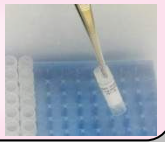

Practical applications of ICSI in mice
Bioresource Engineering Division
RIKEN BRC
Atsuo Ogura, D.V.M., Ph.D.

Bioresource Engineering Division, BRC

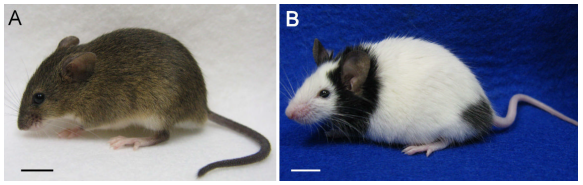
Our specific aims and most recent achievements

Cryopreservation

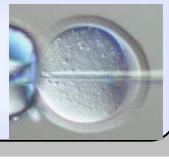


(Presented by Keiji Mochida)

Efficient production of offspring in wild-derived strains of mice *(Biol Reprod in press)*

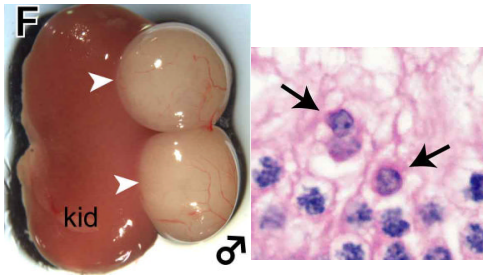


Microinsemination (ICSI)

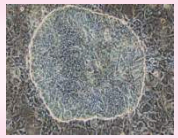


Birth of offspring from ectopically transplanted PGCs *(Biol Reprod 2011)*

ICSI with sperm derived from cultured testes *(Nature 2011)*

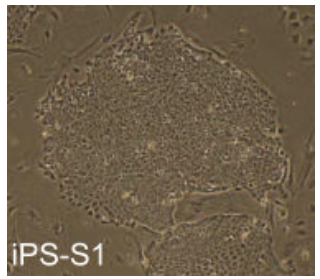


Stem cells

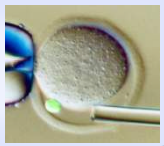


Establishment of rabbit iPS cells *(J Biol Chem 2010)*

Establishment of ES cells from immature oocytes (Stem Cells 2011)



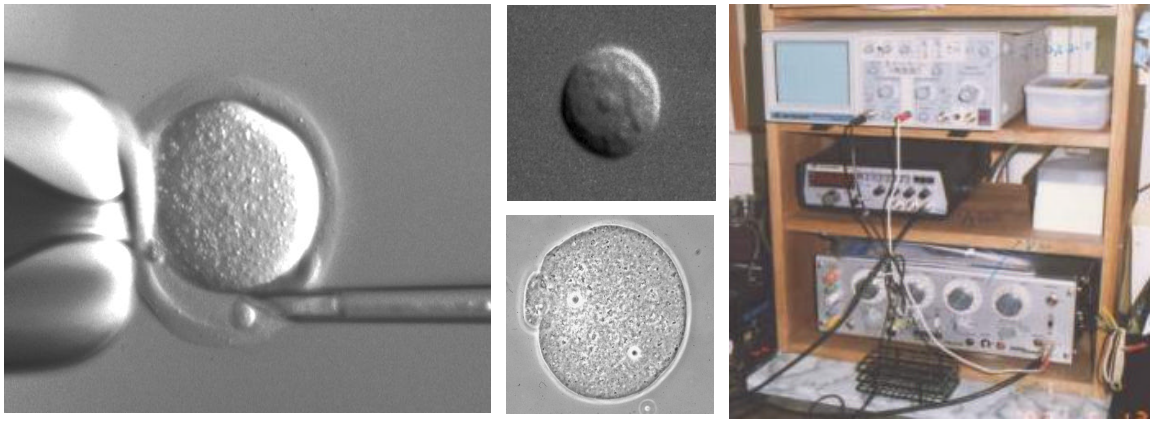
Nuclear transfer



Improvements of efficiency in somatic cell nuclear transfer by correcting aberrant *Xist* expression in cloned embryos *(Science 2010; PNAS 2011)*

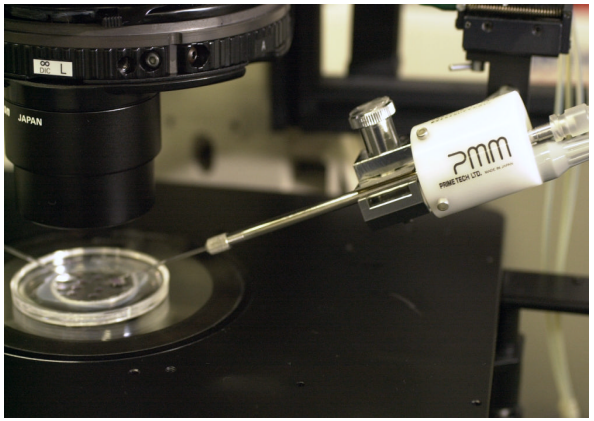
Early mouse ICSI (microinsemination) experiments

Birth of normal young following fertilization of mouse oocytes with round spermatids by electrofusion.
Ogura A et al.
PNAS 91: 7460-7462, 1994.

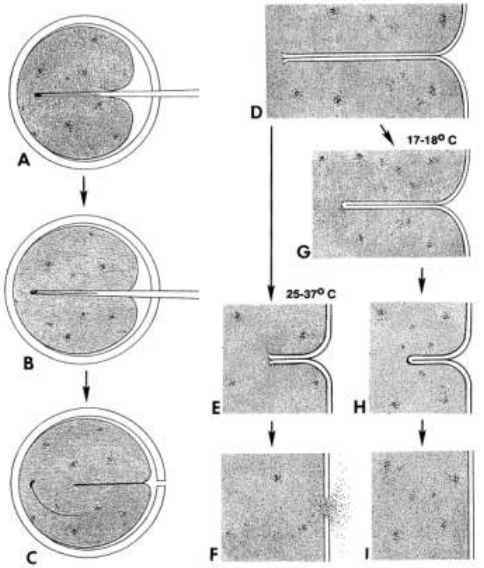


First report on birth of mice following microinsemination

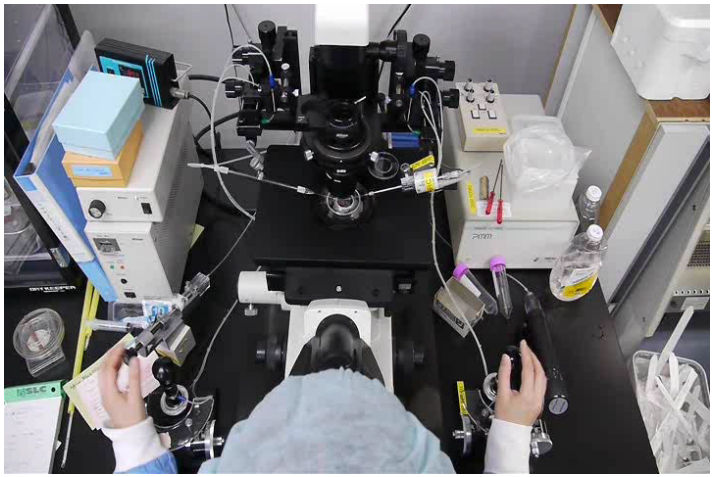
Intracytoplasmic sperm injection in the mouse.
Kimura Y and Yanagimachi R. Biol Reprod 52: 709-720, 1995.



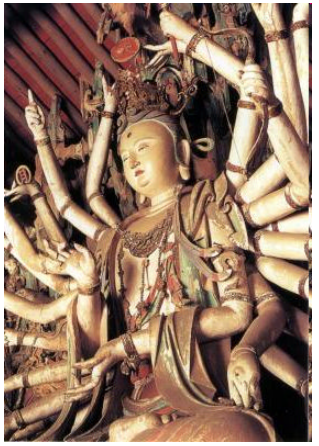
Very efficient Piezo-assisted ICSI in mice



Piezo-assisted mouse ICSI



Piezo-impact unit attached to an injection holder



What factors affect the outcome of ICSI?

Large-scale ICSI experiments for factorial analysis

3 Factors: 5 x 3 x 2 = 30 groups

Genotype
(mouse strain)
ICR
C57BL/6
DBA/2
C3H/He
129^{+Ter}/Sv

The maturity of male germ cells
epididymal sperm
elongated spermatid
round spermatid

Freezing treatment of male germ cells
fresh
freeze-thawing

5 Parameters:
Oocyte survival after injection
Cleavage of oocytes
Implantation
Birth of offspring
Overall efficiency

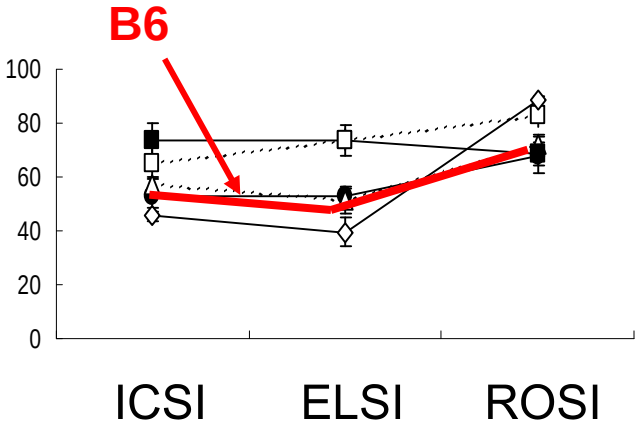
Male germ cells were injected into oocytes from the same strain

Three-way ANOVA from > 100 ICSI experiments

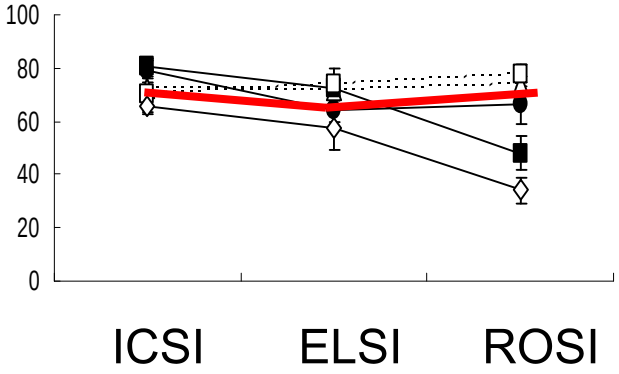
What factors affect the outcome of ICSI?

Data from 5 strains of mice (C57BL/6, DBA/2, C3H, 129, ICR)

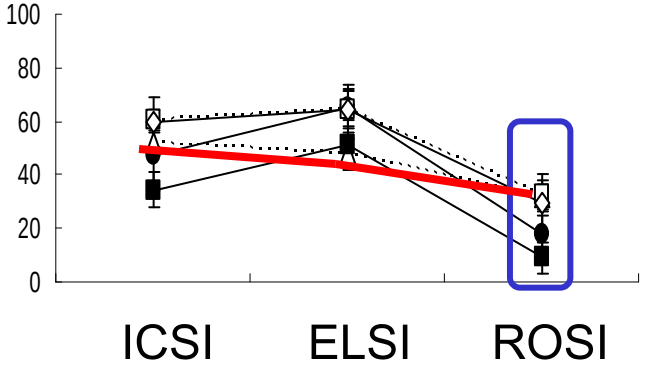
Survival after injection



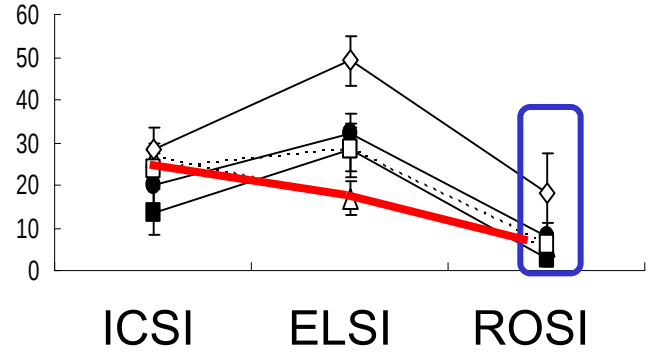
Cleavage



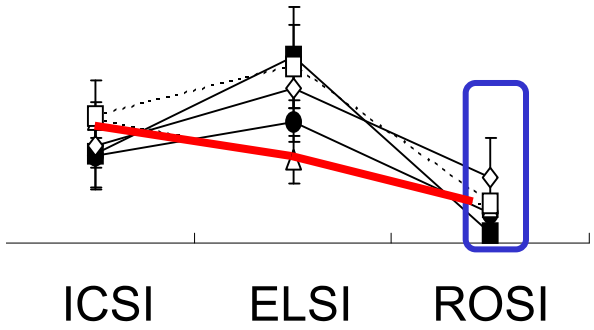
Implantation

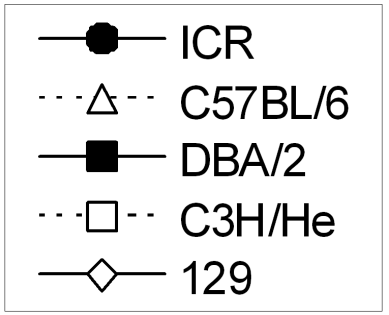


Birth



Overall efficiency





ELSI: elongated spermatid injection; ROSI: round spermatid injection

Ogonuki et al., PLoS One 2010

What factors affect the outcome of ICSI?

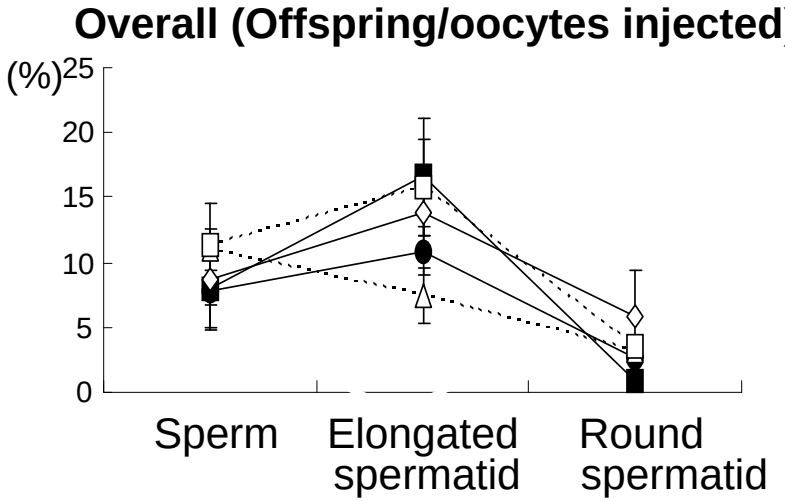
Large-scale ICSI experiments for factorial analysis

Parameters affected by three factors (three-way ANOVA)

- Genotype (strain): Oocyte survival, cleavage, and birth rate.
- Type of germ cells: All (5) parameters
- Freeze-thawing treatment: No effects

Other major findings:

- Overall, elongated spermatids gave better results than spermatozoa.
- DBA/2 oocytes best tolerate the injection stimulus.
- Normal offspring were obtained in all experimental groups (n=30), although the birth rates were often low with frozen-thawed round spermatids.



Ogonuki et al. PLoS One 2010

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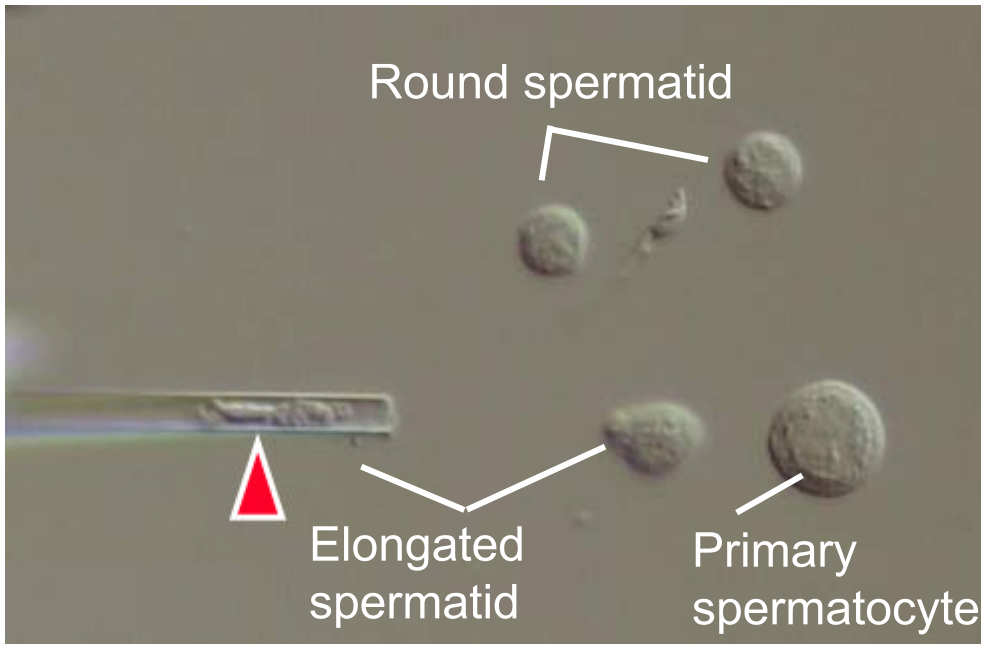


Ogonuki et al. PLoS One 2010

There are many practical applications of microinsemination

Application	Examples
Genetic conservation	Poor reproductive performance Motility failure of frozen sperm due to cryoinjury Simple [illegible] gametes Fertiliz[illegible]yopreserved oocytes
Gene function analysis	Sperm-[illegible]O mice (CD9, Izumo) Cumulus-deficient KO mice
Spermatogonial stem cell transplantation	Allogeneic spermatogonial transplantation Confirmation of clonal origin of spermatogonial colonies
Gene therapy	Rescue of Sl/Sld mice by virus-mediated gene therapy
Production of interspecies F1	C57BL/6 x Spretus
Transgenesis	Sperm-mediated transgenesis by ICSI
Speed congenic	Use of first wave spermatogenic cells
Analysis of sperm factor	Sperm-borne oocyte-activating factor
In vitro manipulation of immature germ cells	ES cell-derived sperm Spermatids derived from in vitro-meiosis PGC ectopic transplantation

Common problems in many repository centers

Applications of ICSI (1) Rescue of infertile strains

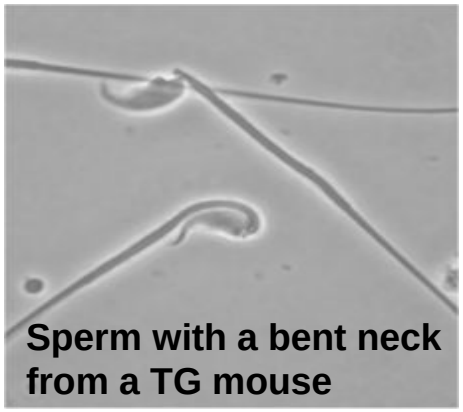
At RIKEN BRC, ICSI has been applied to 35 strains.
They are classified into:

- Fertility problem by unknown causes: 15
- Fertility problem by aging: 4
- Poor motility of frozen sperm for IVF: 12
- Infertile or subfertile TG or mutants: 3
- Delivery of a strain as frozen testes: 1

Of them, 27 strains were successfully propagated.
Unsuccessful 8 cases included:

- Problems in oocytes from sib-females: 7
- Degeneration of sperm from a dead male: 1

Most male-factor infertility cases can be rescued by ICSI



Sperm with a bent neck from a TG mouse

B6;129S-Gtrosa26
Lgh
* DDXC-P
* SMXA-1
C3.KOR-Apoe^{shl}
CXDD-M
STS(tremor)
* NOD.R209-LineT
NOD-Spretus Congenic 18-1
C57BL/6J-TgN(b-actin-hBACE1)rik
Floxed Sox2 mice
mel18-/-
CNR303cp/v11ve1
* KA
* FUBI
Card15KO
129 x B6-G
129 x B6-G
IVCS.129P(B6)-Akr1c18<tm1Mni>
* SMXA-45
* SMXA-33
SMXA-34
NZB.Cg-Cd180<tm1Kmiy>
B6;CB-Rab27a<Gt(pU-21T)58Imeg
FVB129P2(B6)-Mmp2<tm1Ito>
B6C3-Tmem50b<Gt(venusTrap_fJ
B6.Cg-Tg(Scx-GFP)1Stzr
* C57BL/6-Tg(CAG-Nup85)3-3Sush

⋮

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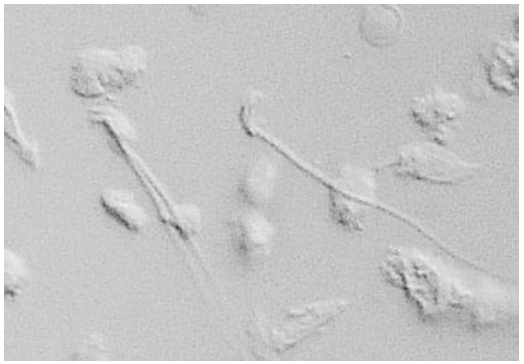
Applications of ICSI (2)　Simple cryopreservation of testis or body

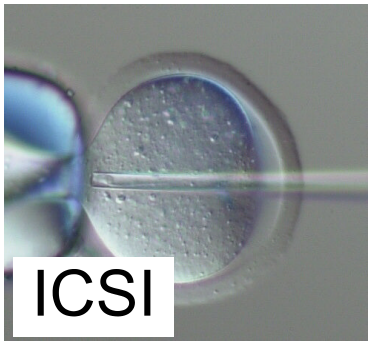
If the ICSI technique is available, cryopreservation of sperm or male germ cells may be simplified.

Epididymides and testes

Store at –80°C

Thawing

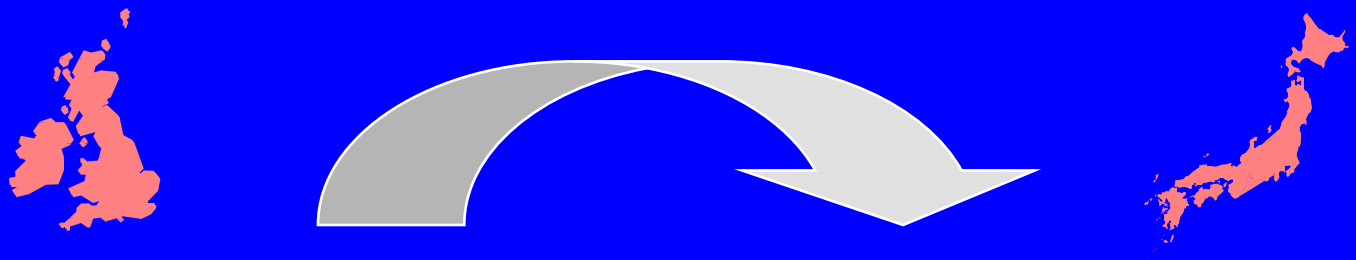




ICSI

Birth (about 30% per ET)

International transportation of frozen testes



Cryopreservation of whole testes from B6 males at MRC, U.K.

Microinsemination at RIKEN BRC, Japan

Cells used	medium	Recipient n	Recipient No. pregnant	No. transferred (Total)	No. (%) implant		No.(%) offspring	
		4	4	50	34	(68.0)	11	(22.0)
		2	2	20	19	(95.0)	13	(65.0)
		1	1	10	3	(30.0)	1	(10.0)
		4	3	39	12	(30.8)	7	(17.9)

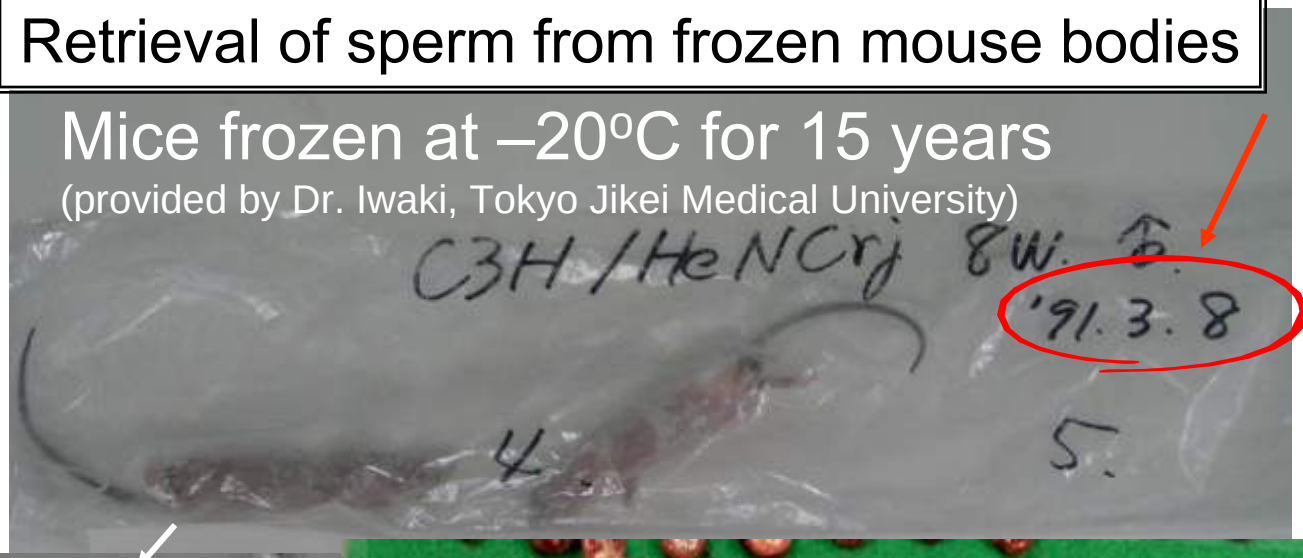
C57BL/6 offspring born at BRC, Japan, using sperm retrieved from testes frozen at MRC, U.K.

Ogonuki et al., PNAS 103: 13098-13103, 2006 in collaboration with Dr. Martin Fray

Retrieval of sperm from frozen mouse bodies

Mice frozen at –20ºC for 15 years

(provided by Dr. Iwaki, Tokyo Jikei Medical University)



Strain of male	No. oocytes that survived inejction	No. (%) oocytes cleaved	No. embryos transferred	Recipient females		No. (%) implant	No. (%) offspring
				No. used	No. pregnant		
BALB/c-nude	106	81 (76.4)	81	6	4	42 (51.9)	17 (21.0)
C3H/He	108	97 (89.8)	97	7	4	34 (35.1)	12 (12.4)

Ogonuki et al., PNAS 103: 13098-13103, 2006

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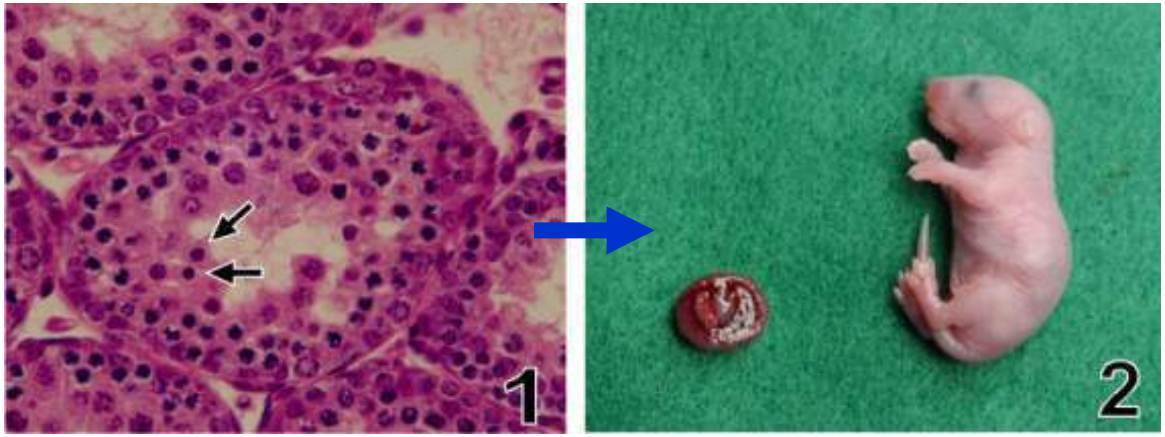
Applications of mouse ICSI (3)

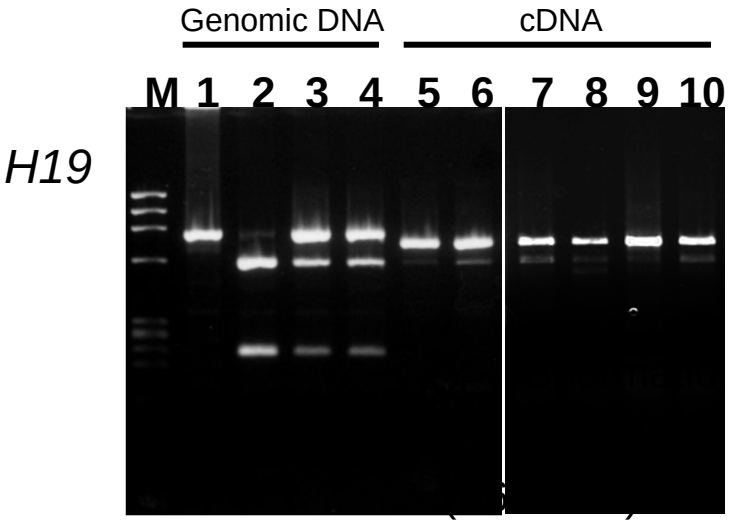
Use of "first wave" spermatids for high-speed congenics



First wave round spermatids in a **17 day-old** testis

A pup born from a 17 day-old spermatid

Application to high-speed congenic strategy



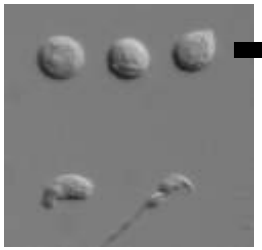
Normal monoallelic expression of imprinted genes (*H19, Igf2, Meg3, Igf2r*)

Miki et al., JRD 2004

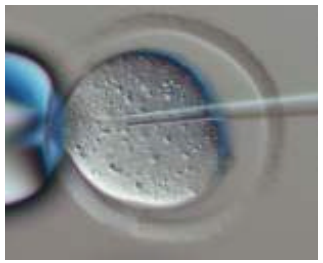
High-speed breeding of mice using immature male germ cells

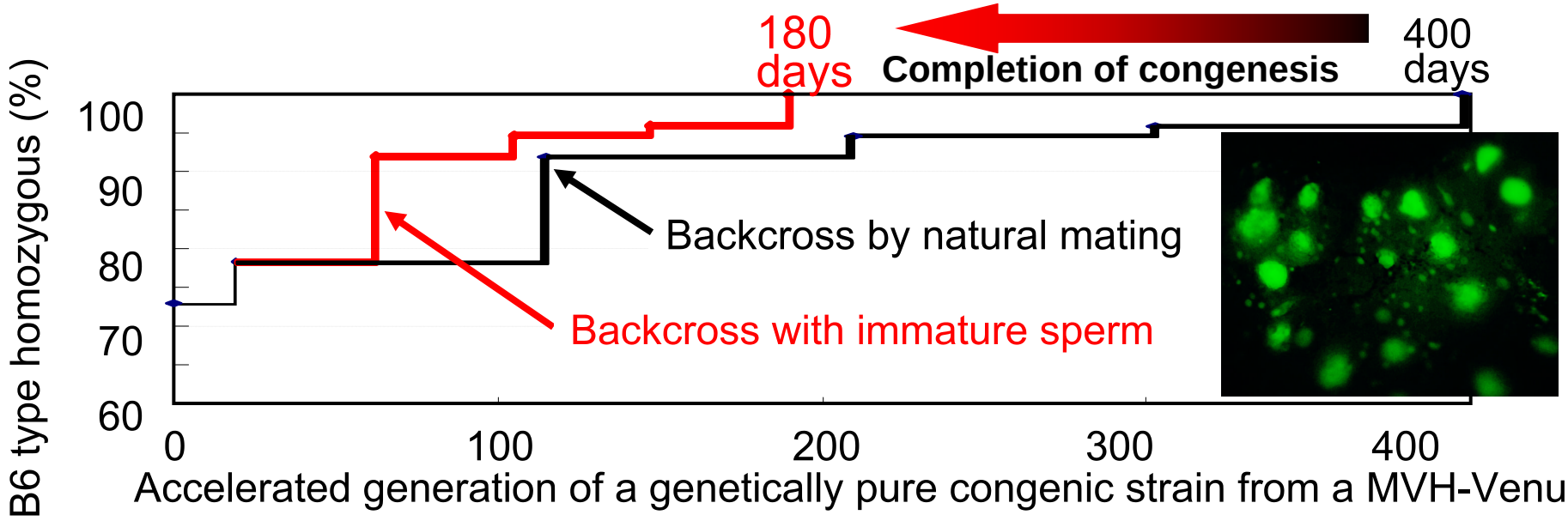
The most rapid generation turnover by sexual reproduction in mammals (40 days)

This **22** day-old male becomes a father



Injection of immature sperm into oocytes

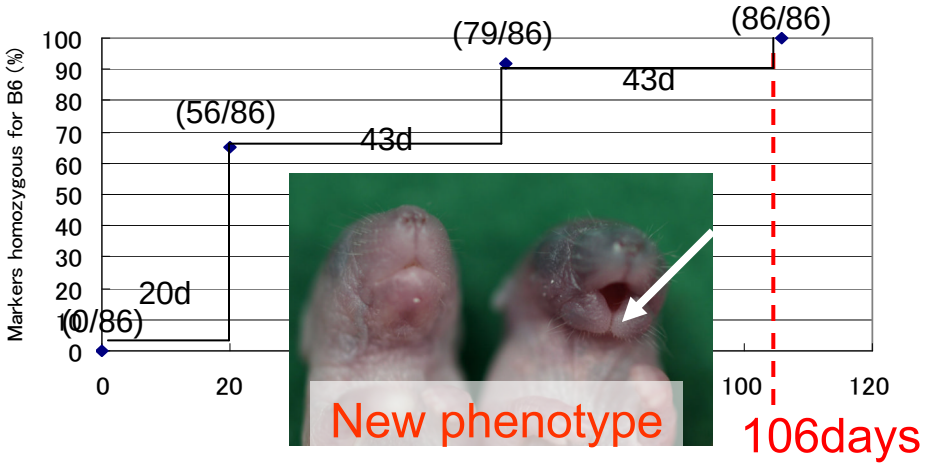




Accelerated generation of a genetically pure congenic strain from a MVH-Venus transgenic male with a mixed genetic background *Ogonuki et al., PLoS One 2009*

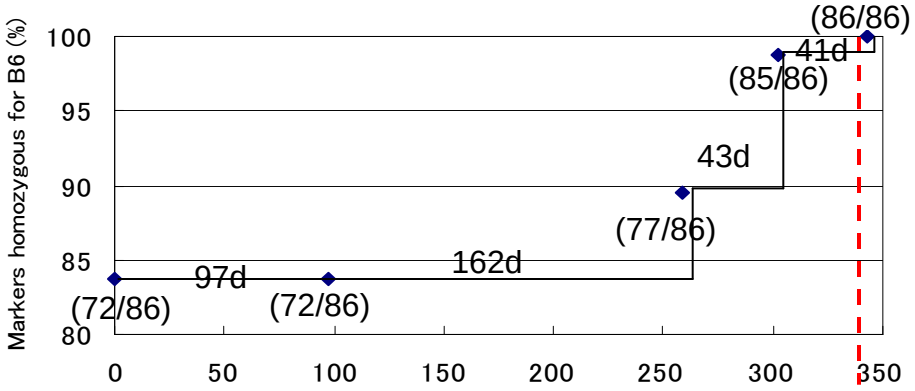
Congenic backcross breeding by round spermatid injection

Knockin (*Ednra*^{EGFP}) ⟶ C57BL/6



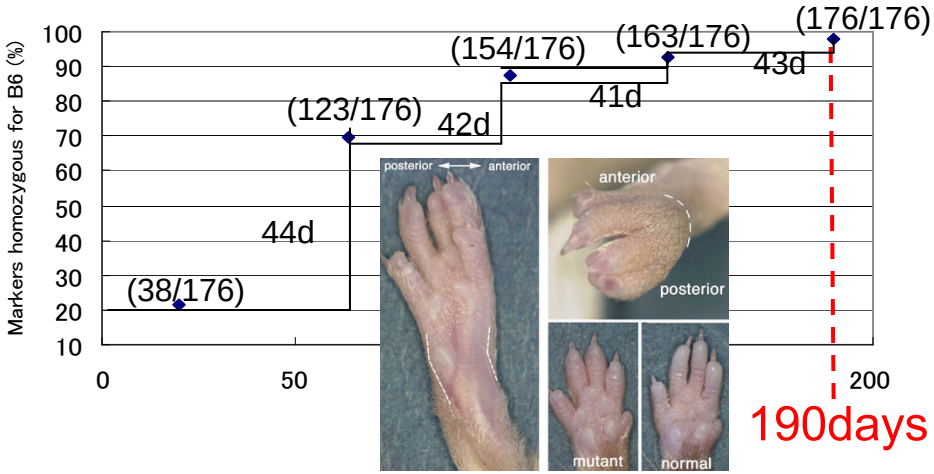
106days

Knock-out (*RELMb*) ⟶ C57BL/6



343days

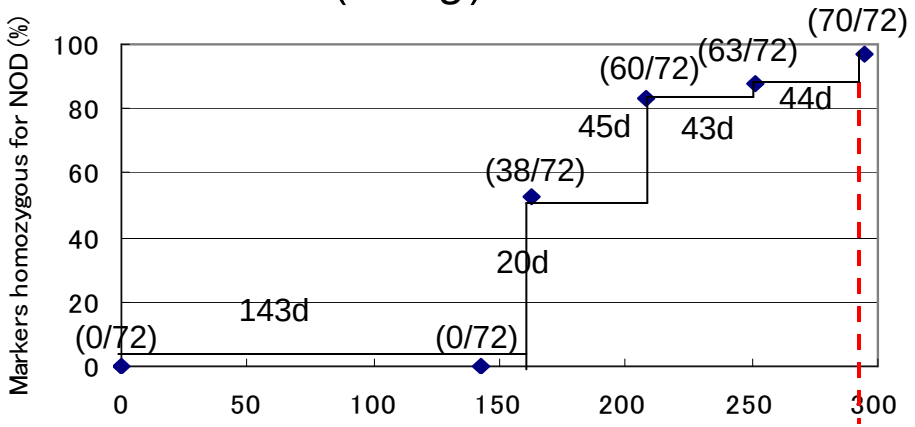
ENU mutant (*Gdf5*) ⟶ C57BL/6



190days

Expected phenotype

Knockin (h*Kitlg*) ⟶ NOD-scid



318days

Ogonuki et al., PLoS One 2009

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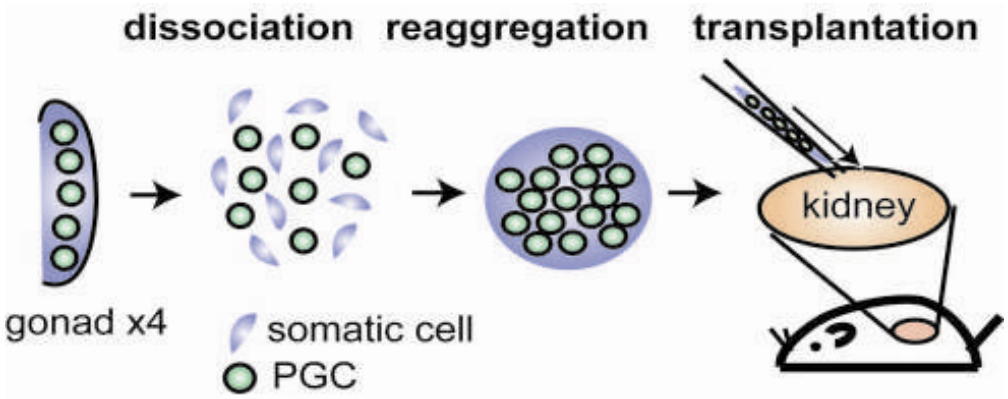
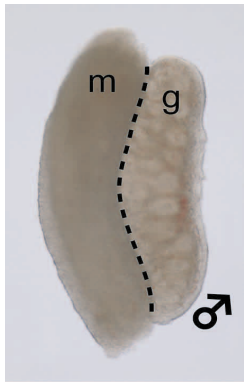
Ectopic transplantation of primordial germ cells

Expecting their future use as a germ cell resource with the proliferating ability



12.5 dpc gonads

♂



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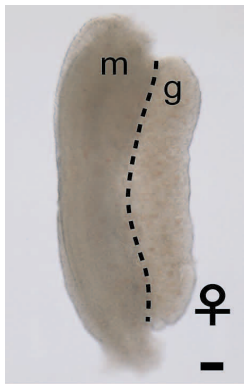
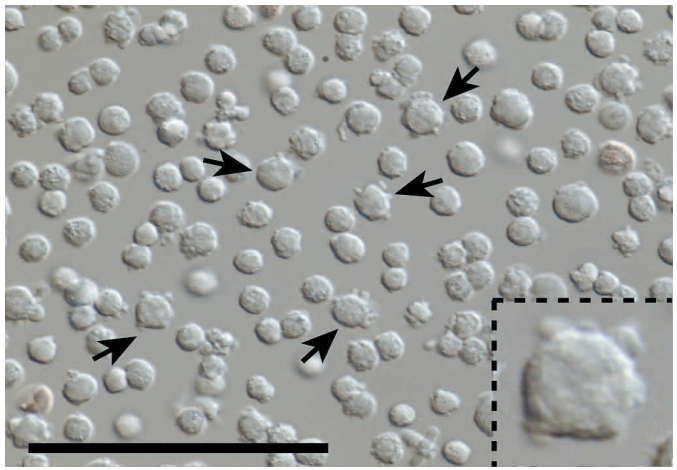
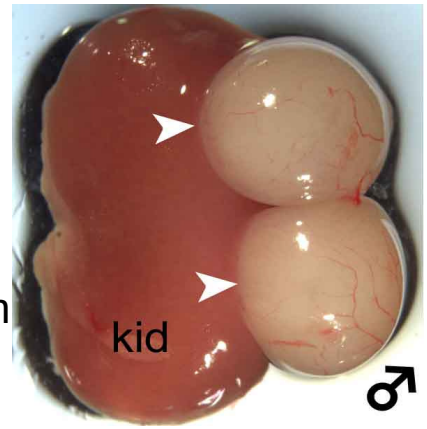


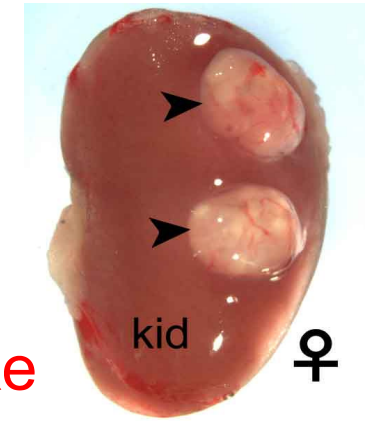
Dissociation by enzyme



Aggregation and transplantation

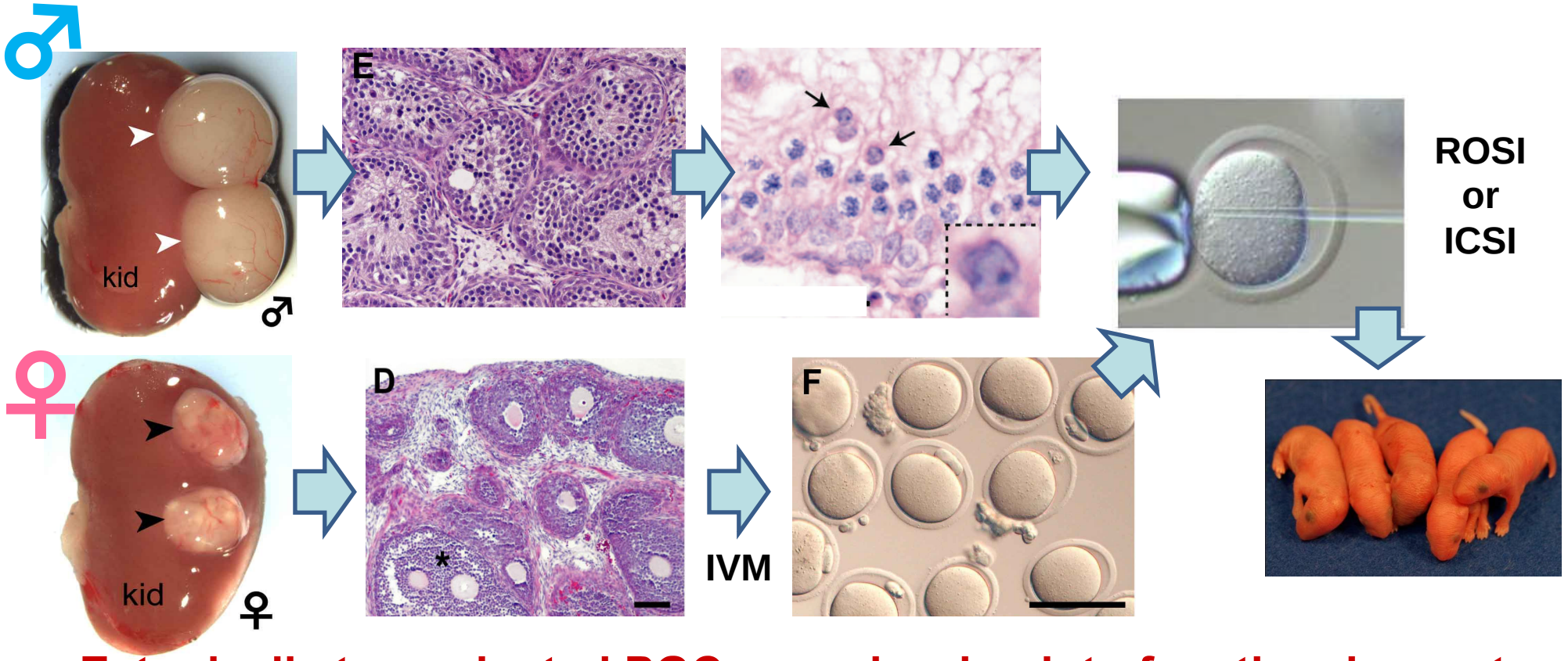
4 weeks after transplantation





PGCs, together with somatic cells, ectopically formed testis- and ovary-like tissues under the kidney capsule.

Use of PGC-derived gametes for microinsemination



Ectopically transplanted PGCs can develop into functional gametes
→ Remarkable flexibility of PGC development

If future technology allows female PGCs to proliferate in vitro, they would become unlimited sources of oocytes.

Matoba and Ogura, Biol. Reprod. 2011

Conclusions

1. Although ICSI in mice needs some skills and experiences, it is a very powerful tool for conservation of mouse strains in mouse repository centers. ICSI can rescue most, if not all, of infertility or IVF failure caused by factors of the male side.

2. Statistical analysis of a large-scale ICSI experiments revealed that elongated spermatid injection is comparable to or even better than epididymal sperm injection in the efficiency of producing offspring.

3. There are many practical applications of ICSI in mice, which may contribute to advancements of mouse genetics and other biomedical research fields.

We would be happy to accept your lab members for ICSI training.
Please contact me at ogura@rtc.riken.go.jp .

For the details of our lab: http://www.brc.riken.go.jp/lab/kougaku/indexE.html