IVF using vitrified unfertilized oocytes

CSIC Main Campus, Madrid, Spain, 7-8 May 2012
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Center for Animal Resources and Development

Mon	Tue	Wed	Thu	Fri	Sat	Sun
PMSG			hCG	IVF	ET/Freezing	
hCG		IVF	ET/Freezing			PMSG

Advantages of cryopreservation of unfetilized oocytes

1) It's not necessary to administer PMSG and hCG.
(You do not need to go to a mouse room in the weekends and holidays.)

Mon	Tue	Wed	Thu	Fri	Sat	Sun
IVF	ET/Freezing					
	IVF	ET/Freezing				
		IVF	ET/Freezing			
			IVF	ET/Freezing		

You can carry out in vitro fertilization when needed!

2) Female mice for donors of oocytes do not need to be kept.

Simple vitrification of unfertilized oocytes

Use of frozen-thawed oocytes for efficient production of normal offspring from cryopreserved mouse spermatozoa showing low fertility.
Sakamoto W, Kaneko T, **Nakagata** N.
Comp Med. 2005 55(2):136-9.

Production of chimeric mice from cryopreserved blastocysts.
Nakao K, **Nakagata** N, Katsuki M.
Exp Anim. 1998 47(3):167-71.

Simple and efficient vitrification procedure for cryopreservation of mouse embryos
Nakao K, **Nakagata** N, Katsuki M.
Exp Anim. 1997 46(3):231-4.

Use of cryopreservation techniques of embryos and spermatozoa for production of transgenic (Tg) mice and for maintenance of Tg mouse lines.
Nakagata N.
Lab Anim Sci. 1996 46(2):236-8.

Studies on cryopreservation of embryos and gametes in mice.
Nakagata N.
Exp Anim. 1995 44(1):1-8.

Production of normal young following transfer of mouse embryos obtained by in vitro fertilization between cryopreserved gametes.
Nakagata N.
J Reprod Fertil. 1993 99(1):77-80.

Cryopreservation of unfertilized mouse oocytes from inbred strains by ultrarapid freezing.
Nakagata N.
Jikken Dobutsu. 1990 39(2):303-5.

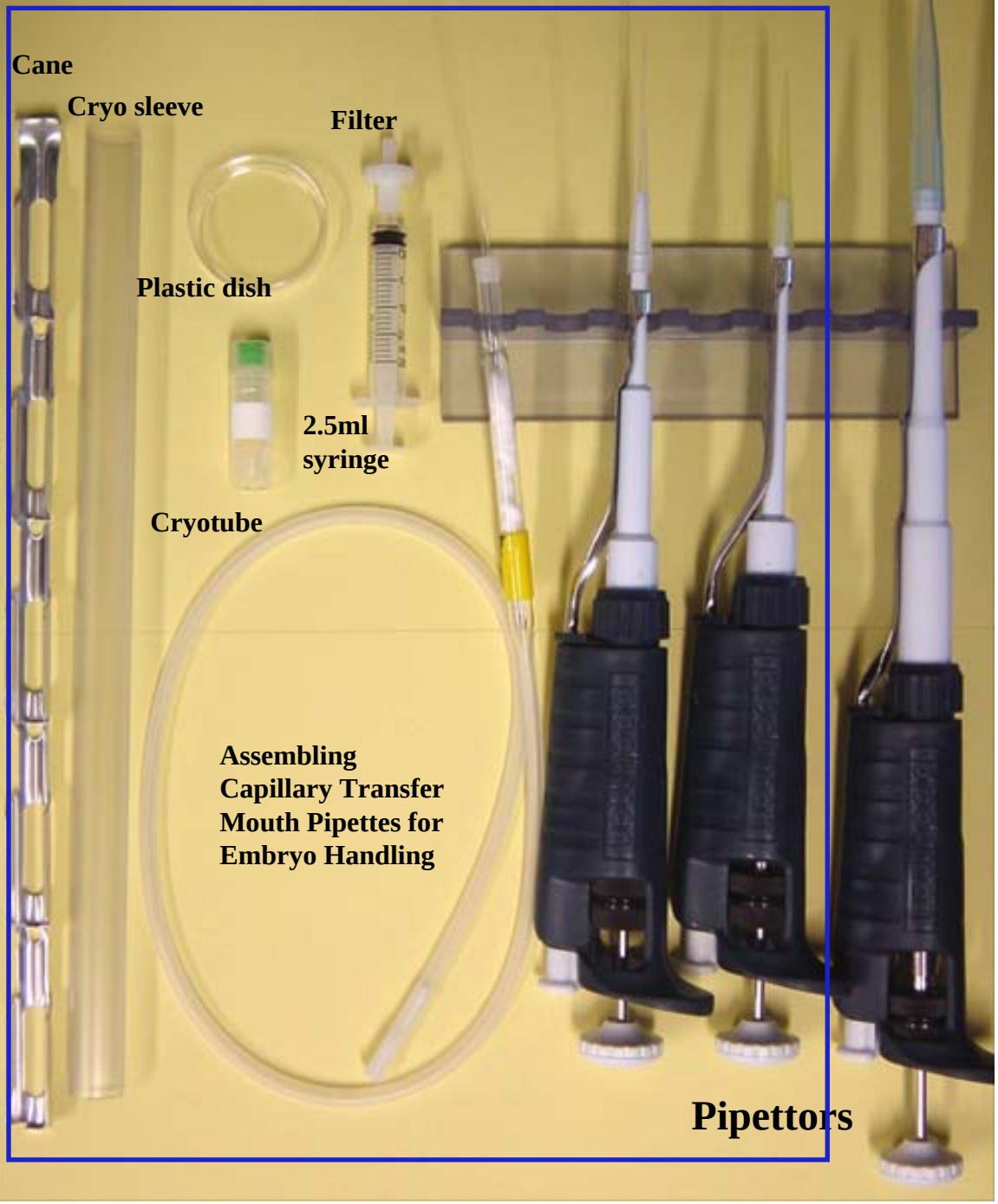
High survival rate of unfertilized mouse oocytes after vitrification.
Nakagata N.
J Reprod Fertil. 1989 87(2):479-83.

Materials and Equipment

1 M DMSO

DAP213

2 M DMSO
1 M acetamide
3 M propylene glycol



Schedule for collection of oocytes

Mice

C57BL/6J

♂ **3 to 6 months of age**

♀ **8 to 12 weeks of age**

Dose：7.5IU/head

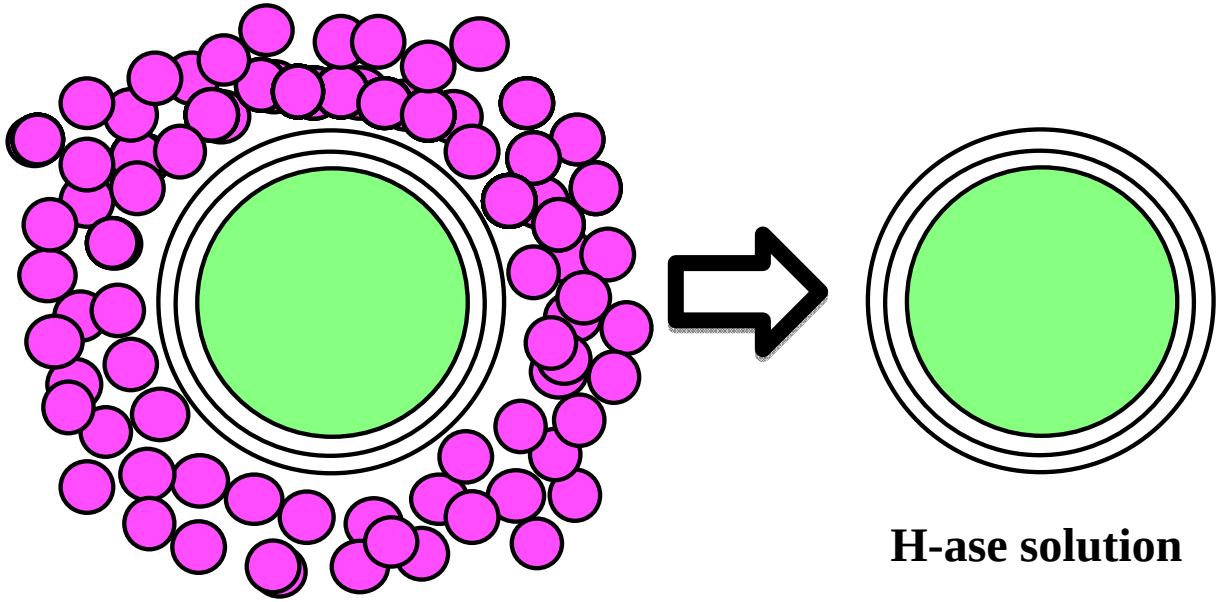
PMSG (17:00~)

48 h

hCG (17:00~)

15 h

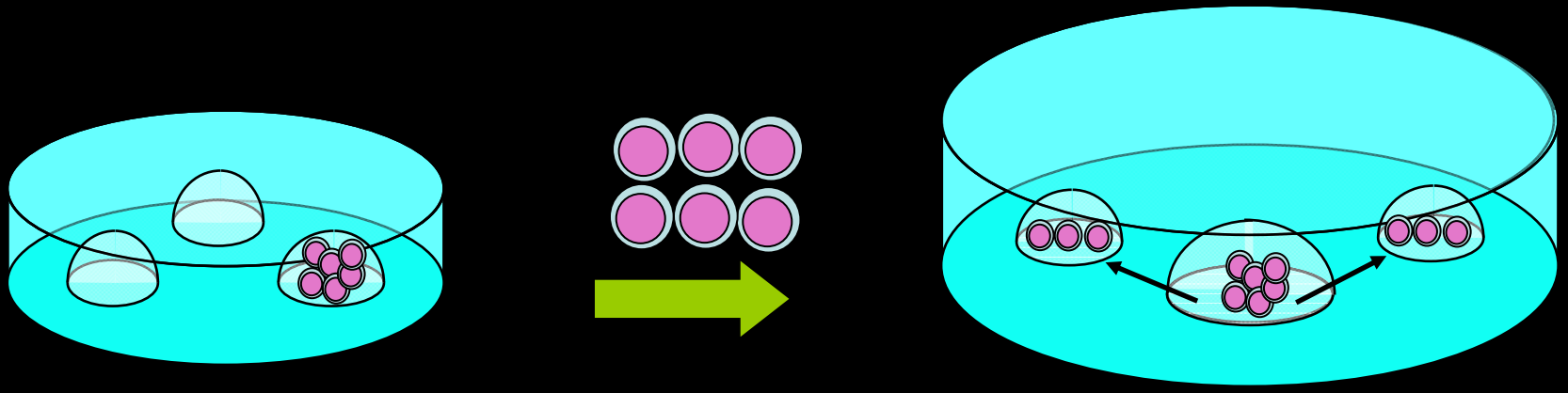
Collection of oocytes (8:00)



Cumulus cells

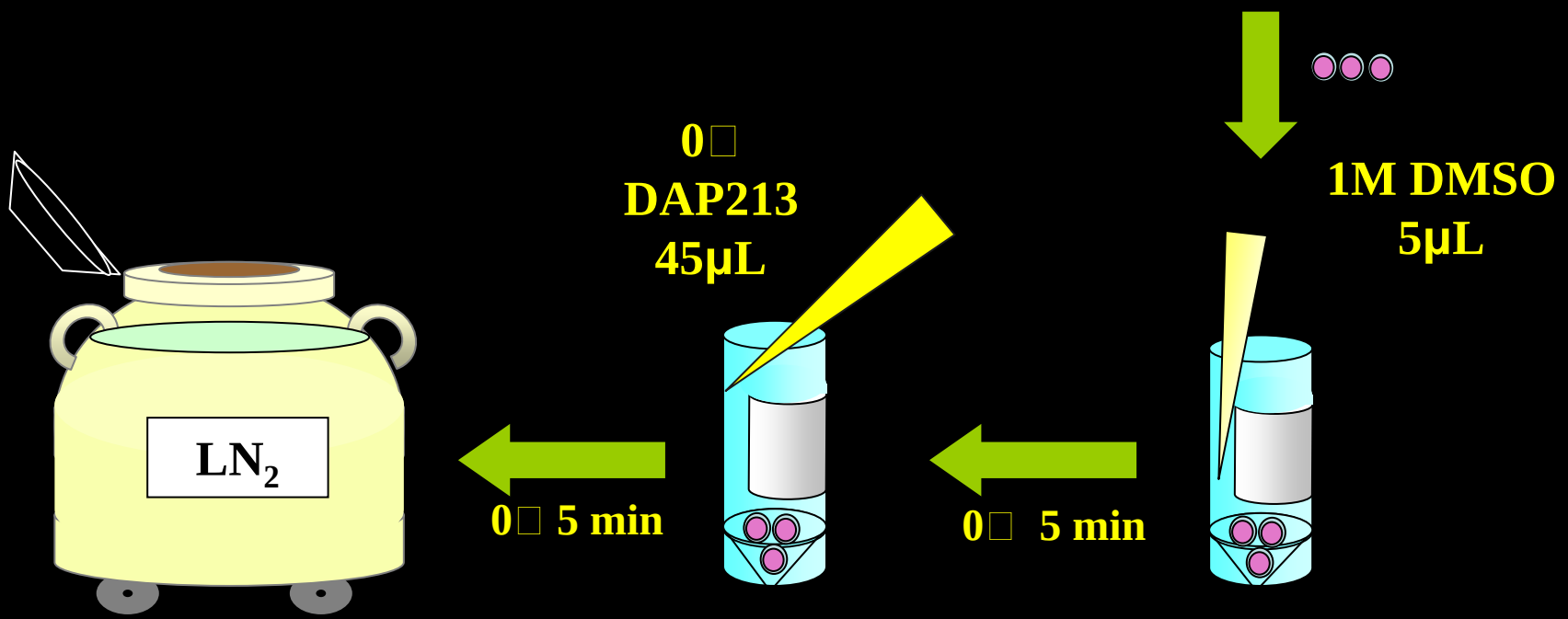
H-ase solution

Simple vitrification



HTF containing 20% FCS

1M DMSO (room temperature)



Materials and Equipment

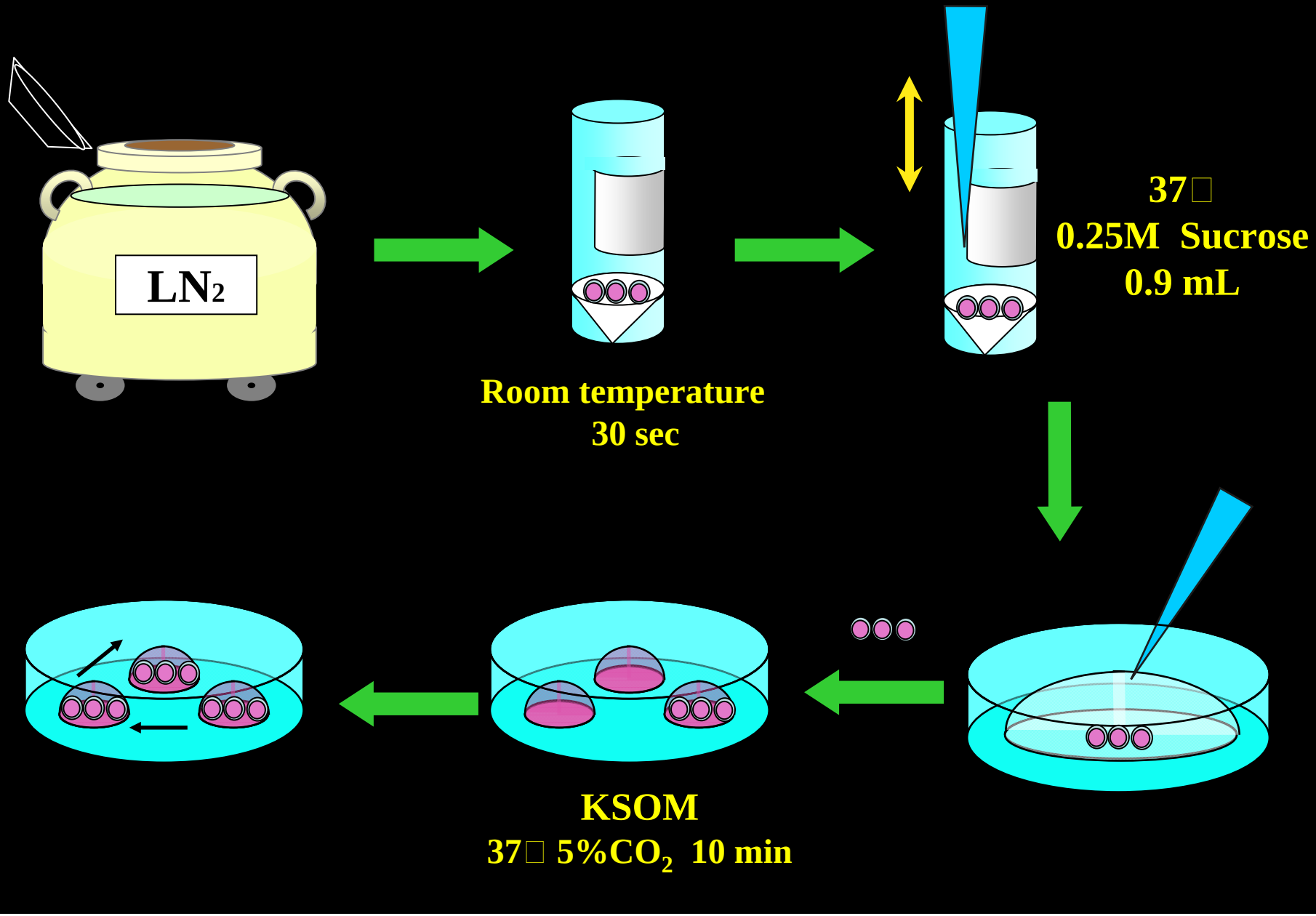
0.25 M Sucrose

Plastic dish

Assembling Capillary Transfer Mouth Pipettes for Embryo Handling

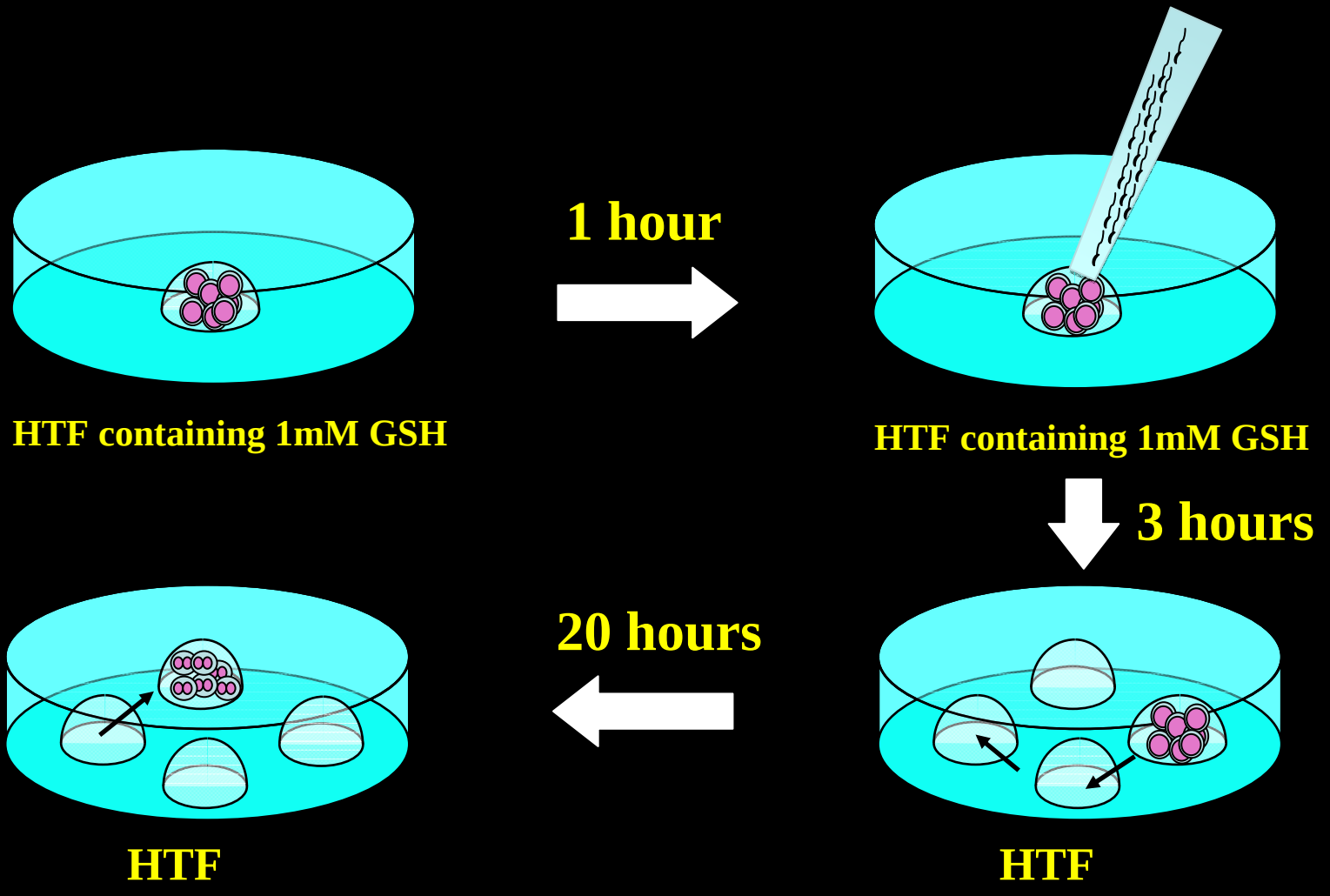
Pipettors

Recovering Vitrified Embryos

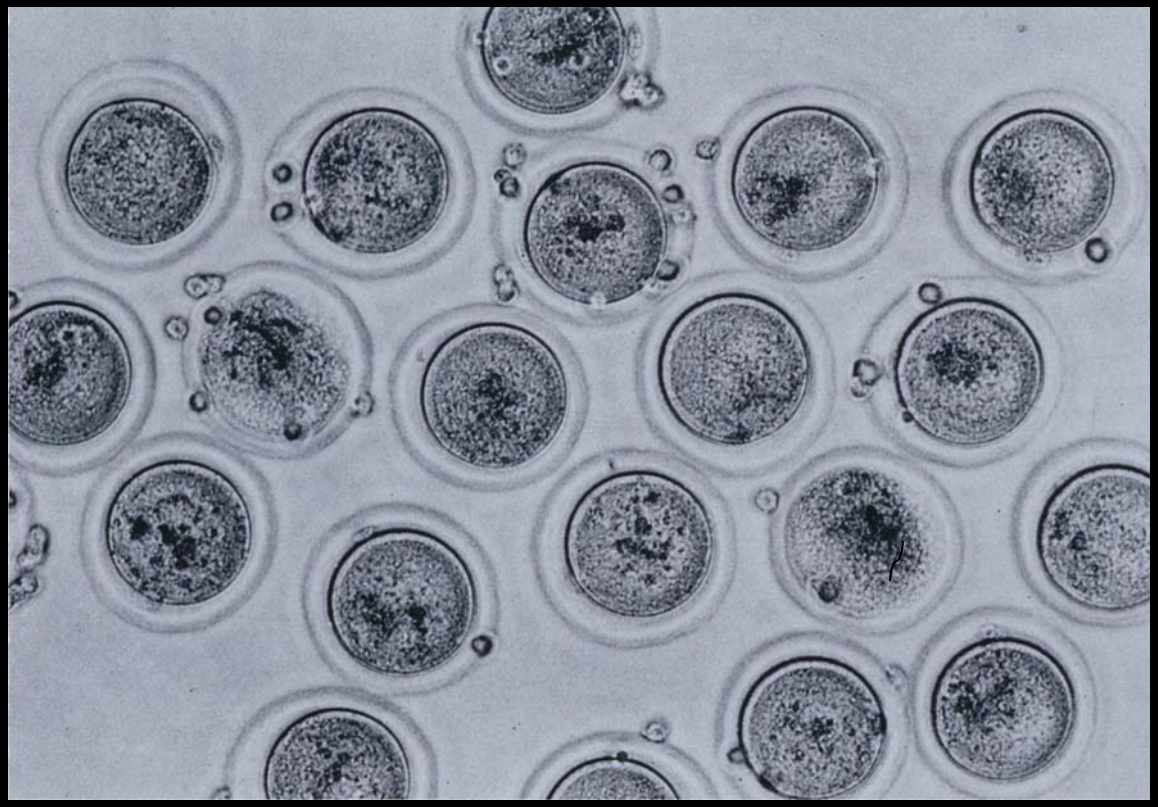


Procedure for IVF using vitrified unfertilized oocytes

Fresh sperm
Cold stored sperm
Frozen sperm
] preincubated in TYH containing 0.75 mM MBCD



Vitrified oocytes after warming



No. of vitrified oocytes	No. of recovered oocytes	No. of morphologically oocytes
2578	2517 (97.6%)	2306 (91.6%)

Fertilization rates using three different oocyte-sperm combinations

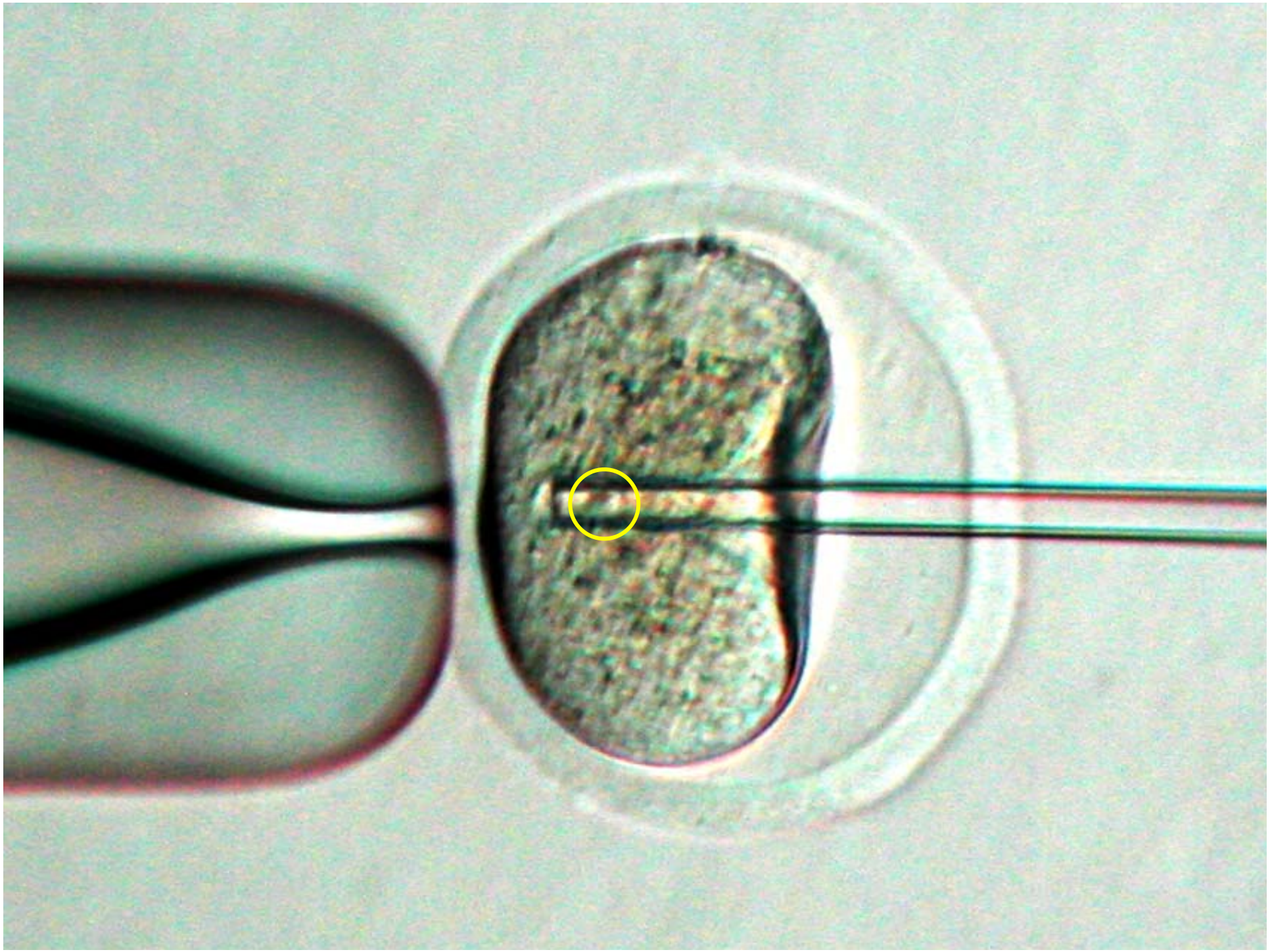
Oocyte	Sperm	No. of inseminated eggs	No. of 2-cell embryos (%)
Vitrified	**Fresh**	**321**	**305 (95.0)**
Vitrified	**Cold stored**	**307**	**245 (79.8)**
Vitrified	**Frozen**	**874**	**746 (85.4)**

Development to blastocysts of 2-cell embryos produced by three different oocyte-sperm combinations

Oocyte	Sperm	No. of 2-cell embryos cultured	No. of blastocysts (%)
Vitrified	Fresh	200	168 (84.0)
Vitrified	Cold stored	125	103 (82.4)
Vitrified	Frozen	306	226 (73.9)

Development to pups of 2-cell embryos produced by three different oocyte-sperm combinations

Oocyte	Sperm	No. of 2-cell embryos transferred	No. of live young (%)
Vitrified	Fresh	100	35 (35.0)
Vitrified	Cold stored	120	55 (45.8)
Vitrified	Frozen	440	188 (42.7)

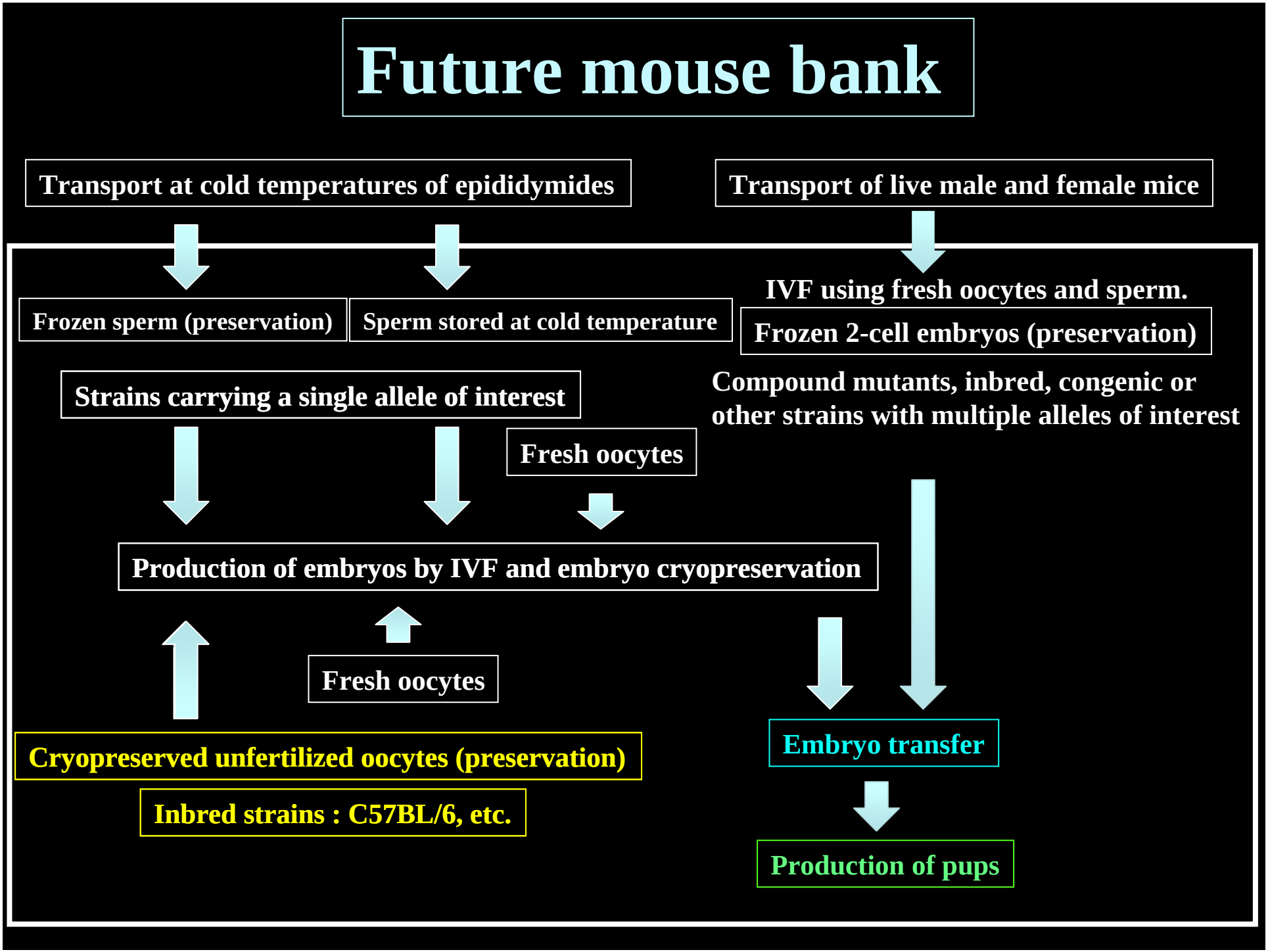


Postimplantation development of embryos derived from frozen-thawed C57BL/6J oocytes injected with frozen-thawed C57BL/6J or transgenic spermatozoa

Strain of frozen sperm	Status of oocytes	No. of oocytes injected	No. (%) of oocytes that survived[a]	No. (%) of oocytes fertilized[b]	No. (%) of embryos that developed to two-cell stage[c]	No. of two-cell embryos transferred	No. (%) of offspring[d]	No. (%) of offspring with transgene[d]
C57BL/6J	fresh	228	121 (53)[e]	118 (98)	116 (98)	106	43 (41)	NA
C57BL/6J	frozen	223	181 (81)[g]	172 (95)	171 (99)	162	68 (42)	NA
Tg A	frozen	30	23 (77)[f]	21 (91)	21 (100)	20	8 (40)	2 (10)
Tg B	frozen	40	36 (90)[g]	35 (97)	35 (100)	35	7 (20)	4 (11)
Tg C	frozen	140	115 (82)[g]	111 (97)	110 (99)	104	17 (16)	4 (4)
Tg D	frozen	82	68 (83)[g]	63 (93)	63 (100)	63	7 (11)	6 (10)

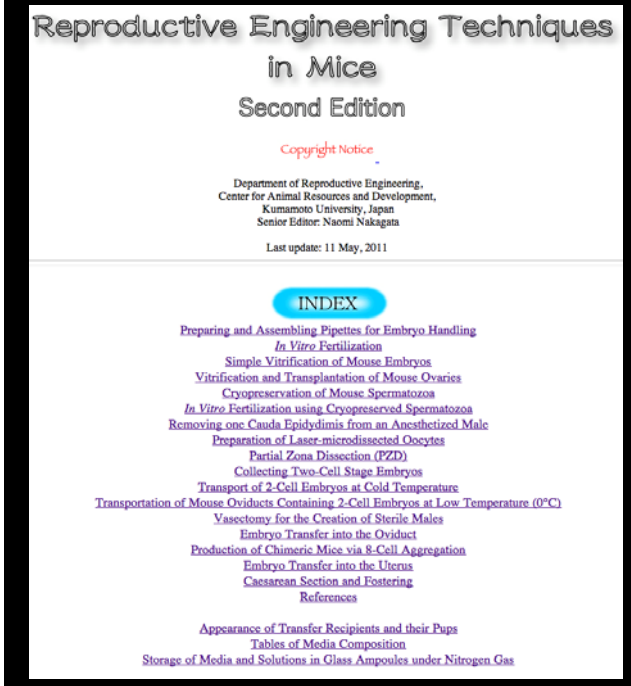
Tg A, B6;D2-Tg(APCS)1lmeg (6); Tg B, C57BL/6J-Tg(MT-hV30M)5lmeg (27); Tg C, B6;CB-Crebbp^{GtAyu3112lmeg} (20); and Tg D, B6;CB-Cdk6^{GtAyu8104lmeg} (1).
Percentages with different letters were significantly different (e versus f, $P < 0.05$, e versus g, $P < 0.005$).
NA, not applicable.
[a]Percentages from no. of oocytes injected.
[b]Percentages from no. of oocytes survived.
[c]Percentages from no. of oocytes fertilized.
[d]Percentages from no. of two-cell embryos transferred.

Future mouse bank



Advantages of the simple vitrification method

1. Very simple
2. Less time-consuming
3. Reduced cost for freezing equipment
4. High survival rate after warming



Reproductive Engineering Techniques in Mice

Second Edition

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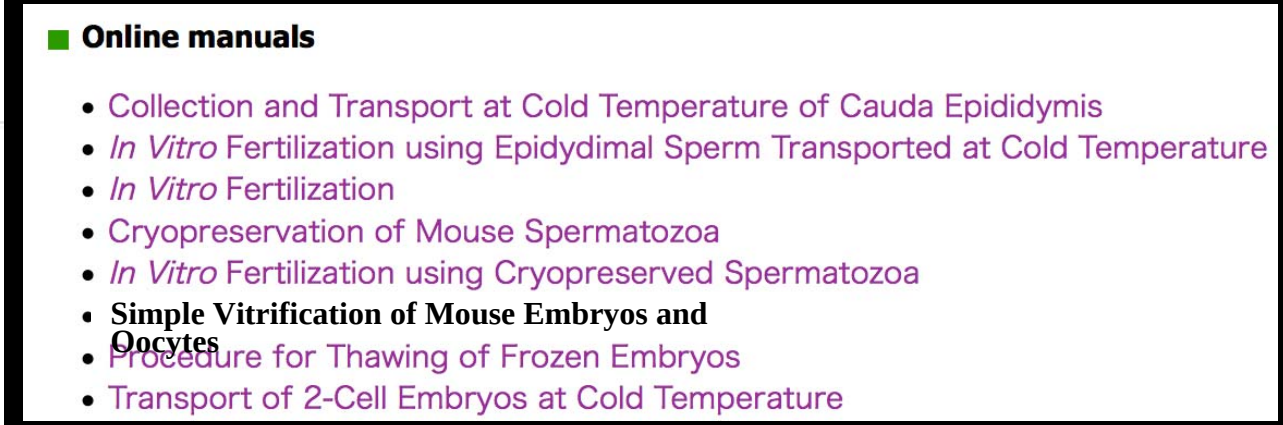
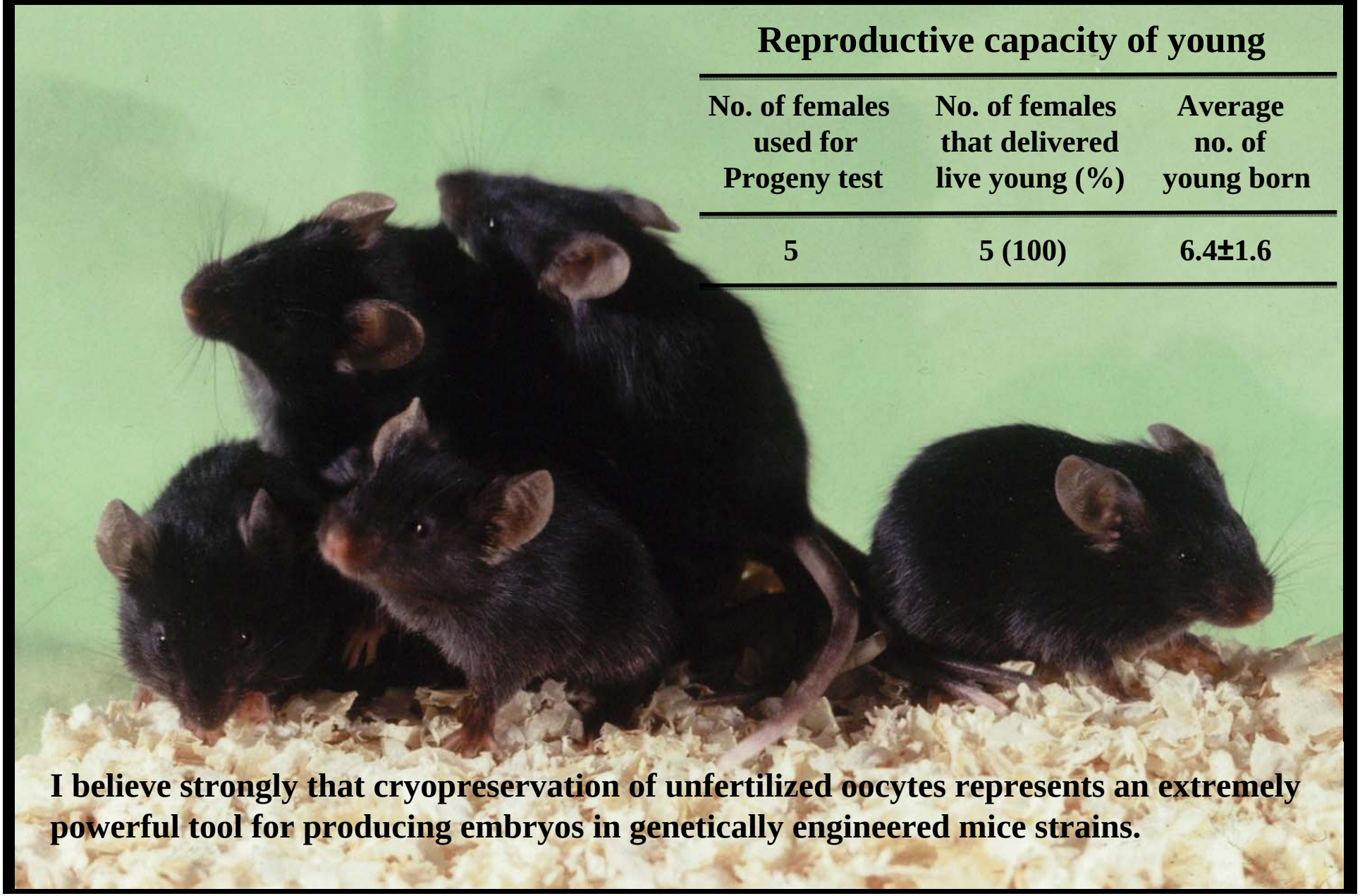


Online manuals

- Collection and Transport at Cold Temperature of Cauda Epididymis
- *In Vitro* Fertilization using Epidydimal Sperm Transported at Cold Temperature
- *In Vitro* Fertilization
- Cryopreservation of Mouse Spermatozoa
- *In Vitro* Fertilization using Cryopreserved Spermatozoa
- **Simple Vitrification of Mouse Embryos and Oocytes**
- Procedure for Thawing of Frozen Embryos
- Transport of 2-Cell Embryos at Cold Temperature

Normal young following transfer of mouse embryos obtained by in vitro fertilization between cryopreserved gametes

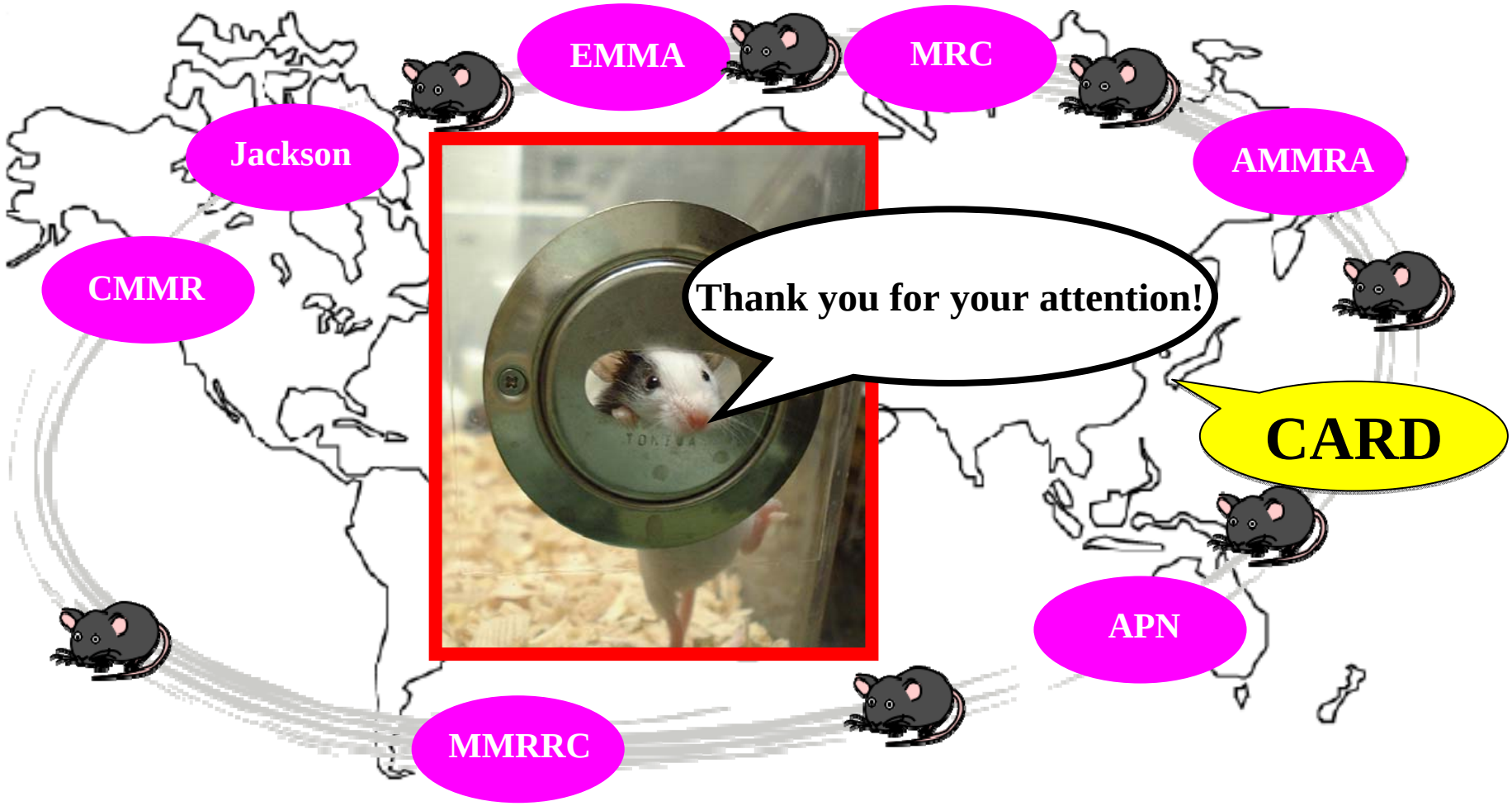


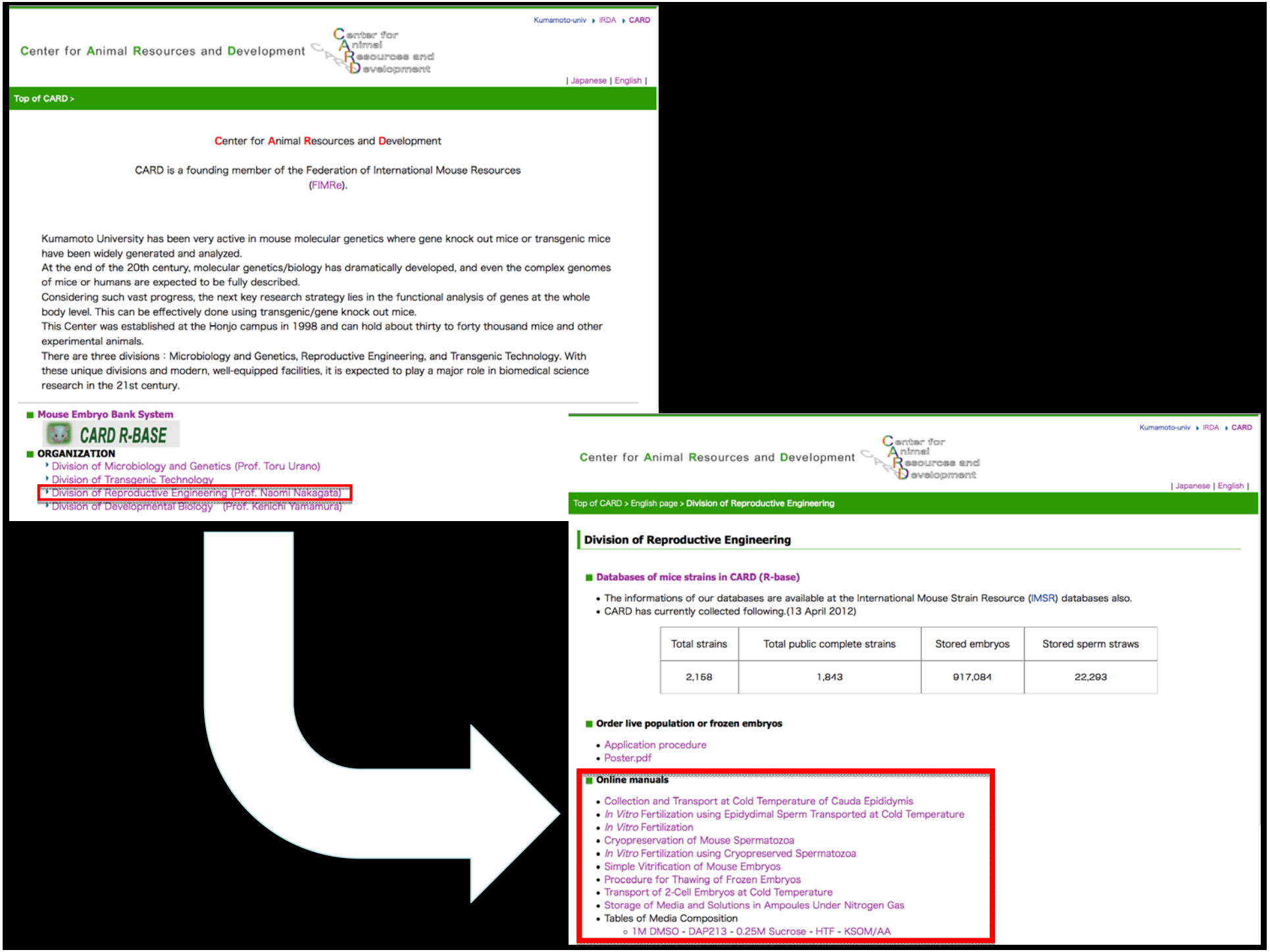
Reproductive capacity of young

No. of females used for Progeny test	No. of females that delivered live young (%)	Average no. of young born
5	5 (100)	6.4±1.6

I believe strongly that cryopreservation of unfertilized oocytes represents an extremely powerful tool for producing embryos in genetically engineered mice strains.

World Hub Centers for Mouse Resource

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Center for Animal Resources and Development
CARD is a founding member of the Federation of International Mouse Resources (FIMRe).
Kumamoto University has been very active in mouse molecular genetics where gene knock out mice or transgenic mice have been widely generated and analyzed.
At the end of the 20th century, molecular genetics/biology has dramatically developed, and even the complex genomes of mice or humans are expected to be fully described.
Considering such vast progress, the next key research strategy lies in the functional analysis of genes at the whole body level. This can be effectively done using transgenic/gene knock out mice.
This Center was established at the Honjo campus in 1998 and can hold about thirty to forty thousand mice and other experimental animals.
There are three divisions : Microbiology and Genetics, Reproductive Engineering, and Transgenic Technology. With these unique divisions and modern, well-equipped facilities, it is expected to play a major role in biomedical science research in the 21st century.
Mouse Embryo Bank System
CARD R-BASE
ORGANIZATION
Division of Microbiology and Genetics (Prof. Toru Urano)
Division of Transgenic Technology
Division of Reproductive Engineering (Prof. Naomi Nakagata)
Division of Developmental Biology (Prof. Kenichi Yamamura)
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Division of Reproductive Engineering
Databases of mice strains in CARD (R-base)
The informations of our databases are available at the International Mouse Strain Resource (IMSR) databases also.
CARD has currently collected following.(13 April 2012)
Total strains
Total public complete strains
Stored embryos
Stored sperm straws
2,158
1,843
917,084
22,293
Order live population or frozen embryos
Application procedure
Poster.pdf
Online manuals
Collection and Transport at Cold Temperature of Cauda Epididymis
In Vitro Fertilization using Epidydimal Sperm Transported at Cold Temperature
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Transport of 2-Cell Embryos at Cold Temperature
Storage of Media and Solutions in Ampoules Under Nitrogen Gas
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