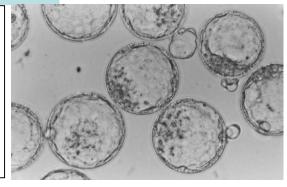
## REPRODUCTIVE BIOLOGY AND DEVELOPMENTAL BIOTECHNOLOGY IN MAMMALS

Reproductive Biology Professor: Imai H., Associate Prof.: Yamada M Assistant Profs.: Minami N. and Nagao Y.

The beginning of life in mammals starts with the union of haploid gametes, the sperm and the ovum, produced by germ cells within the male and female gonads. The fertilized zygote passes through embryonal and fetal stages and is delivered by the process of parturition as a diploid conceptous. Reproductive biology is a field of study which aims at explaining the reproductive process and developing technologies for artificially creating animals with superior traits. The prime focus of our laboratory is on germ-cell development, fertilization, embryo development, establishment of embryonic stem cells, and production of somatic cell cloned and transgenic animals using the modern tools of cell and molecular biology, genetics and developmental biotechnology.

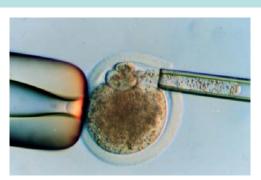
#### Development and differentiation of mammalian eggs

We are aiming at elucidating regulatory mechanisms of development and differentiation of mammalian eggs *in vitro* to efficiently produce embryos of experimental and livestock animals. Major interests are exploring molecular mechanisms of intercellular communication between oocytes and follicle cells, important for acquiring developmental competence of bovine oocytes after fertilization and determining factors regulating pre-implantation development and differentiation and the mechanisms of embryonic genome activation of mouse embryos.



(Mouse fertilized eggs: blastocysts 4 days after *in vitro* fertilization )

#### **Production of somatic-cell cloned animals**



(Somatic-cell nuclear transfer into enucleated oocytes)

It is now possible to produce cloned mammals from somatic cells using nuclear transfer technique. However, the production efficiency is extremely low, with most clones being lost soon after implantation. Very little is known about how differentiated cells used as nuclear donors are reprogrammed in recipient oocytes to acquire totipotency (an ability to create an entire individual) of cloned embryos. We are studying to the mechanisms underlying the reprogramming process of donor cells in enucleated oocytes using molecular and cell biology approaches.

## Establishment of ES cells and production of transgenic animals

Development of techniques to establish totipotent embryonic stem (ES) cells from mammalian embryos are undertaken. Reconstitution of embryos and production of transgenic animals using these ES cells are being studied. We intend to utilize these ES cells in genetic recombination techniques and introduce desirable mutations into gene and further to genome/chromosome level for improving genetic traits of livestock animals.



(Transgenic miniature pigs)

#### Mitochondria and physiological phenotypes of the mouse

The mismatch occurs between mitochondria and nuclear genomes when the mitochondria derived from different species are introduced by microinjection in the cytoplasm or during nuclear transfer techniques. We are aiming at clarifying how the mismatch condition affects physiological phenotypes of the mice. Furthermore, the interaction between mitochondria of different species and cell nuclei is examined using cellular and molecular biological approaches.

#### **Key words**

Germ cell, egg, sperm, fertilization, development, embryonic genome activation, embryonic stem cell, somatic-cell clone, epigenetics, reprogramming, transgenic animal, mitochondria, developmental biotechnology, developmental biology

#### **Recent publications**

## Identification and characterization of an oocyte factor required for development of porcine nuclear transfer embryos.

Miyamoto K, Nagai K, Kitamura N, Nishikawa T, Ikegami H, Binh NT, Tsukamoto S, Matsumoto M, Tsukiyama T, Minami N, Yamada M, Ariga H, Miyake M, Kawarasaki T, Matsumoto K, Imai H.

Proc. Natl. Acd. Sci.USA, 108 (17): 7040-7045. (2011)

# A simple and efficient method for generation of induced pluripotent stem cells using piggyBac transposition of doxycycline-inducible factors and an EOS reporter system.

Tsukiyama, T, Asano R, Kawaguchi T, Kim N, Yamada M, Minami N, Obinata Y and Imai H. Genes to Cells, 16(7): 815-825. (2011)

## Pluripotent stem cells from testis, in "Embryonic Stem Cells - Differentiation and Pluripotent Alternatives", ed. Michael S. Kallos

Goel S. and Imai H.

InTech Publisher, Rijeka, pp. 473-492. (2011) ISBN 978-953-307-632-4

## The Dnmt3b Splice Variant is Specifically Expressed in In Vitro-manipulated Blastocysts and Their Derivative ES Cells.

Horii T, Suetake I, Yanagisawa E, Morita S, Kimura S, Nagao Y, Imai H, Tajima S and Hatada I. J. Reprod. Dev., 57 (5): 579-585 (2011)

### Crucial role of vinexin for keratinocyte migration in vitro and epidermal wound healing in vivo.

Kioka N, Ito T, Yamashita H, Uekawa N, Umemoto T, Motoyoshi S, Imai H, Takahashi K, Watanabe W, Yamada M and Ueda K.

Exp. Cell. Res., 316 (10): 1728-1738. (2010)