

Alternatives to the LN₂ transport - unfrozen and dry-ice shipments

Shipment of unfrozen 2-cell embryos and sperm

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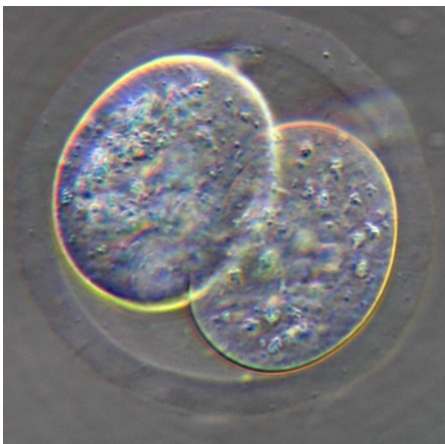
1) Center for Animal Resources and Development (CARD),
Kumamoto University, Japan

2) Mary Lyon Centre, Medical Research Council Harwell, UK

Several ways to transport genetically engineered mice

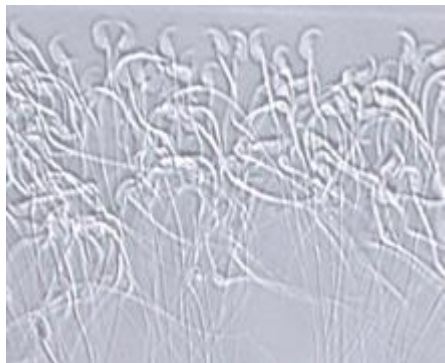


1. Live animals:
Classic



2. Frozen embryos and sperm: Modern

3. Unfrozen embryos and sperm: Latest



Research and Development to improve the functions of mouse resource bank at CARD



Center for Animal Resources and Development (CARD), Kumamoto University, Japan

Shipment of unfrozen materials

1. Cryopreservation of embryos

Exp Animal, 1997

2. Cryopreservation of sperm

Mammalian genome, 2000

Biol Reprod, 2008

Lab Animal, 2010

Biol Reprod, 2011

3. Cryopreservation of oocytes

Cryobiology, 2013

4. Cold-storage of embryos

Cryobiology, 2009

Am Assoc Lab Anim Sci, 2010

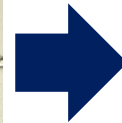
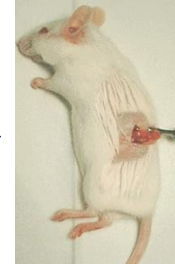
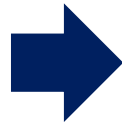
5. Cold-storage of sperm enclosed in cauda epididymides

Cryobiology, 2012

Cryobiology, 2014

Outline of animal production using the cold-stored and transported embryos or sperm

1. Transport of unfrozen 2-cell embryos

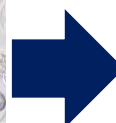
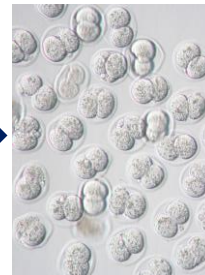
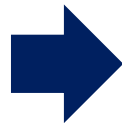


2-cell embryo
From IVF or cryopreservation

Transportation
at 4°C

Embryo transfer

2. Transport of unfrozen sperm (epididymides)



Transportation
at 4°C

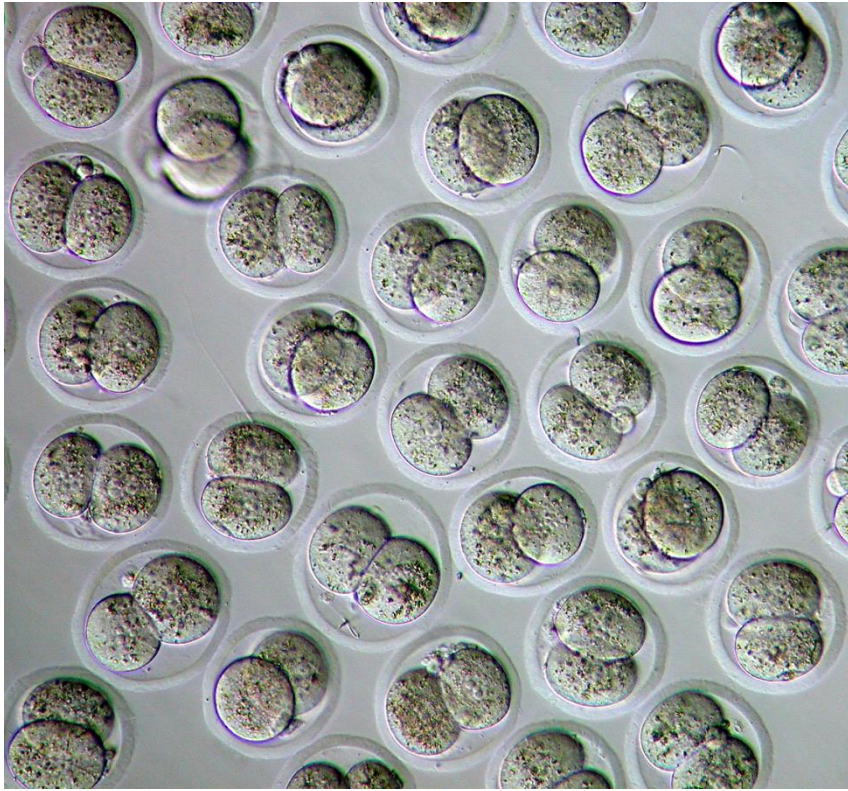
IVF

Embryo transfer

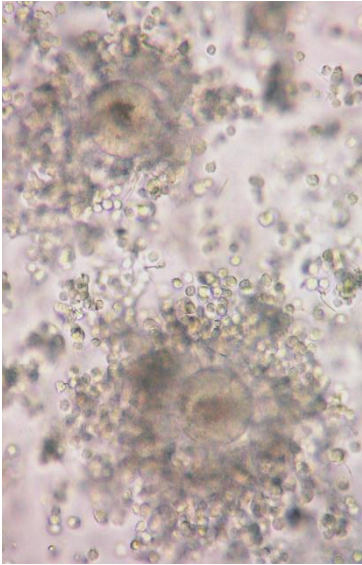


Sperm cryopreservation

1. Transport system of unfrozen 2-cell embryos



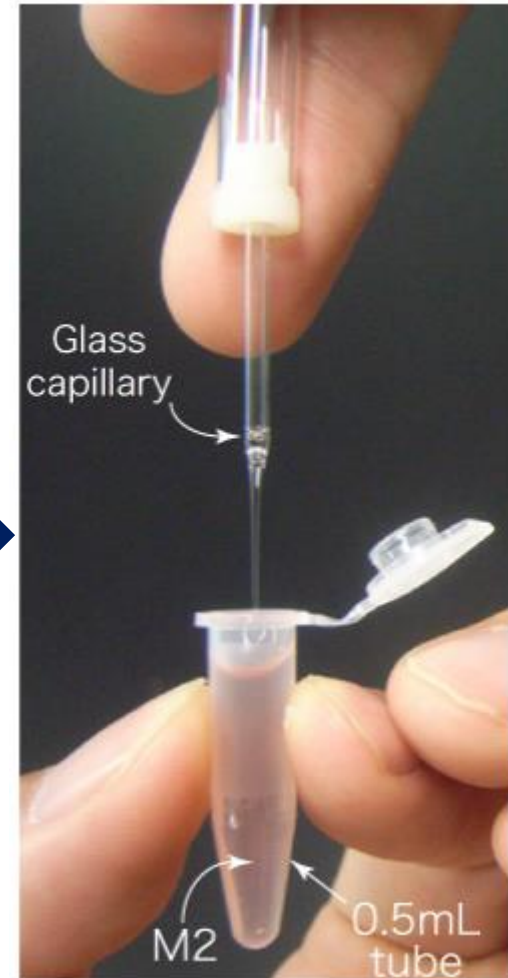
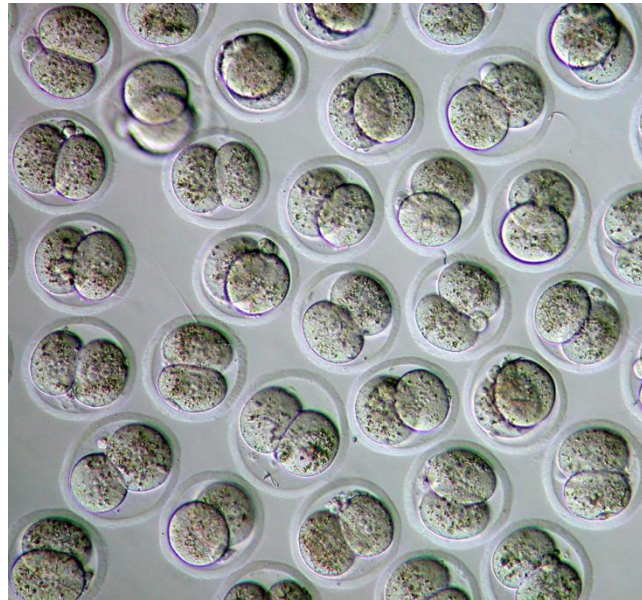
Simple preparations of 2-cell embryos for cold storage



IVF



Cryopreservation



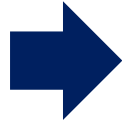
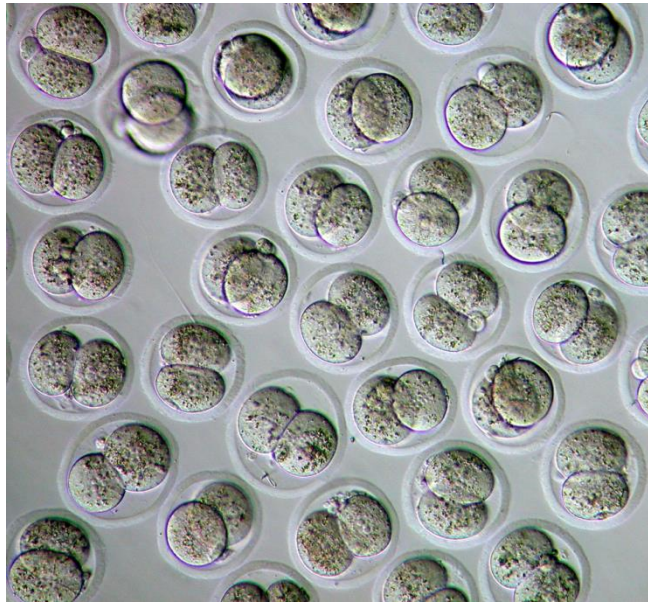
Two-cell embryos maintains developmental ability for 72 hours at 4°C

			No. of survival	No. of	No. of	No. of
	Storage time	No.of examined	2-cell embryos	4-cell embryos	morulae	blastocysts
Media	(h)	2-cell embryos	(%)	at 24 h (%)	at 48 h (%)	at 72 h (%)
control	0	100	100 (100)	97 (97 ± 1.6)	97 (97 ± 1.6)	84 (84 ± 5.5)
PB1	24	78	78 (100)	42 (54 ± 30.9)*	38 (49 ± 30.8)*	25 (32 ± 32.0)*
	48	76	76 (100)	4 (5 ± 7.4)*	2 (3 ± 3.7)*	0 (0)*
	72	77	72 (94 ± 2.5)	2 (3 ± 3.9)*	0 (0)*	0 (0)*
mWM	24	80	80 (100)	73 (91 ± 8.8)	69 (86 ± 1.8)	60 (75 ± 14.1)
	48	80	80 (100)	17 (21 ± 5.3)*	15 (19 ± 5.3)*	4 (5)*
	72	80	79 (99 ± 1.6)	0 (0)*	0 (0)*	0 (0)*
M2	24	76	76 (100)	74 (97 ± 0.2)	65 (86 ± 4.5)	63 (83 ± 7.4)
	48	76	76 (100)	66 (87 ± 2.6)	66 (87 ± 1.3)	48 (63 ± 10.1)
	72	75	75 (100)	66 (88 ± 9.9)	52 (69 ± 10.1)*	32 (43 ± 4.9)*

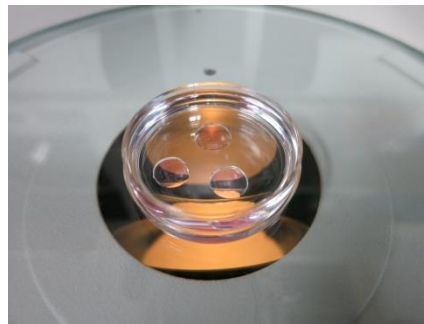
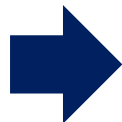
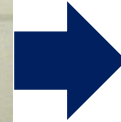
Percent data are presented as Mean ± SD (n = 3).

*Values are significantly different compared to control at 0 h ($P < 0.05$).

The cold-stored 2-cell embryos can be available for embryo transfer or embryo culture to blastocysts



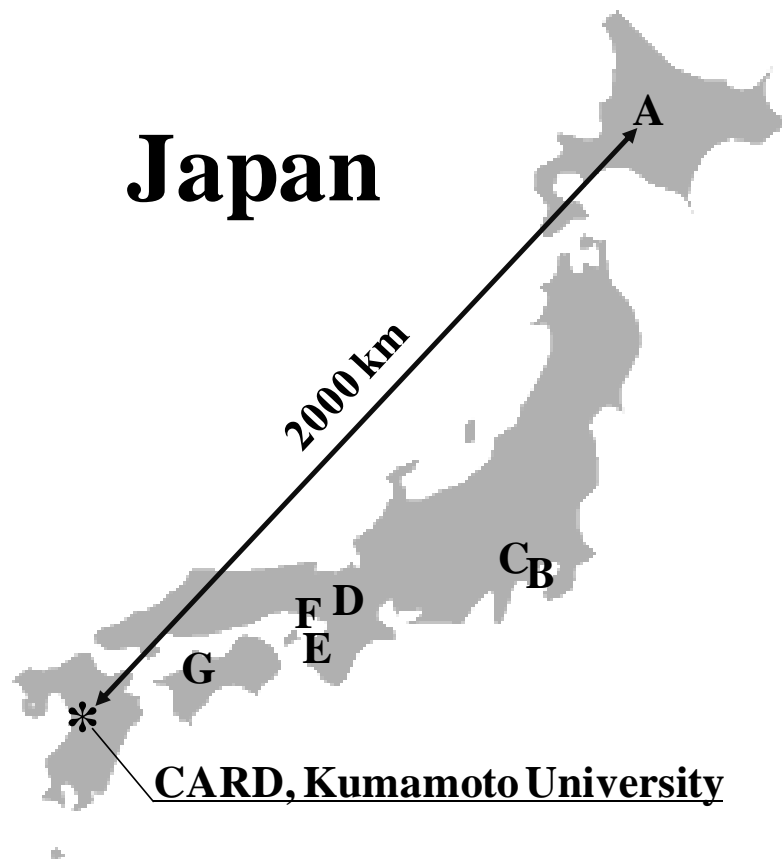
1. Embryo transfer



2. Embryo culture



CARD Embryo Shipment Project I: Domestic shipment in Japan



Shipment environment in Japan

1. Shipment duration: within 2 days everywhere
2. Well-regulated temperature at 4°C
3. Shipment cost: 14 Euro/percel
4. Designated time delivery service

The CARD Cold Transport Kit



Vacuum bottle



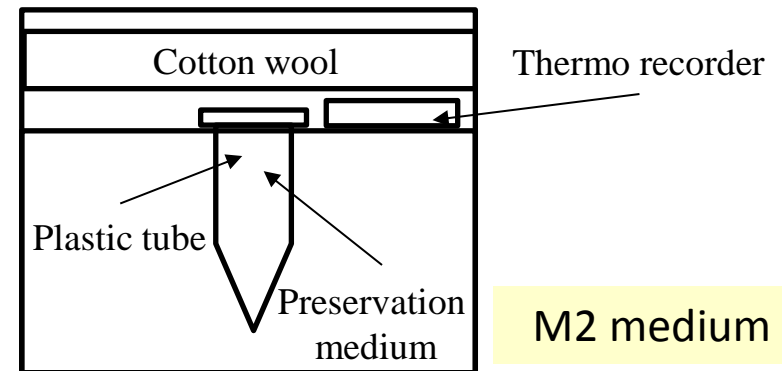
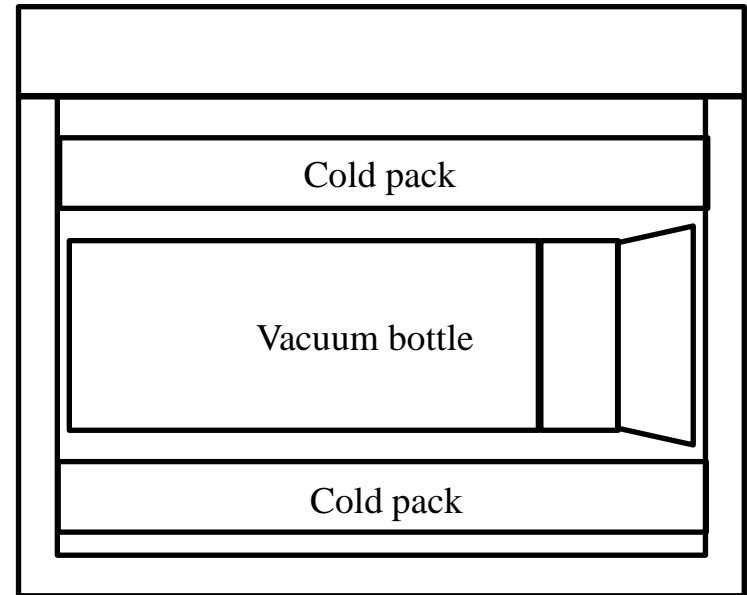
Sample box



Cold pack



Cold pack



Takeo et al. Cryobiology, 2009
Takeo et al. JAALAS, 2010

CARD Embryo Shipment Project I:

Live pups were obtained in all laboratories

Institution	Duration of transport (h)	No. of examined 2-cell embryos	No. of viable 2-cell embryos after transport	No. of 2-cell embryos transferred	No. of recipients	No. of live young
Control	48	74	74 (100)	74	4	36 (49 ± 18.6)
A	47	60	60 (100)	60	3	35 (58 ± 7.6)
B	49	80	80 (100)	80	4	53 (66 ± 10.4)
C	47	80	75 (94 ± 2.0)	75	4	42 (56 ± 18.5)
D	49	60	60 (100)	60	3	25 (42 ± 7.6)
E	50	80	80 (100)	80	4	44 (55 ± 17.8)
F	48	80	80 (100)	80	4	33 (41 ± 16.5)
G	46	80	76 (95 ± 1.5)	76	4	17 (22 ± 8.0)
Total		520	511 (98 ± 2.8)	511	26	249 (49 ± 17.3)

Percent data are presented as Mean ± SD (n = 3-4).

*Values are significantly different compared to control without transport ($P < 0.05$).

CARD Embryo Shipment Project II: International transport

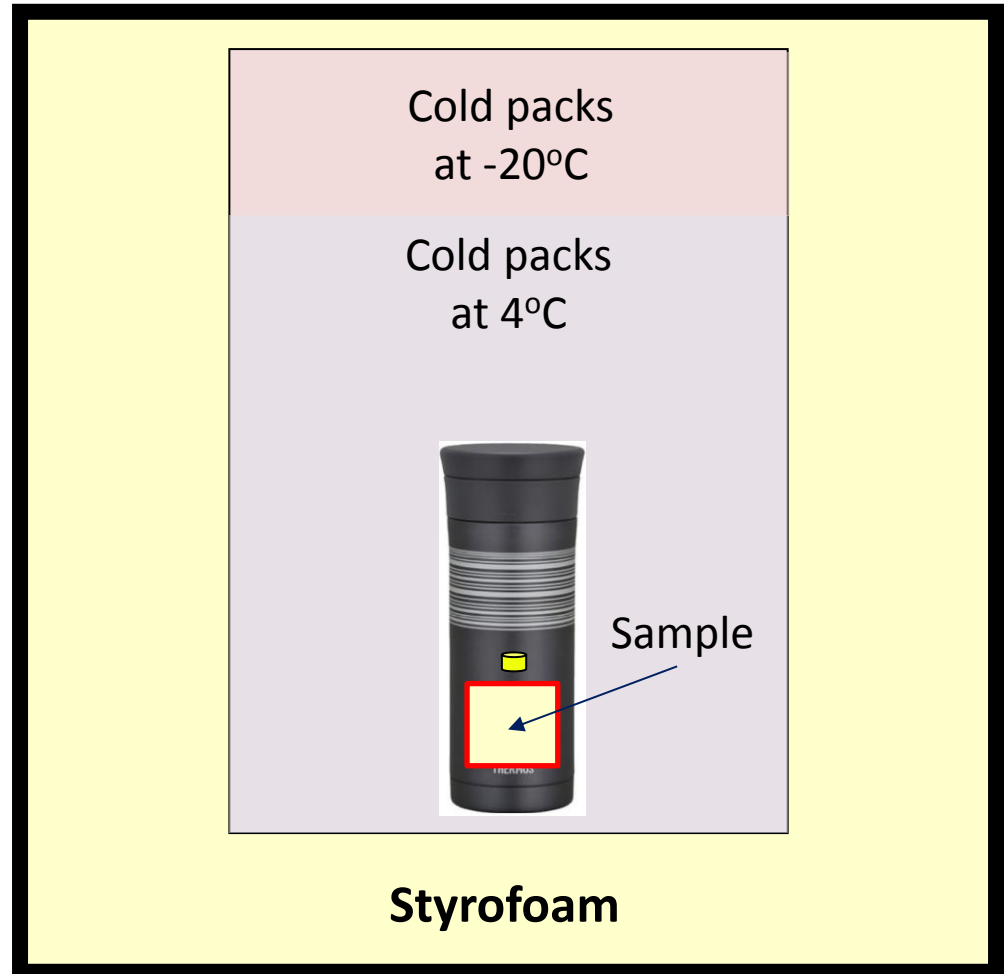


Transport box for international shipment

Domestic transport

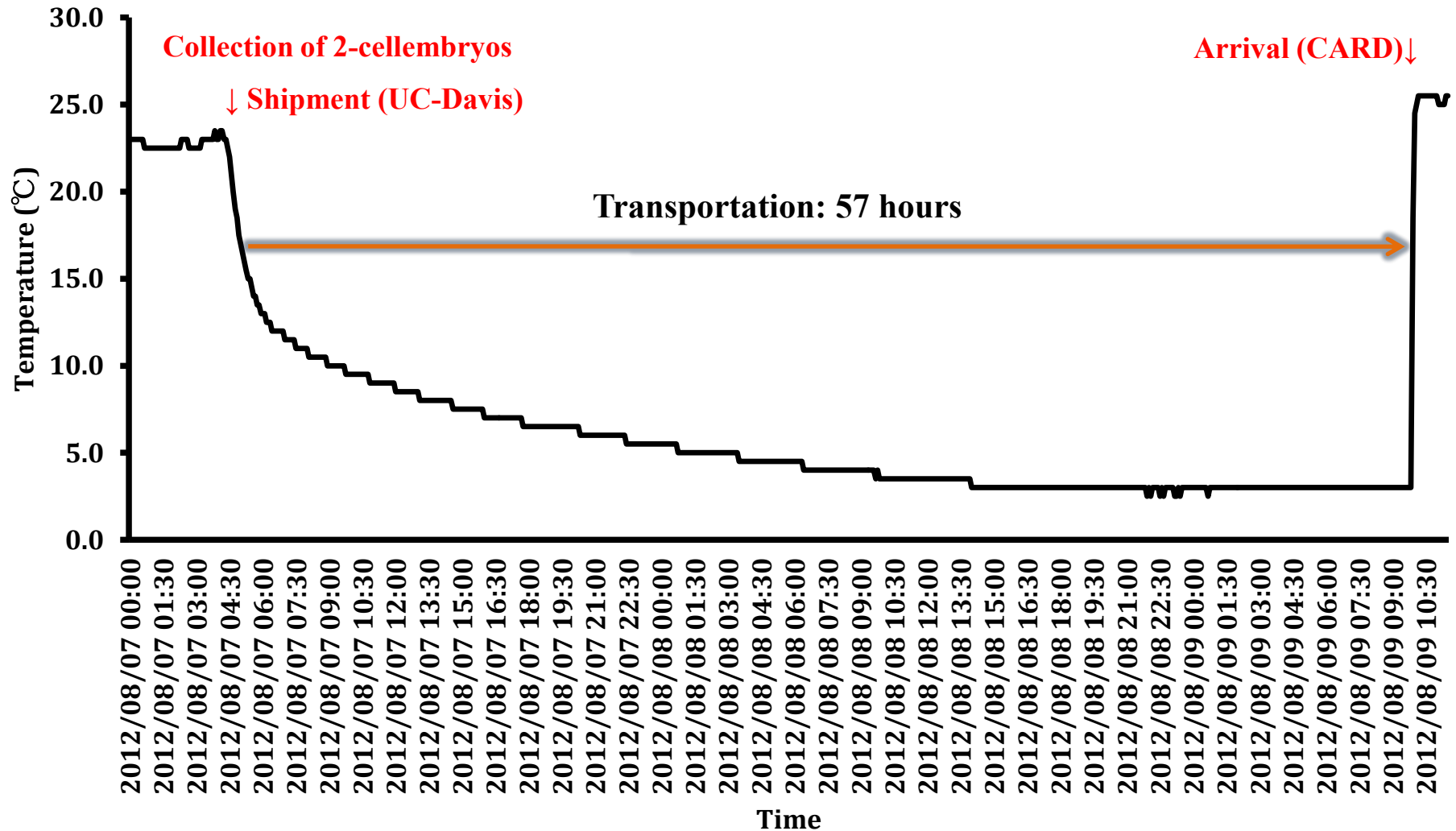


International transport



☐ : Temperature data logger
(Thermochron iButton Cat.No.DS1921G
; Maxim Integrated Products)

Temperature profile during the shipment of 2-cell embryos



CARD Embryo Shipment Project II:

Most of embryos survived after the shipment

Transport	No. of transported embryos	No. of survival embryos	No. of survival embryos	(%)
Non transport	200	200	200	100
UC Davis → CARD	240	238	237	98.8

CARD Embryo Shipment Project II:

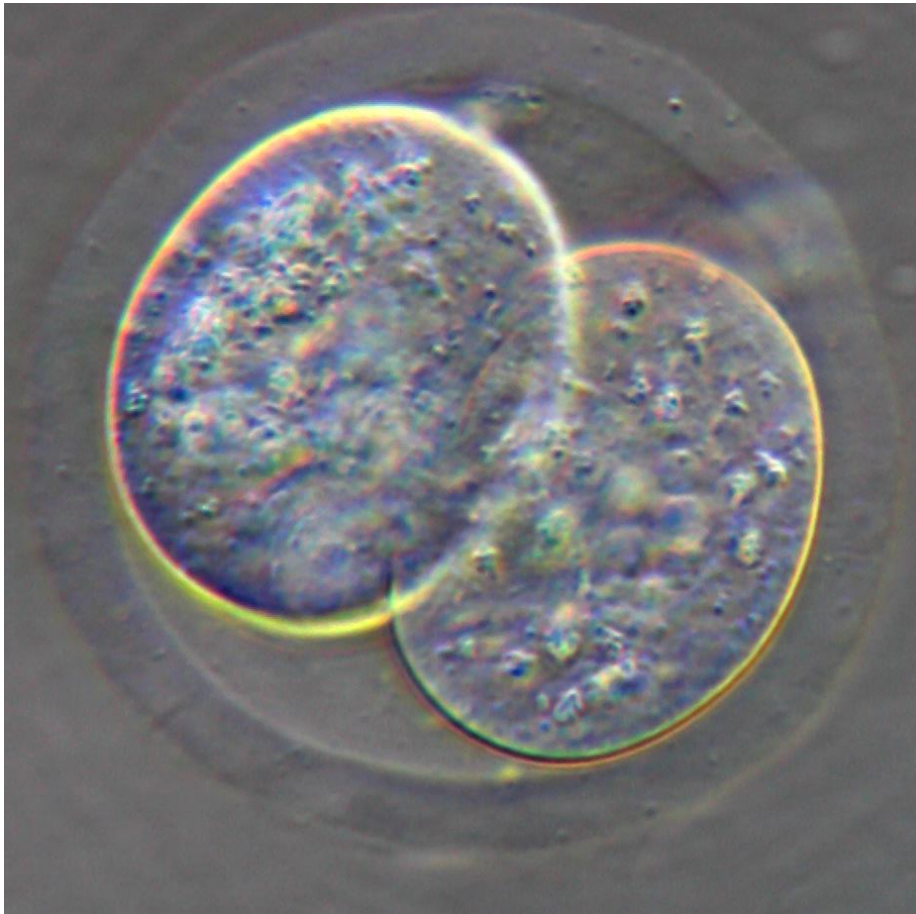
The embryos developed to live pups

Transport	No. of embryos transferred	No. of recipients	No. of pups	(%)
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Non transport	100	5	45	45.0
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UC Davis → CARD	120	6	54	45.0
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Shipment of unfrozen 2-cell embryos



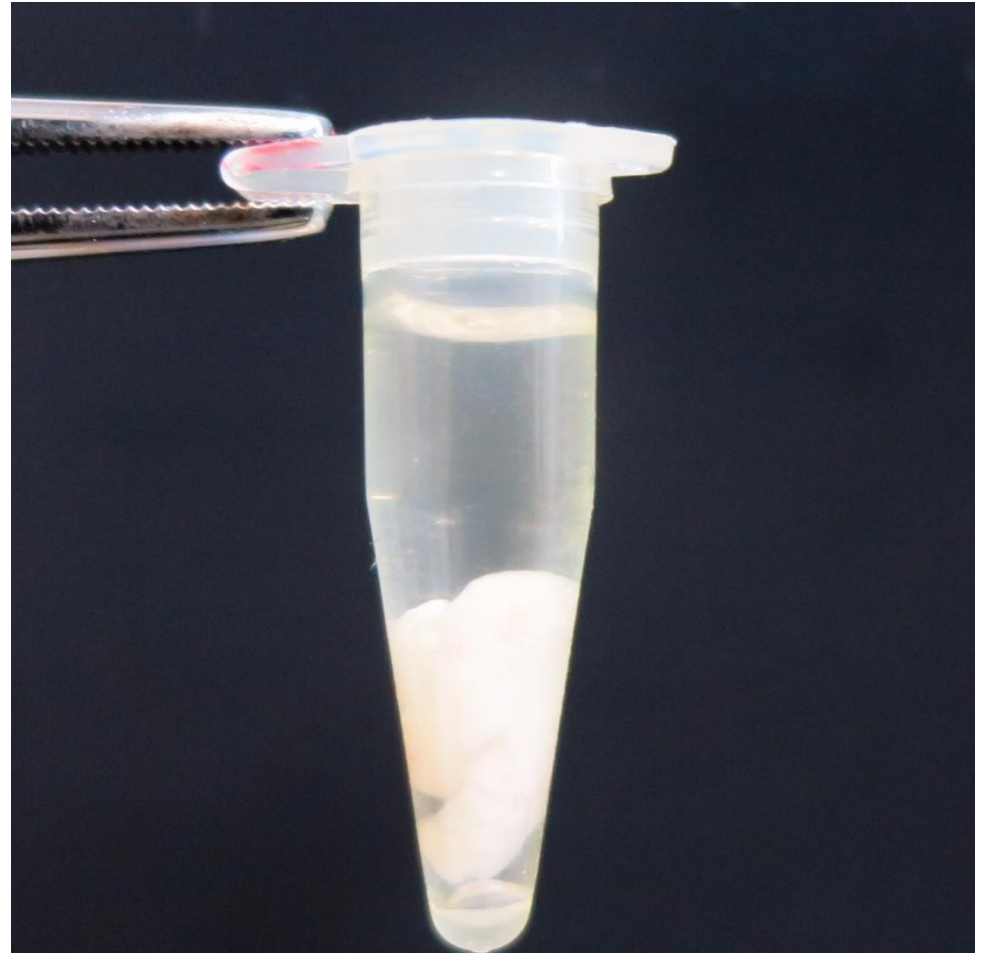
【Essential conditions】

- Refrigerated temperatures:
Around 4°C
- Cold storage medium :
M2 medium
- Duration of preservation:
Within 72 hours

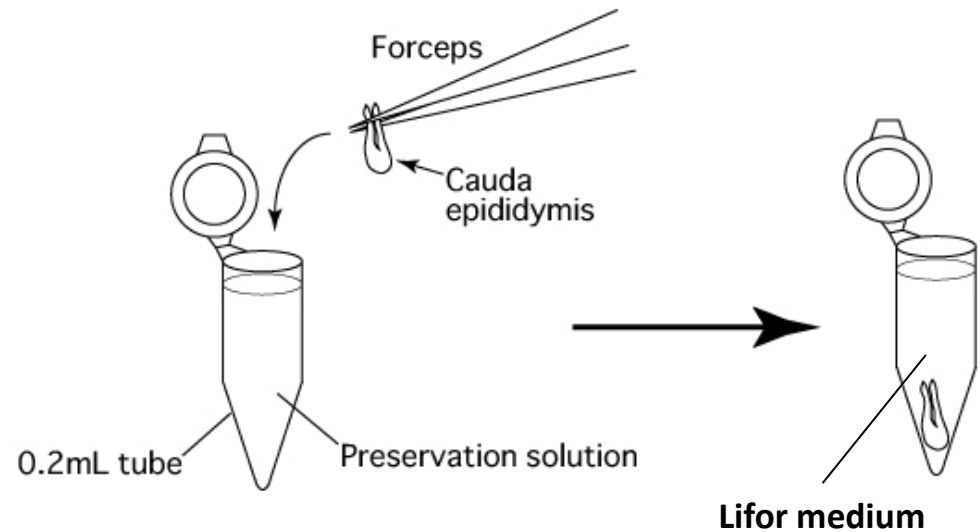
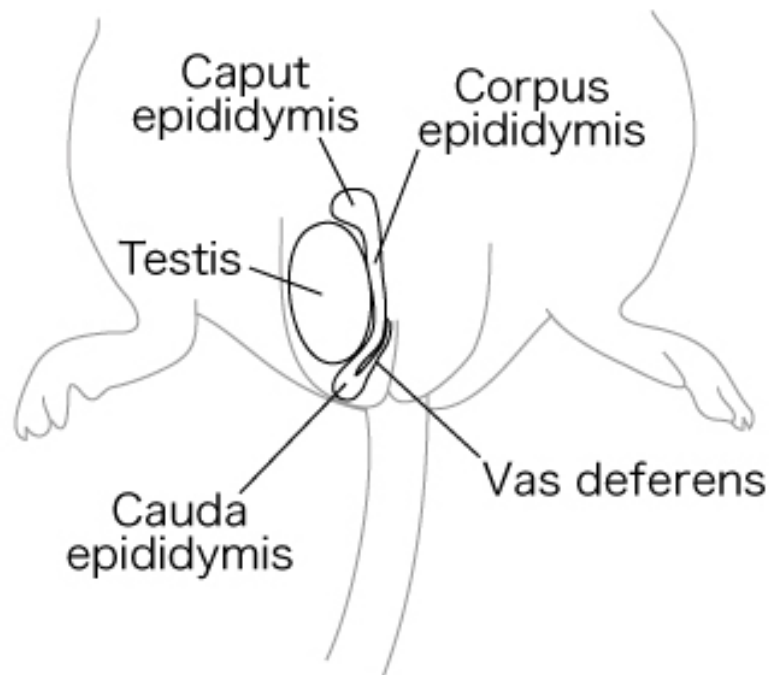
2. Transport system of unfrozen sperm enclosed in cauda epididymides



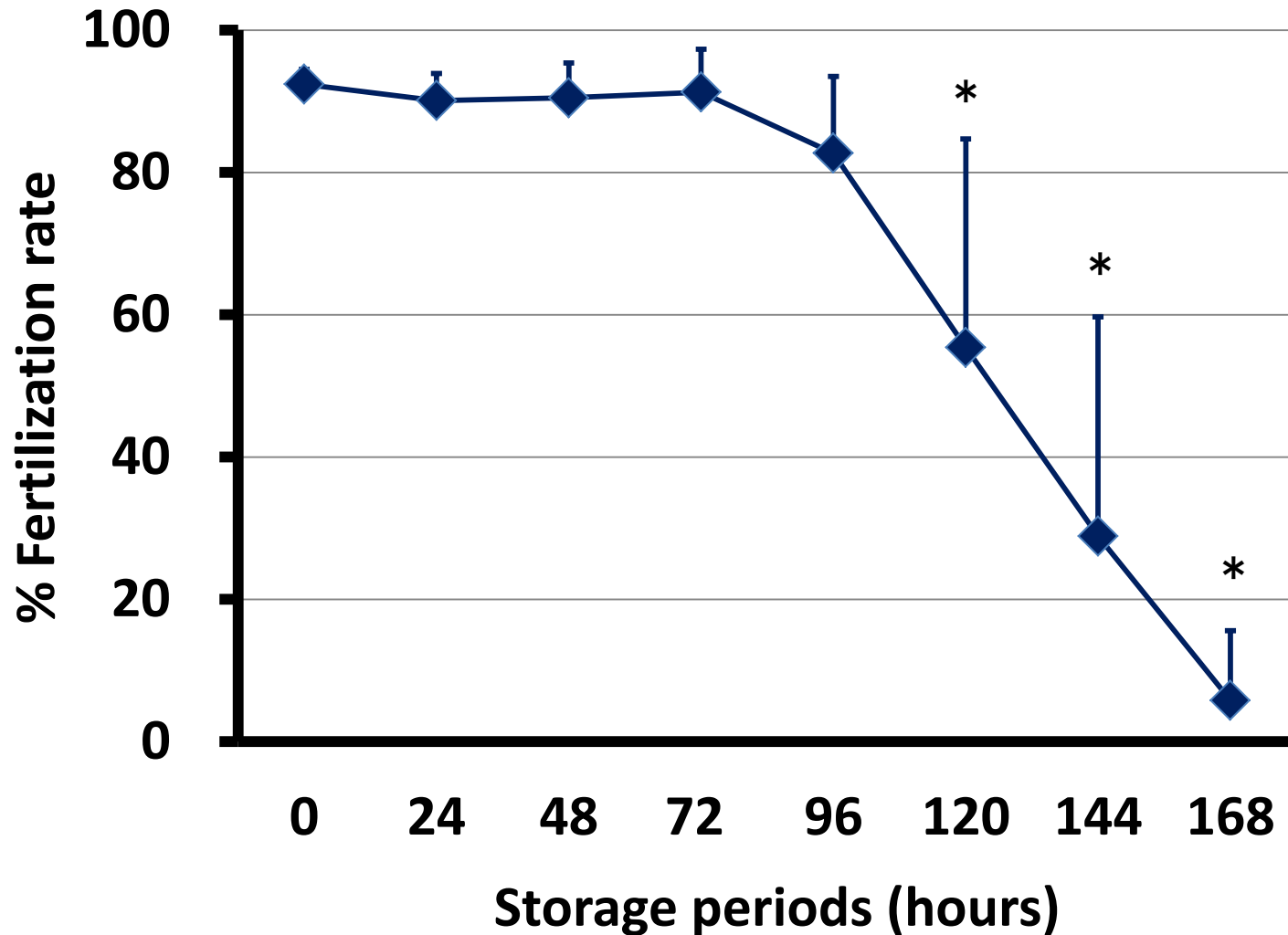
Cauda epididymis



Preparation of cauda epididymides for cold storage



Cold-stored sperm indicated high ability of fertilization for 96 hours at 4°C



CARD Sperm Transport Project I: Domestic transport



The CARD Cold Transport Kit

CARD Sperm Transport Project:

High fertilizing and development rates were obtained

		in vitro			in vivo		
Sperm	No. of inseminated oocytes	No. of 2-cell embryos (%)	No. of cultured 2-cell embryos	No. of blastocysts (%)	No. of transported embryos	No. of recipients	No. of live young (%)
non-transported	884	851 (96.3±2.8)	120	112 (93.3±1.4)	120	6	48 (40.0±6.6)
transported	927	893 (96.3±2.0)	120	118 (98.3±2.9)	119	6	39 (32.8±7.5)



Asahikawa Medical University



CARD, Kumamoto University

CARD Sperm Transport Project II: International transport

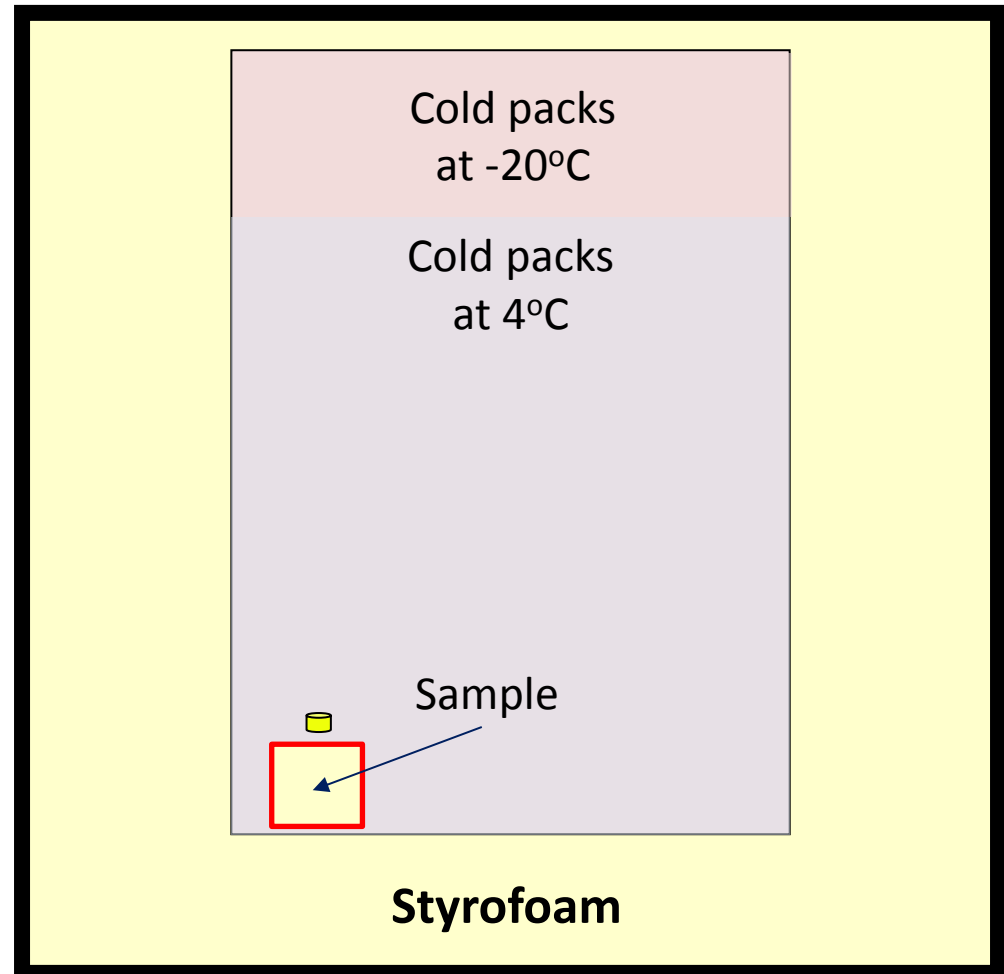


Transport box for international shipment

Domestic transport

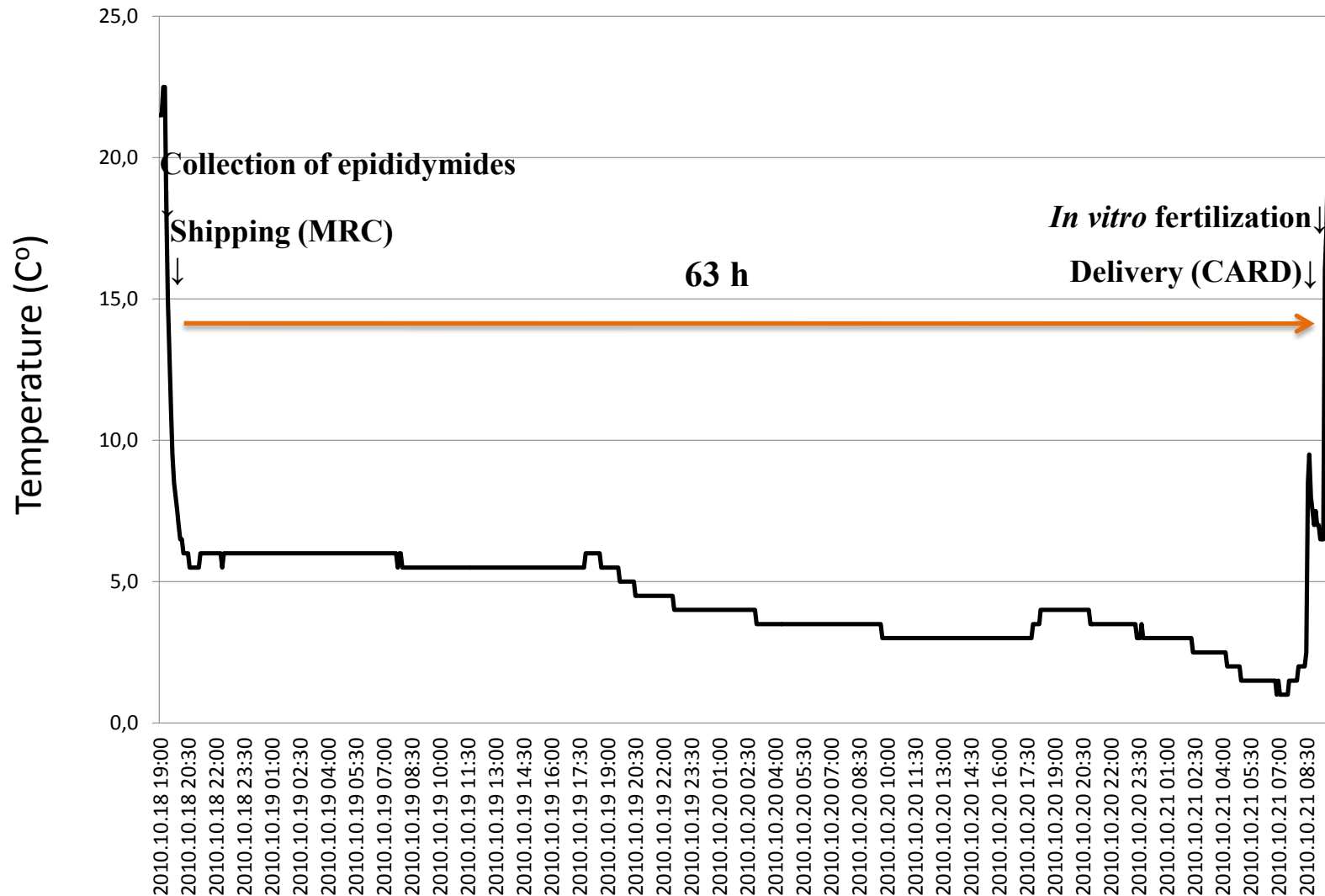


International transport

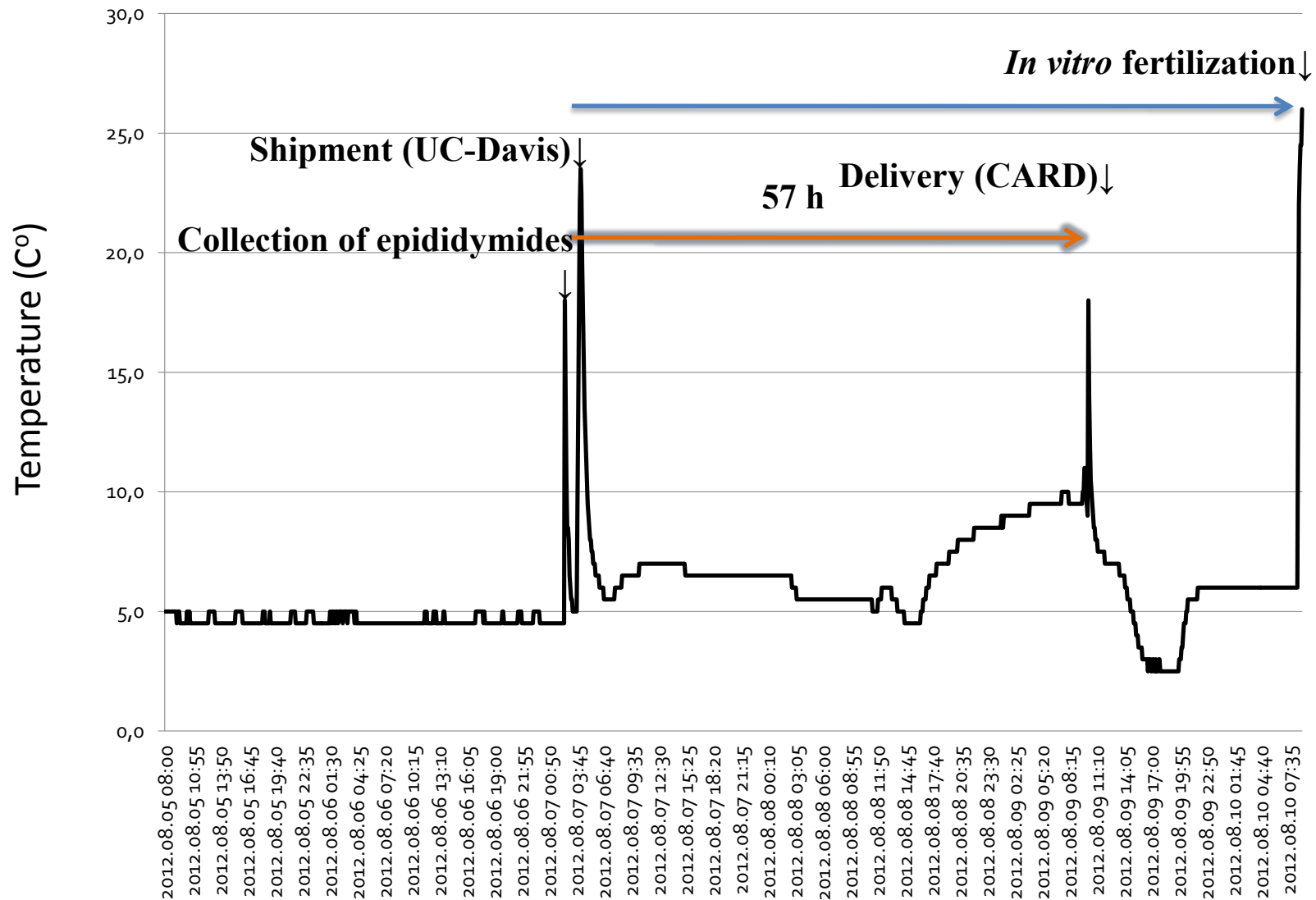


☐ : Temperature data logger
(Thermochron iButton Cat.No.DS1921G
; Maxim Integrated Products)

CARD Sperm Transport Project II: From MRC Harwell to CARD



CARD Sperm Transport Project II: From UC Davis to CARD



CARD Sperm Transport Project II:

Transported sperm indicated high fertilizing ability

Transport	No. of oocytes	No. of 2-cell embryos	(%)
MRC Harwell → CARD	311	232	74.6
UC Davis → CARD	292	280	95.9

CARD Sperm Transport Project II:

Live pups were obtained from transported sperm

Transport	No. of embryos transferred	No. of recipients	No. of pups	(%)
MRC Harwell → CARD	120	6	61	50.8
UC Davis → CARD	200	10	111	55.5



Shipment of unfrozen sperm enclosed in cauda epididymides



【Essential conditions】

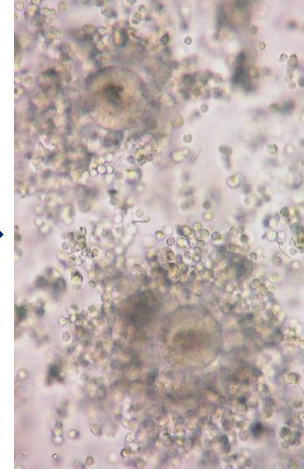
- Refrigerated temperatures:
Around 4°C
- Cold storage medium :
Lifor® medium
- Duration of preservation:
Within 96 hours

We have developed novel applications for the shipment of unfrozen sperm

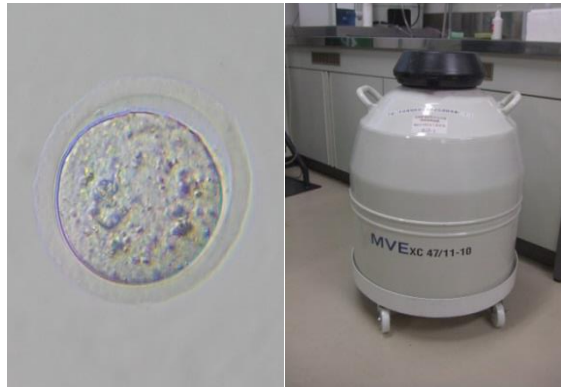
Cold-stored sperm



1. Sperm cryopreservation



IVF

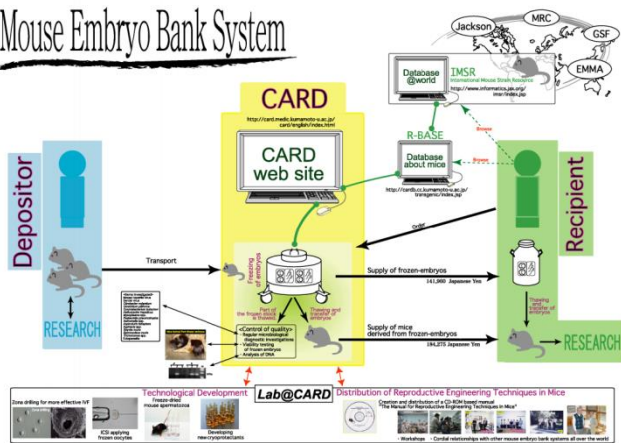


2. IVF with cryopreserved oocytes

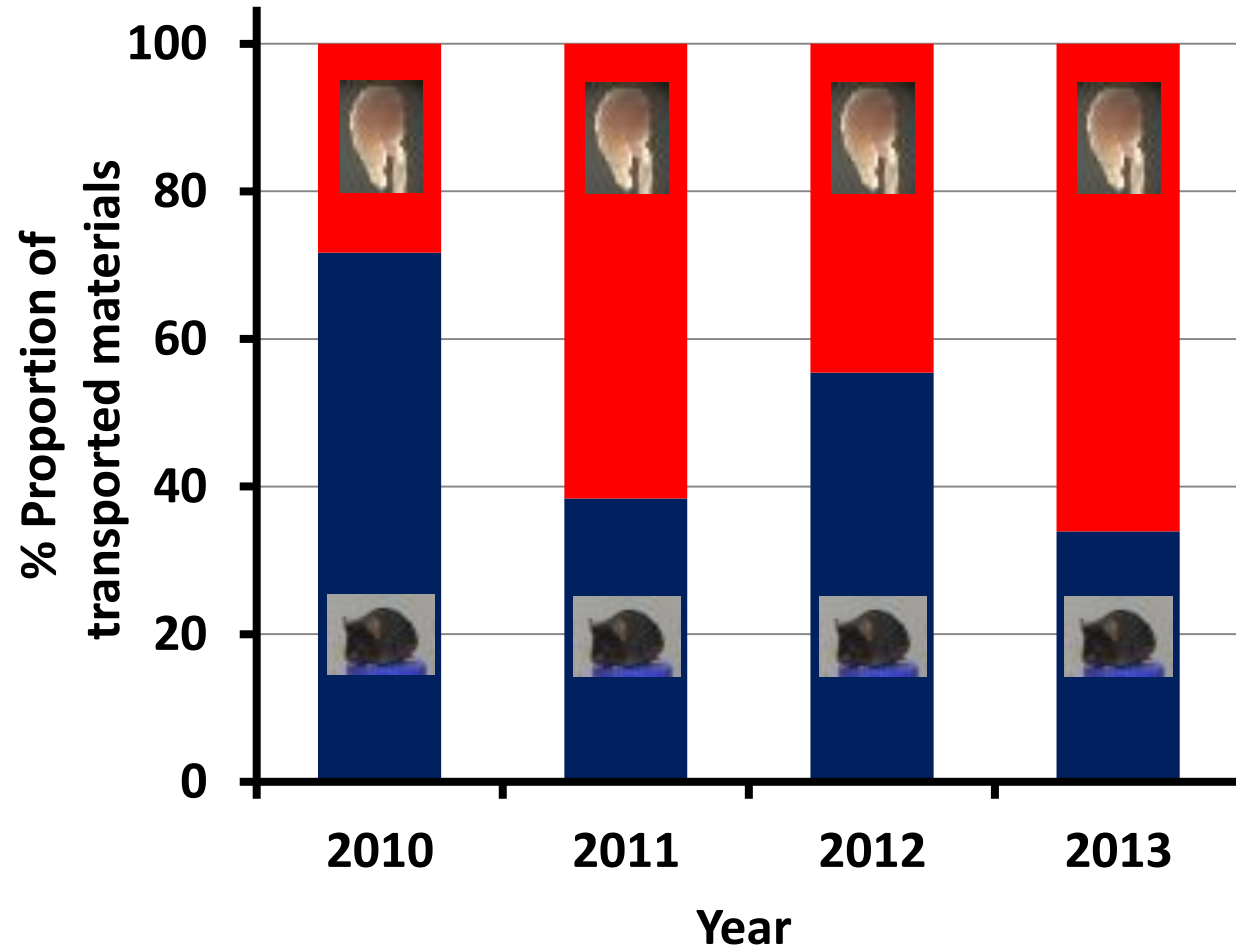
High flexibility
of IVF schedule

Shipment of unfrozen sperm to archive genetically engineered mice have been increasing at CARD

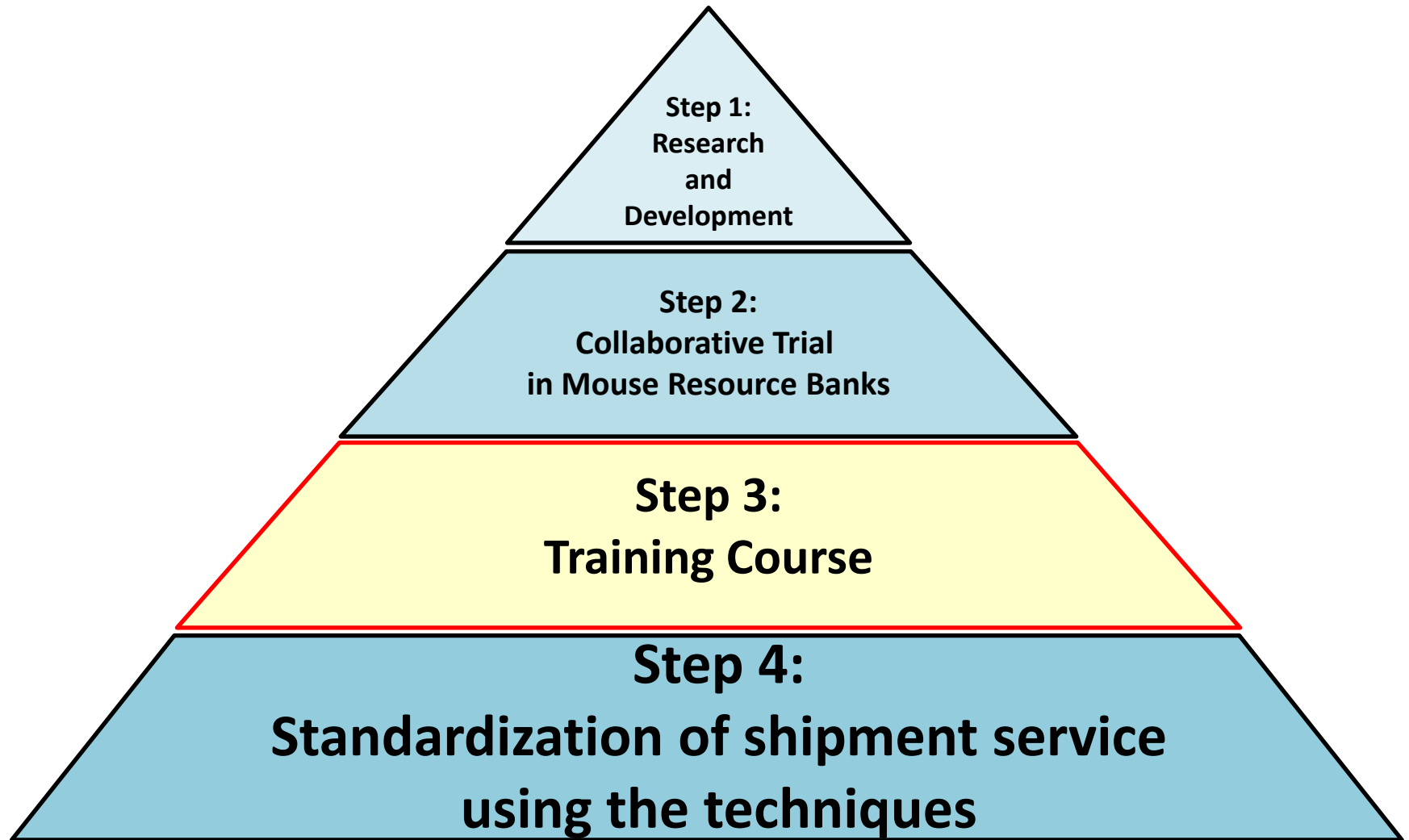
Mouse Embryo Bank System



CARD,
Kumamoto University



To promote the exchange of mouse mutant resources using the innovative techniques in scientific community...



CARD Cryocourse for sharing the technique in scientific community

Number of participants in the CARD cryocourse

2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	Total
6	6	12	12	12	25	14	26	27	24	28	41	96	104	433



Venue

1. CARD, Kumamoto University, Japan.
2. Shanghai Laboratory Animal Center, Chinese Academy Of Sciences, Shanghai, China.
3. College of Life Sciences, Peking University, Beijing, China.
4. Bio-Evaluation Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Chungbuk, Korea.
5. Biological Resource Center, A*STAR, BIOPOLIS, Singapore.
6. National Laboratory Animal Center, Taipei, Taiwan
7. National Institutes for Food and Drug Control (NIFDC), Beijing, China
8. CNB-CSIC, Madrid, Spain

CARD-CNB Cryocourse in CNB-CSIC, Madrid, Spain in 7-11 October 2013

Center for Animal Resources and Development

Center for
Animal
Resources and
Development

CNB
CENTRO NACIONAL DE BIOTECNOLOGÍA



CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

CARD-CNB MOUSE SPERM AND EMBRYO CRYOPRESERVATION COURSE



24 participants from 16 countries:

Australia
Brazil
Canada
Czech Republic
Denmark
Finland
France
Israel
Italy
the Netherlands
Portugal
Spain
Sweden
Taiwan
the UK
the USA

CARD-RPCI Cryocourse will be held in Roswell Park Cancer Institute in this autumn

Center for Animal Resources and Development

Center for
Animal
Resources and
Development



THE UNIVERSITY OF TEXAS
MD Anderson
Cancer Center
Making Cancer History®

CARD-RPCI MOUSE SPERM AND EMBRYO CRYOPRESERVATION COURSE



Roswell Park Cancer Institute, Buffalo, NY, USA

September 15th-19th, 2014

(Monday to Friday, 5-day hands-on practical course)

1. Isolating unfertilized mouse oocytes
2. Isolating and cold storage/shipping of mouse cauda epididymis
3. Freezing/thawing mouse sperm and IVF using CARD frozen sperm and legacy sperm
4. Fresh mouse sperm and IVF
5. Cold Storage sperm and IVF
6. Freezing/thawing 2-cell IVF-derived mouse embryos
7. Vitrification of mouse oocytes and embryos
8. IVF of vitrified mouse oocytes
9. Ovary transplantation/ovary freezing
10. Embryo transfer techniques in mice (oviduct, uterus via NSET)

INFRAFRONTIER cryocourse will be helpful to establish efficient system for exchange of genetically engineered mice



Venue: MRC Harwell, UK

Date: 2015-

Duration: 5 days



Or

CARD, Japan



1. Isolating unfertilized mouse oocytes
2. Isolating and cold storage/shipping of mouse cauda epididymis
3. Freezing/thawing mouse sperm and IVF using CARD frozen sperm and legacy sperm
4. Fresh mouse sperm and IVF
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6. Freezing/thawing 2-cell IVF-derived mouse embryos
7. Vitrification of mouse oocytes and embryos
8. IVF of vitrified mouse oocytes
9. Embryo transfer techniques in mice (oviduct, uterus via NSET)

or more...

Acknowledgements

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Kinchen Kristy



CSIC-CNB, Spain

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NLAC, Taipei

Dr. Chun-Keung Yu
Dr. Leo Wang
Dr. Chan-Kai Chou



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Yuki Sakai
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INFRAFRONTIER
mouse disease models



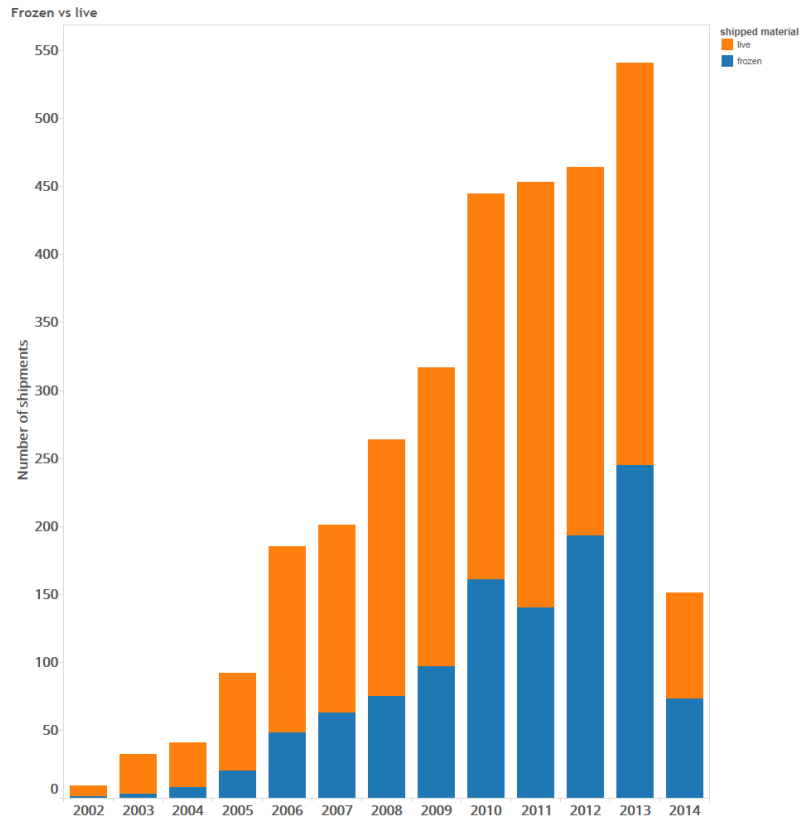
www.transtechsociety.org
International Society for Transgenic Technologies

LN₂ transport alternatives - why

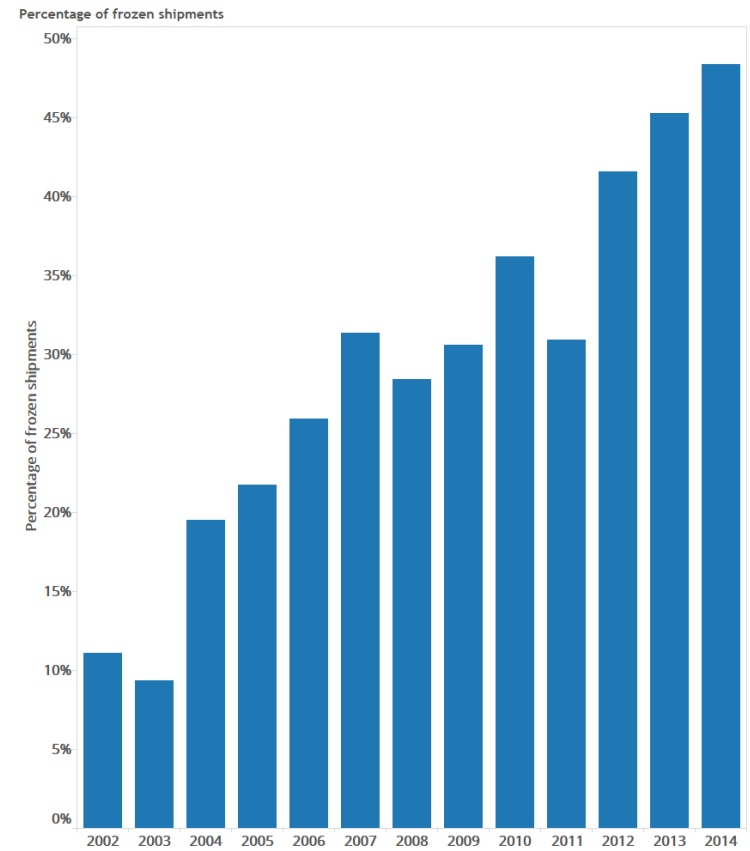
- The laboratories need to:
 - Fast & flexible exchange process
 - Reduce costs
 - More alternatives to live animal transportation



Frozen vs live mice shipments

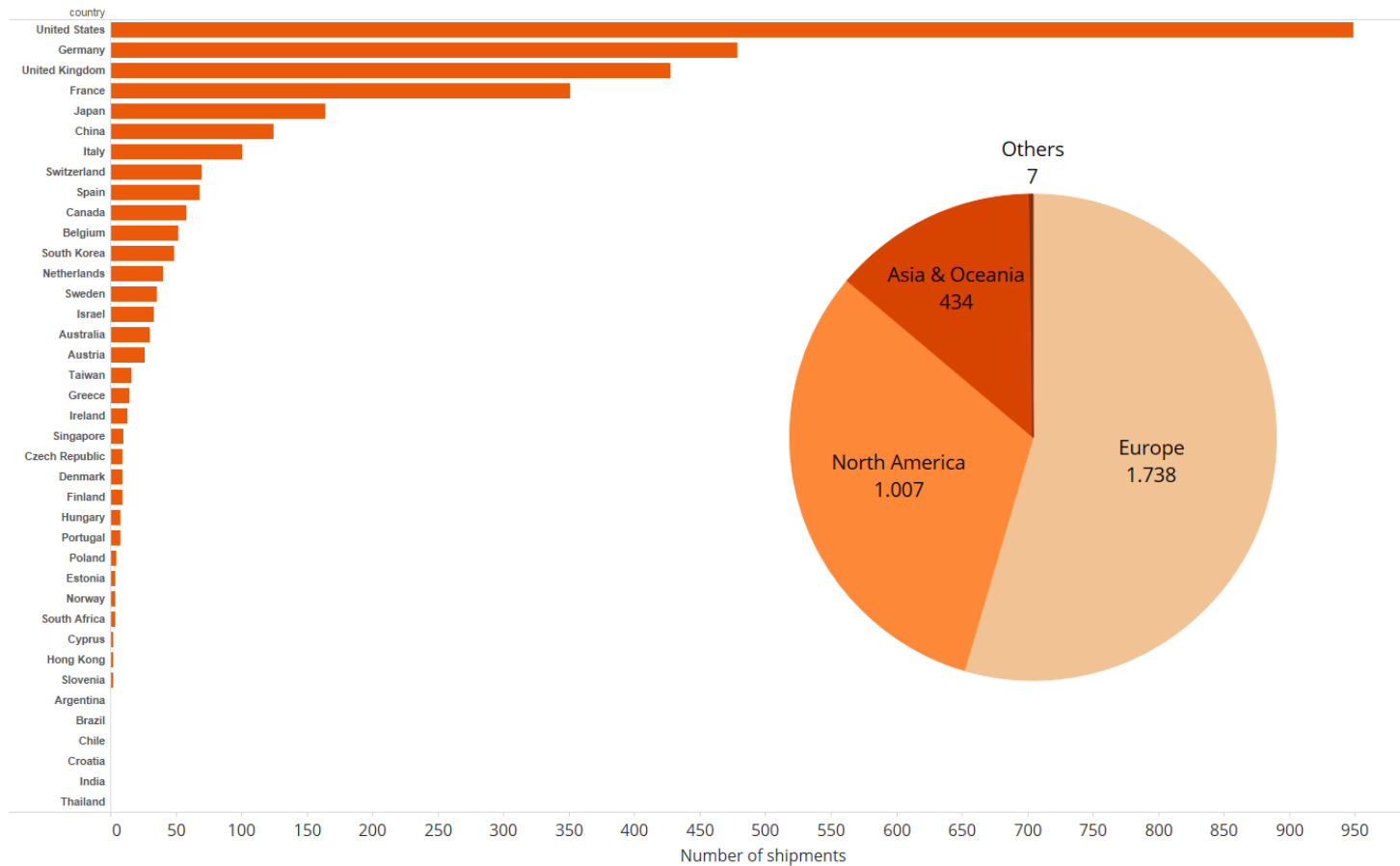


Sum of Number of shipments for each processed date Year. Color shows details about shipped material. The view is filtered on processed date Year, which excludes Null and 2001.



% of Total Number of shipments for each processed date Year. Color shows details about shipped material. The view is filtered on processed date Year, which excludes Null and 2001. Percents are based on each column of the table.

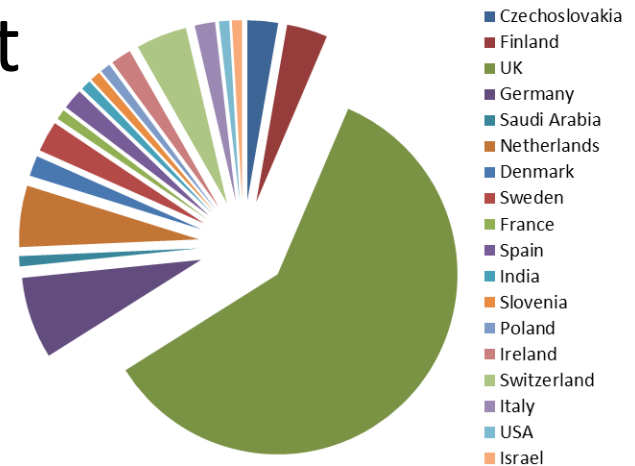
EMMA perspective - shipments



Sum of Number of shipments for each country. The data is filtered on processed date Year, which excludes Null.

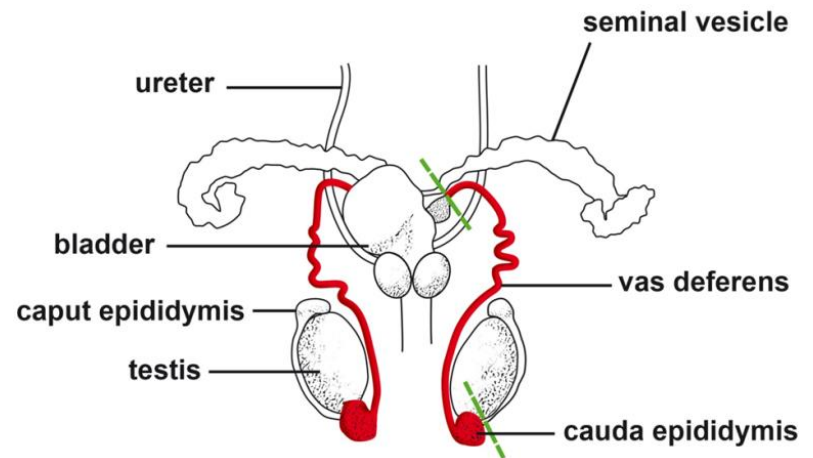
Promoting gamete transfer

- Technology transfer - 3Rs/simplicity/cost
- Training - EMMA/Jax/CARD and others
 - Over 150 training places/year
 - Substantial world wide demand for training
- Training alone is insufficient
- New approach



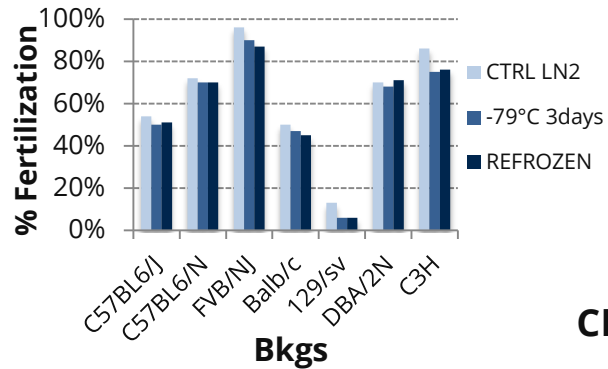
Innovative solutions

- Frozen sperm on dry ice
- Frozen/thawed embryos at refrigerated Temps
- Epididymides at refrigerated Temps
- Vitrified embryos on dry-ice
 - Mochida et al 2013

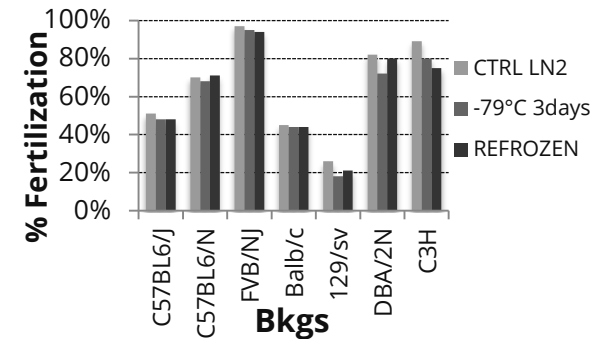


Transporting sperm on dry-ice

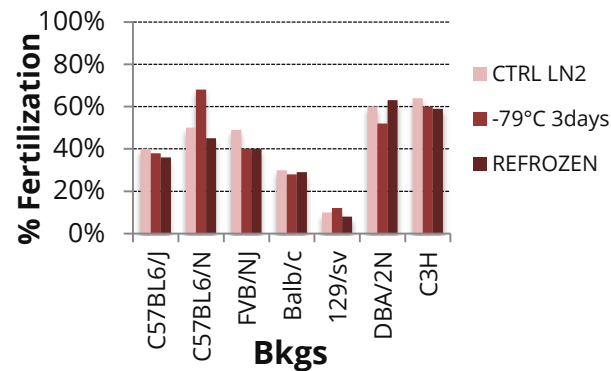
gCPA-MBCD-mHTF



CPA+MTG-Cook



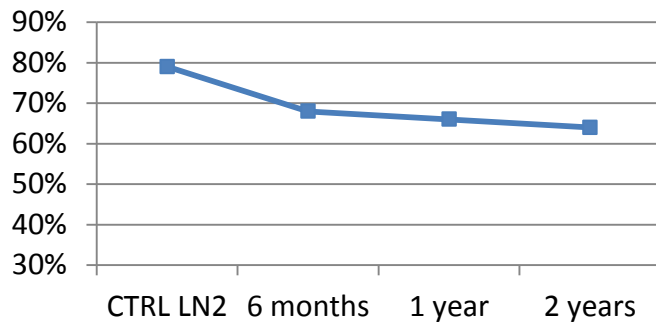
CPA+Rescue Protocol



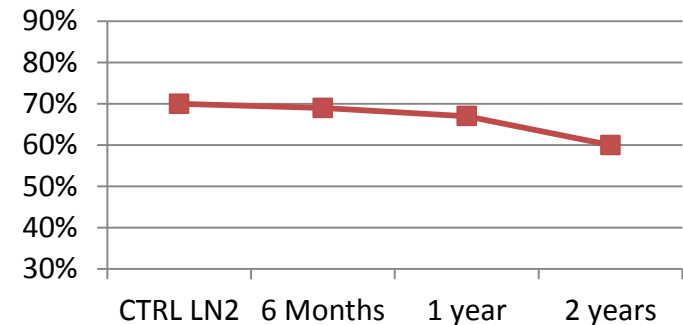
- Marcello Raspa - CNR

Storing sperm on dry-ice – 2 years

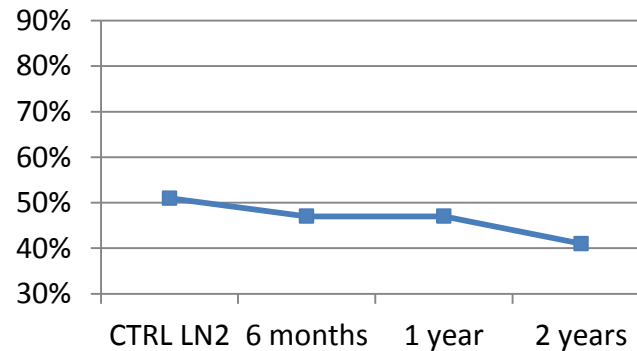
B6D2F1



B6N



B6J



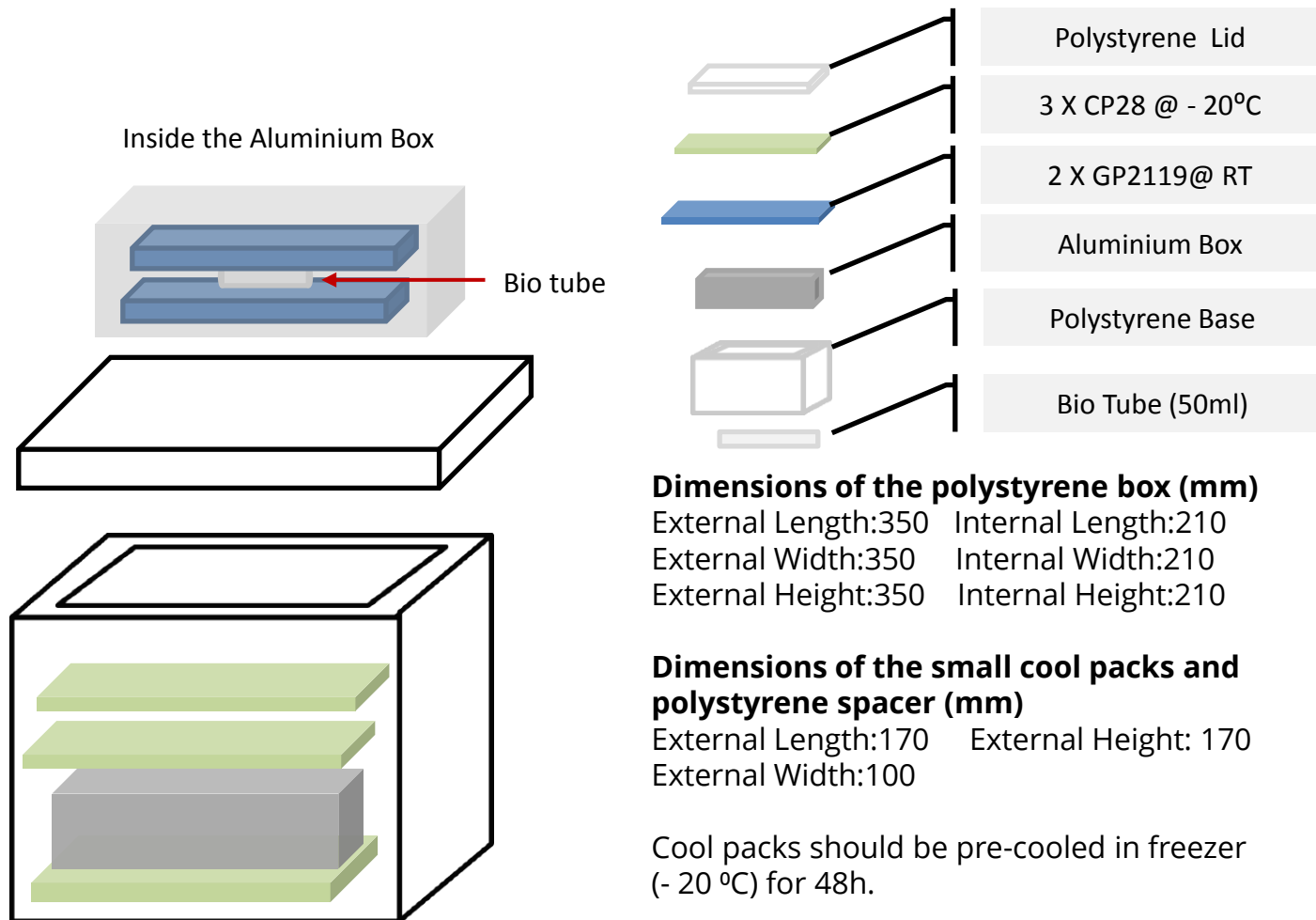
- Marcello Raspa - CNR

Refrigerated transport options

- CARD transfer chamber used in Asia
- External refrigeration required long journeys

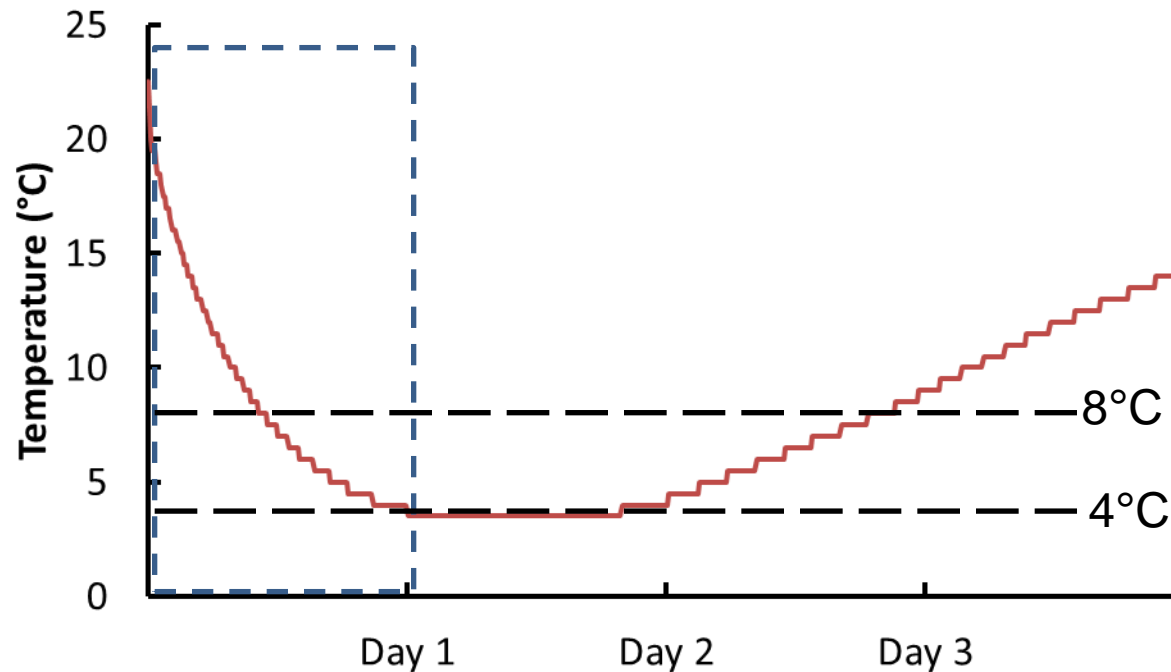


Air-Sea containers - option



Temp profile for Air-Sea's box

- £33.00 each



Epididymal transportation - Lifor

- Lifor - organ transport medium
- Sphingosine-1-phosphate supplement
- Sperm can be frozen for later use

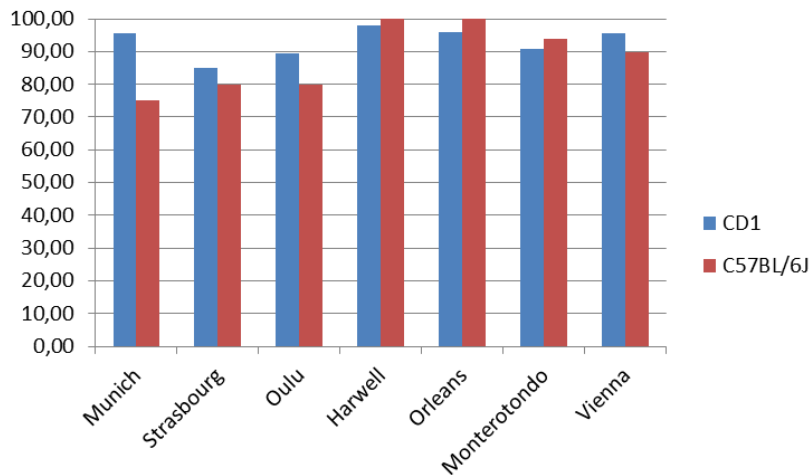
Freshly harvested sperm used for IVF after holding period

Institute	Time before test	Holding media	No. oocytes used	No. 2-cells	Fertilisation (%)
ICS	65h	oil	176	71	40.34
	65h	Lifor	222	152	68.47
MRC	72h	oil	60	18	30.00
	72h	Lifor	94	62	65.96

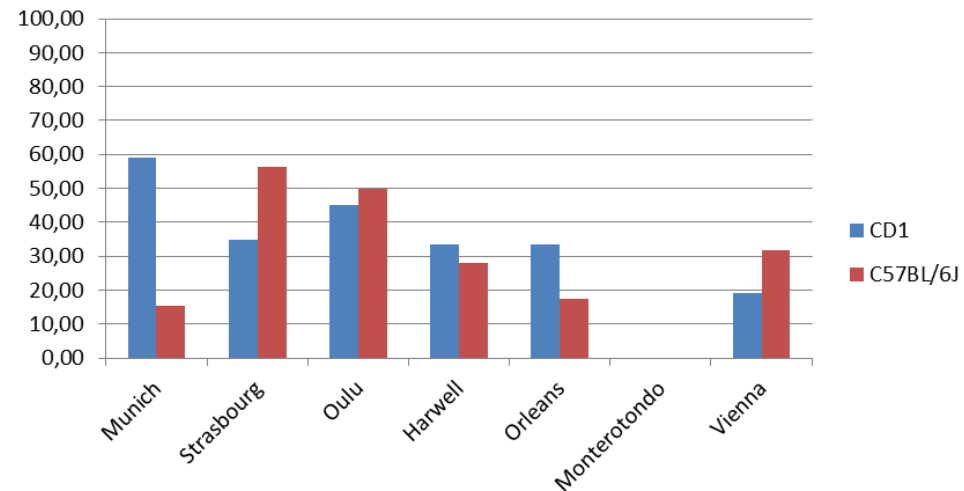
Exchanging unfrozen embryos

- Embryos sent from Madrid to 7 x EMMA nodes
- Refrigerated Temp
- Luis Montoliu

% embryo recovered after shipment



% pups born after transfer



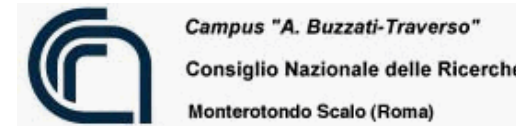
Non-conventional shipments - EMMA

Exported from	Receiving country	Sperm on dry ice	Frozen/thawed embryos
MRC	UK	0	1
MRC	USA	1	0
MRC	UK	3	0
MRC	Asia	1	0
MRC	Europe	2	0
NLAC	MRC	1	0
CNR	Europe	3	0
ICS	Europe	1	0

- LN₂ alternatives need to be promoted aggressively by all repositories

Acknowledgements

- Marcello Raspa – CNR
- Lluís Montoliu – CNB
- Susan Marschall –HMGU
- Toru Takeo – CARD
- Mo Guan – MRC
- EMMA-Technical working group



Spanish National Biotechnology Centre



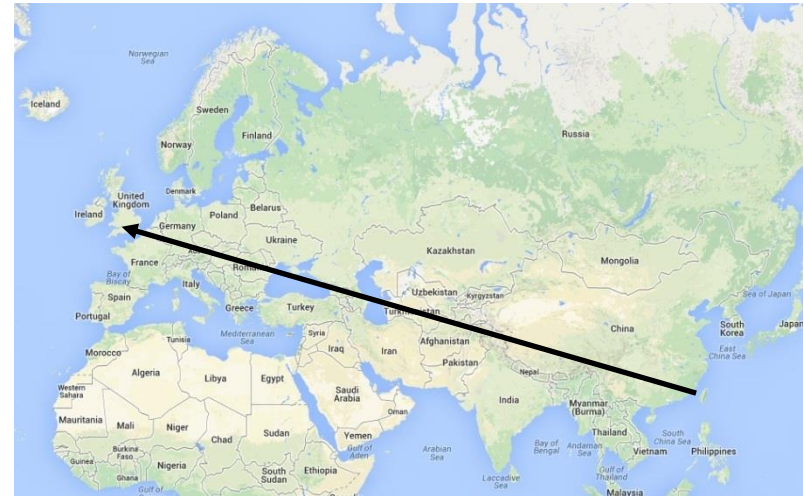
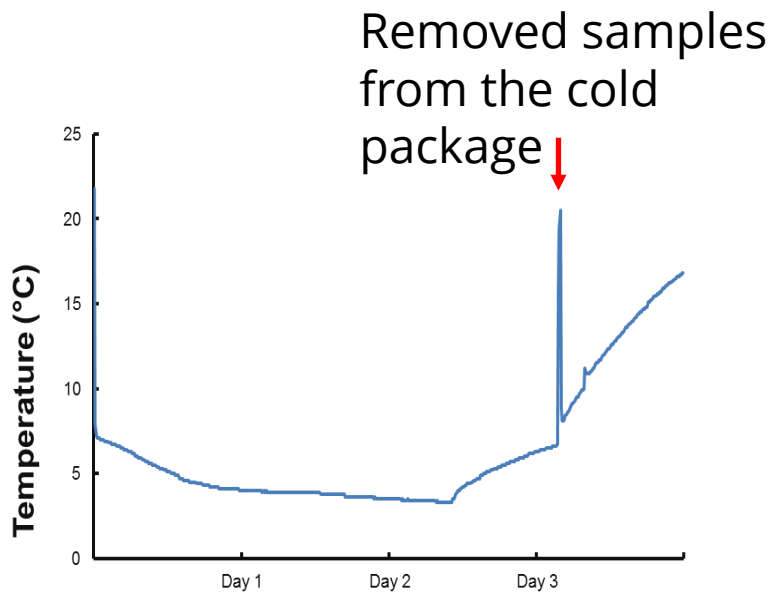
HelmholtzZentrum münchen
German Research Center for Environmental Health

Center for Animal Resources and Development

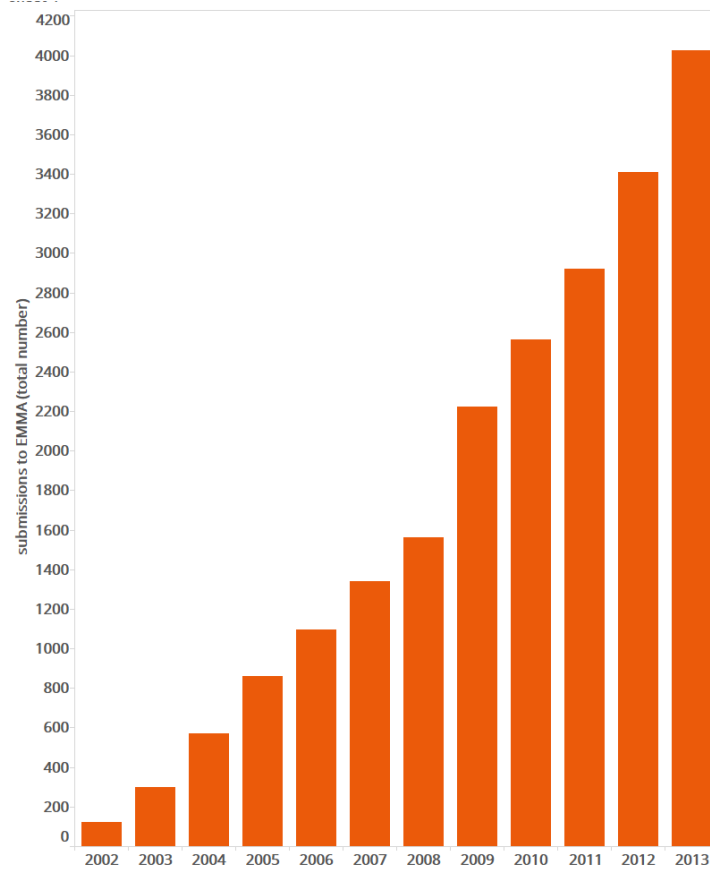
The logo for the Center for Animal Resources and Development (CARD) is located to the right of the text. It features the acronym "CARD" in large, colorful letters (C in blue, A in green, R in red, D in yellow) with the full name "Center for Animal Resources and Development" written in smaller text around it.

Taiwan to MRC-Harwell

- Epididymides sent with 76hrs holding time
- >50% IVF fertilisation rate



Strain submissions into EMMA



Sum of submissions to EMMA (total number) for each year_submitted. The view is filtered on year_submitted, which excludes 2014.

