et al., 2003; Toyooka et al., 2003). Despite these efforts, the generation of healthy individuals from PGCs derived from ESCs has not been achieved. This is partly due to the inappropriate differentiation of ESCs into cells that have an epiblast-like state. Differentiation of ESCs into epiblast has thus far been accomplished by either monolayer culture without LIF, a critical growth factor for self-renewal of ESCs, or formation of embryoid bodies that largely mimic early post-implantation development.

We have recently developed an in vitro culture system in which ESCs systematically differentiate into epiblast-like cells (EpiLCs) and then PGC-like cells (PGCLCs) (Hayashi et al., 2011). The manner of differentiation from ESCs to PGCLCs via EpiLCs reproduces the manner of PGC specification in vivo. Compared with developing PGCs in vivo, the identical patterns of the transcriptome and of epigenetic reprogramming are observed in PGCLCs. PGCLCs are fully potent, since they differentiate into spermatozoa and in turn the fertilized eggs with the spermatozoa give rise to healthy individuals. These results demonstrate that this in vitro culture system allows the production of a huge number of PGCLCs almost equivalent to nascent PGCs. This will make it possible to perform several types of experiments, for example biochemical analysis, which had been limited due to an insufficient number of PGCs in vivo. In the International Symposium on Animal Biotechnology, I would like to discuss how we developed the in vitro culture system, and show perspectives including possible applications.

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Japanese and American Models for Commercializing University Discoveries

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Abstract

On the surface Japan and the United States have similar legal frameworks and university institutions for commercializing university discoveries. However, Japan relies primarily on established companies to develop university discoveries and bring them to market, while America probably relies mainly upon new companies (known as startups or university ventures). The reasons for science and engineering entrepreneurship being stronger in America than Japan are complex and relate to social and institutional factors and government policies.[1] Nevertheless, the ways universities and industries interact tends to reinforce the dominant role of established companies in Japan and the importance of startups in the US. In Japan, collaborative or joint research and co-application (co-ownership) of university discoveries is the dominant mechanism of technology transfer. In America, co-application of patents is rare. Established companies do engage in collaborative research with universities, but if they want exclusive rights to a university discovery, they have to negotiate a separate license. However, the vast majority of exclusive licenses from American universities are made to startups. Startups are probably the most important initial (first stage) development partner for American university inventors.

To describe the above in a bit more detail: Prior to 1998, Japanese university researchers had a tradition of transferring their discoveries directly to companies. The regulations governing this process and the laws that limited outside activities by university faculty made transfer to a startup extremely difficult. Thus the companies that cooperated with university researchers were almost all established companies. How the companies chose to develop the university discoveries was entirely up to them. Obligations to pay royalties for the discoveries, obligations to show proof they were developing the discoveries, pressure from venture capitalists or other outside inventors, were all absent. There was no pressure from outside parties to develop what was essentially a free asset.

Between 1998 and 2004, Japan enacted a series of laws that gave universities the right to own discoveries made by their faculty. The informal system of direct pass-through of intellectual property (IP) rights from professor to company was replaced by a system of contractual collaborative/joint research. However, echoing the pre-1998 situation when professors attributed most inventions they made to donation funding from companies, Japanese university faculty today tend to attribute a majority of their discoveries to contractual joint research with established companies.[2] Also, almost all discoveries attributed to joint research happen to list a company researcher as a co-inventor. The professor and his company counterpart make the final decisions whom to name as inventors, again echoing the relationship-based system of transfer from professors to companies before 1998. Co-invention by university and company inventors gives the university and company the right to co-apply for (i.e. to co-own) a patent. Thus about half of all patented Japanese university discoveries are co-owned by universities and companies—most of these companies being large established Japanese companies. Under the patent laws of most countries (the USA being the main exception) co-ownership means each co-owner is free to use the invention as it wishes, but neither co-owner may transfer its rights to a third party without the other co-owner's permission. In other words, the company co-owner has the equivalent of a perpetual, royalty-free, exclusive license with no development obligations. If it chooses not to develop a university discover, there is nothing the university or the faculty inventor can do. Japanese companies like this arrangement. By-in-large universities and inventors do not seem to mind. The companies provide research funding (though the amounts are modest compared to the US or UK). The system

of transfer is relatively non-bureaucratic and hassle-free. The university generally does not interfere in relations between the professor and the company—just as it did not interfere prior to 1998.

What is curious, however, is why rates of industry co-ownership of university discoveries are so much higher than countries such as Germany, the UK and Canada whose IP ownership laws are similar to Japan's.
Of course, startups can also play this game of monopolizing a large number of discoveries from a professor's laboratory, but so far they rarely do.

In any case, university technology transfer/licensing offices (TLOs/TTOs) generally do not manage joint research inventions. The non-joint research inventions that they are left to manage (roughly half of all reported inventions) are generally farther from commercialization than the joint research inventions. This is probably one of the main reasons that Japanese university revenue (royalties) from technology transfer is less than 1 percent that of US universities.

The reason that such a large proportion of US university technology transfer is to startups is also curious. One might guess that this simply reflects a more favorable entrepreneurial environment in the US compared to Japan. However, just as in Japan, university technology management policies in the US may reinforce the tendency of the surrounding innovation ecosystem to favor established companies or startups. Many American universities have a long history of owning and managing their researchers' inventions.[3] American universities have a much longer history of treating faculty IP as a source of revenue (i.e., license royalties). However, they also have a longer history of trying to make sure that university discoveries are developed for public benefit. US universities demand much higher royalties than Japanese universities. They are probably also much stricter in including development obligations in exclusive license contracts. Sometimes these development obligations simply take the form of high annual royalties—the theory being that if an invention is not valuable to a company, it will not pay the royalties and the university can then relicense it to a company that really wants to develop it. American may be relatively more willing to license to startups because they know the startups are under great pressure from their investors (venture capital, etc.) to develop the discoveries quickly, and that if development falters, these investors will try to liquidate the startup quickly and the university patents can be re-licensed. Also American universities have been increasingly willing to take a larger proportion of their start-ups stock (rather than cash) in exchange for a license. In other words, they may feel that by licensing to a startup they are making a good bet that the startup is most likely to develop the discovery rapidly and that by owning stock they can also gain significant income if the company is successful.

This concern with preventing companies from locking up university discoveries through exclusive licenses (or co-ownership) is also shared by UK universities, which have evolved a separate system to deal with this problem, known as the Lambert Model Agreements. Basically, the Lambert Model Agreements say that, in the case of a joint research invention, if a company simply wants the right to use the invention, it does not have to pay much in addition to what it has already paid for the research. However, if it wants to exclusively control a university invention it must pay the full economic cost of the research (staff salaries, infrastructure costs, etc.). In contrast to the US and UK, Japan does not seem concerned that companies are using the joint research mechanism to monopolize a large proportion of taxpayer funded research without development incentives.

[1] Although the fact that entrepreneurship was fairly vibrant in Japan between the end of WWII even into the 1980s suggests that it can be revived in Japan.

[2] Although this is not the case for biomedical inventions, most of which are attributed to independent university research.

[3] Although in the case of inventions made with government funding, the government generally held this right until the Bayh-Dole amendments to US patent law in 1980.

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Improvement of Germline Transmission in Chimeric Chickens

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ABSTRACT

To produce germline chimeric chickens, elimination of primordial germ cells (PGC) from recipient embryos before transfer of donor PGC will be one of the most effective methods. The administration of a busulfan solubilized sustained-release emulsion was used to improve the efficiency of endogenous PGC depletion in early chicken embryos. Busulfan was solubilized in N,N-dimethylformamide (DMF) and diluted 10 fold in PBS(–). The sustained-release emulsion was prepared by mixing equal amounts of busulfan solubilized solution and sesame oil using a filter. A dose of 100 μg was found to provide the best outcome both the number of endogenous primordial germ cells (PGCs) in embryonic gonads (0.6 % of control value) and hatchability (36.4 %) for preparing partially sterilized embryos to serve as recipients for transfer of exogenous PGCs. Immunohistochemical analysis showed that the proportion of donor PGCs in busulfan treated-embryos was significantly higher than that in controls (98.6 % vs. 6.4 %). Testcross analysis revealed that the germline transmission rate in busulfan treated-chickens was significantly higher than that in controls (99.5 *vs*. 6.0 %). Of eleven chimeras, 7 produced only donor-derived progenies, suggesting that these have yielded only donor-derived spermatozoa or ova in recipient's testis or ovary, respectively. This novel germline replacement technique provides not only potential applications for the conservation of endangered and/or rare avian species, but also for generation of transgenic individuals.

Key words: busulfan, germline chimeras

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Neuroendocrine Control in Animal Production:
Morphological Aspects of Neuroendocrine Control in Intestinal Motility

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Intestinal motility is regulated by the nervous system which has both intrinsic and extrinsic origins and the endocrine system which is distributed in the intestinal mucosal epithelium as single cells. Intrinsic nervous system in the intestine is divided into two system, submucosal and myenteric plexuses. Nitric oxide is the free radical gas and one of the important neurotransmitters regulating the intestinal motility. Numerous endocrine cells are scattered in the intestinal mucosal epithelium and release hormones regulating the intestinal functions and motility. Neurotensin, a 13 amino acid neuropeptide, is one of major hormones involved in the control of the intestinal motility. This hormone is released by the food intake and contracts the intestine. In this presentation, the distribution of NADPH-diaphorase (maker enzyme of nitrergic nerves)-positive neurons in the chicken cecum and the influence of food intake on neurotensin-containing endocrine cells in the chicken small intestine are shown.

Table 1. Number of nicotinamide adenine dinucleotide phosphate diaphorase (NADPHd)-positive cells per ganglion in the submucous (SMP) and myenteric (MP) plexuses of the chicken caecum

	Proximal	Middle	Distal
SMP	25.4 ± 3.9	25.1 ± 2.6	26.5 ± 4.0
MP	23.1 ± 4.0	22.4 ± 2.4	23.3 ± 2.8

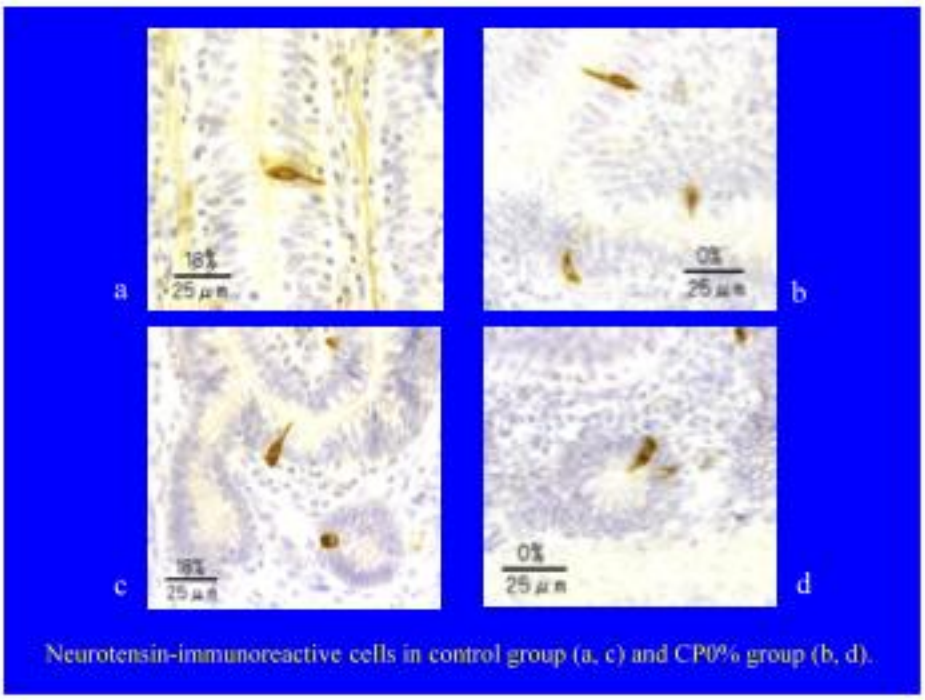
Mean ± standard deviation (SD) from 60 (middle and distal) and 30 (proximal) whole-mount preparations of six birds.
No significant differences were detected.

Table 2. Number of ganglia containing nicotinamide adenine dinucleotide phosphate diaphorase (NADPHd)-positive cells per 1 cm^2 in the submucous (SMP) and myenteric (MP) plexuses of the chicken caecum (number/cm^2) in whole-mount preparations

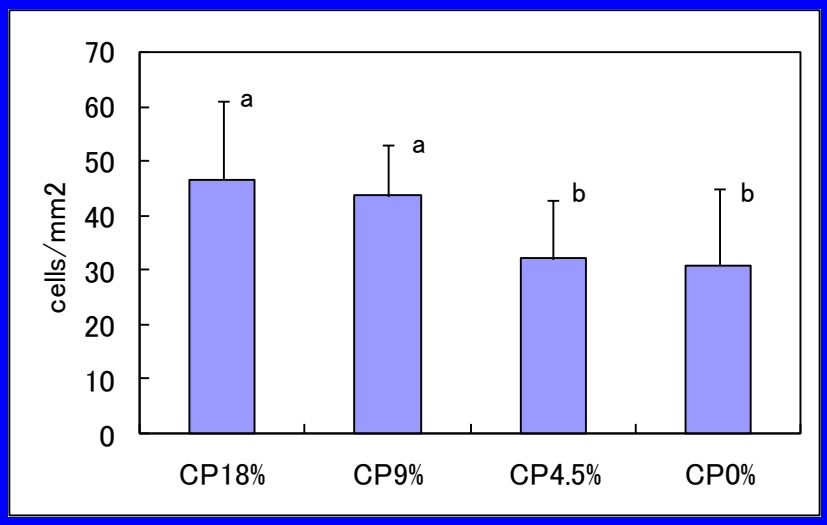
	Proximal	Middle	Distal
SMP	118.1 ± 25.2^a	117.5 ± 9.4^a	117.3 ± 25.5^a
MP	52.8 ± 28.3^b	56.1 ± 12.6^b	58.0 ± 11.0^b

Mean ± standard deviation (SD) from 60 (middle and distal) and 30 (proximal) whole-mount preparations of six birds.
Significantly different between a and b in the same column ($P < 0.05$).

Data from Hiramatsu et al., (1999) Anat. Hist. Embryol. 28:345-349.



Neurotensin-immunoreactive cells in control group (a, c) and CP0% group (b, d).



Effect of CP concentration in the diet on the frequency of neurotensin-immunoreactive cells of the chicken ileum. n=60. ($p<0.001$).

Reference:

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Academic and research Positions:
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2006.10. Senior Scientist, Tokyo Metropolitan Institute for Neuroscience, Japan
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Research Awards:
2006 Fellows Award for Research Excellence, National Institutes of Health
2004 Research Award of Japanese Society of Animal Science

Molecular Mechanisms of Neuronal Connectivity
~Introduce the *Drosophila* Technology for Neuroscience Research~

Shinichi Yonekura

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Abstract

An animal relies on the complex and stereotyped interneuronal connections in its brain to properly respond to environmental stimuli. The establishment of the proper connectivity in the nervous system requires specific target selection between individual presynaptic and postsynaptic cells. How this is achieved during development remains a central issue in neurobiology. With the potential to selectively manipulate the genetics in a cell type-specific manner, as well as its stereotyped and relatively simple anatomy and development, the *Drosophila* visual system has long been a peerless model system for identifying molecules and signaling pathways involved in the early steps of setting of the visual circuitry (for instance, the early steps involving the specification, differentiation, and initial guidance of photoreceptors and their projections). Each of adult *Drosophila's* prominent eyes contain ~800 photosensory organs, called "ommatidia," which each consist of eight photoreceptor neurons (R1-R8) tidily arranged in an ordered array. The axons from R1-R6 cells, which are tuned to green light, project to the first optic ganglion, the lamina layer, while R8 cells, most responsive to blue light, target their axons deeper to second optic ganglion, the M3 layer of the medulla. R7 cells, somewhat of an odd ball, detect the ultraviolet (UV) wavelength, and their axons extend the farthest into the M6 layer of the medulla.

To identify particular molecule in the guidance or targeting of axonal projections, genetic mosaic animals were created in which their retinas alone were homozygous for randomly mutagenized chromosomes, while the remaining tissues were wild-type. We screened these mutant lines using behavioral assays for UV responsiveness (UV/visible light choice assay), and *Drosophila N-cadherin* mutants were thus isolated (Ting et al. 2005). *Drosophila* N-cadherin (CadN) is an evolutionarily conserved, atypical classical cadherin, which has a large complex extracellular domain and a catenin-binding cytoplasmic domain. To determine the functional domains of CadN, we conducted a structure-function analysis focusing on its in vitro adhesive activity and in vivo function in R7 growth cones. We found that the cytoplasmic domain of CadN is largely dispensable for the targeting of R7 growth cones, and it is not essential for mediating homophilic interaction in cultured cells (Yonekura et al. 2007). Instead, the cytoplasmic domain of CadN is required for maintaining proper growth cone morphology. These data suggest that CadN mediates homophilic adhesive interactions between R7 growth cones and medulla neurons to regulate layer-specific target selection.

The CadN locus contains three modules of alternative exons (7a/b, 13a/b, and 18a/b) and undergoes alternative splicing to generate multiple isoforms. Using quantitative transcript analyses and green fluorescent protein-based cell sorting, we found that during development CadN alternative splicing is regulated in a temporal but not cell-type-specific fashion (Yonekura et al. 2006). In particular, exon 18b is predominantly expressed during early developmental stages, while exon 18a is prevalent at the late developmental and adult stages. In vitro quantitative cell aggregation assays revealed that all CadN isoforms mediate homophilic interactions, but the isoforms encoded by exon 18b have a higher adhesive activity than those by its alternative, 18a. CadN alternative splicing might provide a novel mechanism to fine-tune its adhesive activity at different developmental stages or to restrict the use of high-affinity 18b-type isoforms at the adult stage.

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Cryopreservation of Porcine Oocytes and Zygotes

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Introduction

Preservation of rare breeds and precious individuals has a great importance for maintaining genetic diversity in domestic animals. Gamete cryopreservation has been proven to be a useful tool for gene banking in mammals. In pigs, semen cryopreservation has been established; however, gene banking of porcine oocytes is still considered to be a great challenge. Large amount of intracellular lipid droplets make porcine embryos and oocytes the most sensitive to low temperatures among those of farm animals (Nagashima et al., 1994). Traditional slow freezing techniques fail to preserve viability of oocytes in pigs (Didion et al., 1990). Vitrification or "glass like solidification" using high cooling and warming rates combined with high concentrations of cryoprotective agents can prevent ice-crystal formation in oocytes during cooling. The application of different vitrification techniques has resulted in survival of porcine oocytes after low temperature preservation ranging between 30 % and 80% in the last decade (Zhou and Li, 2009). Nevertheless, this technique reduces developmental competence of oocytes. Furthermore, after cryopreservation of oocytes assisted reproductive technology (ART) such as in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) is necessary to generate embryos from oocytes. In pigs ART still results in high frequencies of abnormal fertilization such as polyspermy or the failure of male pronucleus formation further reducing developmental competence of cryopreserved oocytes (Kikuchi et al., 2008). As a result, live piglets from cryopreserved oocytes have not been obtained yet. In this study, we aim to present our experiences on pig oocyte cryopreservation which may be useful for future establishment of standard cryopreservation protocols for porcine oocytes.

Materials and Methods

We have applied *solid surface vitrification* (SSV) (Dinnyes et al., 2000) for pig oocyte cryopreservation because it enables the fast preservation of oocytes/embryos in large quantities with low financial cost. SSV was applied for in vitro matured oocytes, in vitro matured and fertilized oocytes (zygotes) and immature cumulus-oocyte complexes as well. Live or dead status was evaluated by staining with fluorescein diacetate. Meiotic and developmental competence of vitrified oocytes/zygotes was evaluated after in vitro maturation (IVM), IVF, in vitro embryo culture and (in zygotes) by transfer into surrogate mothers.

Results

Early results have demonstrated that survival rates of vitrified oocytes are improved by their nuclear progression from the immature stage to matured stage and even further after fertilization. Vitrification of oocytes after IVM at the metaphase-II stage (M-II) resulted in reasonable survival rates reaching approximately 70%; however, the fertilization and developmental competence of these oocytes was greatly reduced by vitrification. Antioxidant defense ability in oocytes was found to be compromised by vitrification. Furthermore, vitrification induced spontaneous oocyte activation in nearly half of the survived oocytes. As a result, sperm penetration and male pronucleus formation rates were significantly reduced in vitrified oocytes after IVF (Somfai et al., 2007).

In contrast, when IVM oocytes were vitrified 10 h after IVF at the pronuclear stage, the survival and developmental rates were dramatically improved. The transfer experiments of vitrified zygotes resulted in the production of live piglets (Somfai et al., 2009).

Vitrification of cumulus enclosed immature oocytes at the germinal vesicle (GV) stage resulted in a low (approximately 25%) survival rate. On the other hand, during IVM and IVF the surviving oocytes had similar competence for nuclear and cytoplasmic maturation and fertilization to those of the non-vitrified control oocytes. Although development rates were reduced, the quality of blastocysts developed from vitrified immature oocytes was similar to that of the control oocytes (Somfai et al., 2010). Our recent results suggest insufficient permeation of ethylene glycol as a major factor causing membrane damage of immature porcine oocytes during vitrification. On this basis, further improvement of immature oocyte cryotolerance is expected by the optimization of permeable cryoprotectant treatment.

Conclusions

Our results demonstrate that although vitrification of porcine IVM oocytes is characterized by high survival rates, due to cryopreservation-induced damage their cytoplasm fails to support male pronucleus formation in a manner that cannot be restored until sperm penetration. Rapid improvement of cryo-tolerance after fertilization suggests that organelle and membrane structure specific at MII stage may be responsible for low cryo-tolerance of unfertilized oocytes. On the other hand, oocytes vitrified at the GV stage seem to have some ability to recover from cryo-injuries during IVM. Nevertheless, this approach is still stricken with low survival rates. Low numbers of transferable embryos still greatly limit the success of this technology to produce live offspring. Cryopreservation and transfer of zygotes seem to be an advantageous way for embryo preservation because 1) stresses of embryo culture can be avoided and 2) monospermic (normal) zygotes can be selected according to the number of pronuclei for cryopreservation and culture.

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2008.3. Shinshu University Faculty of Agriculture Award
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2009.9. 2009 SRD Outstanding Presentation Award
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2009.9. Best Presentation Award in 111th annual Meeting of JSAS
(from Japanese Society of Animal Science)
2010.9. The Journal of Poultry Science Outstanding Paper Award
(from Japanese Poultry Science Association)
2010.9. Best Poster Award in the Czech-Japan Joint Symposium for Animal Reproduction
(from Society for Reproduction and Development)
2011.4. Shinshu University Distinguished Service Award
(from Shinshu University)
2011.7. Shinshu PAFS Award
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Genetic Conservation at the Cellular Level in Chicken

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Introduction:

In birds, cryopreservation of germplasm is challenging because development of reproductive technologies lags far behind that for many mammals. Techniques for freezing ova and embryos, though used widely for some domestic animals are not available for birds because of the yolky structure of the egg. In 1993, a technique for producing live offspring from isolated Primordial Germ Cells (PGCs), which are the embryonic precursors of germline cells, by their transplantation into developing embryos has been established in chicken (Tajima *et al*. *Theriogenolgy* 1993). In combination with transplantation, cryopreservation of PGCs can be used as a means of gene banking, especially for those species in which the cryopreservation of germplasm is challenging such as avian species. However, the collection of PGCs for cryopreservation and germline chimera-production involves the sacrifice of donor embryos, which have the potential to hatch out under ordinary circumstances. Though live offspring could be produced from cryopreserved PGCs in one of the leading breeds of chicken (Naito *et al*. *J Reprod Fertil* 1994), inconsistency in the PGC-collection makes them impractical when applied to endangered and/or rare breeds.

In avian species, unlike in other species, PGCs use the developing vascular system as a vehicle to transport them to the future gonadal region (Nakamura *et al*. *Poult Sci* 2007). It is prospected that this unique accessibility of avian PGCs during early development provides the opportunity to combine the reproduction of living birds with genetic conservation at the cellular level. Here, the above prospect was applied to the genetic conservation of a rare chicken breed.

Materials & Methods:

Gifujidori fowl (GJ), which was designated a Japanese natural treasure in 1941, was used as a conserved model of rare breed. A total of 88 fertilized GJ eggs were cultured in surrogate eggshells (Perry *Nature* 1988) to determine their exact developmental stage and to enable precise manipulation. Male and female PGCs were isolated from the blood of 2d-old embryos of GJ by Nycodenz gradient density centrifugation (Zhao & Kuwana *Br Poult Sci* 2003) and then cooled to -80°C at 1°C/min. Finally, PGCs were plunged into liquid nitrogen and stored for at least 6 mo until transplantation. After blood collection, the residual GJ embryos were continued to culture up to hatching to maintain them as living birds.

Embryos from two lines of White Leghorn (24HS, ST) were used as recipients for germline chimera production. Frozen–thawed GJ PGCs (80–127) were intravascularly injected into the recipient embryos at 2d with the same sexes of donors and recipients. After sexual maturity, genetic crossing analysis with manipulated GJ (maintained as living birds) was demonstrated. To regenerate live GJ offspring using eggs and spermatozoa derived from frozen–thawed PGCs, germline chimeric hens and roosters were mated.

Results & Discussion:

The hatching rate of the manipulated GJ embryos (25/88; 28%) was slightly lower than that of untreated GJ embryos (18/42; 43%), but the difference was not statistically significant ($\chi^2 = 2.1$). Of 25 hatchlings (14 males, 11 females), 12 (6 males, 6 females) survived to sexual maturity. Fertility test showed that they had normal reproductive ability when compared to untreated GJ, though blood containing PGCs had been removed as much as possible at the early development.

The total number of recovered GJ PGCs after freeze–thaw was 1057 in males and 1213 in females. The average

rate of recovery of GJ PGCs after freeze–thaw was 47.4±2.1% in males (4 vials) and 51.7±2.1% in females (4 vials). Though all recovered PGCs were used for germline transmission in this study due to the limitation of cell number, about 85% of frozen-thawed chicken PGCs were viable and biologically functional when they were cryopreserved by the procedures described here (Nakamura *et al*. *J Poult Sci* 2011).

The successful production of live offspring from donor PGCs depends on the selection of an appropriate recipient. This is a chicken breed or line with a high egg-laying performance and a low number of the endogenous PGCs. Both 24HS and ST can produce over 240 eggs per year (GJ, 100 eggs per year). The concentration of PGCs in the blood of recipient 24HS embryos was three times higher than in recipient ST embryos (46.5±1.0 *v*. 15.5±1.0; $P<0.05$). Testcross analysis showed that offspring originating from frozen-thawed PGCs of GJ in ST recipients were obtained with a higher efficiency than those originating from GJ PGCs in 24HS recipients (23.3% *v*. 3.1%; $P<0.05$). Therefore, the difference in germline transmission rates must be a reflection of the number of endogenous PGCs circulating at the time of transplantation. In addition to this, the number of donor PGCs settled in the gonads of ST recipient embryos at 6d was significantly difference between 3 chicken breeds, when the same number of PGCs had been transplanted (Nakamura *et al*. *J Poult Sci* 2011). These data confirm that the efficiency of germline transmission is affected by the combination of donor and recipient breeds or lines.

Six live GJ progenies were successfully regenerated from frozen-thawed PGCs by crossing male and female germline chimeras in ST group. Moreover, our group succeeded to augment population of GJ from these regenerated progenies. For practical application of PGC-mediated genetic conservation system in chicken, improvement of the germline transmission of donor PGCs is a critically important issue. Removal of the endogenous PGCs of recipient embryos prior to transplantation appears to be the most powerful approach. Administration of drug such as busulfan or irradiation of X-ray removes the endogenous PGCs and increases the germline transmission of donor PGCs (Nakamura *et al*. *Biol Reprod* 2010, unpublished data).

Conclusions:

This is the first study to use a limited number of chicken embryos and achieve the combined preservation of two different types of functional genetic materials, living birds and PGCs.

Special Lecture

Sarah Marie Cummings,
Representative Director, Masuichi-Ichimura Sake Brewery

1991-92 Exchange Student at Kansai University of Foreign Studies
May 1993 Graduated from Pennsylvania State University
June 1993 Upon graduation volunteers at Nagano Winter Olympics
June 1994 Joined Obusedo Corporation and launched the Management Information Office
January 1996 Certified as a kikizake-shi (sake sommelier)
July 1997 Took part in the restructuring of Masuichi-Ichimura Sake Brewery
February 1998 Assistant Olympic attache to the Nagano Winter Olympics British team; plans and executes hospitality program
October 1998 Opens Masuichi Club/The Club restaurant
Appointed a director of Obusedo and Masuichi-Ichimura Sake Brewery
August 2001 Launches Obusession Cultural Salon, always held on the date which matches the number of The month (Jan. 1, Feb. 2, etc.), an event which continues to the present
December 2001 Named Nikkei Woman of the Year 2002
July 2003 Launches Obuse mini Marathon, on Marine Day. "In ocean-less Obuse, let's make waves." Organized in just three months.
April 2004 Named director of newly created cultural affairs department
May 2004 Appointed as the Representative Director of the Japan Sake Brewers Association (the first woman ever to assume this position)

Poster Session

Graded organization of collagen fibrils in the equine superficial digital flexor tendon

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Abstract

Tendons transfer the forces generated by the muscles to bones and contribute toward storing and recovering the energy required for smooth locomotion. The viscoelastic property of tendons operates in parallel with the force produced by the muscle and enhances muscle performance. The equine superficial digital flexor tendon (SDFT) is long and located in the posterior aspect of the forelimbs. Its anatomical characteristics form the physical basis for the athletic potential of horses. Connective tissues such as tendons and ligaments are primarily composed of collagen fibrils. By using ultramorphological and biochemical methods, we analyzed the regional differences between the three parts of the SDFT, namely, the myotendinous junction (MTJ), middle metacarpal (mM), and osteotendinous junction (OTJ). Cross-sectional images showed unique distributions of collagen fibrils of varying diameters in each region. Small collagen fibrils (diameter <100 nm) were distributed predominantly in the MTJ region, and the OTJ region was relatively rich in large collagen fibrils (diameter >200 nm). In the mM region, the collagen fibrils were intermediately distributed between the MTJ and OTJ. The results indicate a graded arrangement of collagen fibrils in the tendon. Type I, III, and V collagens have been shown to be components of the collagen fibrils in tendons. Collagen fibrils are hybrid fibrils formed by interactions between these collagens. An increase in the relative ratio of type III or V collagens to type I collagen apparently regulates the collagen fibril diameter negatively. Type V collagen was detected preferentially in the MTJ region. These findings indicated that different regions of the equine SDFT perform distinct functions.

Long term culture and characterization of chicken primordial germ cells

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【Introduction】

Primordial germ cells (PGCs) are the precursors of the germ cell lineage and are restricted to the formation of sperm and eggs in the adult animals. Therefore, PGCs have been considered as one of the valuable cells for clarification of germ cell development. In the chicken, PGCs has a specific property that temporarily circulates in bloodstream at early embryo. It makes possible the PGCs to isolate and purify from embryonic blood and subsequent *in vitro* culture. In the present studies, establishment of culture system and characterization of the cultured PGCs were performed.

【Materials and Methods】

Feeder cells for the culture of PGCs were prepared from Buffalo Rat Liver (BRL) cells. Whole blood cells were collected from White Leghorn (WL), Barred Plymouth Rock (BPR) and Silkey (SL) embryos at stage 13-15 (Hamburger and Hamilton, 1951), and each blood samples were seeded into a gelatin-coated 48 well tissue culture plate containing MITC-treated BRL feeder layer. Then, about 0.5-1μl blood samples were subjected for sexing by PCR with a pair of W-chromosome specific primers. PGCs culture medium used was described in van de Lavoir *et al.* (2006), with some modifications. Over 40 to 200 days, the cultured PGCs were analyzed immunohistochemistory with VASA and SSEA-1 antibodies were performed.

【Results and Discussion】

At day 1 of culture, the number of PGC-like cells (PGC-LCs) seemed to be very few in all samples. During 10-20 days, most of the blood cells died and PGC-LCs visualized that weakly-attached on BRL feeder layer proliferated. At 40 days, number of PGC-LCs largely increased into 500-1000 fold as compared to 10 days. These cells expressed VASA and SSEA-1, suggested that the cultured cells were PGCs. However, proliferated cells found to be male PGCs alone. It was suggested that the condition could be culture only male PGCs and necessity to develop novel conditions to culture female PGCs was considered.

Male PGCs cultured for long term expressed VASA and SSEA-1 that maintained characteristics of the PGCs between all lineages. During 40 to 200 days of the culture, the expression levels were not different. From this, it was considered that these cultured PGCs could proliferate endlessly *in vitro* and could generate germ line chicmeric chickens. In addition, we got successful proliferation of male PGCs in the chicken. It was concluded that the established strategies could be applied for generation of transgenic chickens, conservation of endangered birds and clarification of germ cell development.

Avian Stem Cells for Regeneration of Muscular Dystrophy Chickens by Means of Germline Chimeras

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Abstract

The offspring were generated from germline chimera between muscular dystrophy chicken; NH-413 strain (donor), and White Leghorn L-M strain (recipient). Phenotype and symptom of type-I offspring were quite similar to that of the NH-413 strain. In type-II offspring, feather color showed mixture of white and brown and the symptom was not dominantly indicated.

Sexually matured males and females of the type-I were mated each other. Chickens manifesting completely same phenotype to that of the donor NH-413 strain; brown feather color and symptoms of muscular dystrophy, were regenerated from the mating. These results suggested that complete regeneration of the muscular dystrophy chickens could be generated by fertilization of spermatozoa and ovum derived from donor cells. Statistics such as fertility, hatchability and survival rate of these regenerated offspring were significantly increased as compared to that of the original NH-413 strain.

From these results, it was concluded that the novel strategies to regenerate chickens with muscular dystrophy was developed.

Isolation of Adipose-tissue derived stem cells and the application for blood vessel regeneration in the chicken

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Introduction: In resent years, tissue engineering with bone marrow cells is used for clinical treatments. Bone marrow donor has a lot of risks. Therefore we focused on fat tissue which is harvested easily and profusely from living donor. Adipose-tissue derived stem cells(ASCs) have potential to differentiate into bone, cartilage, fat and other tissues. Chickens are used as a model for applied biological studies. Because of its in ovo embryonic development rather than utero development, chicken is an important model organism for embryology and differentiation studies. However, ASCs have not been isolated from chickens. In this study, we explored ASCs from White Leghorn chickens and differentiation inducted cells into fat, osteoblast, cartilage cells.

Materials and Methods: Adipose-tissues were obtained from abdominal fats of chickens. Then adipose-tissues were digested to single cells with collagenase. After digestion, cells were expanded in Serum Free Media with b-FGF. Incubated cells were used for the differentiation assays and RT-PCR. Cells inducted differentiations were analyzed with cytologigal staining and RT-PCR. Isolated cells as donor were injected into the recipient embryos whose blood vessels were degenerated by UV irradiation.

Results and Disucussion: Adipose-tissue derived cells are showed a fibroblast-like morphology. Gene expressons of Adipose-tissue derived cells were positive for PouV and CD29, CD44; negative for CD34, CD45. After Adipogenic and Osteogenic differentiation induction, cells were stained with the Oil Red O dye and ALP stain. Osteogenic and Chondrogenic differentiation inducted cells were expressed Runx2 and collagen2. Consequently, present study sugessted that multipotent stem cells were isolated from abdominal fat tissue in White leghorn. It was suggested that the isolated cells could regenerate the blood vessels.

Effect of abnormal UPR on lipid accumulation of adipocyte

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Recent studies suggested that obesity may be a disease related to endoplasmic reticulum (ER) dysfunction. The majority of cells cope with the increased ER load by activating the unfolded protein response (UPR). The reasons for ER stress and UPR activation in the obese state are not fully understood. Furthermore, it is largely unknown how ER dysfunction affects the physiological functions in adipocytes at molecular basis. The aim of this study was thus to understand the relationship between UPR and fat accumulations.

In order to block the UPR system, we used missense mutation (P56S) in the gene-encoding vesicle-associated membrane protein binding protein B (VAPB) which has been identified in a familial form of ALS (amyotrophic lateral sclerosis). GFP or RFP-tagged wild type VAPB (WT-VAPB) and P56S-VAPB constructs were generated and expressed in 3T3-L1 cells. In order to characterize the role of VAPB in the IRE1/XBP1 pathway of 3T3-L1, an XBP1-Venus construct was used as a reporter of IRE1/XBP1 activation. We confirmed that WT-VAPB had a clear induction of IRE1 activity, and this property was significantly impaired in P56S-VAPB-expressing cells. Next, we quantified the intracellular lipid accumulation in both WT and P56S-VAPB expressing 3T3-L1 cells using BODIPY 493/503. Our result indicated that lipid contents in P56S-VAPB cells were lower than that in WT-VAPB cells at 6 days after stimulation, whereas both were same at 3 days after stimulation.

These results suggested that abnormal UPR affected the lipid accumulation in adipocyte. Now, we are investigating expression level of the gene, related to lipid accumulation, in both WT and P56S-VAPB cells.

Which molecules are associated with axonal transport via stress related molecule "milton"?

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【Background】

Based on genome-wide screening, we identified milton as a stress associated molecule. Transgenic mice over-expressing mutant milton had abnormal feeding behavior after acute restraint stress. Previous paper suggested that milton was associated with anterograde transport of mitochondria in neuronal cell. Milton is thought to be an adaptor protein for axonal transport. But, little is known about which molecules are associated with transport by milton. As we hypothesize that the exposure stress affects axonal transport, we try to find out the molecules that is associated with axonal transport.

【Result and Discussion】

As axonal transport requires protein-protein interactions, we conducted yeast two-hybrid assay to identify molecules that bind to milton. We found out that several encoded proteins such as Adipor1, Siah2, Gad1 and Sorbs1 were likely to bind to milton. Therefore, exposure stress may disrupt axonal transport and neuron function. In the future, we should investigate the effect of these molecules on neuronal function.

Abnormal UPR impaired C2C12 myotube formation

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Abstract

The process of skeletal muscle differentiation is characterized by mononucleated myoblasts exiting cell cycle and fusing to form multinucleated myotubes. Recent study suggested that endoplasmic reticulum (ER) stress increases myofiber formation. However, the role of ER stress for myotube formations is still largely unknown. The aim of this study was thus to determine the relationship between UPR (unfolded protein response) and myotube formation using C2C12. In order to block the UPR system, we used missense mutation (P56S) in the gene-encoding vesicle-associated membrane protein binding protein B (VAPB) which has been identified in a familial form of ALS (amyotrophic lateral sclerosis). GFP or RFP-tagged wild type VAPB (WT-VAPB) and P56S-VAPB constructs were generated and expressed in C2C12 cells. The typical ER localization pattern of a weblike distribution within the cytosol extending from the nuclear surface was shown for the WT-VAPB construct, whereas P56S-VAPB constructs produced VAPB protein aggregates within cells, as reported previously (in neuronal cell). In order to characterize the role of VAPB in the IRE1/XBP1 pathway of C2C12, an XBP1-Venusconstruct was used as a reporter of IRE1/XBP1 activation. We confirmed that WT-VAPB had a clear induction of IRE1 activity, and this property was significantly impaired in P56S-VAPB-expressing cells. Moreover, we found that overexpression of P56S-VAPB prevented myotube formation, whereas the overexpression of WT-VAPB or empty vector had no effect. Although there were morphological differences, no significant difference showed on the number of cells between P56S-VAPB-expressing myotubes and other control myotubes. This result suggested that UPR inhibition impaired myotube formation without affecting myoblast cell fusion. We hypotheses that IRE1-XBP1 pathway was required for myotube formation. Now, we are trying to plug up a hole between the UPR pathway and myotube formation.

Animal Biotechnology and Poverty Alleviation: Bangladesh Perspective

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Abstract

Animal biotechnology has only limited potentiality to alleviate poverty in Bangladesh, because it does not address the main reasons for poverty such as weak infrastructures, bad governance, erratic and extreme climate, lack of education and unfavourable terms of trade. Looking at the main characteristics of the predominantly small-scale animal farming in Bangladesh, the potentials and limitations for biotechnological applications in food processing, forage improvement, animal breeding and animal health would be discussed. Indigenous biotechnology under the control of livestock farmers can be beneficial, whereas—with the exception of some animal health technologies—large-scale and 'high-tech' applications of biotechnology have shown little potential to alleviate poverty. Indeed, these applications can have the opposite effects. Rather than pouring an undue amount of human and financial resources into further refinement of advanced animal biotechnology, Bangladeshi livestock researchers should develop their own research agenda that addresses the real problems of resource-poor small-scale livestock keepers and poverty alleviation.

Key words: Animal biotechnology, poverty alleviation, Bangladesh, Research priorisation

The Japanese Black cattle sire "Eijyu" bread in Nagano Prefectural Animal Industry Experimental Station

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Breeding background:

"Eijyu" was born in Nagiso-cho, Nagano. Eijyu was ranked as top among the 65 sires in 2003 for estimated breeding values on dressed carcass, marbling and rib eye area due to the progeny tests. In October 2007, Eijyu was selected as a special sire for Nagano prefecture. Eijyu was awarded in 9th Japanese black cattle for the high performance contest in Yonago city. Generation of the offspring with high performance on dressed carcass, marbling and rib eye area will be expected using Eijyu as the sire. Also, Eijyu has the SCD genes which is considered to increase the amount of oleic acid for good flaver. As for the pedigree, the father of Eijyu is Hirashigekatsu and the mother is Saeka; a daughter of Fukuyasu 9/165. For the breeding value, 6 on dressed carcass is rated as very high. In our station, value for the taste is developed due to marbling and oleic acid. The valued beef is qualified as premium meet. In October 2007, Eijyu was selected as a special sire for Nagano prefecture. Eijyu will be subjected for 10th Japanese black cattle contests in Nagasaki city to be held October 2012. Frozen semen and embryos could be distributed in our station.

“Shinshu-ogon Shamo” bread in Nagano Prefectural Animal Industry Experimental Station

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“Shinshu-ogon Shamo” was bread in Nagano Prefecture Animal Industry Experimental Station on 2005. According to JAS law, Jidori is defined as the chickens containing genetic background of native chicken breed for more than 50%. A famous Jidori chickens includes Hinai of Akita, Kuji of Ibaraki and Nagoya of Aich. "Shinshu-ogon Shamo" was registered as an trademark of Nagano prefecture in 2006 and has been promoted for the usage. "Shinshu-ogon Shamo" has been bread by crossing Shamo as sire with Nagoya as dam, thus containing 100% of native genetic background. In Nagano prefecture, “Shinano-dori” has been bread by crossing Shamo as sire with White Plymouth Rock as dam which contained 50% native genetic background. "Shinshu-ogon Shamo" has fewer variation in the phenotype as compared to “Shinano-dori”, gentle, suitable body size and small amount of abdominal fat. The body weight of "the Shinshu-ogon Shamo" at 14 weeks is about 2.9 and 2.1 kg, and at 18 weeks 3.6 and 2.4 kg in males and females, respectively. Their feed conversion rates are 3.35 and 3.98, redspectively. When slaughtered at 126 days, breast meat performance; water content, drip, heating, showed better record in “SHinshu-ogon Shamo “ than “Shinano-dori”. Meat color with the spectrum colorimeter significantly lower a*(red degree) in male, and it tended to be gloomy as for the color both in males and females. The female color that a meaningful difference was accepted together by L*(luminosity), b*(yellow degree), and a male female had dark male color tended to be thin with the breast meat. The energy per 100 g of eatable breast meat is lower than "young chicken" and in "Shinano-dori" published in Japanese foods standard. Smaller fatty and energy is one of the attractive points of Shinsyu-ogon Shamo. As for the test in 126 days, "the Shinshu-ogon Shamo"was evaluated to be more soft than that of “SHinano-dori”. Our experimental station promotes the more usage of “shinshu-ogon Shamo. Breeding standard of the “Shinshu-ogon Sham was established. the Shinshu gold The shipment number of the birds of 2010 is approximately 35,000 of and will be planed to increase amount of production more in future.

CROSSBREEDING OF INDIGENOUS JAPANESE CATTLE FOR BEEF PRODUCTION

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Mr. Nisbet is a graduate of the Faculty of Agriculture, University of Alberta, Canada (BSc) and Nagoya University, Faculty of Agriculture (Animal Science, MSc), where he researched the crossbreeding of Japanese cattle. Currently, Mr. Nisbet is an editor and rewriter of life science manuscripts for a number of universities in Japan.

Wagyu is the most famous beef cattle breed in Japan, but they are not the only breed to be found in Japan. The modern version of Wagyu cattle has its roots in the Meiji Period when many things were imported and incorporated into a modernizing Japan. European beef cattle breeds, such as Brown Swiss, Shorthorn, Devonshire and Angus were imported and crossbred with local indigenous cattle that had mainly been used for farm work; since the eating of four legged animals had been prohibited. Crossbreeding had a significant effect on the overall size and muscle physiology that we see in the modern Wagyu while retaining his trait of significant marbling.

There are however, still a few isolated breeds of indigenous cattle. Mishima and Kuchinoshima cattle have been isolated on the islands that give them their name and being few in number are protected to various degrees by the government and the islanders that keep their tradition. The island of Mishima is in the Sea of Japan off the coast of Yamaguchi prefecture which had a long history of whaling and only used their cattle for farming the steep hillsides. While whale meat was a staple food, they would never consider slaughtering one of their precious cattle for food. Most of the domestic beef production in Japan is actually a by-product of the dairy industry. All Holstein male calves and most of the female calves from dairy farms are sold at auction on weaning to supply the beef cattle feeding sector. Only female calves from each farm's top producing cows are retained in a replacement program and all other calves are sold as a secondary source of income. While Wagyu cattle remain exclusive and expensive, dairy cattle provide a consistent source of reasonable beef to the market.

While Mishima numbers remain very small, the use of biotechnology including artificial insemination and sex selection will enable these cattle to remain a prized genetic resource in Japan for years to come. The opportunity exists to use the domestic diary industry as a production base to offer dairy farmers a more valuable product by breeding their cows with semen from Mishima bulls. The resulting carcasses have proven to be significantly larger than Mishima cattle and to produce superior marbling; bringing a higher market price to the producers.

This model of production offers a classic win-win situation by widening the awareness and promotion of the ancient, indigenous Mishima cattle and a source of income from the sale of semen to a growing domestic market. It offers dairy farmers a value-added opportunity to breed their cows with Mishima seed stock and capture a better return for the same calves they would normally sell. The feeding industry would gain a source of high quality calves that produce a superior carcass but at significantly lower prices than traditional Wagyu calves, and receive a higher wholesale market price for their finished cattle. Finally, the consumers in Japan would benefit from high quality, marbled beef at retail prices significantly lower than the luxury Wagyu product.

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