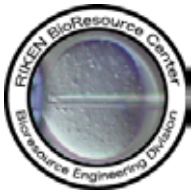
Bioresource Engineering Division



Head, Atsuo OGURA

Goal

To develop genetics-related techniques, especially those essential for maintenance and supply of laboratory mice and stem cell lines at a high quality in RIKEN Bioresource Center.

Activities

I. Development of mouse somatic nuclear transfer techniques
II. Development of microinsemination techniques
III. Development of reliable cryopreservation techniques for mouse embryos or gamates
IV. Development of new stem cell lines

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Specific aim

I. Development of mouse somatic nuclear-transfer techniques

1) Cloning mice from hematopoietic stem cells

It is often assumed that tissue-specific stem cells can be used as efficient donors in cloning experiments. However, we have found that embryos reconstructed from hematopoietic stem cells (HSCs) show very poor development *in vivo* and *in vitro* compared with those reconstructed from cumulus cells. Gene expression analysis of two-cell HSC-cloned embryos revealed that five of the six zygotically activated genes examined had failed to activate. Importantly, a comparison with cumulus clones suggested that Hdac1 (histone deacetylase-1), a key gene in subsequent embryonic gene activation, was specifically repressed in HSC clones. These results suggest that the genomes of HSCs are less plastic than we imagined, at least in terms of their reprogrammability in the ooplasm after nuclear transfer.

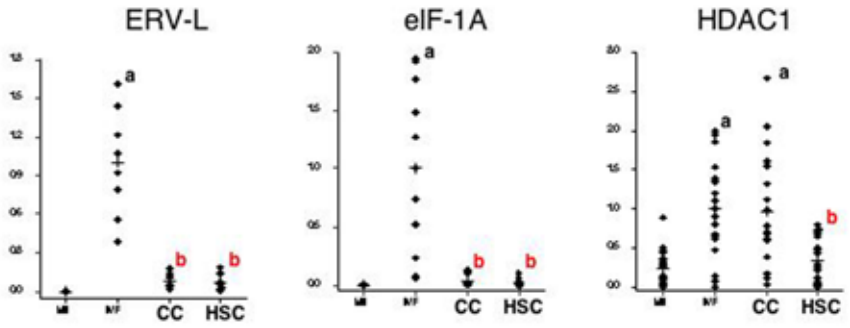

Figure 1. Repressed expression of zygotic genes in cumulus cell (CC) and hematopoietic stem cell (HSC) clone embryos.

2) Interspecies nuclear-transfer cloning using monkey somatic cells

The quality of recipient oocytes is the critical factor for successful somatic cell cloning. However, it is often difficult to prepare several high-quality fresh oocytes at a time in some species. We examined the feasibility of interspecies nuclear transfer using monkey somatic cells and rabbit oocytes. About 50%–70% of the reconstructed oocytes developed to the 2–4-cell stage, irrespective of the cell type, whereas only 1% developed to blastocysts. When cytoplasts prepared from monkey somatic cells were introduced into reconstructed oocytes, the efficiency of their development into blastocysts was significantly improved ($P < 0.005$).

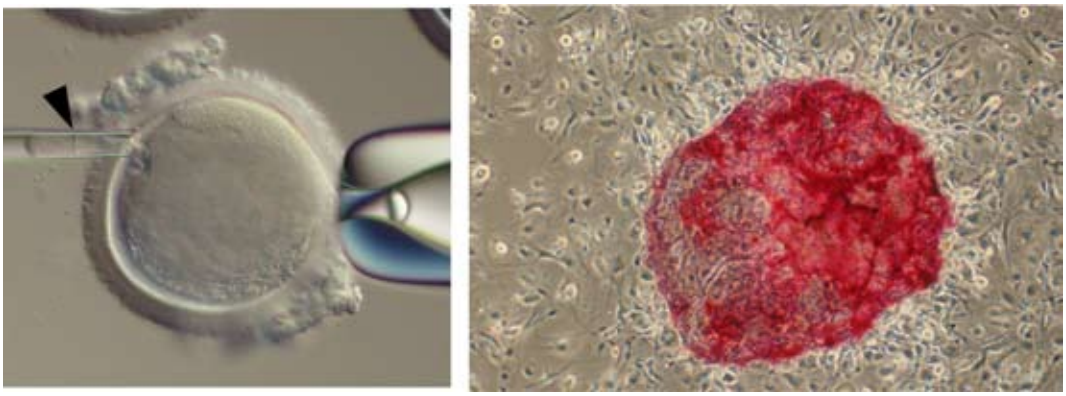

Figure 2. Enucleation of a recipient rabbit oocyte (Left). Arrowhead indicates the oocyte chromosomes. An ES-like cell colony derived from the inner cell mass of a rabbit-monkey interspecies nuclear transfer blastocyst (Right)

II. Development of microinsemination techniques

1) Screening for sterility-related genes in mutant mice

To screen for dominant male-sterility-related genes, male germ cells from sterile males among the progeny (G1) of mutagen (N-ethyl-N-nitrosourea)-treated mice were used for microinsemination. The G2 mice thus obtained after natural matings were then examined. We concluded that sterility was not attributable to genetic causes because all the offspring examined were fertile. We are now investigating the offspring from a second sterile line.

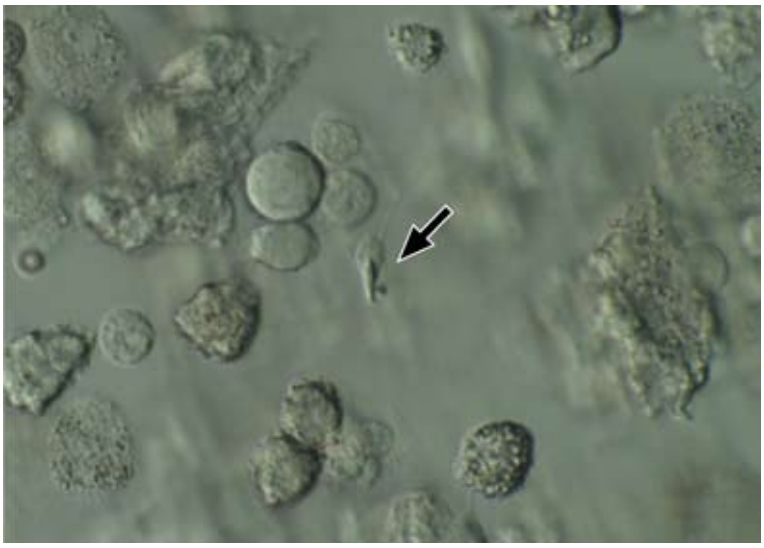

Figure 3. An elongated spermatid retrieved from a testis of a sterile G1 male (arrow). Only a few spermatids were found the testicular cell suspension but offspring were successfully obtained following microinsemination.

2) Microinsemination using male germ cells from epididymides and testes stored in freezers

The fertilization capacity of male germ cells retrieved from epididymides and testes stored at -80ºC up to one year without cryoprotectant was examined using a microinsemination technique. Microinsemination was performed with epididymal/testicular spermatozoa and round spermatids by direct injection into oocytes. Normal pups were obtained irrespective of the method of cryopreservation or the cell type used. For transportation experiments, frozen testes within a polypropylene cryotube were placed in a polystyrene foam case filled with dry ice and were transported from the U.K. to Japan by air and land. Microinsemination was performed after the testes were thawed and the spermatogenic cells collected. Normal pups were obtained after embryo transfer. These results indicate that male mouse germ cells retain their nuclear integrity even after the epididymides/testes are frozen in freezers. Because this cryopreservation technique is very simple and allows storage at –80°C, it may allow us to transport male mouse germ cells internationally on dry ice, even when the senders are not specialists in cryopreservation.

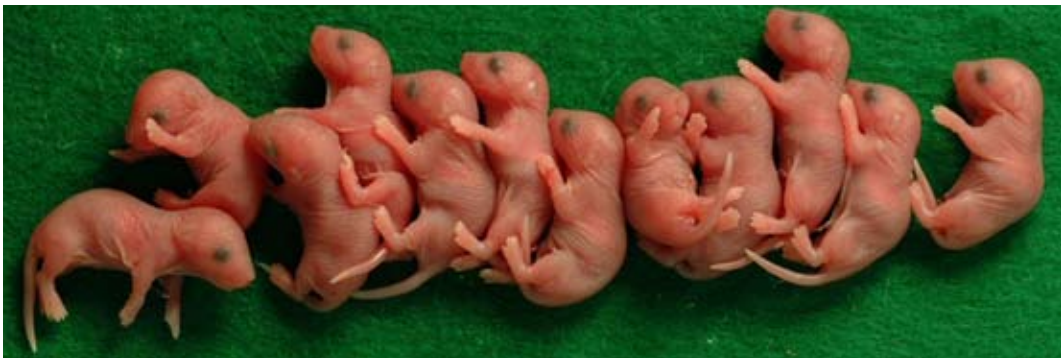

Figure 4. Mice born after microinsemination with spermatids retrieved from testes which had been frozen at MRC (U.K.) and transported to BRC on dry ice.

3) Production of offspring from GS cells or mGS cells

The contribution of embryonic stem (ES)-like cells (mGS cells) derived from germline stem (GS) cells to normal embryonic development was examined by their microinjection into blastocysts. The chimeric mice thus obtained were used for microinsemination experiments to confirm the germline transmission capacity of mGS cells. After transfer of the microinsemination-derived embryos, donor-origin pups were obtained. In another study, we assessed the normality of GS cells that had been cultured under anchor-free conditions for a long period. Following microinsemination with round spermatids of cultured GS cell origin, normal pups were obtained. This indicates that GS cells can be cultured under floating-culture conditions without loss of their germline stem-cell capacity.

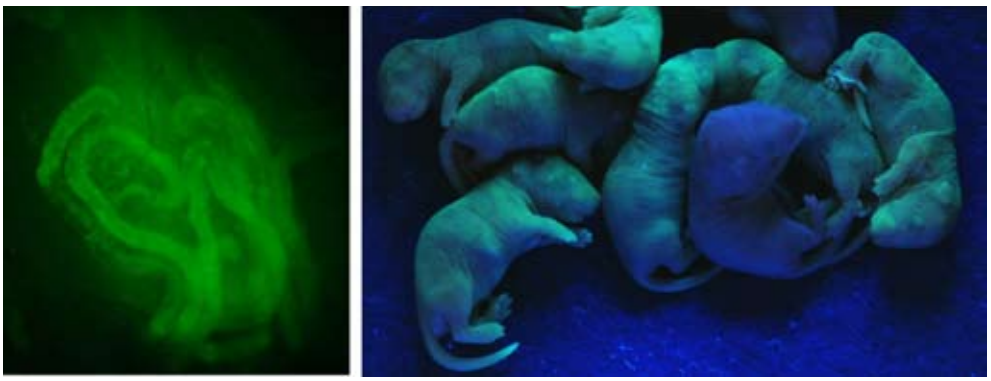

Figure 5. GS-cell derived spermatogenic cells that had colonized and differentiated in the seminiferous tubules of the host testis (green fluorescence tubules in the left panel). GS-cell derived pups born following microinsemination (green mice in the right panel).

4) Microinsemination with primary spermatocytes by single nuclear transfer

We found that cumulus-free in vitro maturation (IVM) in mice can be considerably improved by using an IVM medium consisting of MEM and TYH media (1:1 mixture). A high rate (23.8%) of development from germinal vesicle oocytes to full-term fetuses following in vitro fertilization and embryo transfer to foster mothers was achieved using this medium. When this IVM system was applied to metaphase I (MI) oocytes injected with spermatocytes, offspring were first obtained without cytoplasmic replacement at MII.

III. Development of reliable cryopreservation techniques for mouse embryos/gametes

1) Efficient cryopreservation of mouse embryos of different strains

In our routine protocol, embryos cryopreserved (vitrified) in EFS40 solution are thawed in 0.5 M sucrose –PB1 medium and equilibrated in the same medium for 5 min. This thawing step requires great skill and experience. We have found that embryos can be handled more easily after thawing if they are thawed and equilibrated for 3 min in 0.75 M sucrose–PB1 medium. The rates of embryos retrieved and the rates of embryos with normal morphology were significantly improved to 98%–100% and 95%–100%, respectively, and this was consistent among all the mouse strains tested (C57BL/6, BDF1, BALB/c, and FVB).

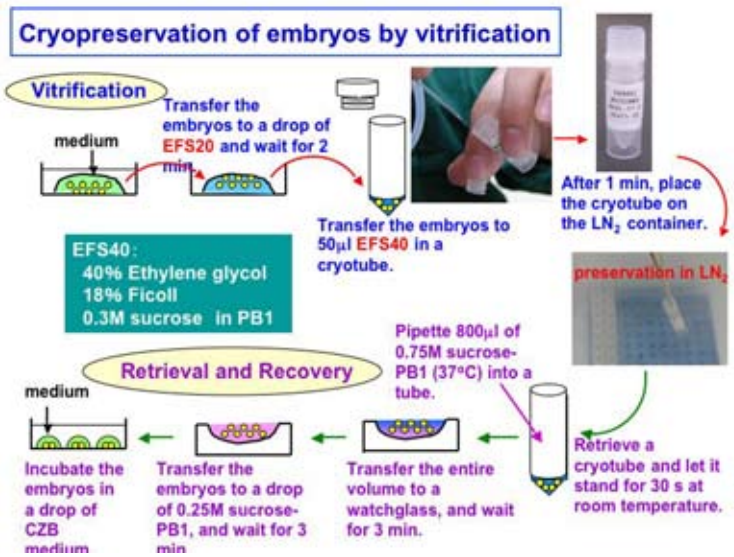

Figure 6. Cryopreservation of embryos by vitrification

2) Development of sperm cryopreservation techniques

The freezing rate for mouse sperm was optimized for their most efficient cryopreservation. When sperm were frozen with 18% raffinose solution as cryoprotectant at rates between –10 °C/min and –450 °C/min, about 20% of motile sperm were retrieved after thawing. No motile sperm were retrieved after freezing at a rate of –0.7 °C/min or –3000 °C/min. The freezing rate was most critical when the temperature decrease was between the refrigeration temperature (4 °C) and –50 °C.

3) Development of oocyte cryopreservation techniques

Mature unfertilized oocytes from BDF1 mice were cryopreserved in 16% DMSO + 16% ethylene glycol + Ficoll + sucrose–PB1 in a cryo-top rather than a cryotube after equilibration in 8% DMSO + 8% ethylene glycol–PB1 for 3 min. Ninety-eight percent of oocytes were morphologically normal after recovery in 0.5 M sucrose. When the surviving oocytes were treated in 2.5 M $SrCl_2$–CZB medium to determine their

developmental capacity, 94% and 69% of oocytes developed parthenogenetically to the two-cell and blastocyst stages, respectively.

IV. Development of new stem cell lines

We have established ES cells and nuclear-transfer embryonic stem (ntES) cells from several mouse strains, as experimental models for human regenerative medicine and the conservation of mouse genetic resources. We also aim to develop methods of gene targeting, and the effective derivation of GS cells and embryonic germ (EG) cells in laboratory animals.

1) Establishment of mouse ES cell lines

Generally, mouse ES cell lines are established from 129 strains. If ES cell lines can also be established from strains other than these 129, they would provide a valuable resource for biomedical research. We have established 37 ES cell lines from six mouse strains including C57BL/6 and F1 hybrids between wild and laboratory strains. Furthermore, 32 ntES cell lines have been established from five donor cell types. Their pluripotency and germline transmission capacity are now being investigated.

2) Establishment of germline stem cells

We are establishing new GS cell lines from mice, rabbits, and monkeys. We have successfully established GS-like cell colonies from newborn gonadal cells from both male and female rabbits. These cells produce stem-cell marker proteins and have alkaline phosphatase activity. Their ability to differentiate into germ cells will be examined with in vivo experiments. Preliminary experiments have revealed that they can be transfected with viral vectors, but further technical improvements are necessary.

3) Characterization of oocytes and stem-like cells isolated from neonatal mouse ovaries

We have found that oocytes collected from neonatal ovaries can develop in vitro for up to 18 days in the presence of stem cell factor and start to degenerate thereafter. They produce a normal-looking zona pellucida and express the markers of growing oocytes. Without treatment with stem cell factor, round colonies consisting of stem-like cells were formed. After they were transplanted into ovaries, some successfully proliferated and preferentially colonized the cortex. More detailed histological examinations are now underway.

4) Derivation of EG cell lines

Embryonic germ cells are multipotent stem cells that are derived from the primordial germ cells of embryos at 8.5–12.5 days post-coitus (dpc). Because of the difficulties involved in establishing EG cell lines from post-12.5-dpc embryos, we have generated EG cell lines from 14.5-dpc female embryos. These EG cell lines exhibit stem-cell marker expression and multipotency. It will be interesting to understand the character of the constituent cells of developing mouse gonads. Further characterization will be undertaken.

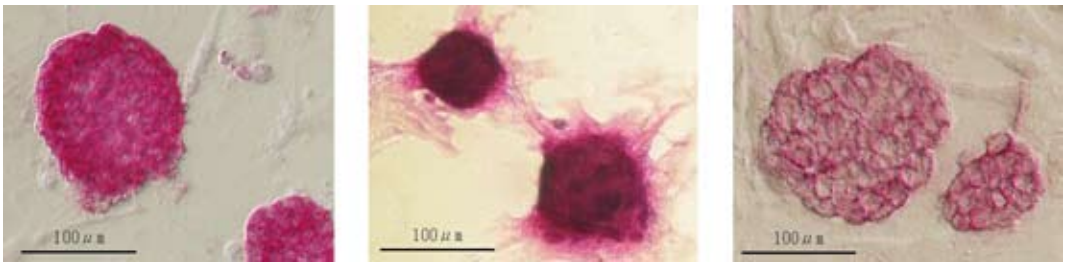

Figure 7. Staining for the alkaline phosphatase activity in mouse ntES cells, rabbit GS-like cells, and mouse EG cells (from the left).

Publications

Original Papers (*Peer reviewed Journal)

1. Kanatsu-Shinohara, M., Miki, H., Inoue, K., Ogonuki, N., Toyokuni, S., Ogura, A. and Shinohara, T.: "Germline niche transplantation restores fertility in infertile mice", Hum. Reprod. 20, 2376-2382 (2005).*

2. Kwon, J., Mochida, K., Wang, Y. L., Sekiguchi, S., Sankai, T., Aoki, S., Ogura, A., Yoshikawa, Y. and Wada, K.: "Ubiquitin C-terminal hydrolase L-1 is essential for the early apoptotic wave of germinal cells and for sperm quality control during spermatogenesis", Biol. Reprod. 73, 29-35 (2005).*

3. Ogonuki, N., Inoue, K., Miki, H., Mochida, K., Hatori, M., Okada, H., Takeiri, S., Shimozawa, N., Nagashima, H., Sanakai, T. and Ogura, A.: "Differential development of rabbit embryos following microinsemination with sperm and spermatids", Mol. Reprod. Dev. 72, 411-417 (2005).*

4. Chuma, S., Kanatsu-Shinohara, M., Inoue, K., Ogonuki, N., Miki, H., Toyokuni, S., Hosokawa, M., Nakatsuji, N., Ogura, A. and Shinohara, T.: "Spermatogenesis from epiblast and primordial germ cells following transplantation into postnatal mouse testis", Development 132, 117-122 (2005).*

5. Inoue, K., Wakao, H., Ogonuki, N., Miki, H., Seino, K., Nambu-Wakao, R., Noda, S., Miyoshi, H., Koseki, H., Taniguchi, M. and Ogura, A.: "Generation of cloned mice by direct nuclear transfer from natural killer T cells", Curr. Biol. 15, 1114-1118 (2005).*

6. Kanatsu-Shinohara, M., Miki, H., Inoue, K., Ogonuki, N., Toyokuni, S., Ogura, A. and Shinohara, T.: "Long-term culture of mouse male germline stem cells under serum- or feeder-free conditions", Biol. Reprod. 72, 985-991 (2005).*

7. Kanatsu-Shinohara, M., Ogonuki, N., Iwano, T., Lee, J., Kazuki, Y., Inoue, K., Miki, H., Takehashi, M., Toyokuni, S., Shinkai, Y., Oshimura, M., Ishino, F., Ogura, A., and Shinohara, T.: "Genetic and epigenetic properties of mouse male germline stem cells during long-term culture", Development 132, 4155-4163 (2005).*

8. Miki, H., Inoue, K., Kohda, T., Honda, A., Ogonuki, N., Yuzuriha, M., Mise, N., Matsui, Y., Baba, T., Abe, K., Ishino, F. and Ogura, A.: "Birth of mice produced by germ cell nuclear transfer", Genesis 41, 81-86 (2005).*

9. Miyamoto, Y., Kuramitsu-Miyamoto, K., Iwanaga, E., Uchio-Yamada, K., Yamaguchi-Yamada, M., Ogura, A., and Manabe, N.: "Effect of human erythropoietin (hEPO) treatment on anemia in ICR-derived glomerulonephritis (ICGN) mice", Exp. Anim. 54, 181-184 (2005).*

10. Mochida, K., Ohkawa, M., Inoue, K., ValdezJr, D. M., Kasai, M. and Ogura, A.: "Birth of mice after in vitro fertilization using C57BL/6 sperm transported within epididymides at refrigerated temperatures, Theriogenology 64, 135-143 (2005).*

11. Mochida, K., Wakayama, T., Takano, K., Noguchi, Y., Yamamoto, Y., Suzuki, O., Matsuda, J. and Ogura, A.: "Birth of offspring after transfer of mongolian gerbil (Meriones unguiculatus) embryos cryopreserved by vitrification", Mol. Reprod. Dev. 70, 464 - 470 (2005).*

12. Ogura, A., Ogonuki, N., Miki, H., and Inoue, K.: "Microinsemination and nuclear transfer using male germ cells", Int. Rev. Cytol. 246,189-229 (2005).*

13. Tanemura, K., Ogura, A., Cheong, C., Gotoh, H., Matsumoto, K., Sato, E., Hayashi, Y., Lee, H. W. and Kondo, T.: "Dynamic rearrangement of telomeres during spermatogenesis in mice", Dev. Biol. 281, 196-207 (2005).*

14. Yamagata, K., Yamazaki, T., Yamashita, M., Hara, Y., Ogonuki, N., and Ogura, A.: "Noninvasive visualization of molecular events in the mammalian zygote", Genesis 43, 71-79 (2005).*

15. Yamaguchi-Yamada, M., Manabe, N., Kiso, M., Goto, Y., Mori, T., Sakata, C., Anan, S., Nagao, M., Yamamoto, Y., and Ogura, A.: "Dysfunction of erythropoietin-producing interstitial cells in the kidneys of ICR-derived glomerulonephritis (ICGN) mice", J. Vet. Med. Sci. 67, 891-899 (2005).*

16. Fulka-Jr, J., Miyashita, N., Nagai, T., and Ogura, A.: "Do cloned mammals skip a reprogramming step?", Nat. Biotechnol. 22, 25-26 (2004).*

17. Goto, Y., Manabe, N., Uchio, K., Yamaguchi-Yamada, M., Inoue, N., Yamamoto, Y., Ogura, A., Nagano, N., and Miyamoto, H.: "Augmented cytoplasmic Smad4 induces acceleration of TGF-beta1 signaling in renal tubulointerstitial cells of hereditary nephrotic ICGN mice with chronic renal fibrosis; possible role for myofibroblastic differentiation", Cell Tissue Res. 315, 209-221 (2004).*

18. Inoue, K., Ogonuki, N., Yamamoto, Y., Takano, K., Miki, H., Mochida, K., and Ogura, A.: "Tissue-specific distribution of donor mitochondrial DNA in cloned mice produced by somatic cell nuclear transfer", Genesis 39, 79-83 (2004).*

19. Kai, M., Irie, M., Okutsu, T., Inoue, K., Ogonuki, N., Miki, H., Yokoyama, M., Migishima, R., Muguruma, K., Fujimura, H., Kohda, T., Ogura, A., Kaneko-Ishino, T., and Ishino, F.: "The novel dominant mutation Dspd leads to a severe spermiogenesis defect in mice", Biol. Reprod. 70, 1213-1221 (2004).*

20. Kamei, Y., Miura S., Suzuki M., Kai Y., Mizukami J., Taniguchi T., Mochida K., Hata T., Matsuda J., Aburatani H., Nishino I. and Ezaki O.: "Skeletal muscle FOXO1 (FKHR) transgenic mice have less skeletal muscle mass, down-regulated Type I (slow twitch/red muscle) fiber genes, and impaired glycemic control", J. Biol. Chem. 24, 41114-41123 (2004).*

21. Kanatsu-Shinohara, M., Inoue, K., Lee, J., Yoshimoto, M., Ogonuki, N., Miki, H., Baba, S., Kato, T.,

Kazuki, Y., Toyokuni, S., Oshimura, M., Heike, T., Nakahata, T., Ishino, F., Ogura, A. and Shinohara, T.: "Generation of pluripotent stem cells from neonatal mouse testis", Cell 119, 1001-1012 (2004).*

22. Manonmani, P., Okada, H., Ogonuki, N., Uda, A., Ogura, A., Yoshida, T. and Sankai, T.: "Fertilization and preimplantation development of mouse oocytes after prolonged incubation with caffeine", Reprod. Med. Biol. 3, 245-251 (2004).*

23. Miki, H., Inoue, K., Ogonuki, N., Mochida, K., Nagashima, H., Baba, T. and Ogura, A.: "Cytoplasmic asters are required for progression past the first cell cycle in cloned mouse embryos", Biol. Reprod. 71, 2022-2028 (2004).*

24. Miki, H., Lee, J., Inoue, K., Ogonuki, N., Noguchi, Y., Mochida, K., Kohda, T., Nagashima, H., Ishino, F., and Ogura, A.: "Microinsemination with first-wave round spermatids from immature male mice", J. Reprod. Dev. 50, 131-137 (2004).*

25. Nakamura, T., Yao, R., Ogawa, T., Suzuki, T., Ito, C., Tsunekawa, N., Inoue, K., Ajima, R., Miyasaka, T., Yoshida, Y., Ogura, A., Toshimori, K., Noce, T., Yamamoto, T., and Noda, T.: "Oligo-astheno-teratozoospermia in mice lacking CCR4-associated factor 1, a novel regulator of RXRb", Nat. Genet. 36, 528-533 (2004).*

26. Ohgane, J., Wakayama, T., Senda, S., Yamazaki, Y., Inoue, K., Ogura, A., Marh, J., Tanaka, S., Yanagimachi, R., and Shiota, K.: "The Sall3 locus is an epigenetic hotspot of aberrant DNA methylation associated with placentomegaly of cloned mice", Genes Cells 9, 253-60 (2004).*

27. Uchio, K., Manabe, N., Yamatuchi-Yamada, M., Goto, Y., Yamamoto, Y., Ogura, A., and Miyamoto, H.: "Changes in the localization of type I, III and IV collagen mRNAs in the kidneys of hereditary nephrotic (ICGN) mice with renal fibrosis", J. Vet. Med. Sci. 66, 123-128 (2004).*

28. Yamaguchi-Yamada, M., Manabe, N., Uchio-Yamada, K., Akashi, N., Goto, Y., Miyamoto, Y., Nagao, M., Yamamoto, Y., Ogura, A., and Miyamoto, H.: "Anemia with chronic renal disorder and disrupted metabolism of erythropoietin in ICR-derived glomerulonephritis (ICGN) mice", J. Vet. Med. Sci. 66, 423-431 (2004).*

29. Yamaguchi-Yamada, M., Manabe, N., Goto, Y., Anan, S., Miyamoto, K., Miyamoto, Y., Nagao, M., Yamamoto, Y., and Ogura, A.: "Improvement of anemia associated with chronic renal failure by recombinant human erythropoietin treatment in ICR-derived glomerulonephritis (ICGN) mice", J. Vet. Med. Sci. 66, 883-886 (2004).*

30. Inoue, K., Ogonuki, N., Mochida, K., Yamamoto, Y., Takano, K., Kohda, T., Ishino, F. and Ogura, A.: "Effects of Donor Cell Type and Genotype on the Efficiency of Mouse Somatic Cell Cloning", Biol. Reprod. 69, 1394-1400 (2003).*

31. Kanatsu-Shinohara, M., Ogonuki, N., Inoue, K., Miki, H., Ogura, A., Toyokuni, S. and Shinohara, T.: "Long-Term Proliferation in Culture and Germline Transmission of Mouse Male Germline Stem Cells", Biol. Reprod. 69, 612-616 (2003).*

32. Kanatsu-Shinohara, M., Ogonuki, N., Inoue, K., Ogura, A., Toyokuni, K., Kogishi, T., Honjo, T. and Shinohara, T.: "Allogeneic offspring produced by male germ line stem cell transplantation into infertile mouse testis", Biol. Reprod. 68, 167-173 (2003).*

33. Kanatsu-Shinohara, M., Ogonuki, N., Inoue, K., Ogura, A., Toyokuni, S. and Shinohara, T.: "Restoration of fertility in infertile mice by transplantation of cryopreserved male germline stem cells", Hum. Reprod. 18, 1-8 (2003).*

34. Ogonuki, N., Tsuchiya, H., Hirose, Y., Okada, H., Ogura, A. and Sankai, T.: "Pregnancy by the tubal transfer of embryos developed after injection of round spermatids into oocyte cytoplasm of the cynomolgus monkey (Macaca fascicularis)", Hum. Reprod. 18, 1273-1280 (2003).*

35. Ogonuki, N., Mochida, K., Inoue, K., Matsuda, J., Yamamoto, Y., Takano, K. and Ogura, A.: "Fertilization of Oocytes and Birth of Normal Pups Following intracytoplasmic Injection with Spermatids in Mastomys (Praomys coucha)", Biol. Reprod. 68, 1821-1827 (2003).*

36. Ogura, A., Ogonuki, N., Inoue, K. and Mochida, K.: "New microinsemination techniques for laboratory animals", Theriogenology 59, 87-94 (2003).*

Oral Presentations

1. Miki, H., Ogonuki, N., Inoue, K., Yamamoto, Y, Noguchi, Y., Takano, K., Mochida, K., Ogura, A.: "Microinsemination with first-wave spermatogenic cells from immature male mice", 29th Ann. Conf. of Int. Embryo Transfer Soc., Auckland, New Zealand, Jan. (2003).

2. Ogura A.: "Cloned mice", 2003 Pre-Conf. Symp. on Mechanisms Regulating Developmental Plasticity, Auckland, New Zealand, Jan. (2003).

3. Ogura A.: "New microinsemination techniques for laboratory animals", 29th Ann. Conf. of Int. Embryo Transfer Soc., Auckland, New Zealand, Jan. (2003).

4. Inoue, K., Ogonuki, N., Miki, H., Noda, S., Kim, J.-M., Aoki, F., Miyoshi, H. and Ogura, A.: "Development of embryos cloned from hematopoietic stem cells", 37th Ann. Meet. of the Soc. for the Study of Reproduction, Vancouver, Canada, August (2004).

5. Miki, H., Inoue, K., Kohda, T., Honda, A., Ogonuki, N., Mise, N., Matsui, Y., Abe, K., Ishino, F., Ogura, A.: "In vitro development of embryos cloned from primordial germ cells in mice", The First Workshop of the Asian Reproductive Biotechnology Society, Ho Chi Minh City, Vietnam, Apr. (2004).

6. Miki, H., Inoue, K., Ogonuki, N., Mochida, K., Nagashima, H., Baba, T., Ogura A.: "Cytoplasmic asters are required for progression of the first cell cycle in mouse cloned embryos", 37th Ann. Meet. of the Society for the Study of Reproduction, Vancouver, Canada, Aug. (2004).

7. Ogura, A.: "ICSI in laboratory animals", Current Status and Perspectives in Reproductive Biology and Biotechnology, Kyoto, Japan, September (2004).

8. Honda, A., Hirose, M., Hiura, H., Sugimoto, M., Yuzuriha, M., Abe, K., Kono, T., Shinohara, T., Ogura, A.: "Derivation of growing oocytes from the neonatal mouse ovary in vitro", International Symposium on Germ Cells, Epigenetics, Reprogramming and Embryonic Stem Cells, Kyoto, Japan, November (2005).

9. Inoue, K.: "Cloning mice from differentiated and undifferentiated cells", The Biology and Practice of Mammalian Cloning, Cold Spring harbor Laboratory, USA, November (2005).

10. Mochida, K.: "An embryo/gamete cryopreservation program at the RIKEN Bioresource Center", The 2nd Asian Reproductive Biotechnology Conference, Bangkok, Thailand, November (2005).

11. Ogonuki, N.: "Application of microinsemination techniques to mouse genetics", 2nd Asian Reproductive Biotechnology Conference, Bangkok, Thailand, November (2005).

12. Ogonuki, N., Inoue, K., Miki, H., Hirose, Y., Okada, H., Shimozawa, N., Takeiri, S., Nagashima, H., Sankai, T. and Ogura, A.: "Differential development of rabbit embryos following microinsemination using sperm and spermatids", 31st Annual Conference of International Embryo Transfer Society, Copenhagen, Denmark, January (2005).

13. Ogura A.: "Cloning Mice from Differentiated and Undifferentiated Cells", International Symposium on Gem Cells, Epigenetics, Reprogramming, and Embryonic Stem Cells, (MEXT Grant "Germ Cells"), Kyoto, Japan, November (2005).

14. Ogura, A.: "Nuclear transfer using germ cells", Mammalian Oogenesis and Epigenetic Modification, Kisarazu, Japan, October (2005).

15. Miki, H., Ogonuki, N., Inoue, K., Baba, T., Ogura, A.: "Improvement of cumulus-free oocyte maturation in vitro and its application to microinsemination with primary spermatocytes in mice", 32nd Annual Conference of International Embryo Transfer Society, Orland, USA, January (2006).