Importance of preservation of gametes and gonadal tissue for porcine genetic resources

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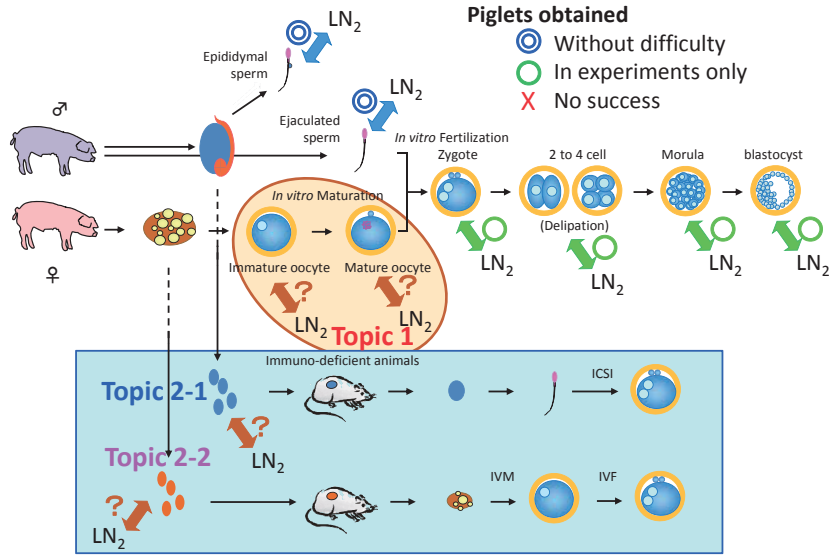
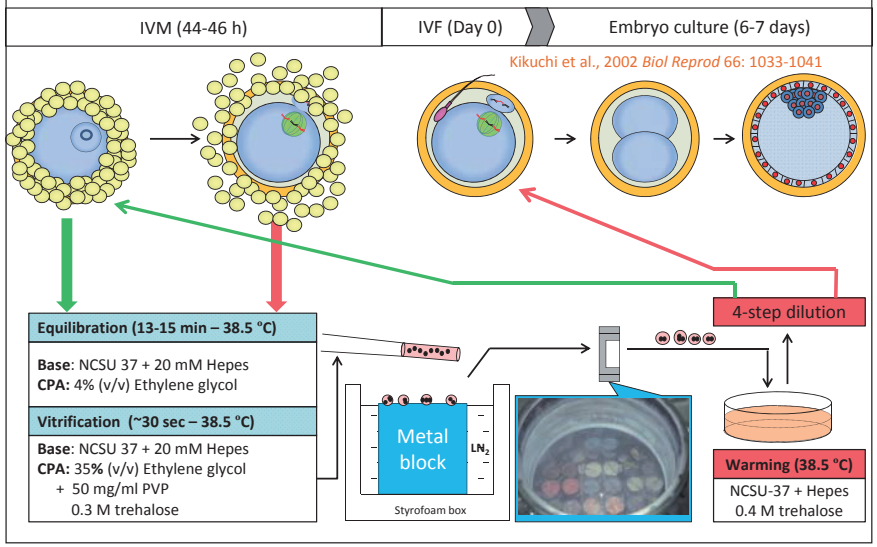
National Agriculture and Food Research Organization

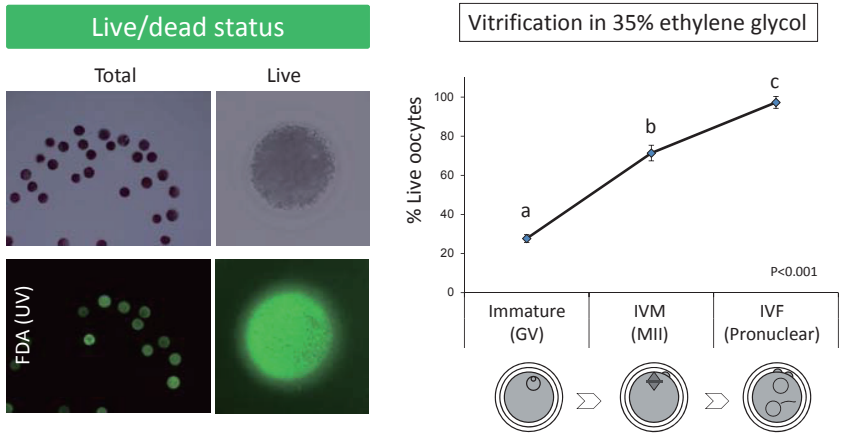
Status of the procedures used to cryopreserve porcine gonadal tissue, gametes and embryos



Solid Surface Vitrification (SSV)



Survival

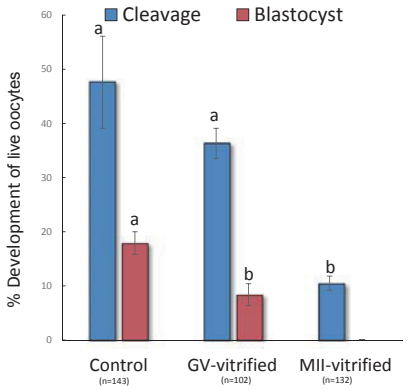


Somfai et al., J Reprod Dev 2012; 58: 14-17

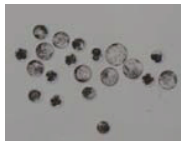
Oocyte survival increases by nuclear progression

In vitro development

IVF/IVC

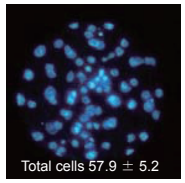


Blastocyst quality (total cells)

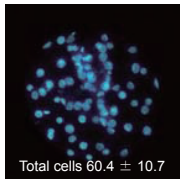


Day 6 IVC: control

Day 6 IVC: GV-vitrified



Blastocyst from control (Day 7)



Blastocyst from a GV-vitrified oocyte (Day 7)

Egerszegi et al., Cryobiology 2013; 67: 287–292

Somfai et al., Theriogenology 2010; 73:147-156

Oocytes vitrified at GV stage have competence to form high quality blastocysts

Piglet production

Table 1. Production of piglets by the transfer of in vitro-derived blastocysts obtained from vitrified oocytes on Day 5 (Day 0 = IVF)

Recipient	Total vitrified oocytes	No. embryos transferred	Pregnancy	Gestation (days)*	Total No. of piglets born (live)	Gender	Average body weight of piglets at birth (kg)$
#1	567	16	+	115	4 (4)	♂: 2 / ♀: 2	1.5 ± 0.04
#2	1235	27	+	114	6 (6)	♂: 3 / ♀: 3	1.52 ± 0.05
Overall	1802	73			10 (10)	♂: 5 / ♀: 5	

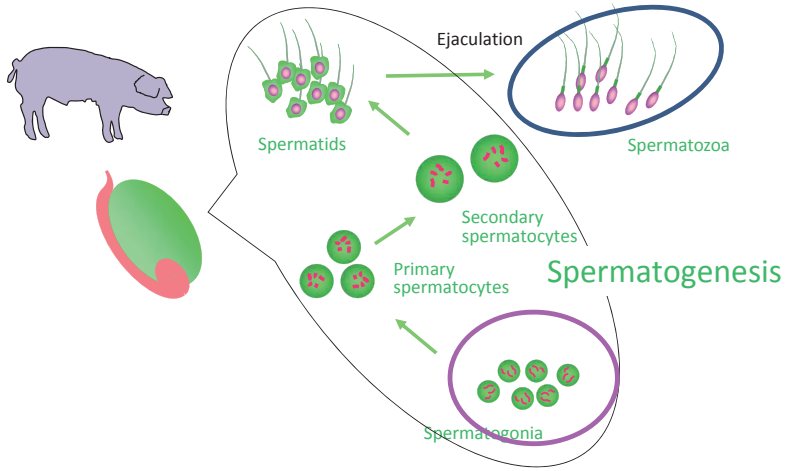
*Recorded from the second day after hCG injection. $Data are presented as means ± SEM.

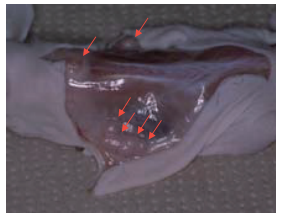
Conclusion/Oocyte cryopreservation

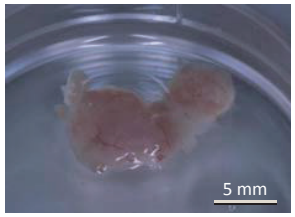
- MII stage: the survival is high but oocytes have limited chance for recovery > high frequencies of fertilization anomalies.
- **GV stage:** the survival is low, but surviving oocytes have the ability to recover > normal fertilization, good quality blastocysts and piglet production.

2-1. Cryopreservation of Testicular tissue

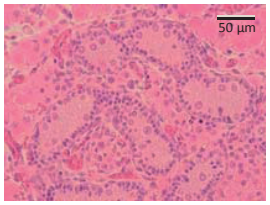
Utilization of testicular tissues

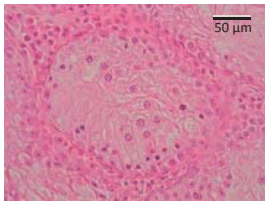


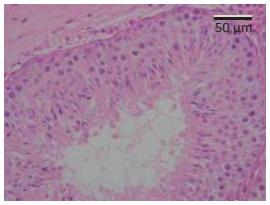




Xenografts (Day 125)







5 days old piglet

Before xenografting

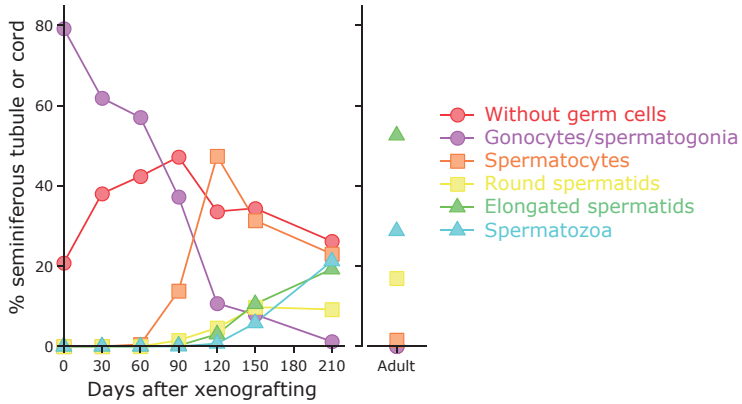
The seminiferous cords contain only gonocytes and spermatogonia.

Day 78 Day 118

After xenografting

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

Generation of sperm cells

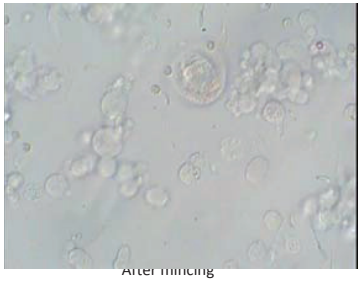


Percentage of seminiferous cord or tubule crosssection after xenografting. Histological sections of testicular tissues grafted into mice were prepared 0–210 days after xenografting. Those from adult boars were also prepared.

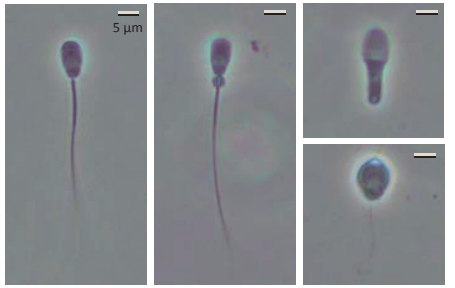
Kikuchi et al., Anim Sci J 2011;72:2-9.

Sperm cells from the xenografted tissue

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



After mincing



Spermatozoon (used for ICSI)

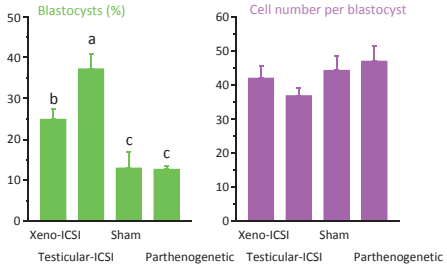
Spermatozoon with cytoplasmic droplet

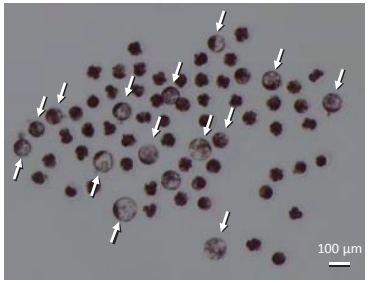
Immature sperm cells

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%)			

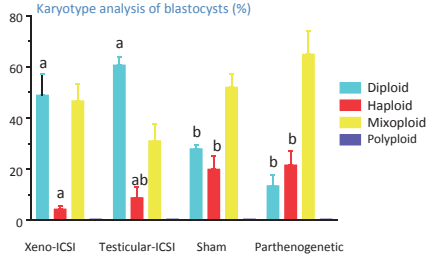
Nakai et al., Theriogenology 2009;72:2-9.

In vitro development after ICSI





Blastocysts (Day 6)



Embryo transfer experiment

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

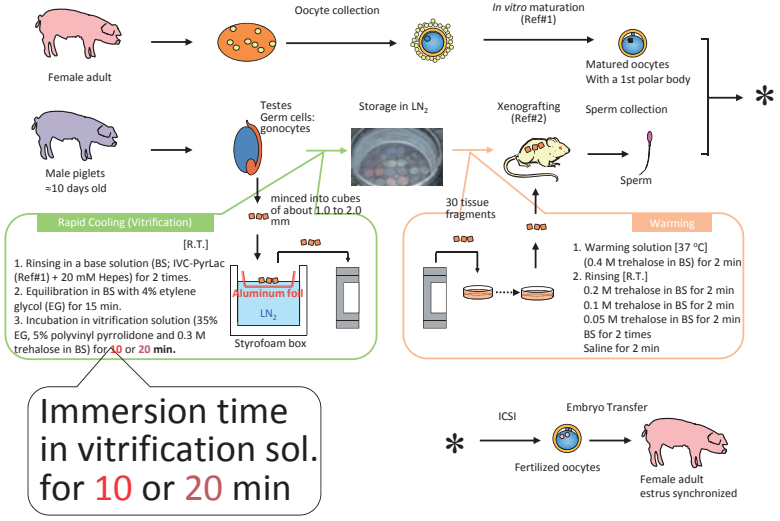
Piglet production

Recipient #8 ♂4+♀1

Recipient #14 ♂1

Nakai et al., Reproduction 2010;139: 331–335.

Cryopreservation of testicular tissue



Piglet production

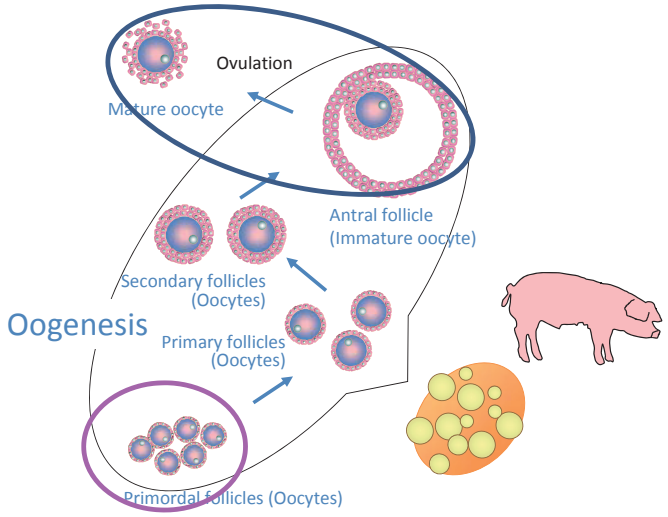
Table. Transfer to synchronized recipients of porcine oocytes injected with sperm from cryopreserved xenografts.

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	–	

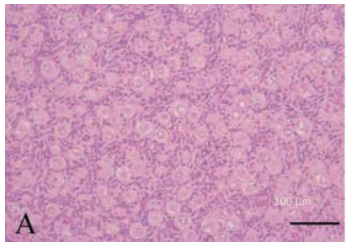
Kaneko et al., PLoS One. 2013;8: e70989.

2-2. Cryopreservation of ovarian tissues

Utilization of female germplasm

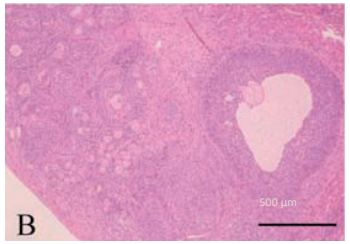


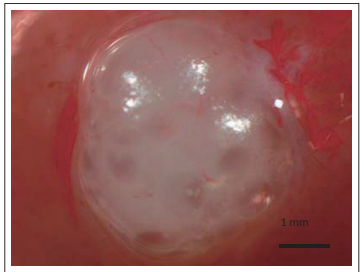
Follicular development



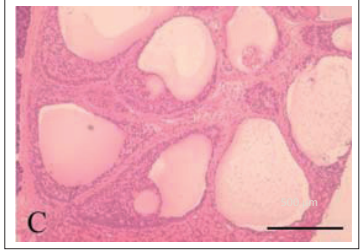
Before grafting

-eCG (60 day)





eCG-60



Kaneko et al., Biol Reprod 2003; 69: 1488-1493.

In vitro maturation

Table 2. Number and meiotic competence of porcine oocytes recovered from host mice.

Group[1]	No. of mice	No. of grafts recovered[2]	No. of oocytes recovered[2]	No. of oocytes per mouse[3]	No. of oocytes per grafts[3]	No. of oocytes larger lager than 115 μm[4]	No. of oocytes in MII stage[4]
–eCG	7	51	122	17.4 ± 5.8[a]	2.4 ± 0.5[a]	7 (2.4 ± 0.7[a])	6 (0.8 ± 0.4[a])
eCG-10	7	56	261	37.3 ± 7.7[ab]	4.7 ± 1.0[b]	40 (5.7 ± 1.0[a])	13 (1.9 ± 0.4[a])
eCG-30	5	39	295	59.0 ± 13.4[bc]	7.6 ± 0.9[c]	40 (8.0 ± 3.4[a])	14 (2.8 ± 1.1[a])
eCG-60	7	57	573	81.9 ± 5.7[c]	10.1 ± 0.7[d]	212 (30.3 ± 3.9[b])	98[5] (14.0 ± 1.0[b])

[1]The eCG-10, -30, and –60 groups received 5 IU eCG at 10, 30, or 60 days, respectively, after vaginal cornification and the grafts were examined 48 later; the –eCG group received no hormone treatment and grafts were examined 10 days after vaginal cornification.
[2]Total number of grafts or oocytes recovered
[3]Values are mean ± SEM per mouse or per graft.
[4]The number of oocytes in each category is represented by the total number followed by (mean ± SEM per mouse).
[5]Value includes the number of oocytes with the first polar body that were subjected to IVF
a–cValues in the same column without common superscripts are significantly different ($P < 0.05$).

In vitro fertilization

2	No. of mature oocytes* inseminated	No. (%) of oocytes fertilized**	No. (%) of monospermic oocytes
eCG-60	20	11 (55.0)	9 (45.0)

* Oocytes with a visible first polar body
**Oocytes with male pronuclear and female pronuclei after 2nd polar body extrusion

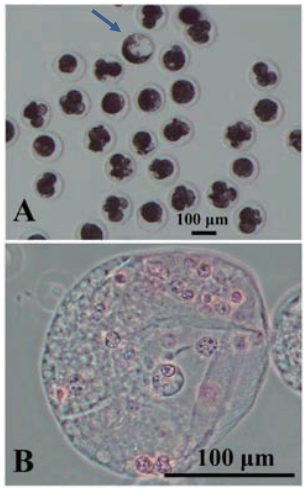
Kaneko et al., Biol Reprod 2003; 69: 1488-1493.

In vitro development

In vitro development of porcine oocytes recovered from host mice that had received hormonal treatment

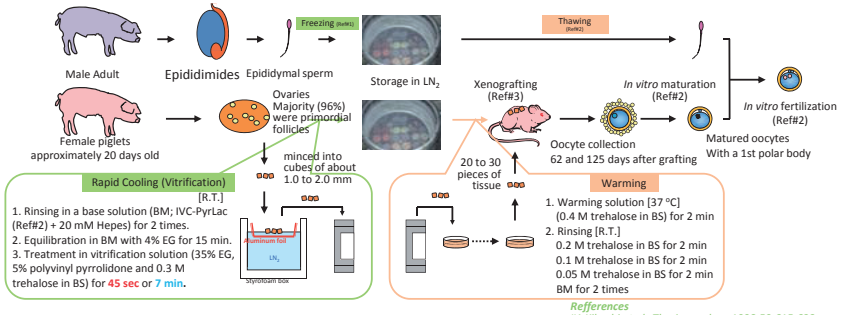
Group	No. of mature oocyte* for IVF	No. of oocytes developed to blastocyst (Cell number)
eGG-2	31	0
eGG-2	100	0
FSH-7	115	1 (23)
FSH-14	163	1 (16)
FSH-14EA	113	1 (30)

*Oocytes with a visible first polar body



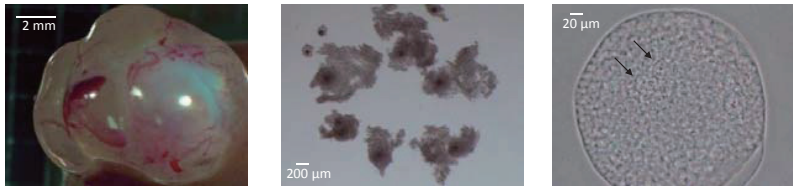
Kaneko et al., Reproduction 2006;131: 279-288.

Cryopreservation of Ovarian tissue



Refferences
#1 Kikuchi *et al.*, Theriogenology 1998;50:615-623.
#2 Kikuchi *et al.*, Biol Reprod 2002;66:1033-1041.
#3 Kaneko *et al.*, Reproduction 2006;131:279-288.

Results



Ovary 145 days after xenografting

In vitro maturation and fertilization

Maturation and fertilization of oocytes collected from vitrified and xenografted ovarian tissue

Group*	Total	Matured oocytes	Sperm penetration	FPN+MPN formation
45-sec	39	18% (7/39)	83% (5/6)	100% (5/5)
7-min	49	33% (16/49)	88% (14/16)	100% (14/14)

*The period treated with vitrification solution.
Data were not different statistically between groups.

Conclusion/ gonadal tissues cryopreservation

1) Testicular tissue

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

2) Ovarian tissues

Hopefully also in near future