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NARO

Utilization of Porcine Testicular Tissues after Cryopreservation and Grafting into Nude Mice

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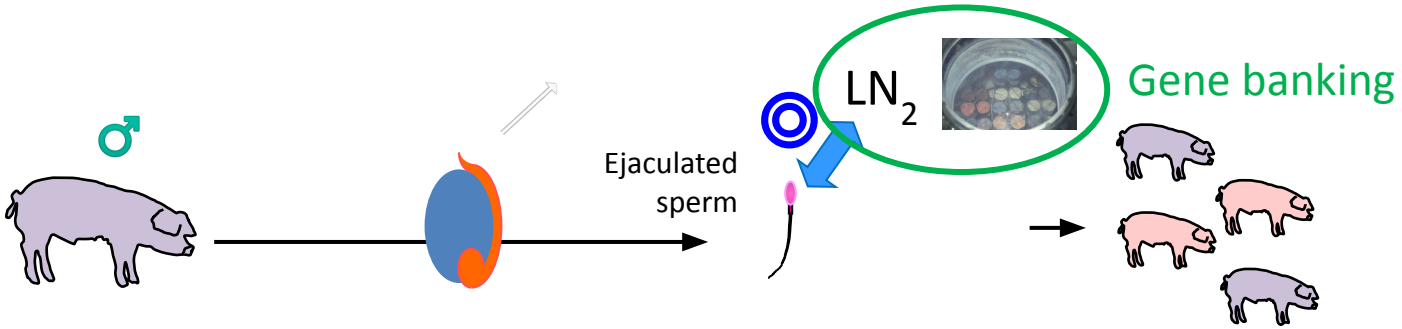
NARO

Status of the procedures used for sperm freezing



Piglets obtained

◎ Without difficulty

Gene bank

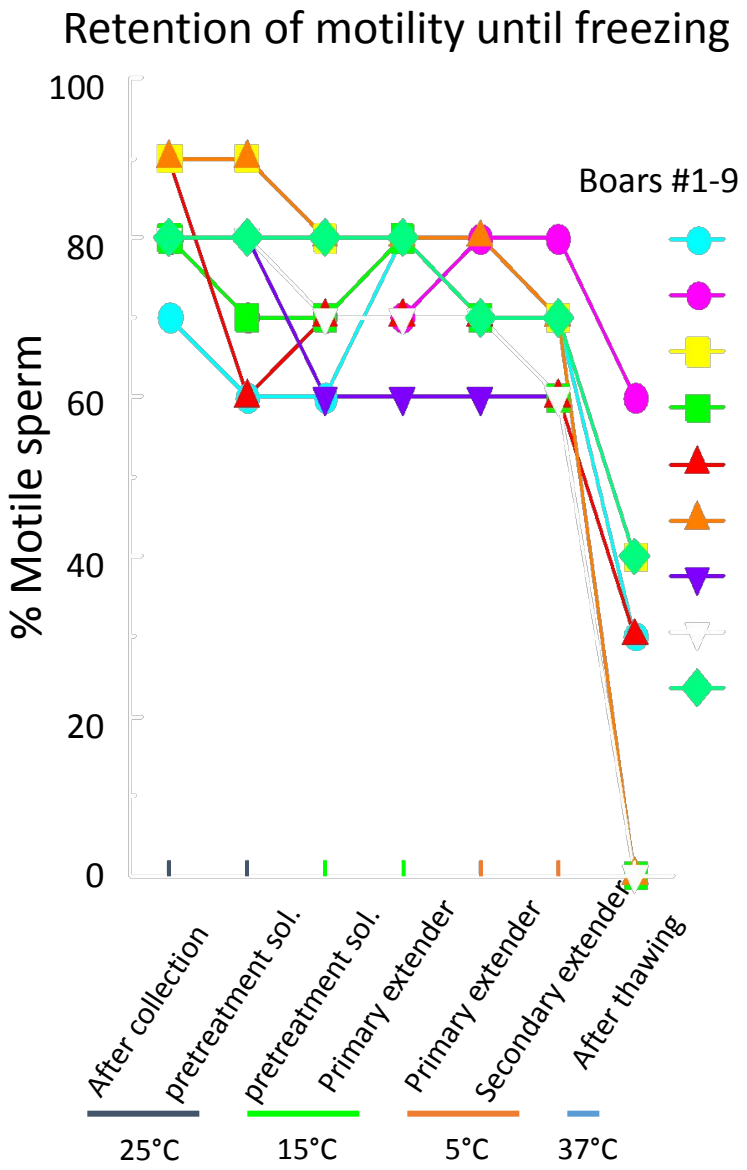
Genebank Project, MAFF/NIAS/NARO

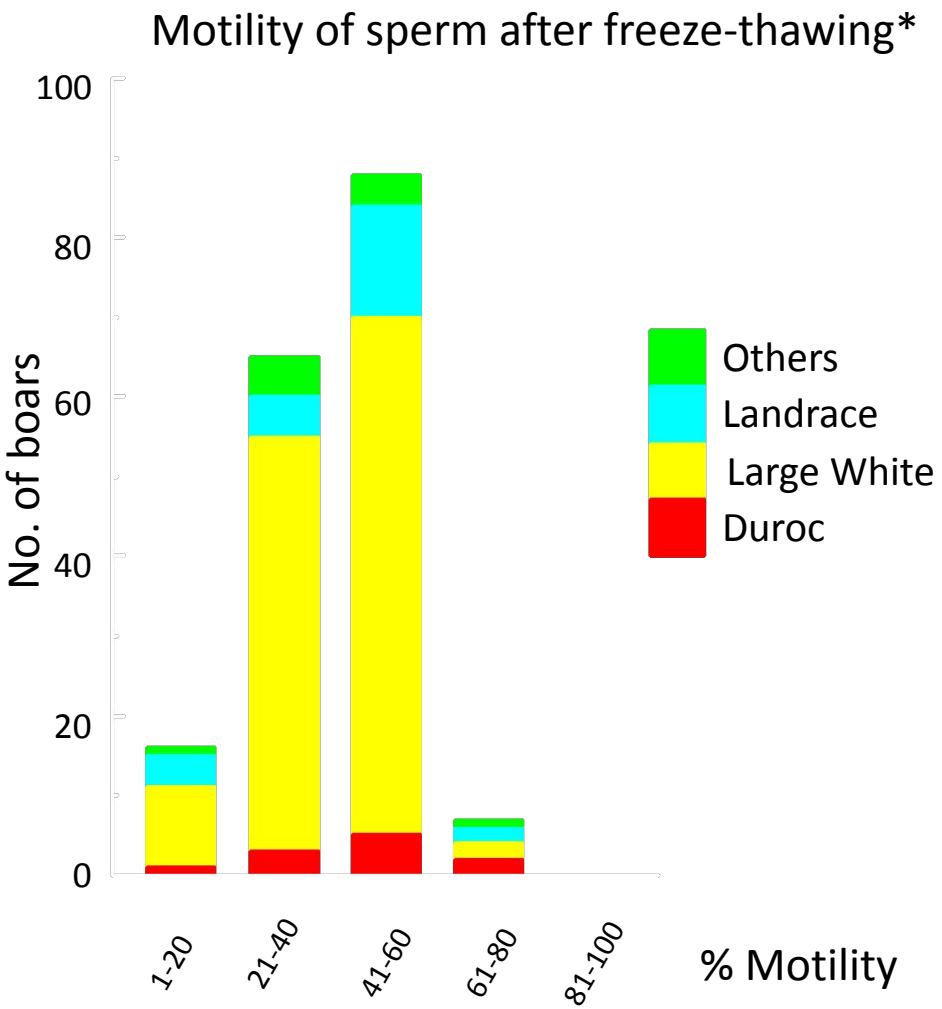
・Livestock and poultry sperm are cryopreserved in the Genebank project of NARO (Agrobiological Resource Genebank).

・In pigs, the fertilizing ability of thawed sperm differs between individuals and lots, and some of them have poor motility and cannot be fertilized or conceived even if artificial insemination or in vitro fertilization is performed.＝difference in cryotolleance

・Establishment of fertilization and production of piglets by intra-cytoplasmic sperm injection (ICSI) is expected as a necessary and indispensable technology for the maintenance and utilization of genetic resources.

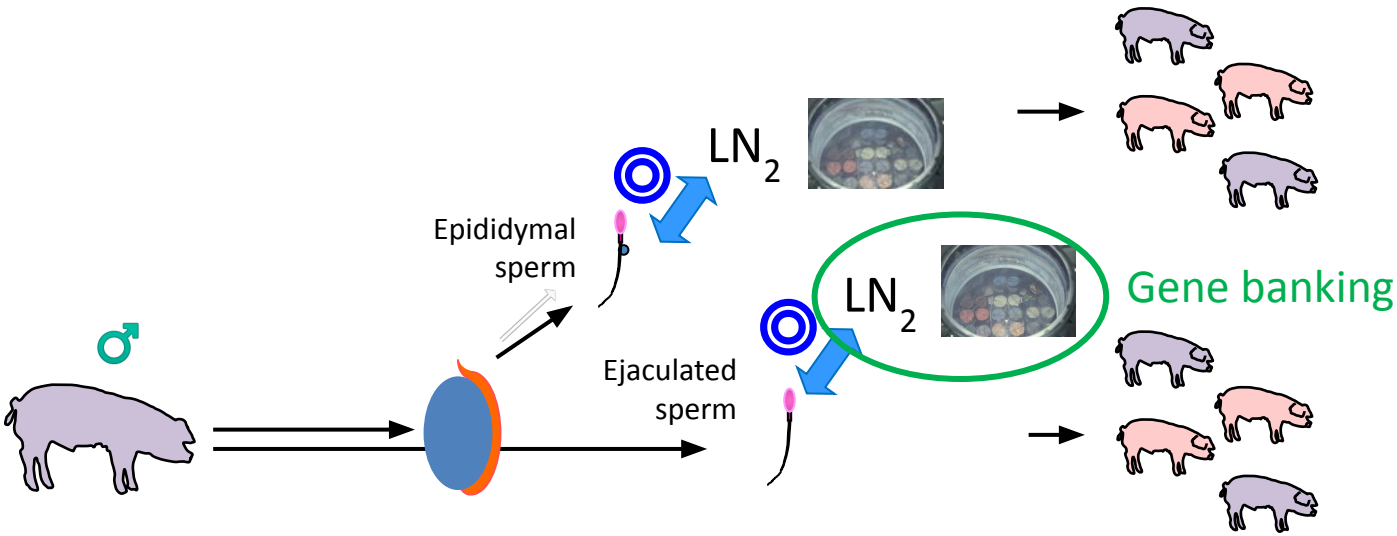
Motility of frozen-thawed sperm





*A total of 176 boars were investigated. Sperm with greater than 60% motility before freezing were froze. Sperm showing 0% after thawing were previously removed. (MAFF & NIAS Gene Bank, 1990-2002)

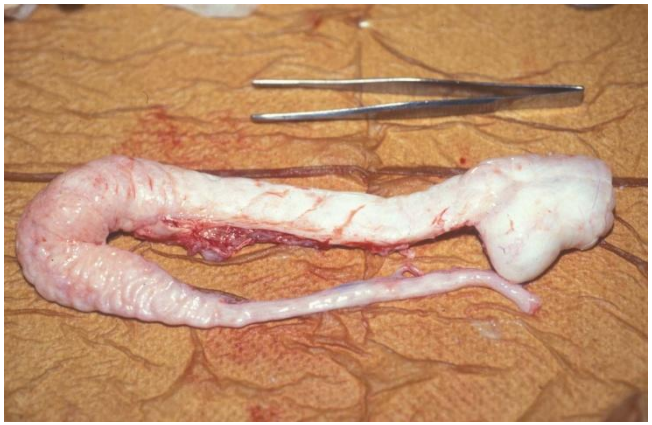
Status of the procedures used for sperm freezing

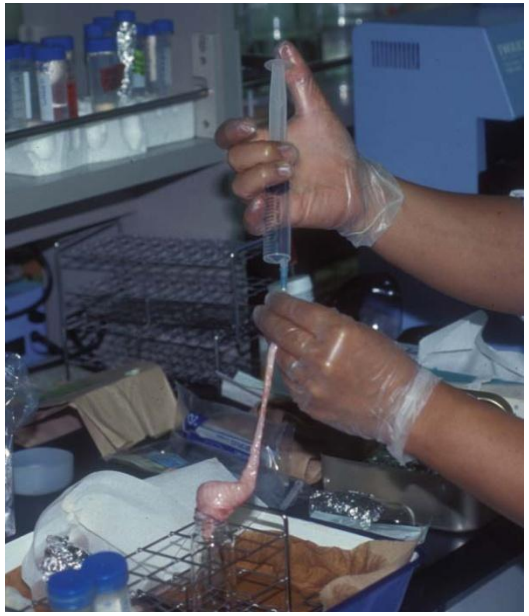


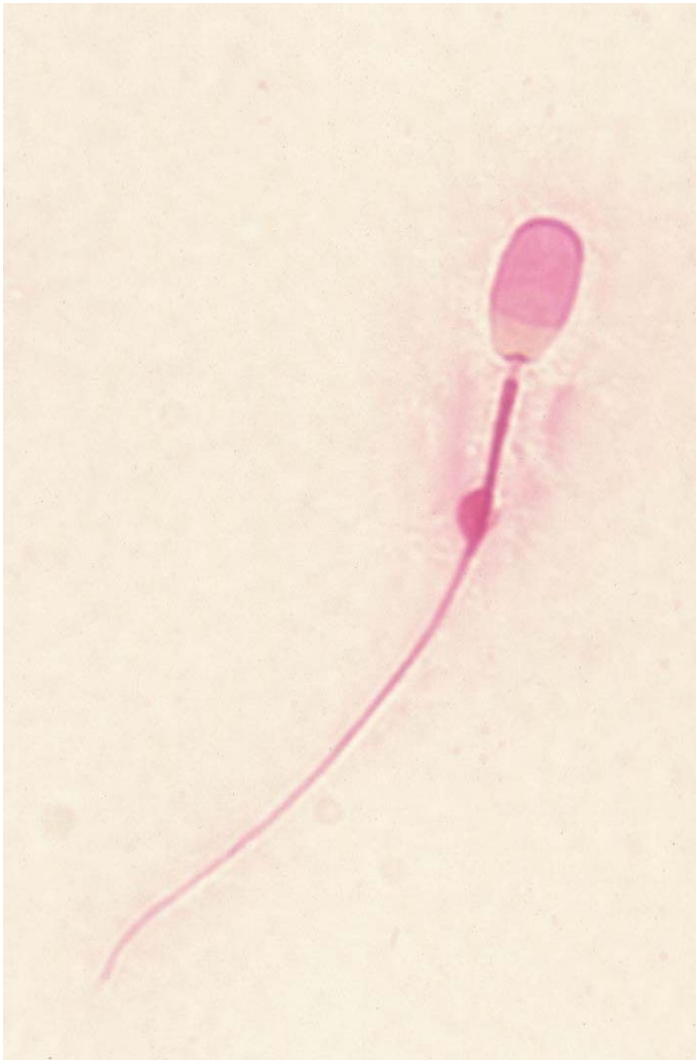
Piglets obtained

Without difficulty

Collection of epididymal spermatozoa







Sperm from cauda epididymis show better freeze-tolerance than ejaculated sperm.

Table 1. Sperm motility (%) in fresh and frozen-thawed ejaculated semen and in epididymal semen in 3 boars

	Boar 1		Boar 2		Boar 3	
	$\bar{x}$ (%)	±SEM	$\bar{x}$ (%)	±SEM	$\bar{x}$ (%)	±SEM
Unprocessed semen	84.2^{ad}	2.0	74.2^{ae}	5.9	77.7^{ae}	5.2
Fresh semen prepared for IVF	74.2^{bd}	4.9	74.2^{ad}	3.8	80.8^{ae}	4.9
Frozen-thawed ejaculated semen	50.0^{cd}	11.0	28.2^{be}	7.5	42.5^{bd}	9.9
Frozen-thawed epididymal semen	76.7^{bd}	2.6	69.2^{ad}	7.4	70.8^{ad}	6.7

[a-c] Values with different superscripts within a column differ significantly between treatments ($P < 0.05$).

[d-e] Values with different superscripts within a row differ significantly between boars ($P < 0.05$).

Rath and Niemann Theriogenology1997

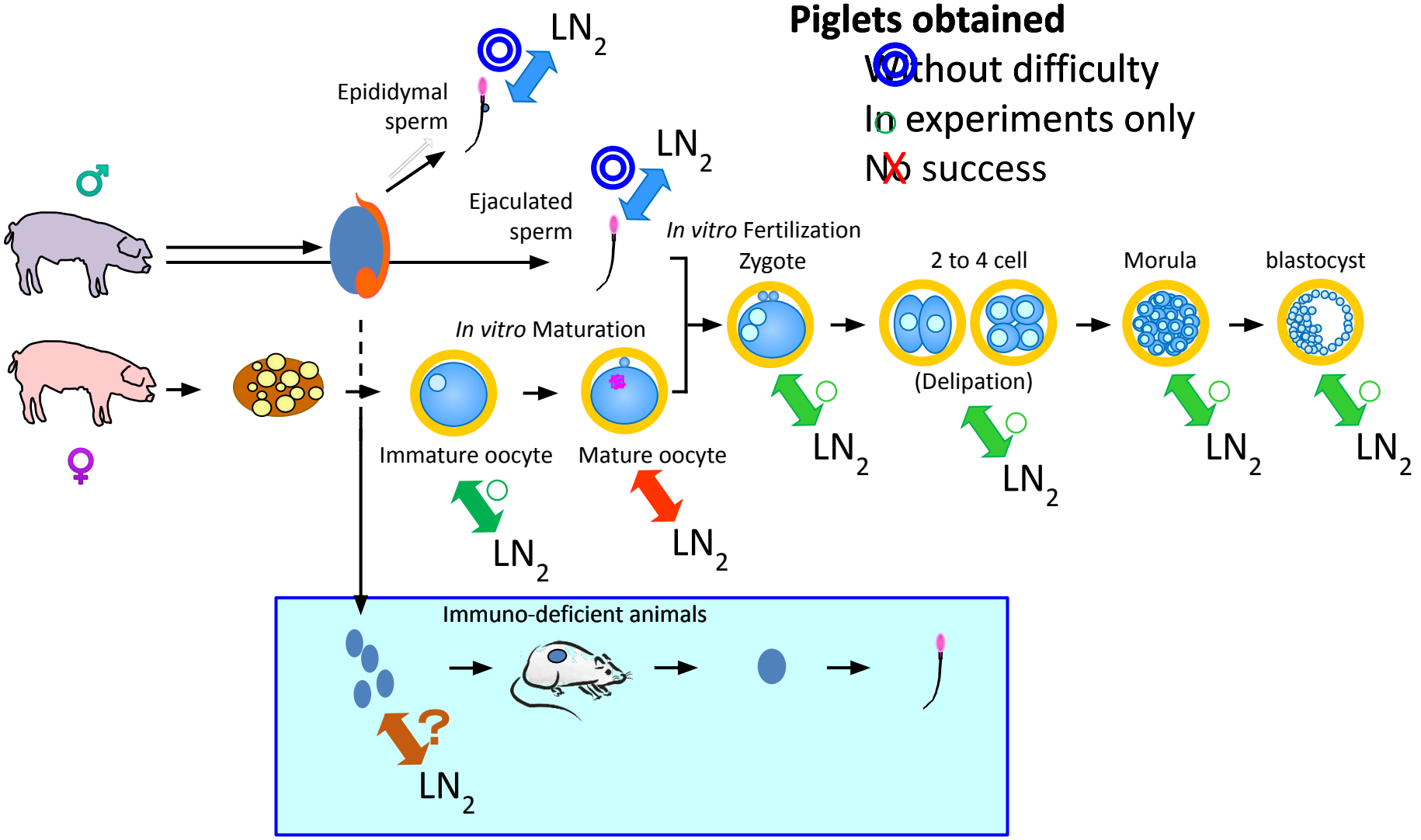
Table 2. Percentage of pronucleus development of in vivo matured porcine oocytes 8 hours after in vitro fertilization with 3 different types of semen

	IVF with					
	Fresh semen		Frozen-thawed ejaculated semen		Frozen-thawed epididymal semen	
	$\bar{x}$ (%)	±SEM	$\bar{x}$ (%)	±SEM	$\bar{x}$ (%)	±SEM
No. of oocytes	73		44		94	
Germinal vesicle	5.5	2.9	4.4	2.9	7.2	2.3
Metaphase II	28.9^{b}	6.8	36.7^{b}	8.2	9.5^{c}	2.4
Degenerated	18.2	4.6	12.8	5.4	10.5	4.2
Penetrated [a]	14.1	5.2	11.5	6.9	9.1	2.3
1 pronucleus	24.4	5.6	20.1	5.4	18.4	3.1
2 pronuclei	8.9	4.8	2.2^{b}	2.2	23.0^{c}	4.7
> 2 pronuclei	0^{b}		12.3^{bc}	7.5	22.3^{c}	5.3

ANOVA: Values with different superscripts within a row differ significantly ($P \leq 0.05$).
[a] Expanded sperm head and corresponding tail in the cytoplasm, no visible pronuclei.

Rath and Niemann Theriogenology1997

Status of the procedures used to cryopreserve porcine gonadal tissue, gametes and embryos



Requirement for xenografting

1. Immuno-deficent animals were purchased commercially

Nude mice (T-cell null) | **Nude rats (T-cell null)** | **SCID mice (T- and B-cell null)** | **ALY mice (Lymph node & Peyer's patch –null)**

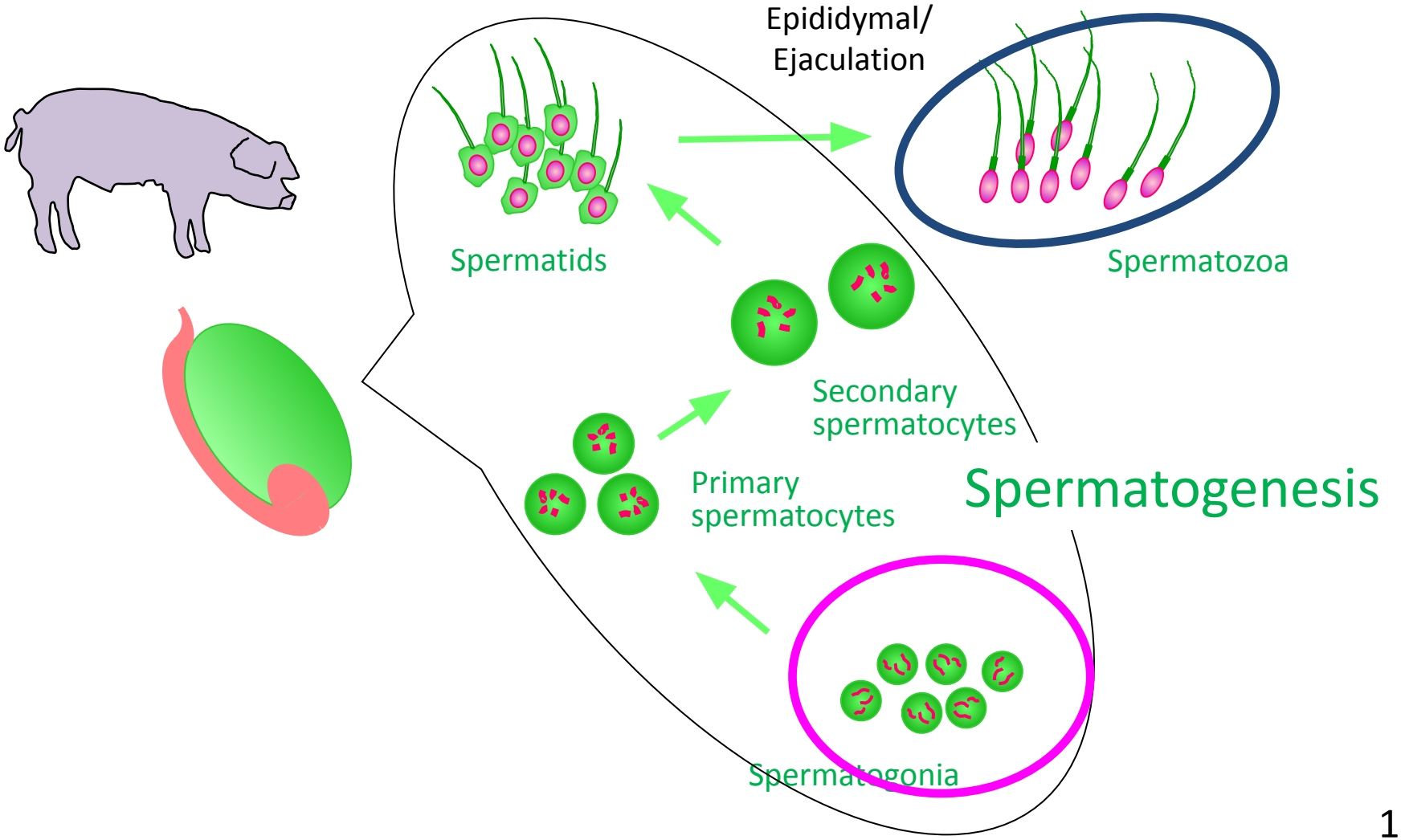
from CLEA Japan. Inc.

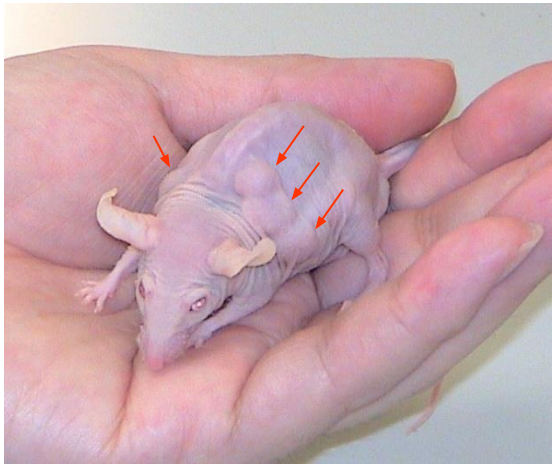
2. In vitro embryo production (IVM/IVF/IVC) is established.

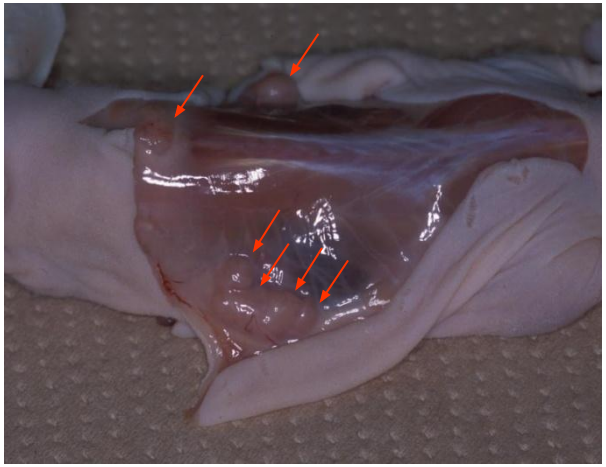
3. Techniques of embryo transfer is also established.

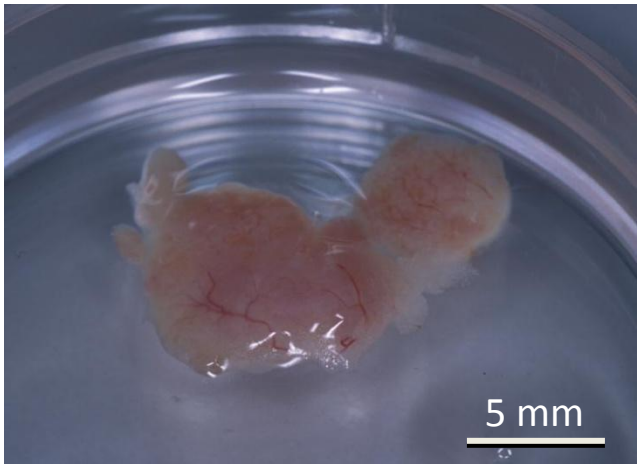
2-1. Cryopreservation of Testicular tissue

Utilization of testicular tissues

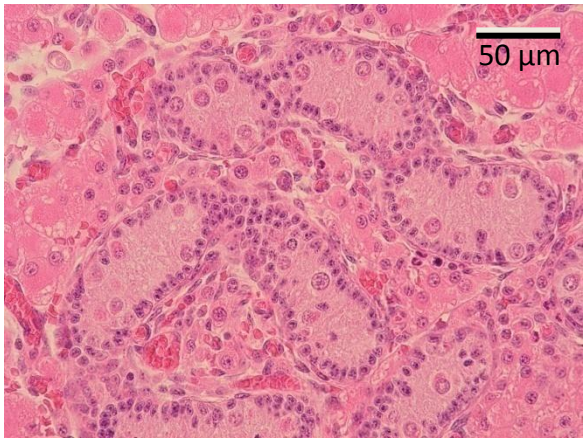


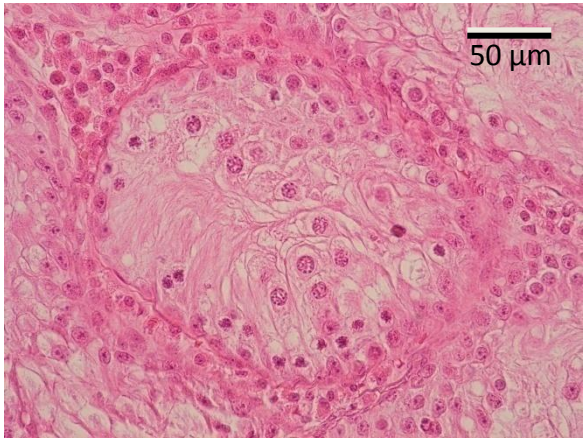


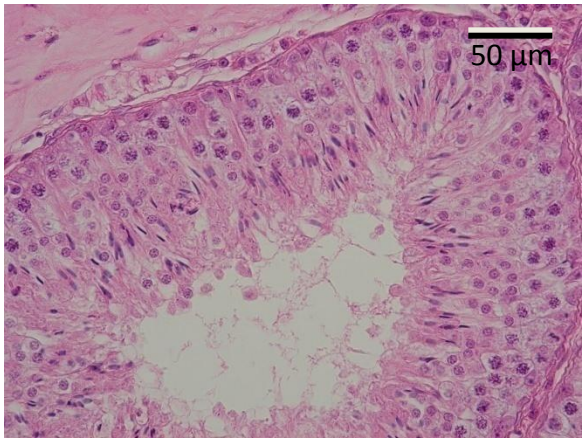




Xenografts (Day 125)







5 days old piglet

Day 78

Day 118

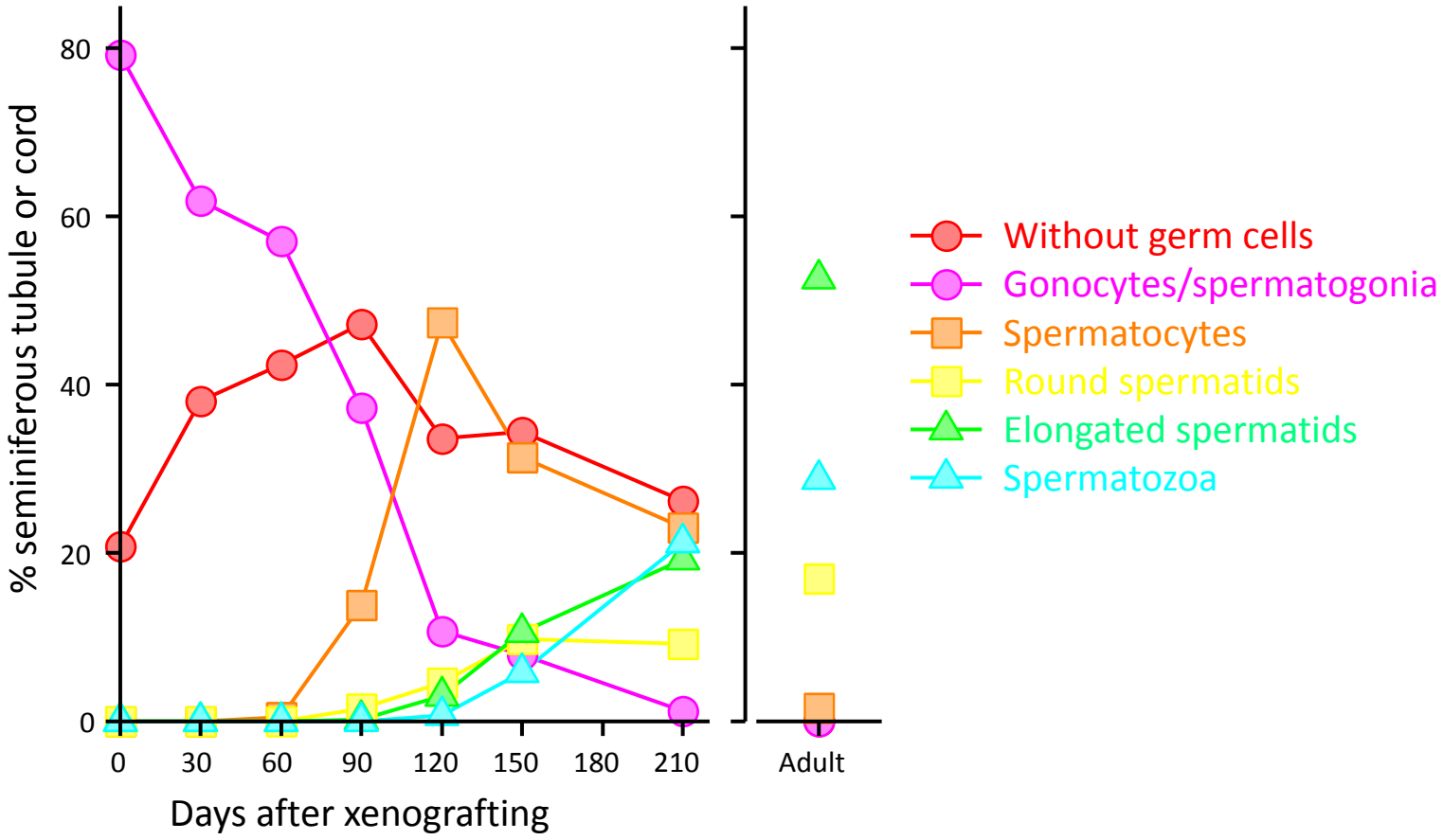
Before xenografting

The seminiferous cords contain only gonocytes and spermatogonia.

After xenografting

Kikuchi et al., Reprod Fertil Dev 2006:18: 247

Generation of sperm cells

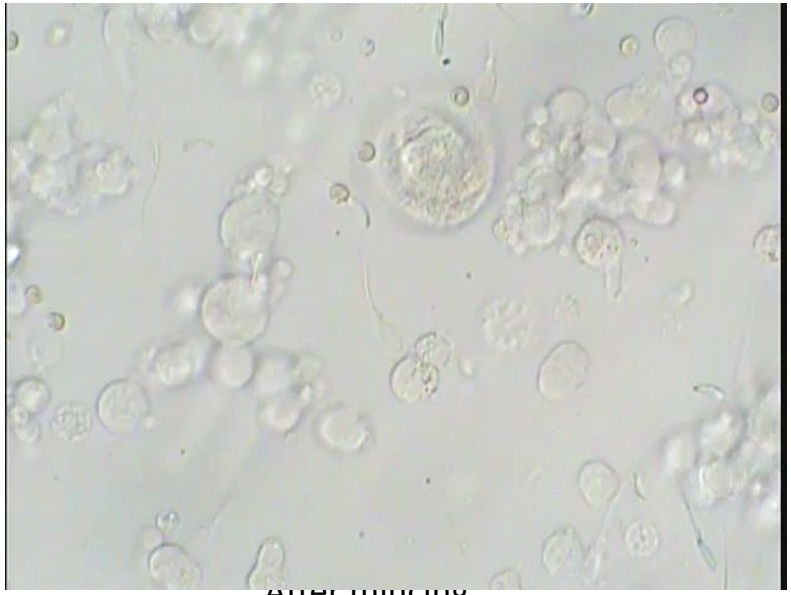


Percentage of seminiferous cord or tubule crosssection after xenografting. Histological sections of testicular tissues grafted into mice were prepared 0–210 days after xenografting. Those from adult boars were also prepared.

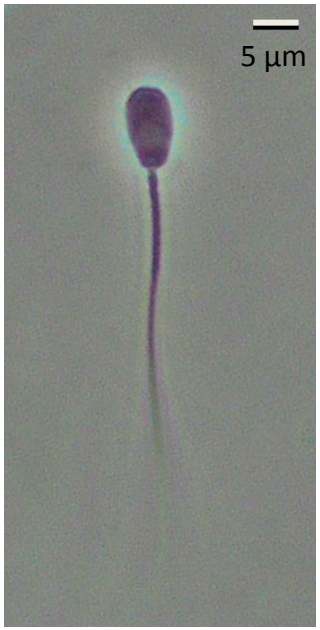
Kikuchi et al., Anim Sci J 2011;72:2-9

Sperm cells from the xenografted tissue

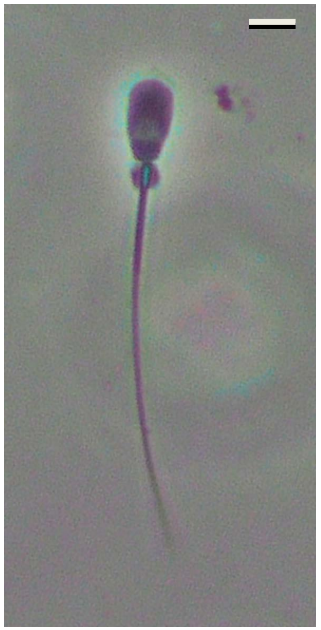
Well-developed xenografts; 27 mice
Sperm cells obtained; 19 mice (70.4%)



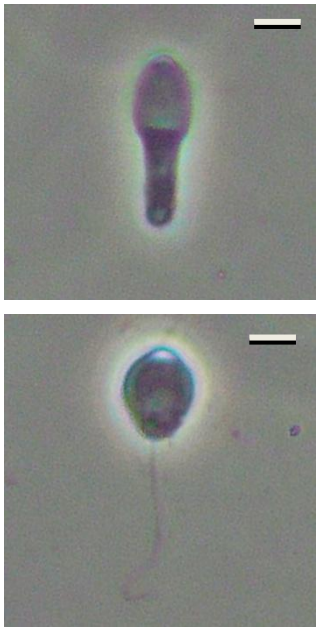
After mincing



Spermatozoon (used for ICSI)



Spermatozoon with cytoplasmic droplet

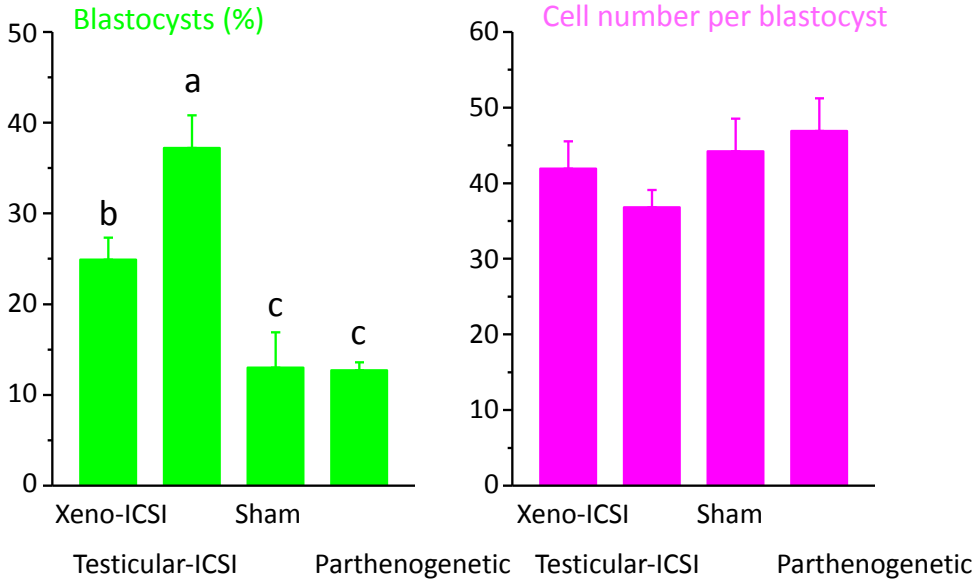


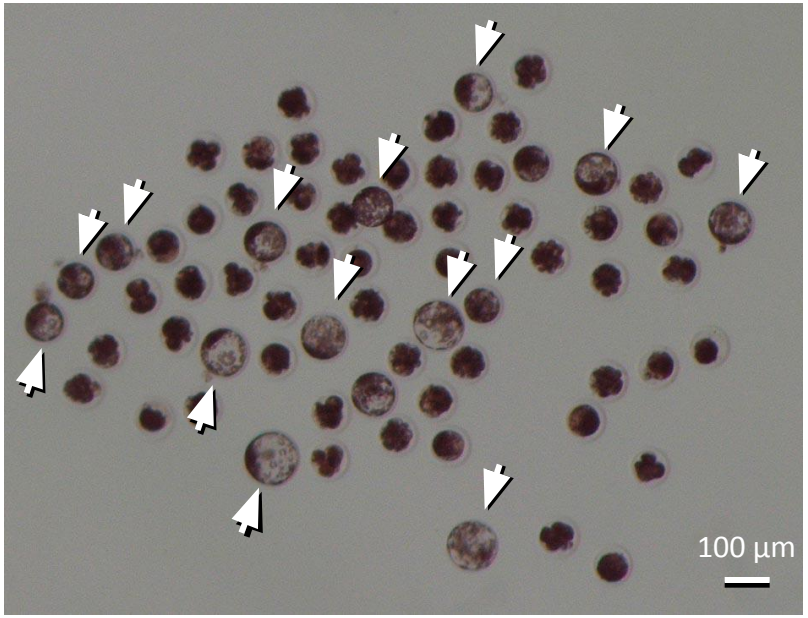
Immature sperm cells

Morphologically normal（w/ head, midpieace & tail）				Abnormal (immature)	Total
	+	–	total		
Cyroplasmic droplet	280 (98.2%)	5 (1.8%)	285 (89.3%)	34 (10.7%)	319
Motility	134 (47.0%)	151 (53.0%)			

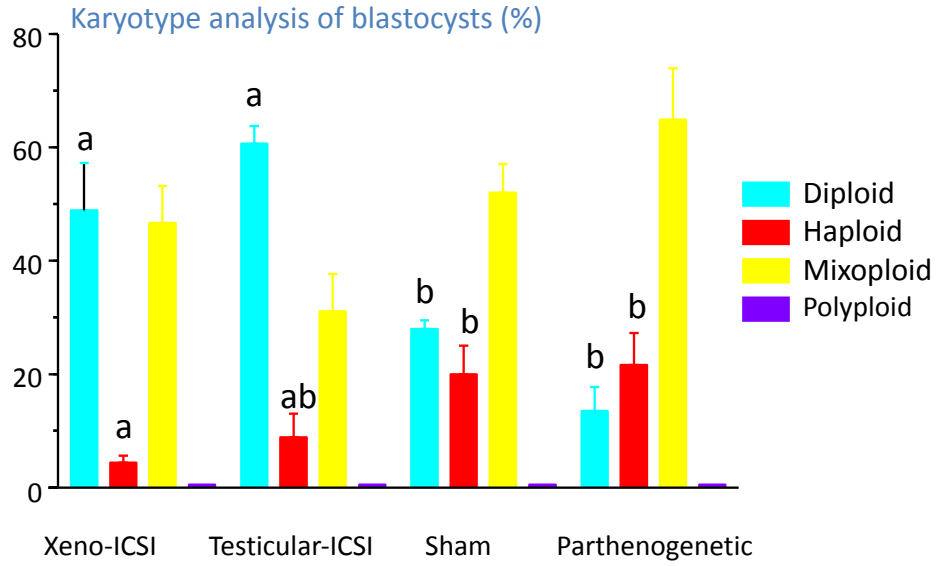
Nakai et al., Theriogenology 2009;72:2-9

In vitro development after ICSI





Blastocysts (Day 6)



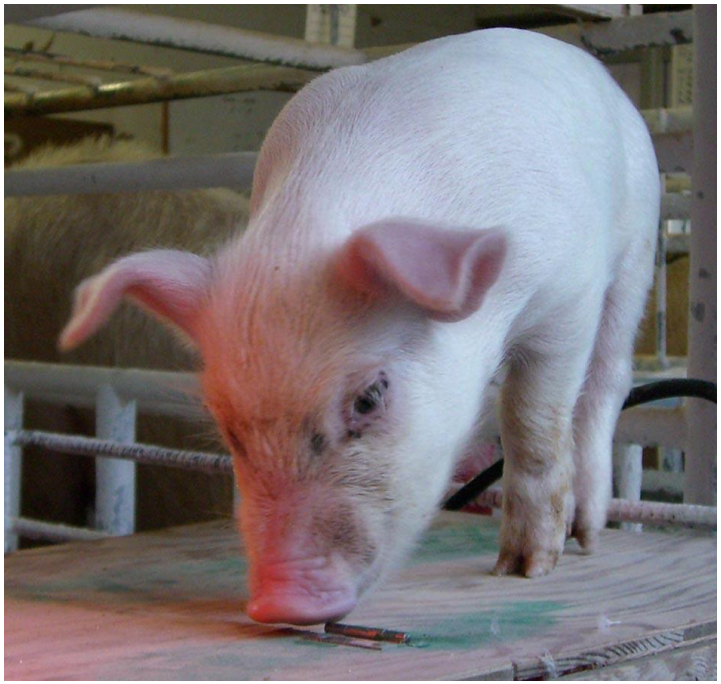
Embryo transfer experiment

Nakai et al., Theriogenology 2009;72:2-9

Piglet production

Recipient #8

♂4+♀1

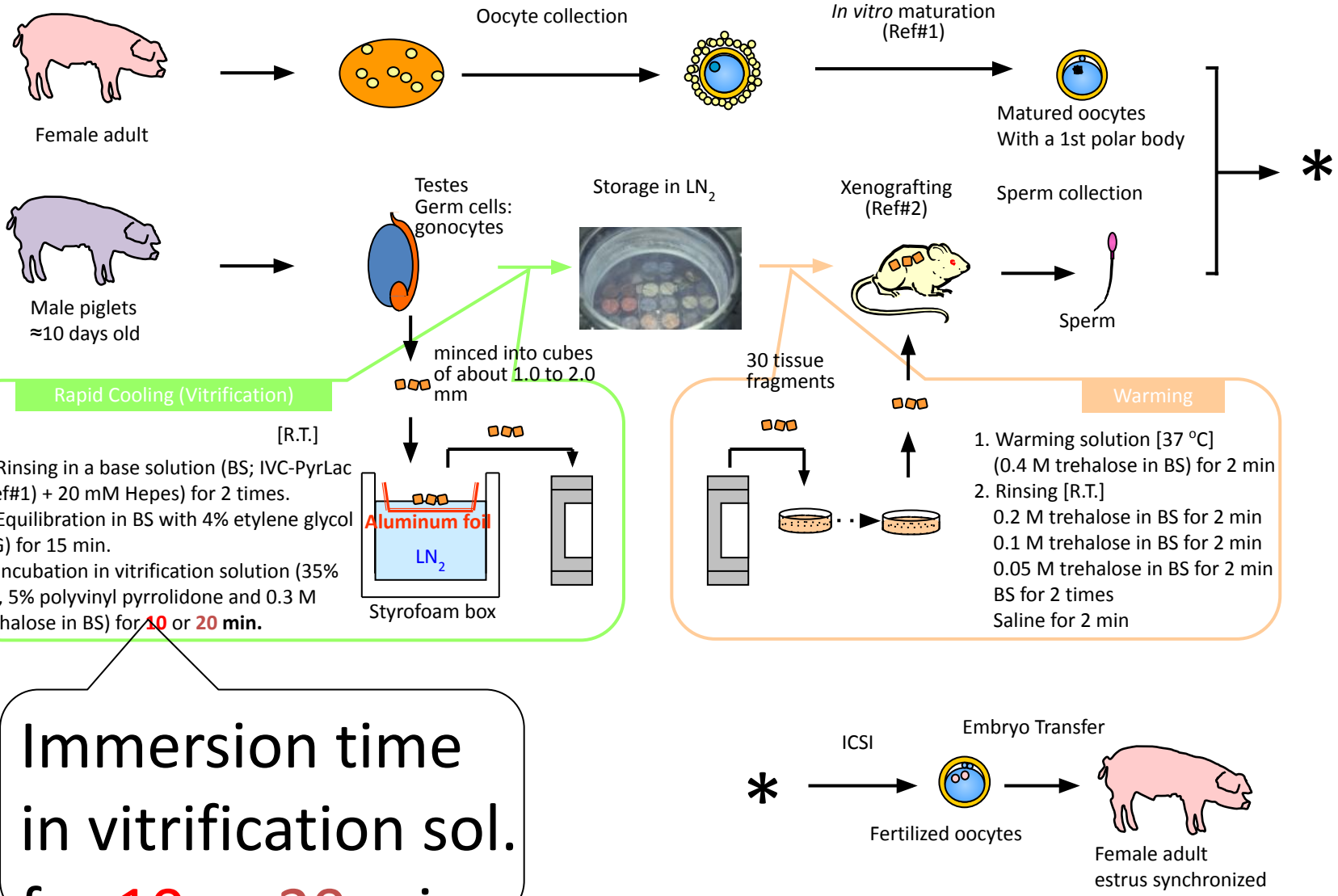


Recipient #14

♂1

Nakai et al., Reproduction 2010;139: 331–339

Cryopreservation of testicular tissue



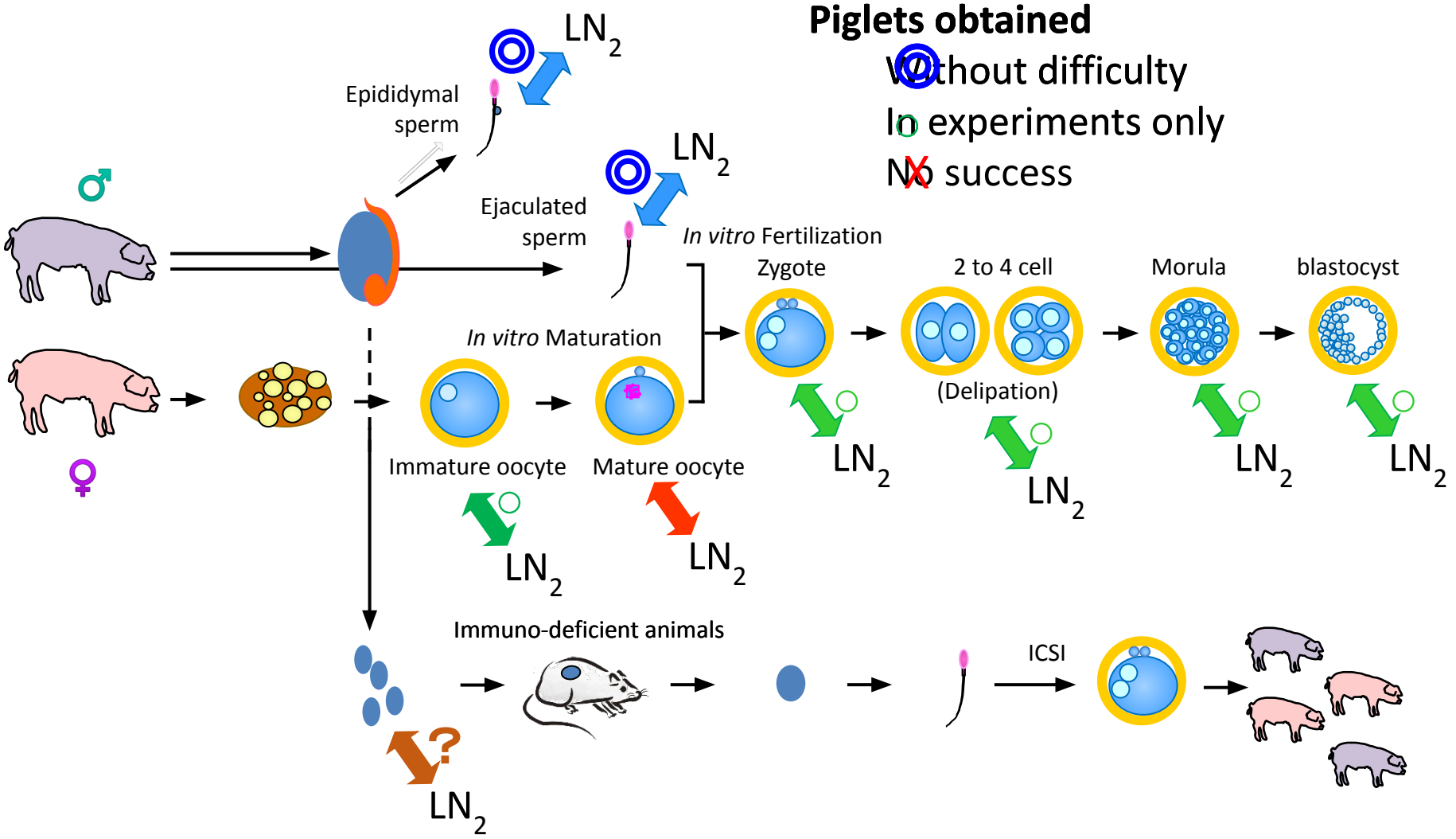
Piglet production

Table. Transfer to synchronized recipients of porcine oocytes injected with sperm from cryopreserved xenografts.

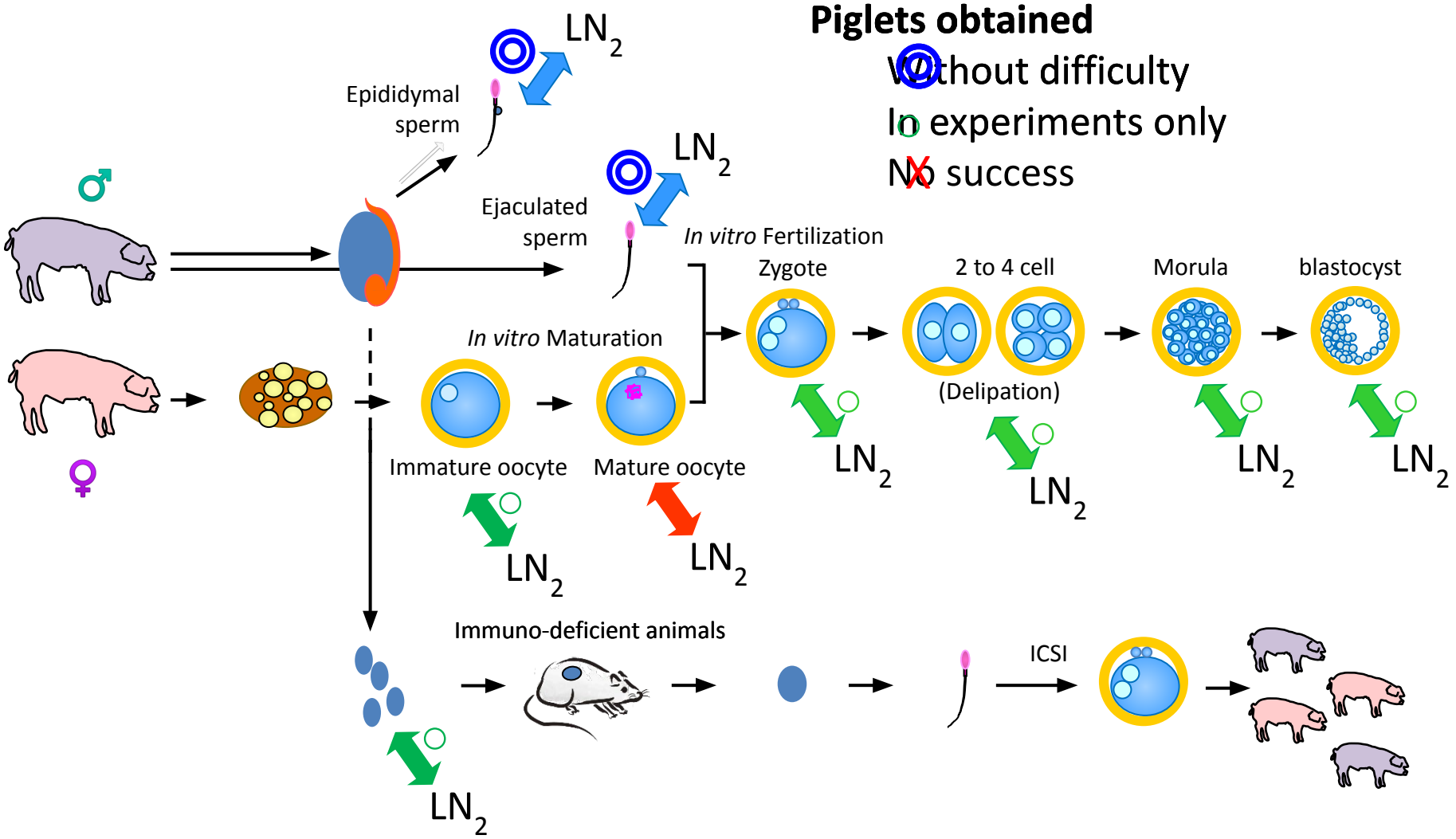
Immersion-time group	Recipient No.	Preservation of testicular tissue before grafting (days)	Sperm collection (days postgrafting)	No. of fertilized oocytes transferred	Pregnancy	No. of piglets born
10-min	1	140	231	74	+	♂;1, ♀;1
	2	188	230	70	–	
	3	585	318	100	–	
	4	587	234	101	–	
20-min	1	188	230	59	–	♂;2 ♀;3
	2	188	254	89	+	
	3	578	291	79	–	
	4	587	248	97	–	

Kaneko et al., PLoS One. 2013;8: e70989.

Conclusion/ Testicular tissue cryopreservation



Conclusion/ Testicular tissue cryopreservation



Xenografting offers a possible way for the effective utilization of vitrified testicular tissue fragments.

Collaborators

NARO

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