Cryopreservation and Transplantation of Human Ovarian Tissue

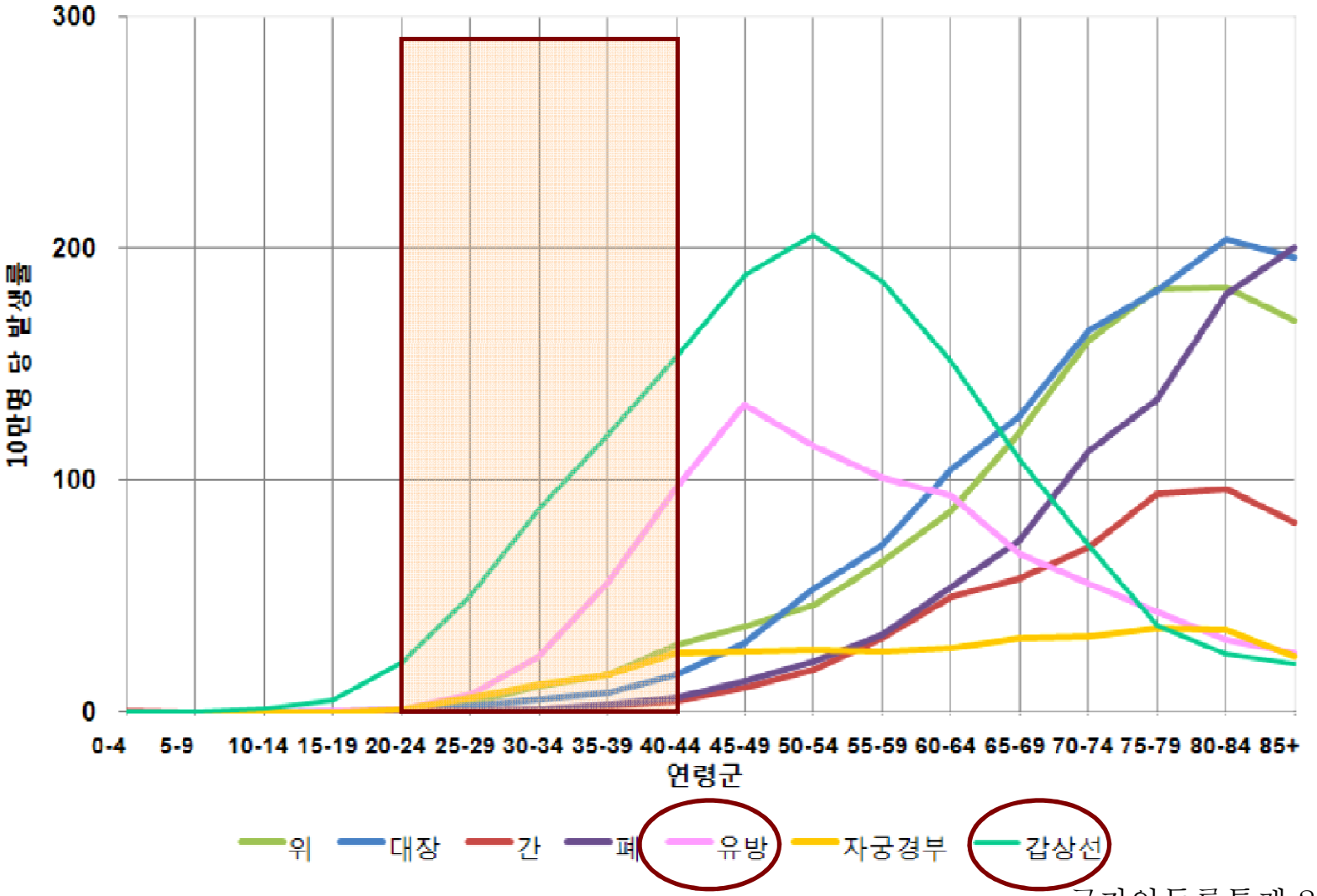
분당서울대학교병원

산부인과 불임연구실

염 혜 원

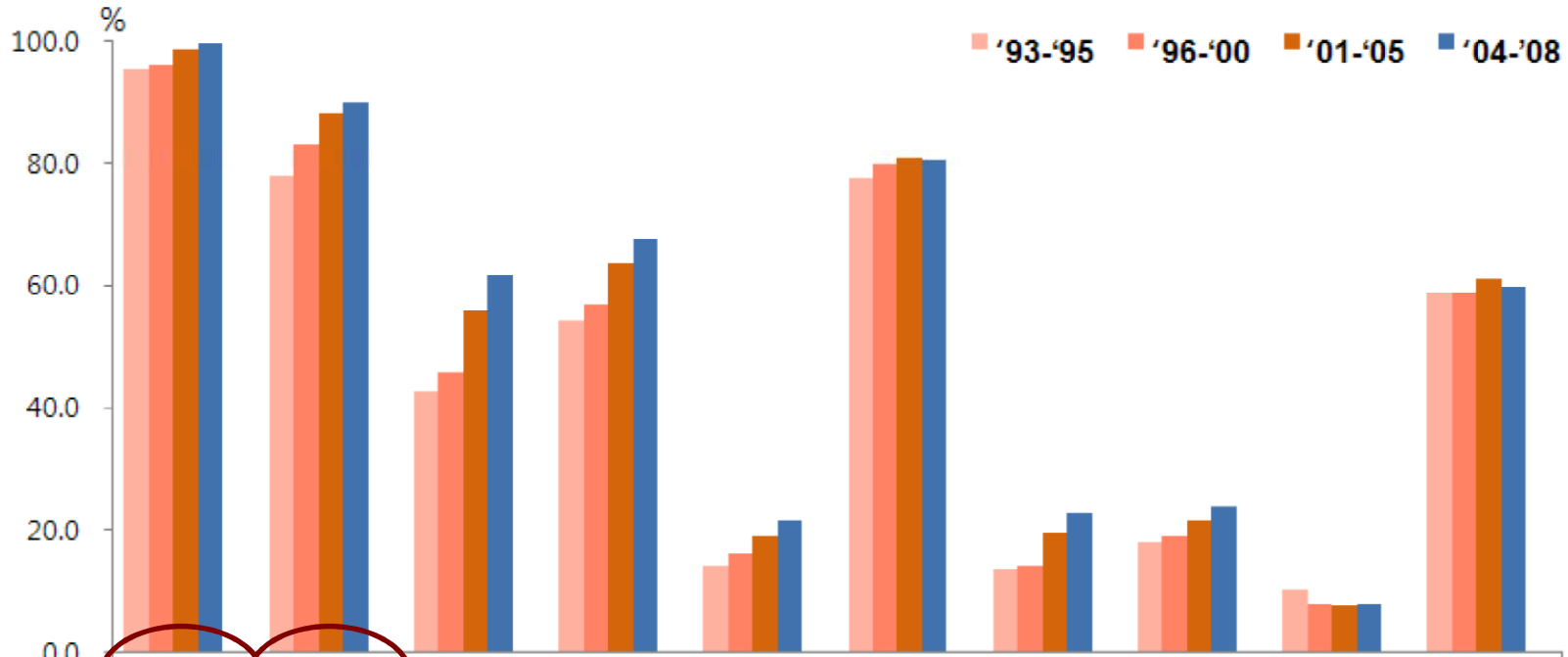
SNUH 분당서울대학교병원
SEOUL NATIONAL UNIVERSITY BUNDANG HOSPITAL

Female Cancer Incidence According to Age



국가암등록통계 2008

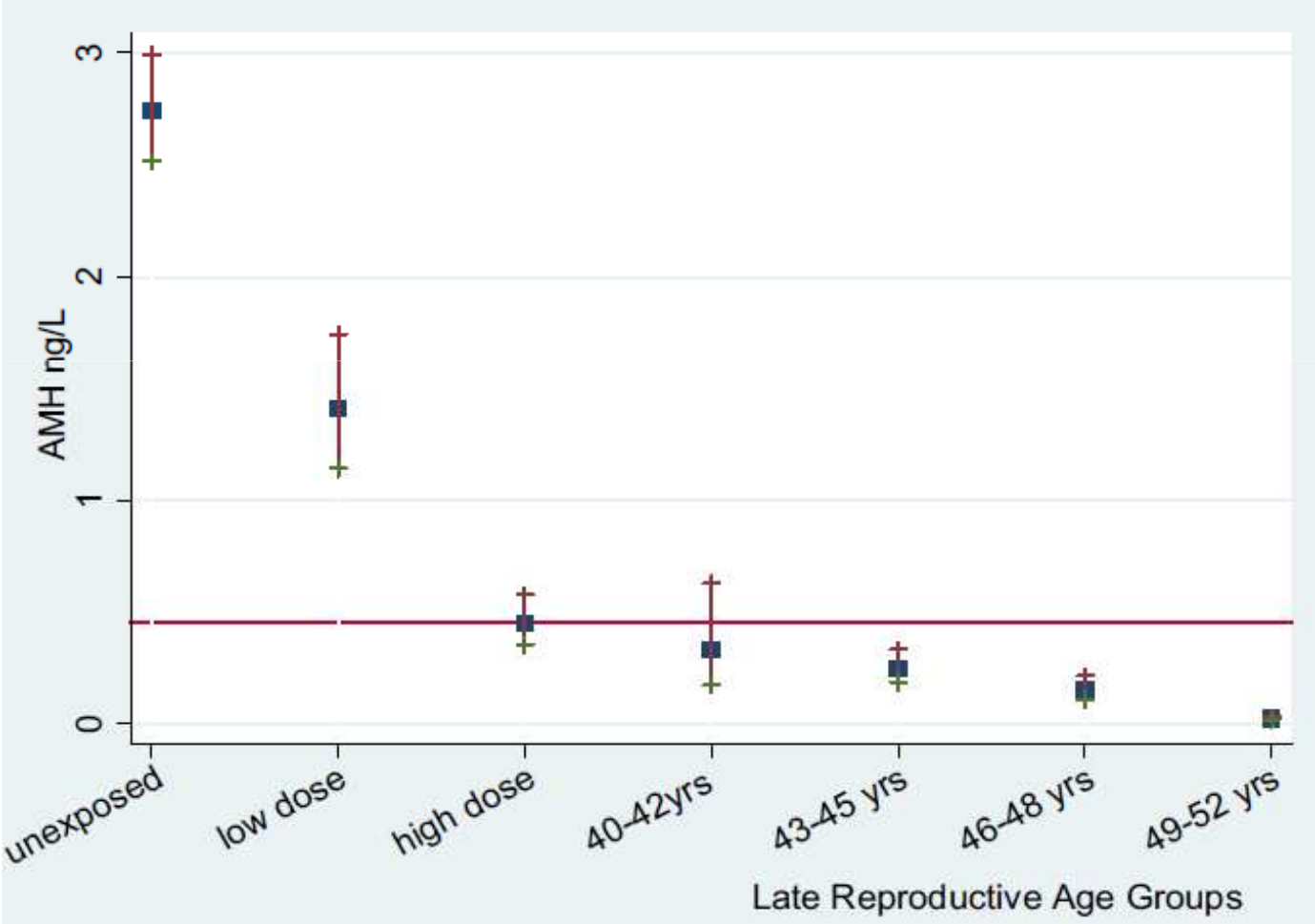
Trends in Female Cancer Survival Rate



	갑상선	유방	위	대장	폐	자궁경부	간	담낭 등*	췌장	난소
'93-'95	95.4	78.0	42.6	54.2	14.2	77.5	13.6	18.0	10.1	58.7
'96-'00	95.9	83.2	46.0	56.8	16.2	80.0	14.2	19.1	8.1	58.9
'01-'05	98.5	88.2	55.9	63.8	19.1	81.0	19.7	21.7	7.5	61.0
'04-'08	99.5	90.0	61.6	67.5	21.5	80.5	22.9	23.9	7.9	59.9
증감	4.1	12.0	19.0	13.3	7.3	3.0	9.3	5.9	-2.2	1.2

국가암등록통계 2008

Ovarian Reserve after Cancer Therapy



Gracia et al., Fertil Steril. 2012;97:134

Fertility Preservation Options

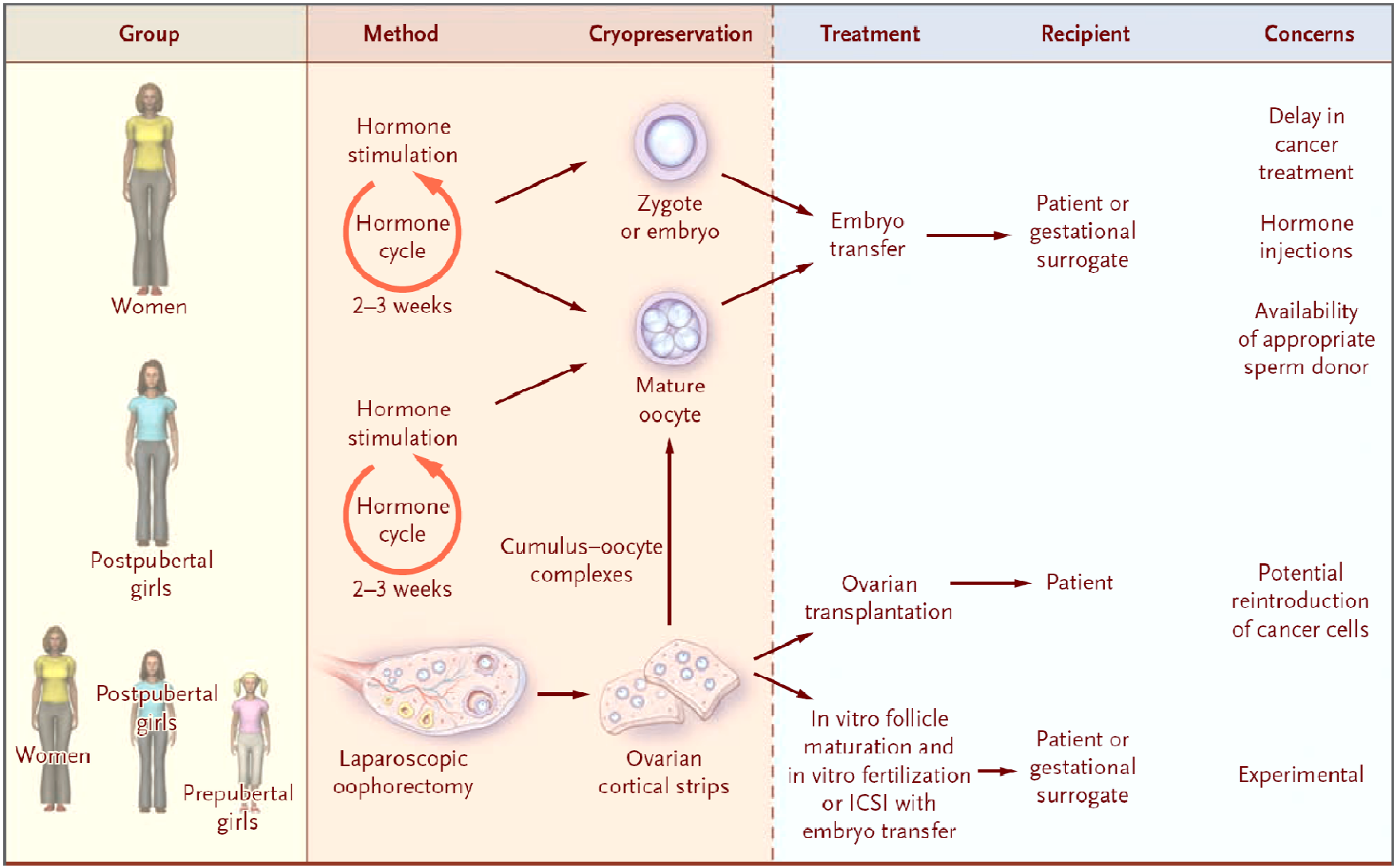
- Ovarian protection (GnRH-a, Oophoropexy)
- Oocyte / Embryo freezing
- **Ovarian tissue cryopreservation**

  → autotransplantation, in vitro culture

  Limitation

  - Cryoinjury : many different cell types → difficult to optimize conditions
  - Ischemic injuries after transplantation → no vascular anastomosis

Options for Female FP



Jeruss and Woodruff. NEJM 2009;360:902

Worldwide Frozen Ovarian Cortex Transplantation Pregnancy

TABLE 2

Series of 20 live births after transplantation of frozen-thawed ovarian cortex.

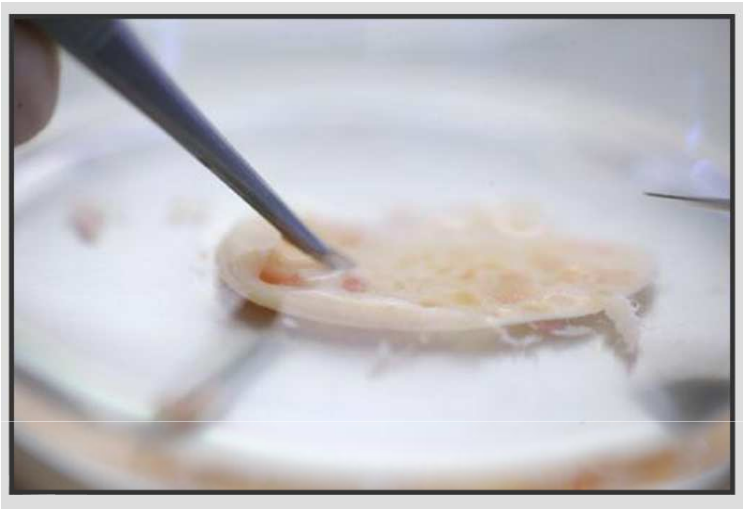
References	Cryopreservation procedure	Graft site	Live birth: Spontaneous	Live birth: IVF
Donnez et al., 2004 (9), 2008 (20), 2011 (4,32)	SF	Peritoneal window (2 steps) Ovarian medulla	+ ++ (+)*	+ (+)*
Meirow et al., 2005 (17)	SF	Beneath the ovarian cortex	–	+
Demeestere et al., 2007 (24)	SF	Ovarian and peritoneal windows (2 steps)	++	–
Andersen et al., 2008 (18); Ernst et al., 2010 (33); Schmidt et al., 2011 (29)	SF	Subcortical ovarian pocket Ovarian medulla	+ +	+ +
Silber et al., 2008 (34), 2010 (35)	SF	Ovarian medulla	+ +	–
Piver et al., 2009 (25); Roux et al., 2010 (11)	SF	Ovarian and peritoneal windows (1 and 2 steps)	+ +	–
Sanchez-Serrano et al., 2010 (36)	SF	Ovarian medulla	–	++ (twins)
Revel et al., 2011 (37)	SF	Peritoneal window	–	+
Dittrich et al., 2012 (38)	SF	Ovarian medulla	–	+
Revelli et al., 2012 (39)	SF	Ovarian medulla	+	

* Parentheses indicate ongoing pregnancy at the present time.

Donnez. Live birth after bilateral oophorectomy. Fertil Steril 2012.

SNUH 분당서울대학교병원 SEOUL NATIONAL UNIVERSITY BUNDANG HOSPITAL

Preparation of Ovarian Tissue for Cryopreservation



ISFP 2011

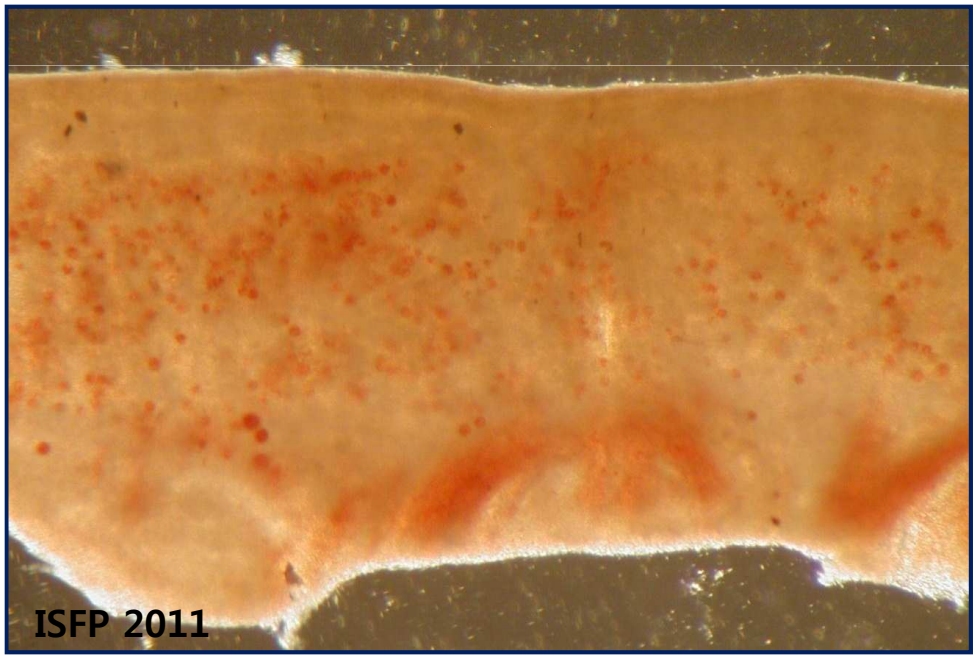
Ovarian Cortex

→ the 1mm outer layer of ovary

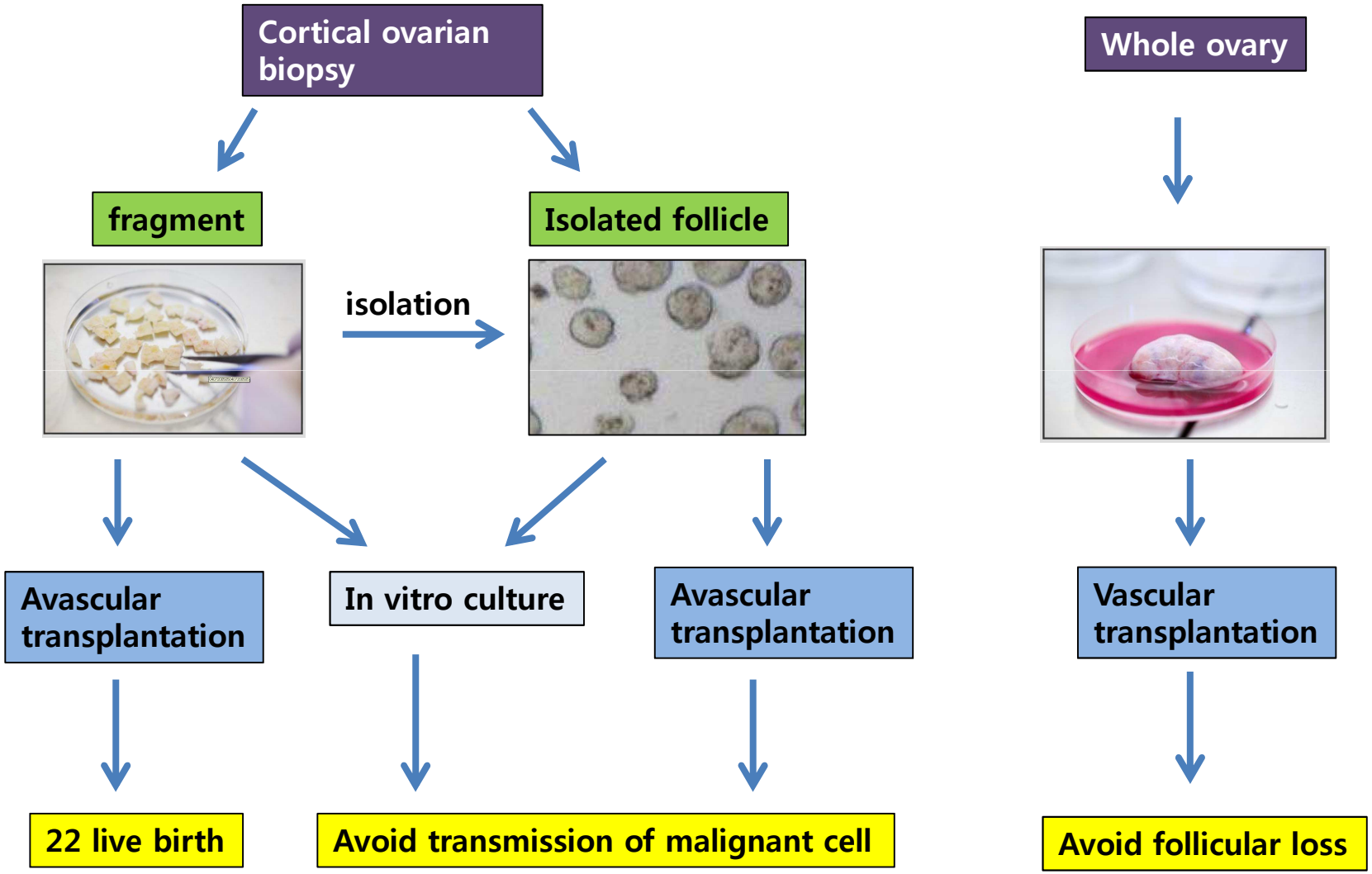
→ contain more than 90 % of the primordial follicles

→ allows effective cryopreservation of follicles

→ active follicles constitute the organ function



Cryopreservation of Ovarian Tissues : 3 Options



Freezing Methods for Reproductive Cells

1) Slow freezing

cryoprotectants : 1.0-1.5 M and low

cooling rates : low (0.3~2°C/min)

2) Vitrification

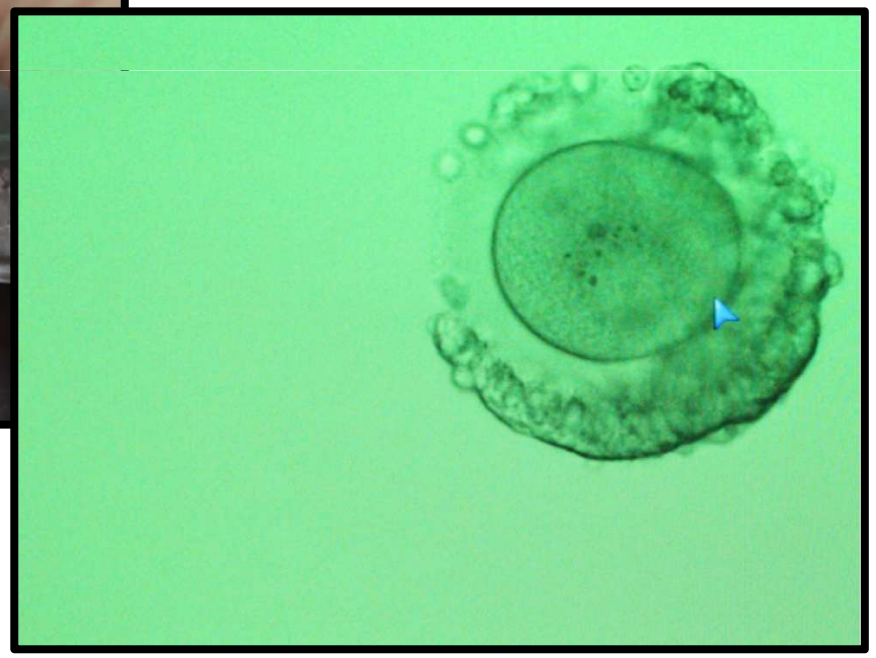
cryoprotectants : 3M and high

cooling rate : high (over 20,000°C/min)

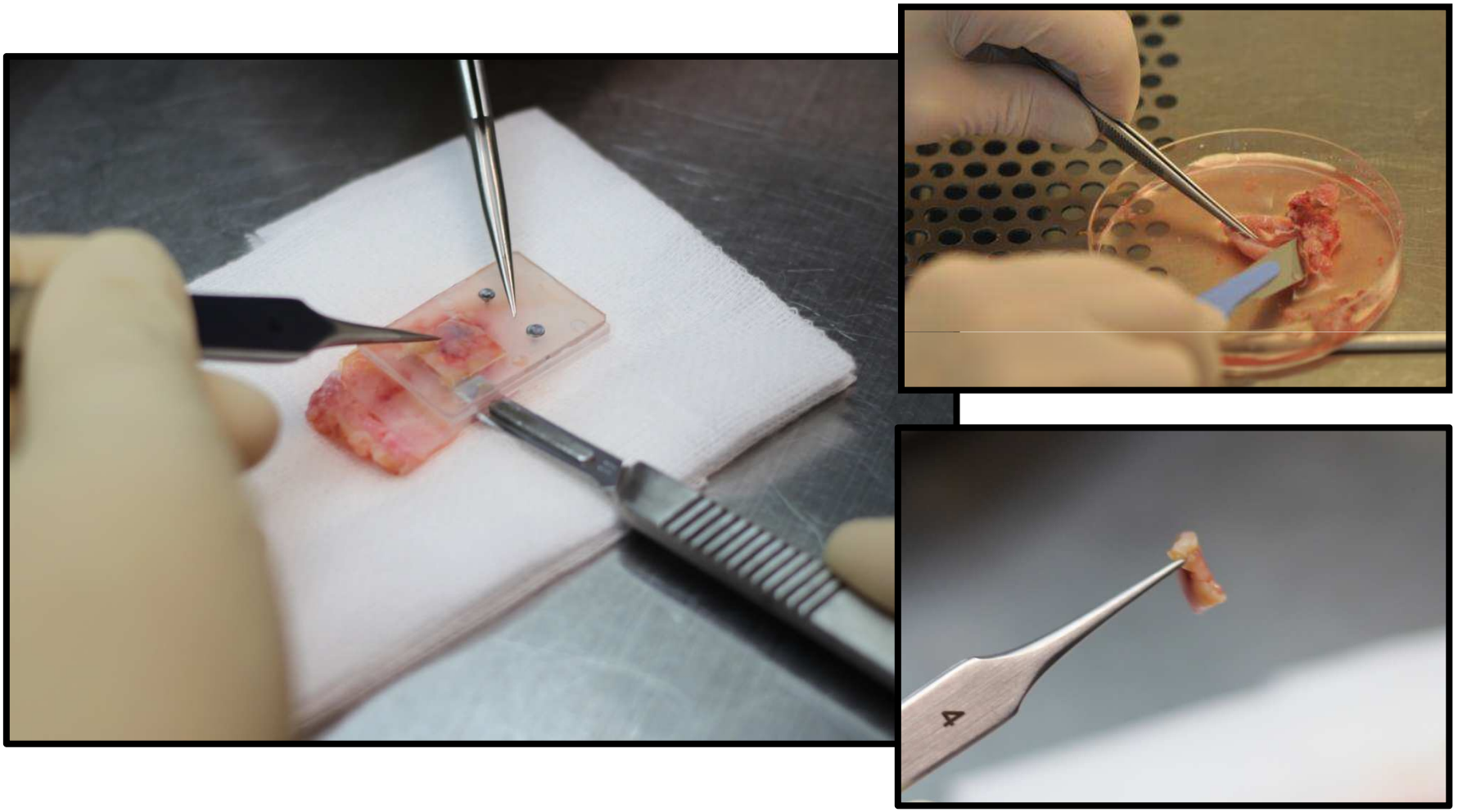
Ovarian Cortex Freezing Protocol

Reference	Slow freezing	Vitrification
Donnez et al. (2012)	1. ovarian tissue cut : 8-10 x 4-5 x 1(mm) 2. Cryoprotectant : 10% DMSO (20-30min, 4℃)	1. ovarian tissue cut : 1 x 1 x 1 (mm) 2. Cryoprotectant : 38% EG + 0.5M trehalose (3min, RT)
Silber et al. (2012)	1. ovarian tissue cut : multiple x 1 (mm) 2. Cryoprotectant 1.5M PROH + 0.1M sucrose (30min, 37℃) 1.5M PROH + 0.2M sucrose (5min)	1. ovarian tissue cut : 10 x 10 X 1(mm) 2. Cryoprotectant 7.5% EG + 7.5% DMSO (25min, RT) 20% EG + 20% DMSO (15min, RT)
SNUBH	1. ovarian tissue cut : 5 x 1-5 x 1(mm) 2. Cryoprotectant 1.5M DMSO + 0.1M sucrose (20min, RT)	1. ovarian tissue cut : 10 x 10 X 1(mm) 2. Cryoprotectant 20% EG (10min, RT) 40% EG ++ 18% Ficoll + 0.3M sucrose (5min, RT)

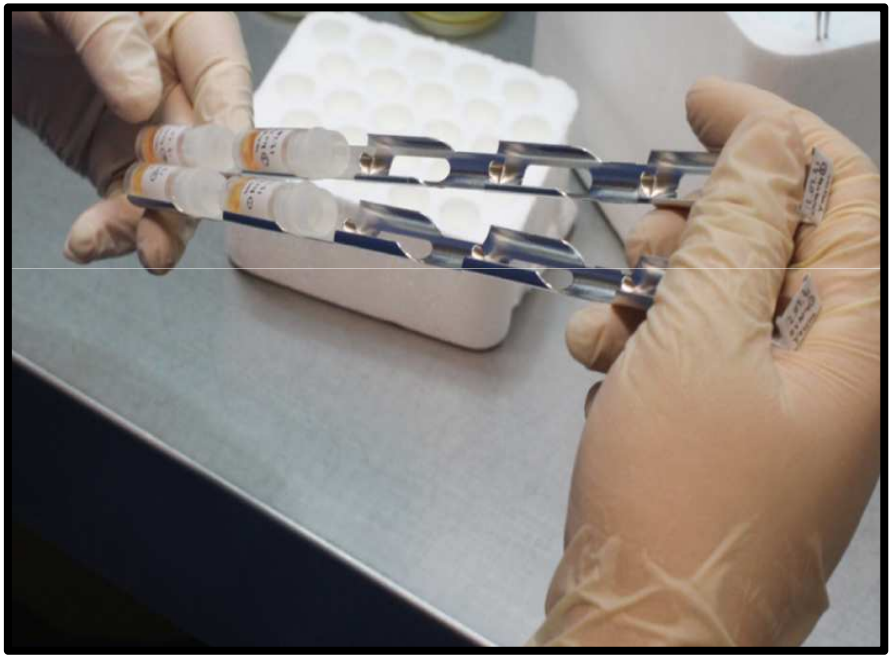
Oocyte Collection From Ovarian Cortex



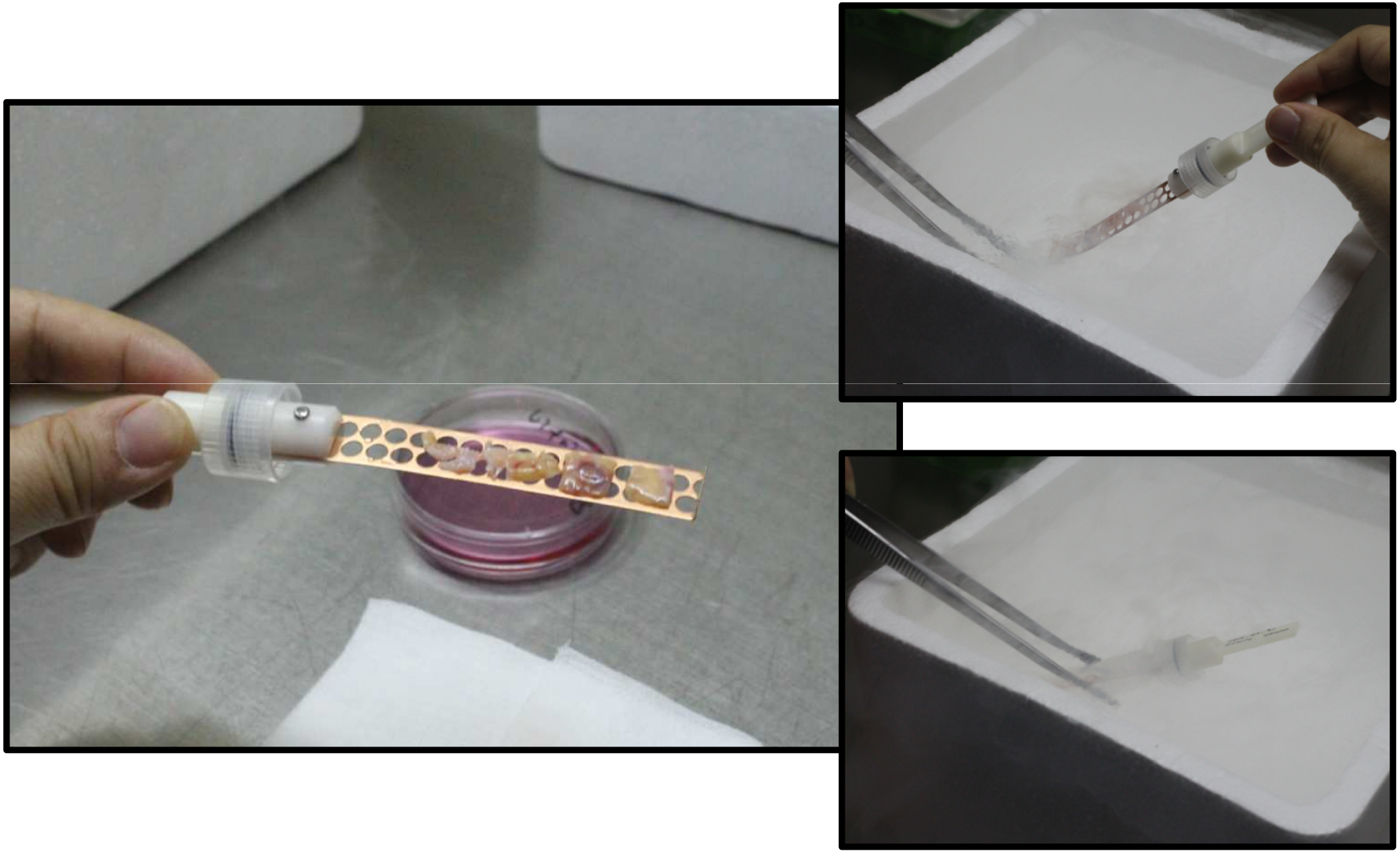
Preparation of Ovarian Tissue for Cryopreservation



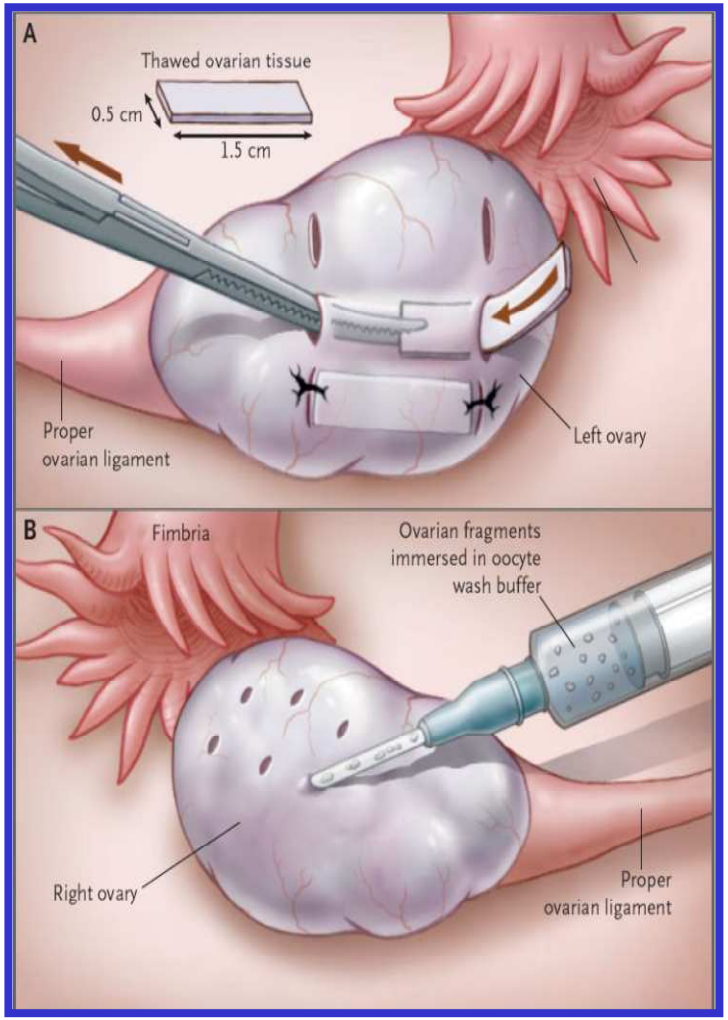
Slow Freezing of Ovarian Cortex



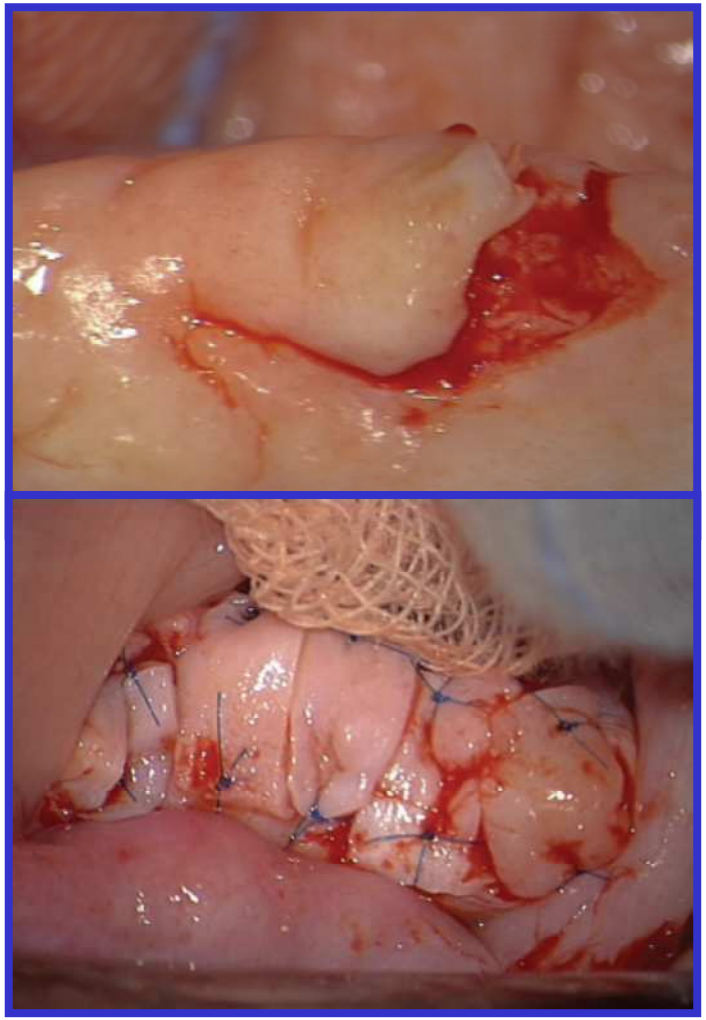
Vitrification of Ovarian Cortex



Transplantation of Human Ovarian Tissue



Meirow et al., NEJM 2005;353:318



Donnez et al., Hum Reprod Update 2006;12:519

Available Tests before Transplantation

- Before grafting schedule :
  1 piece of cryopreserved cortex
  → thawing
  → evaluation of follicular density
  normal : n > 15-20 follicles/mm^3

- **Malignant potential :** type & stage dependent
  Histology, RT-PCR,
  Long-term xenografting (6 months)
  → **So far, no relapse due to grafting out of 56 transplantations. (Dolmans, ISFP 2011)**

Ovarian Tissue Viability Test

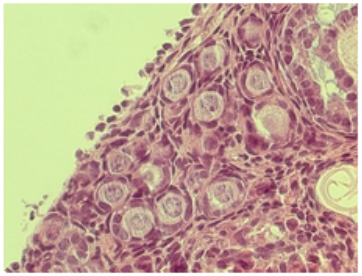
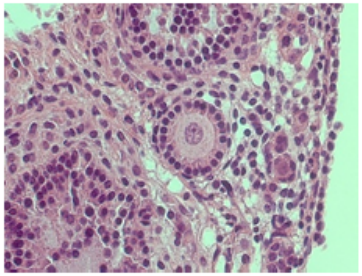
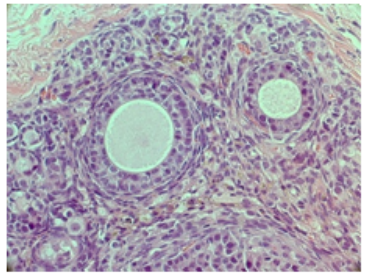
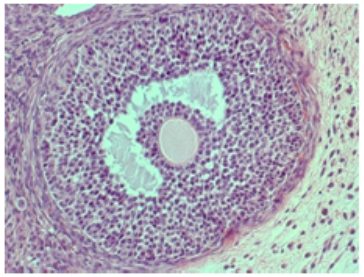
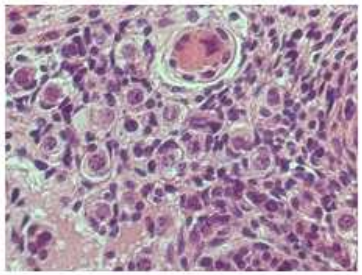
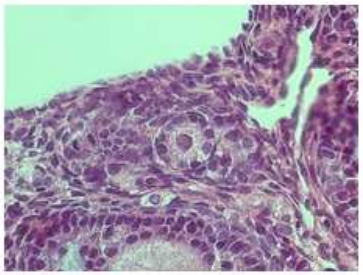
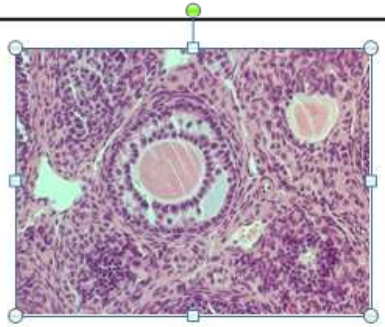
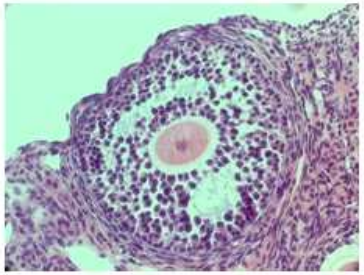
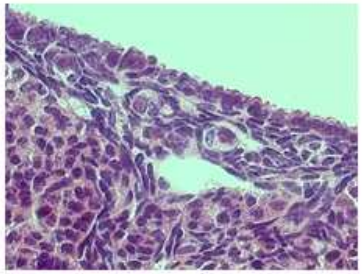
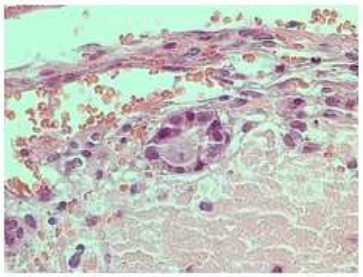
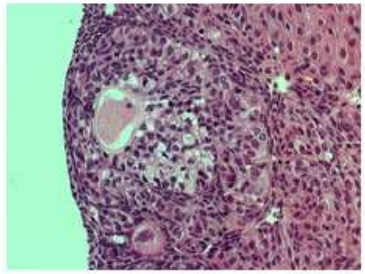
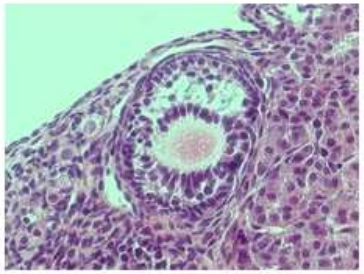
histological signs of cell death

- loss of cell membrane integrity and attachment
- cell shrinkage
- nuclear fading & nuclear shrinkage
- nuclear fragmentation

Live/Dead cytotoxicity kit assay

- **Calcein AM** : live cell, green
- **Ethidium-homodimer 1** : dead cell, red

Morphologic Classification of Mouse Ovarian Follicles (SNUBH OBGY)

	Primordial	Primary	Secondary	Antral
G1				
G2				
G3				

doi:10.1111/j.1447-0756.2010.01496.x J. Obstet. Gynaecol. Res. 2011

Optimal condition of vitrification method for cryopreservation of human ovarian cortical tissues

Hye Jin Chang[1], Jeong Hee Moon[2], Jung Ryeol Lee[2], Byung Chul Jee[2,3], Chang Suk Suh[2,3] and Seok Hyun Kim[3]

[1]Health Promotion Center, Seoul National University Bundang Hospital, [2]Department of Obstetrics and Gynecology, Seoul National University Bundang Hospital, Seongnam, and [3]Department of Obstetrics and Gynecology, College of Medicine, Seoul National University, Seoul, Korea

Results:
336 follicles were cryopreserved and thawed.
Grade 1 follicle : 3.6%, 34.7%, 13.8%, and 20.0% in the 5-min, 10-min, 20-min vitrification, and slow-freezing groups, respectively

Ration of TUNEL positive : 52.1%, 31.5%, 53.1%, and 46.7% in the 5-min, 10-min, 20-min, vitrification and slow-freezing groups, respectively

Conclusions: The 10-min exposure group for vitrification showed better results compared with other conditions and the slow-freezing group.

Duration of fertility after fresh and frozen ovary transplantation

Sherman Silber, M.D.,[a] *Nori Kagawa, Ph.D.,*[b] *Masashige Kuwayama, Ph.D.,*[b] *and Roger Gosden, Ph.D., D.Sc.*[c]

[a] Infertility Center of St. Louis, St. Luke's Hospital, St. Louis, Missouri; [b] Kato Ladies Clinic, Tokyo, Japan; and [c] Center for Reproductive Medicine and Infertility, Weill Medical College of Cornell University, New York, New York

Conclusion(s): Ovarian transplantation in humans is a robust procedure, even after cryopreservation, and vitrification might prove to be more effective than slow freezing. (Fertil Steril® 2010;94:2191–6. ©2010 by American Society for Reproductive Medicine.)

TABLE 2

Survival of small oocytes after enzymatic isolation from ovarian tissues following cryopreservation by vitrification or slow freezing.

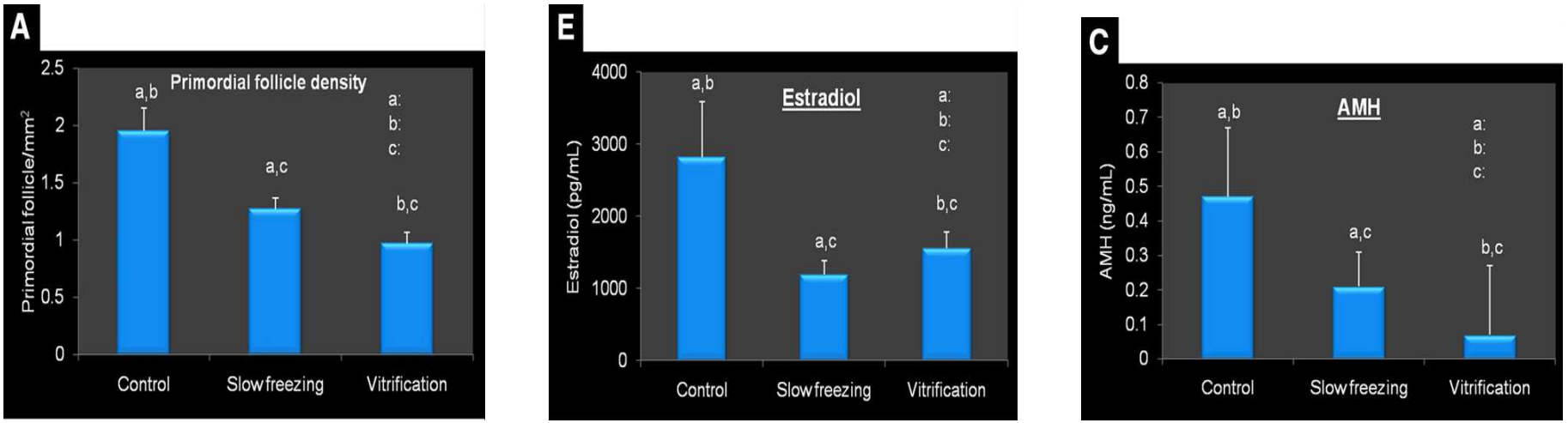
Group	No. of ovaries	No. of oocytes harvested	No. of surviving oocytes (%)
Fresh	2	358	329 (91.9%)[a]
Vitrified	8	1,122	1,000 (89.1%)[a]
Cryopreserved	6	821	342 (41.7%)[b]

[a,b]Groups with the same superscripts are not significantly different ($P>.05$), whereas those with different superscripts are significantly different ($P<.01$).

Silber. Fertility after ovary transplantation. Fertil Steril 2010.

Vitrified human ovaries have fewer primordial follicles and produce less antimüllerian hormone than slow-frozen ovaries

Slow-freezing and vitrification methods of human ovarian tissue cryopreservation were compared in terms of primordial follicle count and in vitro antimüllerian hormone (AMH) and estradiol production. Compared with fresh and slow-frozen ovaries, vitrified ovaries contained statistically significantly fewer primordial follicles and produced statistically significantly less AMH in vitro. Estradiol production from slow-frozen and vitrified ovaries was similar but statistically significantly lower than from fresh cultured strips. (Fertil Steril® 2011;95:2661–4. ©2011 by American Society for Reproductive Medicine.)



Ozgur Oktem, M.D.a, Turkey

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Effect of sphingosine-1-phosphate supplementation on follicular integrity of vitrified–warmed mouse ovarian grafts

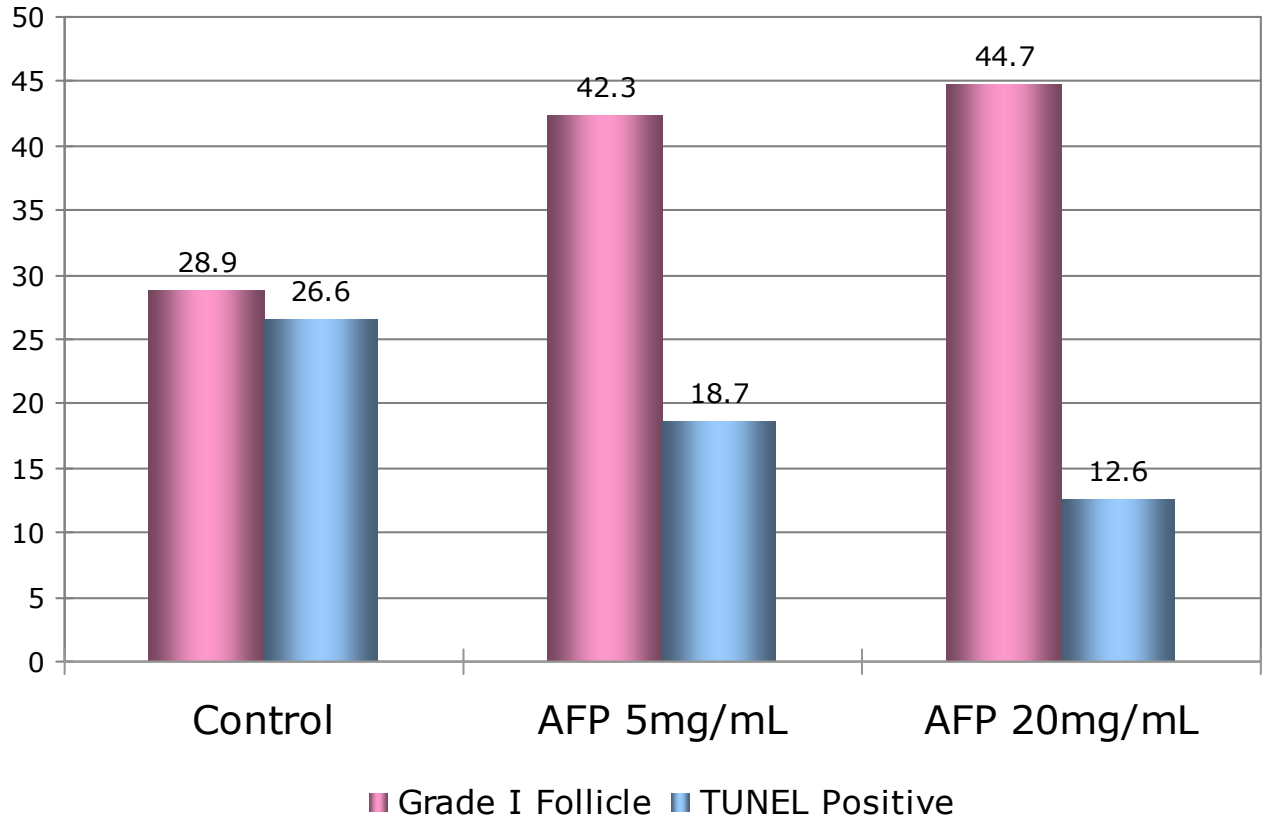
Byung Chul Jee [a,b], Jung Ryeol Lee [a], Hyewon Youm [a], Chang Suk Suh [a,b,c], Seok Hyun Kim [b,c,*], Shin Yong Moon [b,c]

[a] Department of Obstetrics and Gynecology, Seoul National University Bundang Hospital, 300 Gumi, Bundang, Seongnam, Gyeonggi, Republic of Korea
[b] Department of Obstetrics and Gynecology, Seoul National University College of Medicine, 28 Yeongeon, Chongno, Seoul, Republic of Korea
[c] Institute of Reproductive Medicine and Population, Medical Research Center, Seoul National University, 28 Yeongeon, Chongno, Seoul, Republic of Korea

Results: During vitrification and warming, inclusion of 2 μM S1P into the vitrification solution significantly raised the rate of morphologically intact follicles compared to controls (36.6% vs. 30.8%, $p = 0.047$). This protective effect was profound especially in primordial follicles (45.5% vs. 34.6%, $p = 0.034$). After transplantation of vitrified–warmed ovaries, the morphological integrity of primordial follicles was superior in the S1P-treated group (55.0% vs. 39.4%, $p = 0.035$). The rates of non-apoptotic follicles (TUNEL-negative) were similar in the two groups in either non-transplanted or transplanted ovaries.

Conclusion: Inclusion of S1P in the vitrification solution during transplantation of vitrified–warmed ovary had a beneficial effect on preservation of the primordial follicular pool.

AFP Effect on mouse ovarian follicles after vitrification and warming



unpublished

Conclusions

1. Ovarian cryopreservation is now a clinical option .

2. Transplanted frozen/thawed tissue restores ovarian function and maintain function for prolonged periods. But the efficacy is probably not high and refinement needed.

3. Results are encouraging for a continued research effort to reduce..

Cryoinjury

- Optimazation of cryopreservation method
- Supplementation of protective agents

Ischemic injuries after transplantation

- Supplementation of protective agent
- Supplementation of agent for enhancing revascularization

Thank you.