



OOSİT DONDURULMASI

Bio. Semra Sertyel

CENTRUM CLINIK
IVF LABORATUARLARI

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Oocyte cryopreservation
Ne zaman ve nasıl?

Medical Sebepler

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İşlem Günü Yeterli Sperm Bulunamaması

Ülke kanunlarının uygulanımı

Mature oocyte cryopreservation: a guideline

The Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology

Society for Reproductive Medicine and Society for Assisted Reproductive Technology, Birmingham, Alabama

There is good evidence that fertilization and pregnancy rates are similar to IVF/ICSI with fresh oocytes when vitrified/warmed oocytes are used as part of IVF/ICSI for young women. Although data are limited, no increase in chromosomal abnormalities, birth defects, and developmental deficits has been reported in the offspring born from cryopreserved oocytes when compared to pregnancies from conventional IVF/ICSI and the general population. Evidence indicates that oocyte vitrification and warming should no longer be considered experimental. This document replaces the document last published in 2008 titled, "Ovarian Tissue and Oocyte Cryopreservation," Fertil Steril 2008;90:S241-6. (Fertil Steril® 2013;99:37–43.

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COMMITTEE OPINION

Number 584 • January 2014

Committee on Gynecologic Practice

This document reflects emerging clinical and scientific advances as of the date issued and is subject to change. The information should not be construed as dictating an exclusive course of treatment or procedure to be followed.

Oocyte Cryopreservation

ABSTRACT: In 2013, the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology published a joint document, *Mature Oocyte Cryopreservation: A Guideline*, which addresses advances in techniques to freeze human eggs that have resulted in significant recent improvements in pregnancy success. Based on the current state of evidence, modern procedures to cryopreserve oocytes should no longer be considered experimental. The American College of Obstetricians and Gynecologists' Committee on Gynecologic Practice endorses the joint document and encourages its use by Fellows. There are not yet sufficient data to recommend oocyte cryopreservation for the sole purpose of circumventing reproductive aging in healthy women.

Oosit kalitesi ve maturasyonunun etkisi

IVM sonrası elde edilmiş MII oositlerin vitrifikasyonu sonrası %25 oranında Devam etme oranı gözlenmiştir (Chian et al.,2009).

Euploid MII oositlerden gelişim ve devam etme oranı (%96) , blastosist gelişim Oranı (%65) gözlenmiştir (Sher et al., 2008)

MII Oocyte Cryopreservation : ASRM Guideline

TABLE 1

Summary of randomized controlled trials comparing fresh versus vitrified oocytes.

	Cobo 2008 (24)	Cobo 2010 (26)	Rienzi 2010 (25)	Parmegiani 2011 (19)
Patient population	Oocyte donors	Oocyte donors	Infertile patients <43 years of age requiring ICSI with >6 mature oocytes	Infertile patients <42 years of age requiring ICSI with >5 mature oocytes
No. patients	30 vitrification 30 fresh	295 vitrification 289 fresh	40 vitrification 40 fresh	31 vitrification 31 fresh
Mean age at retrieval	26	26	35	35
No. oocytes	231 vitrification 219 fresh	3286 vitrification 3185 fresh	124 vitrification 120 fresh	168 vitrification NA fresh
No. oocytes per retrieval	18.2	11	13	NA
Survival	96.9%	92.5%	96.8%	89.9%
Fertilization rate	76.3 vitrification 82.2 fresh	74% vitrification 73% fresh	79.2% vitrification 83.3% fresh	71% vitrification 72.6% fresh
No. transferred vitrification vs. fresh	3.8 vitrification 3.9 fresh	1.7 vitrification 1.7 fresh	2.3 vitrification 2.5 fresh	2.5 vitrification 2.6 fresh
Day of transfer	3	3	2	2–3
Implantation rate	40.8% vitrification 100% fresh	39.9% vitrification 40.9% fresh	20.4% vitrification 21.7% fresh	17.1% vitrification NA fresh
CPR/transfer vitrification vs. fresh	60.8% (23 vitrification transfers) 100% (1 fresh transfer)	55.4% vitrification 55.6% fresh	38.5% vitrification 43.5% fresh	35.5% vitrification 13.3% fresh
CPR/oocyte thawed	6.1%	4.5%	12%	6.5%

Note: All used vitrification with Cryotop, 15% EG + 15% DMSO + 0.5M sucrose. CPR = clinical pregnancy rate.

Practice Committee. Oocyte cryopreservation. Fertil Steril 2012.

ASRM F&S 2013

Clinical Application of Oocyte Vitrification: Review & Meta-analysis

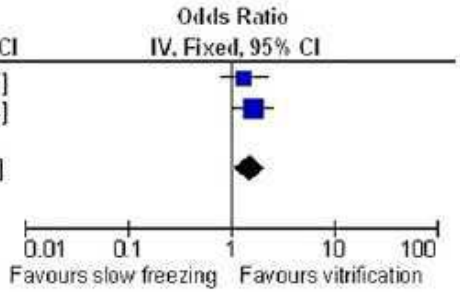
FIGURE 3

Odds ratio of fertilization rate. (**A**) Vitrification versus SF. (**B**) Vitrification versus fresh oocytes. Fixed effects-model.

A Vitrification vs. Slow freezing. Fixed effects model

Study or Subgroup	Vitrification Events	Vitrification Total	Slow Freezing Events	Slow Freezing Total	Weight	Odds Ratio IV, Fixed, 95% CI
Cao 2009	182	268	46	75	41.0%	1.33 [0.78, 2.27]
Smith 2010	200	260	104	155	59.0%	1.63 [1.05, 2.54]
Total (95% CI)		528		230	100.0%	**1.50 [1.07, 2.11]**
Total events	382		150			

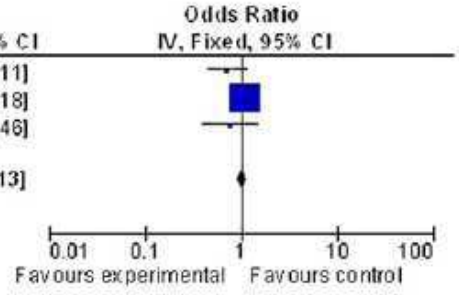
Heterogeneity: $Chi^2 = 0.33$, $df = 1$; $I^2 = 0\%$
Test for overall effect: $Z = 2.36$



B Vitrification vs. Fresh oocytes. Fixed effects model

Study or Subgroup	Vitrification Events	Vitrification Total	Fresh Events	Fresh Total	Weight	Odds Ratio IV, Fixed, 95% CI
Cobo 2008	171	224	180	219	5.5%	0.70 [0.44, 1.11]
Cobo 2010	2256	3039	2334	3185	91.8%	1.05 [0.94, 1.18]
Rienzi 2010	95	120	100	120	2.8%	0.76 [0.40, 1.46]
Total (95% CI)		3383		3524	100.0%	**1.02 [0.91, 1.13]**
Total events	2522		2614			

Heterogeneity: $Chi^2 = 3.60$, $df = 2$; $I^2 = 44\%$
Test for overall effect: $Z = 0.33$



Cobo. Meta-analysis of the use of oocyte vitrification. Fertil Steril 2011.

Cobo &Diaz F&S 2011

Clinical application of oocyte vitrification: a systematic review and meta-analysis of randomized controlled trials.

Cobo A[1], Diaz C.

INTERVENTION(S):
Vitrification of human oocytes vs. slow freezing or fresh oocytes.
MAIN OUTCOME MEASURE(S):
Ongoing pregnancy rate; secondary outcomes were clinical pregnancy rate, implantation rate, embryo development, fertilization rate, and oocyte survival.
RESULT(S):
Five eligible studies were finally included. They involved 4,282 vitrified oocytes, 3,524 fresh oocytes, and 361 slow-frozen oocytes between 2005 and 2009. The rates of ongoing pregnancy, top-quality embryo, embryo cleavage, and fertilization did not differ between the vitrification and the fresh oocyte groups. The oocyte survival rate was higher in vitrified vs. slow-frozen oocytes (odds ratio [OR] 2.46, 95% confidence interval [CI] 1.82-3.32), although heterogeneity between studies was observed. The fertilization rate was higher in vitrified vs. slow-frozen oocytes (OR 1.50, 95% CI 1.07-2.11). Vitrification also resulted in a higher rate top-quality embryo (22.4% vs. 8.0%, OR 3.32, 95% CI 1.37-8.02) and embryo cleavage rate (day 2: 64.6% vs. 47.7%, OR 2.00, 95% CI 1.33-3.00; day 3: 53.0% vs. 33.3%, OR 2.25, 95% CI 1.32-3.85) as compared with slow freezing.
CONCLUSION(S):
Vitrification is an efficient method to preserve oocytes, although more large controlled clinical trials are needed to strengthen this conclusion.

Kontrollü Dondurma ve/veya vitrifikasyon

	Vitrifikasyon	Yavaş Dondurma
Sıvı penetrasyonunun kontrolü	Evet	Hayır
Dehidrasyon oranının kontrolü	Evet	Hayır
İnkübatör dışındaki süre	10 dakika	3 saat
Uzatılmış ısı şoku	Hayır	Evet
Zona Pellucida parçalanması	Hayır	Mümkün
Buz kristalleri oluşumu	Hayır	Mümkün
Malzeme ve masraflar	Ucuz	Pahalı
Cryotolarance	Yüksek	Düşük
Cryotoksisiti	Düşük	Yüksek

Kuleshova ve Lopata 2002, Fertility&Sterility

Kontrollü Dondurma ve/veya vitrifikasyon

Table 1: Results from different oocyte cryopreservation protocols: slow freezing (high-sucrose concentration) and vitrification.

	Vitrification (VIT)	Slow freezing (SF)	Survival	Fertilization	Pregnancy	Miscarriage	Egg donation program
Chen et al., 2005 [44]	—	Yes	75% (119)	67% (80)	33% (7)	0%	Partially
Li et al., 2005 [45]	—	Yes	90% (73/81)	82% (60/73)	47% (7/15)	28% (2/7)	Partially
Kuwayama et al., 2005 [46]	Yes	—	91% (58/64)	81% (52/64)	41% (12/29)	17% (2/12)	No
Borini et al., 2006 [47]	—	Yes	43,4% (306/705)	51,6% (158/306)	19,2% (14/73)	28,6% (4/14)	No
Barritt et al., 2007 [48]	—	Yes	86,1% (68/79)	89,7% (61/68)	75% (3/4)	NS	Yes
Lucena et al., 2006 [49]	Yes	—	96,7% (143)	87,2% (105)	56,5% (13)	NS	Yes
Antinori et al., 2007 [50]	Yes	—	99,4% (328/330)	92,9% (305/328)	32,5% (39/120)	20,5% (8/39)	No
Cobo et al., 2008 [51]	Yes	—	96,9% (224/231)	76,3% (171/224)	65,2% (15/23)	20% (3/15)	Yes
Parmegiani et al., 2008 [52]	—	Yes	75,1% (328/437)	80% (227/328)	19% (16/83)	31,2% (5/16)	No
Cao et al., 2009 [42]	Yes	Yes	SF 61% (75/123)	SF 61,3% (46/75)	ND	ND	No
			VIT 91,8% (268/292)	VIT 67,9% (182/268)	ND	ND	No
Smith et al., 2010 [53]	Yes	Yes	SF 65% (155/238)	SF 67% (104/155)	SF 13% (4/30)	SF 25% (1/4)	No
			VIT 75% (260/349)	VIT 77% (200/260)	VIT 38% (18/48)	VIT 18% (4/18)	No
Rienzi et al., 2010 [54]	Yes	—	97% (120/124)	79,2% (95/120)	30,8% (15/39)	20% (3/15)	No
Cobo et al., 2010 [55]	Yes	—	92,5% (3039)	73,3% (NS)	55,4% (148)	NS	Yes

NS = Data not reported.
ND = Data not calculated, not a study endpoint.

Revelli et al., Obst. &Gyn. Int 2012

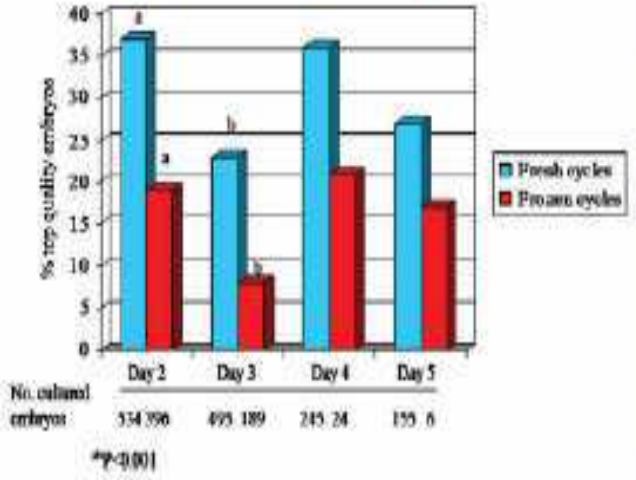
Kontrollü Dondurma

Impact of oocyte cryopreservation on embryo development

M. Cristina Magli, M.Sc., Michela Lappi, B.Sc., Anna P. Ferraretti, M.D., Alessandra Capoti, B.Sc., Alessandra Ruberti, B.Sc., and Luca Gianaroli, M.D.

Reproductive Medicine Unit, Società Italiana Studi Medicina della Riproduzione, Bologna, Italy

Top-quality embryo development after culture to day 2, day 3, day 4, and day 5. Values with same superscripts are significantly different.



Magli. Embryo development from thawed oocytes. Fertil Steril 2009.

Fertilization and embryo development in fresh and frozen cycles according to maternal age.

	≤35 y		≥36 y	
	Fresh cycles	Frozen cycles	Fresh cycles	Frozen cycles
No. cycles	117	120	117	136
Age (mean ± SD) (y)	31.9 ± 4.6	31.7 ± 4.1	38.5 ± 4.6	38.5 ± 4.2
No. inseminated oocytes	351	303	351	316
No. fertilized oocytes (%)	287 (82)[a]	219 (72)[a]	298 (85)[b]	231 (73)[b]
No. zygotes with the configurations A1α, A2α, A1β, and A2β (%)	164 (57)[c]	101 (46)[c]	190 (64)[d]	124 (54)[d]
No. embryos (%)	259 (90)	189 (86)	275 (92)	207 (90)
Day 2—top-quality embryos (%)	91 (35)[e]	33 (17)[e]	106 (39)[f]	41 (20)[f]
Day 3—top-quality embryos (%)	58/255 (23)[g]	10/102 (10)[g]	57/240 (24)[h]	5/87 (6)[h]
Day 4—top-quality embryos (%)	43/128 (34)	4/16 (25)	44/117 (38)	1/8 (13)
Day 5—top-quality embryos (%)	22/78 (28)	1/4 (25)	25/77 (32)	0/2
No. transferred cycles (%)	108 (92)[i]	93 (78)[j]	110 (94)[i]	110 (81)[j]

Evidence-based clinical outcome of oocyte slow cooling

Since 1992, Dr Borini has been Clinical Director of the Assisted Reproduction Centre 'Tecnobios Procreazione', Bologna, Italy, and of a number of associated IVF clinics. After graduating in Medicine and Surgery at the University of Bologna, Bologna, Italy, in 1986, he completed his residency in Obstetrics and Gynaecology at the University of Bologna in 1991. He then attended the University of California Irvine, Irvine, California, as Research Fellow from October 1989 to May 1991. His research areas are embryo implantation, oocyte freezing and multiple induction of ovulation. Dr Borini has published more than 160 research papers and acts as Chairman of CECOS ITALY.

Table 1. Cumulative pregnancy outcome from transfers with fresh and frozen–thawed embryos.

No. of patients	749
No. of transfers with fresh or frozen–thawed embryos	702
No. of embryos transferred (mean ± SD)	1679 (2.2 ± 0.9)
No. of pregnancies	267
Pregnancy rate (%) per embryo transfer	38.0 (267/702)
Pregnancy rate (%) per patient	35.6 (267/749)
Multiple pregnancy rate (%)	31.1 (83/267)
Gestational sacs	366
Implantation rate (%)	21.8 (366/1679)
Abortions (*n*)	37
Abortion rate (%)	13.9

Table 2. Pregnancy outcome using frozen–thawed oocytes.

No. patients with at least one thawing cycle	510
No. of thawing cycles	660
No. of oocytes	
Frozen	5448
Thawed	3238
Survived (%)	2205 (68.1)
Microinjected	1815
Two-pronucleate (%)	1381 (76.1)
No. of embryos cleaved (%)	1268 (91.8)
No. of embryos transferred (%)	1268 (100)
No. of transfers	590
No. of pregnancies	88
Gestational sacs	103
Implantation rate (%)	8.1
Pregnancy rate (%) per patient	17.2 (88/510)
Pregnancy rate (%) per transfer	14.9 (88/590)
Abortions (*n*)	19
Abortion rate (%)	21.6

Table 3. Cumulative pregnancy rate derived from fresh and frozen–thawed oocytes.

No. of patients	749
No. of pregnancies	355
No. of gestational sacs	458
Cumulative pregnancy rate (%) per transfer	47.4 (355/749)
Cumulative implantation rate (%)	15.5 (458/2947)

Taze ve Dondurulup Çözülüp Oositlerin Laboratuvar Sonuçları

human reproduction

ORIGINAL ARTICLE *Embryology*

Embryo development of fresh 'versus' vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study

Laura Rienzi[1], Stefania Romano, Laura Albricci, Roberta Maggiulli, Antonio Capalbo, Elena Baroni, Silvia Colamaria, Fabio Sapienza, and Filippo Ubaldi

G.E.N.E.R.A. Centre for Reproductive Medicine, Clinica Valle Giulia, via de Notaris 2B, Rome, Italy

[1]Correspondence address. E-mail: rienzi@generanet.it

Vitrifikasyon Sonrası Devam Eden Gebelik Oranları

Human Reproduction, Vol.00, No.0 pp. 1–7, 2010
doi:10.1093/humrep/deq046

human reproduction

ORIGINAL ARTICLE *Infertility*

Cumulative ongoing pregnancy rate achieved with oocyte vitrification and cleavage stage transfer without embryo selection in a standard infertility program

Filippo Ubaldi [1], Reno Anniballo [2], Stefania Romano [1], Elena Baroni [1], Laura Albricci [1], Silvia Colamaria [1], Antonio Capalbo [1], Fabio Sapienza [1], Gábor Vajta [3], and Laura Rienzi [1,*]

[1]G.EN.E.RA Centre for Reproductive Medicine, Clirica Valle Giulia, Via G. De Notaris 2, 00197 Rome, Italy [2]Andrology Center 'John McLeod', via F. Petrarca, Naples, Italy [3]James Cook University, Cairns Campus, Cairns, QLD, Australia

Laboratuvar Sonuçları

Survival rate 96.7%

Table III Primary and secondary outcomes measures: fertilization, pronuclear morphology, embryo development and embryo morphology of fresh and vitrified sibling oocytes

	Fresh ICSI	Vitrified/Warmed ICSI (%)	Absolute difference (%) (95% CI)	OR (95% CI)	P
Fertilization (2PN) per sibling oocyte	100/120 (83.3)[b]	95/124 (76.6)[a]	−6.73 (−16.6 to 3.39)	0.65 (0.33 to 1.29)	0.20
Fertilization (2PN) per injected oocyte	100/120 (83.3)[b]	95/120 (79.2)[b]	−4.17 (−14.0 to 5.7)	0.76 (0.37 to 1.53)	0.50
Normal 2PN morphology	96/100 (96.0)[c]	86/95 (90.5)[c]	−5.47 (−13.4 to 1.84)	0.39 (0.08 to 1.49)	0.16
1PN oocytes	3/120 (2.5)[b]	6/120 (5.0)[b]	2.5 (−2.82 to 8.22)	2.05 (0.42 to 12.9)	0.50
3PN	1/120 (0.83)[b]	2/120 (1.66)[b]	0.83 (−3.09 to 5.1)	2.01 (0.10 to 119.9)	1
Degenerated oocytes post-ICSI	1/120 (0.83)[b]	4/120 (3.34)[b]	2.51 (−1.75 to 7.47)	4.08 (0.39 to 203.5)	0.37
Day 2 embryo development	100/100 (100)[c]	93/95 (97.9)[c]	−2.11 (−7.3 to 1.9)	0.0 (0.00 to 0.23)	0.24
Excellent quality embryos	52/100 (52.0)[d]	49/95 (51.6)[d]	−0.43 (−14.2 to 13.3)	0.98 (0.53 to 1.79)	0.90
Good quality embryos	38/100 (38.0)[d]	41/95 (43.2)[d]	5.16 (−8.49 to 18.6)	1.24 (0.67 to 2.28)	0.47
Fair/poor quality embryos	10/100 (10.0)[d]	3/95 (3.16)[d]	−6.84 (−14.6 to 0.42)	0.29 (0.05 to 1.19)	0.10

[a]Percentages, expressed per warmed oocyte.
[b]Percentages, expressed per inseminated oocyte.
[c]Percentages, expressed per 2PN fertilized oocyte.
[d]Percentages, expressed per cleaved oocyte.

Rienzi et al., Human Reproduction 2010

Clinical results

Table II Clinical outcomes of fresh, and warming cycles according to female age.

	Overall	≤34 years	35–37 years	38–40 years	41–43 years
Fresh cycles: clinical outcomes					
No. of cycles	182	72	48	41	21
No. of ET	172/182 (94.5)	66/72 (91.6)	46/48 (95.8)	40/41 (97.6)	20/21 (95.2)
Clinical pregnancy rate per cycle	77/182 (42.3)[a]	32/72 (44.4)	22/48 (45.8)	18/41 (43.9)	5/21 (23.8)
Clinical pregnancy rate per ET	77/172 (44.8)[b]	32/66 (48.5)	22/46 (47.8)	18/40 (45.0)	5/20 (25.0)
Implantation rate	101/435 (23.2)[c]	46/153 (30.0)	29/116 (25.0)[f]	21/112 (18.7)	5/54 (9.2)
Abortion rate	9/77 (11.7)	3/32 (9.4)	2/22 (9.0)	3/18 (16.7)	1/5 (20.0)
Ongoing pregnancy rate per fresh cycle	68/182 (37.4)[d]	29/72 (40.3)	20/48 (41.7)[g]	15/41 (36.6)	4/21 (19.0)
Ongoing implantation rate	90/435 (20.7)[e]	42/153 (27.4)	26/116 (22.4)[h]	18/112 (16.1)	4/54 (7.4)
Warmed cycles: clinical outcomes					
No. of cycles	115	37	30	30	18
No. of ET	111 (96.5)	35/37 (94.6)	29/30 (96.7)	30/30 (100)	17/18 (94.4)
Clinical pregnancy rate per cycle	35/115 (30.4)[a]	17/37 (45.9)	7/30 (23.3)	7/30 (23.3)	4/18 (22.2)
Clinical pregnancy rate per ET	35/111 (31.5)[b]	17/35 (48.6)	7/29 (24.1)	7/30 (23.3)	4/18 (22.2)
Implantation rate	43/266 (16.1)[c]	21/77 (27.3)	8/73 (10.9)[f]	9/75 (12.0)	5/41 (12.2)
Abortion rate	6/35 (17.1)	1/17 (5.9)	3/7 (42.8)	1/7 (14.3)	1/4 (25.0)
Ongoing pregnancy rate per warmed cycle	29/115 (25.2)[d]	16/37 (43.2)	4/30 (13.3)[g]	6/30 (20.0)	3/18 (16.6)
Ongoing implantation rate	35/266 (13.2)[e]	19/77 (24.7)	5/73 (6.8)[h]	8/75 (10.7)	3/41 (7.3)

Data are expressed as absolute and percentage frequency. ET, embryo transfer.
[a,b,c,d,e,f,g,h]$P < 0.05$.

Ubaldi et al., Human Reproduction 2010

Obstetric outcomes

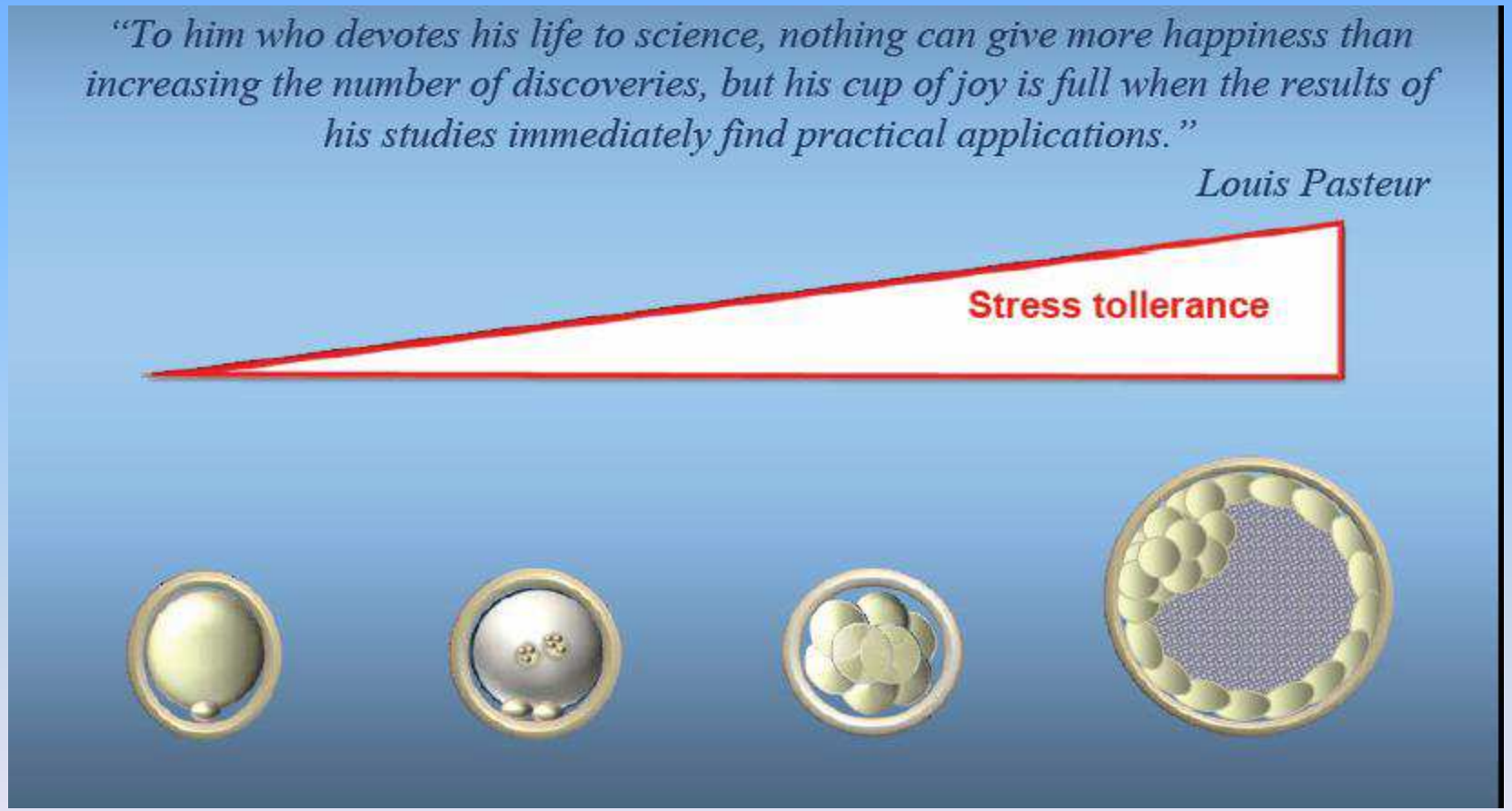
Chian RC, Huang JY, Tan SL, Lucena E, Saa A, Rojas A, Castellón LA, García Amador MI, Montoya Sarmiento JE.

Obstetric and perinatal outcome in 200 infants conceived from vitrified oocytes. *Reprod Biomed online 2008*

Noyes N, Porcu E, Borini A.

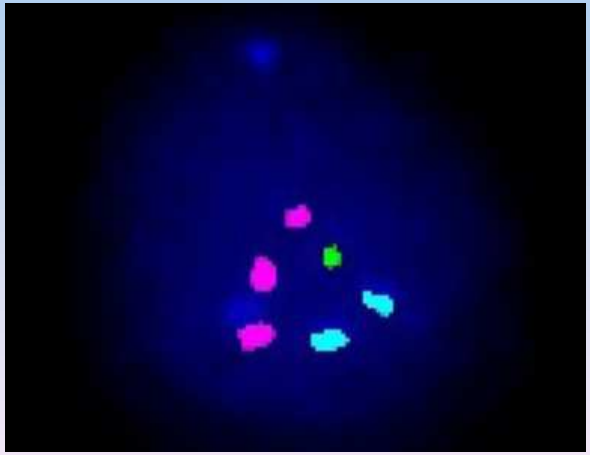
Over 900 oocyte cryopreservation babies born with no apparent increase in congenital anomalies. Reprod Biomed online 2009

Oosit fizyolojisi ve moleküler Göstergelerin Analizi

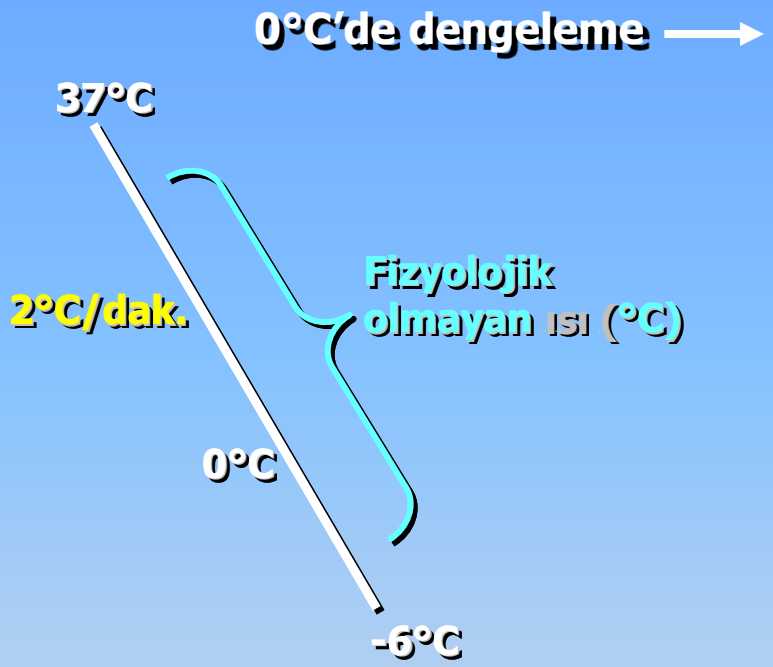


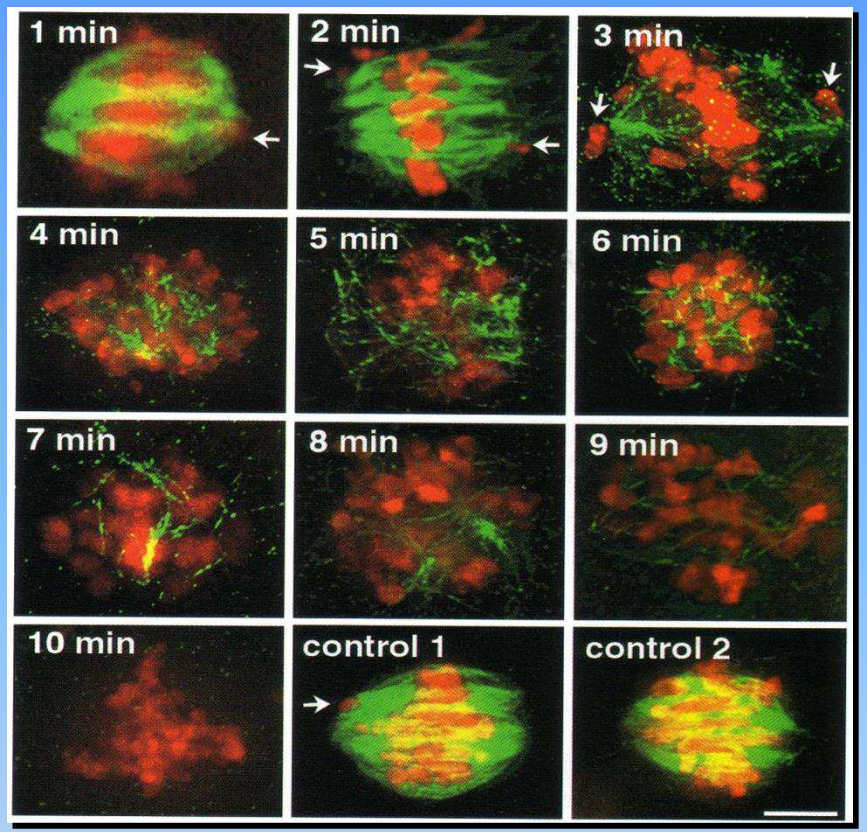
MII oositlerin kriyoprezervasyonunda başarısızlığa neden olan faktörler

- Oosit kalitesi (membran geçirgenliği !!!)
- Klasik IVF sonrası düşük fertilizasyon
- Düşük canlılık oranı (%25-40)
- Yüksek anöploidi
- Embriyoların gelişim özellikleri



Soğuma hasarı





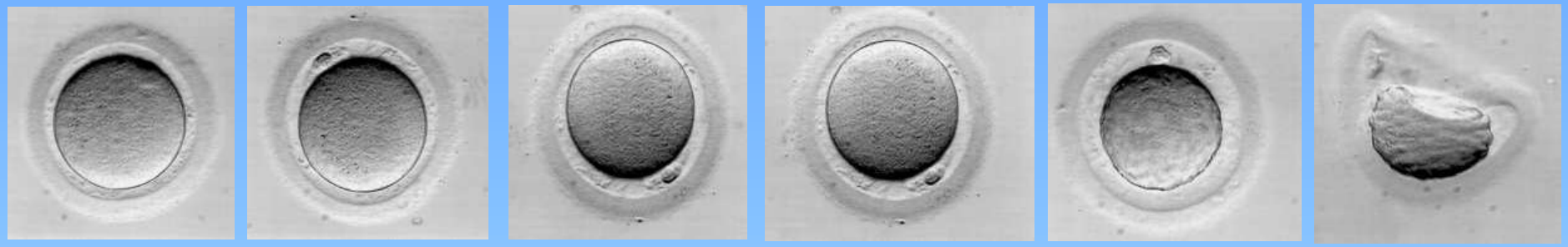
Zenzes FS 2001

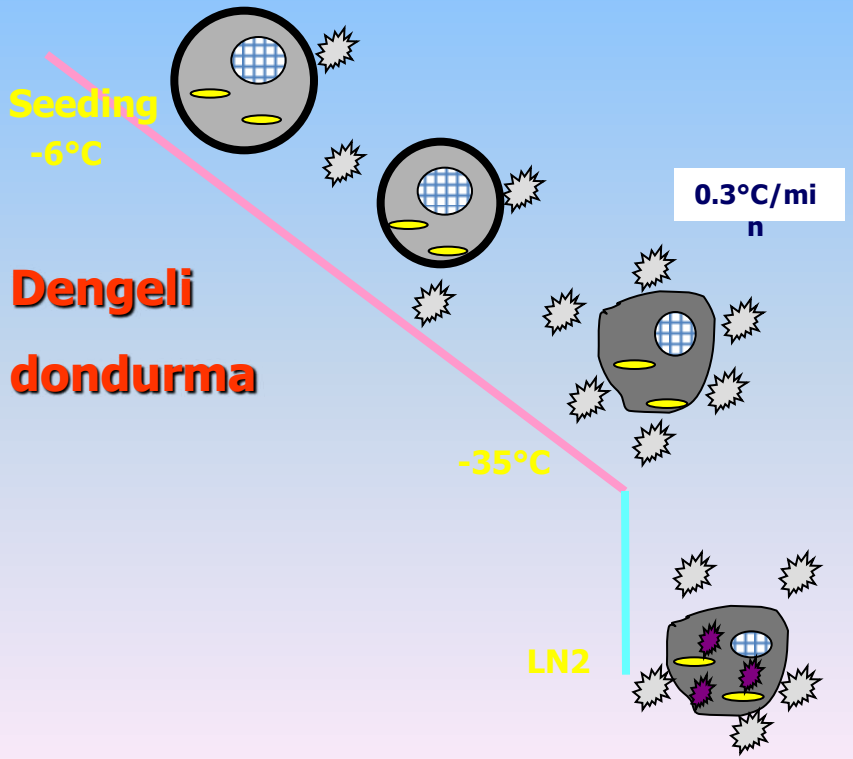
- Tubulin depolimerizasyonunun kaybolması sonucu kromozomların ayrılma hataları (repolimerizasyon anomalisi). **Sonuç:** Anöploidi artışı

- Sitoplazma yapısı, organeller ve moleküler hareketlerde meydana gelen değişiklikler. **Sonuç:** Mayoz, fertilizasyon ve polarizasyon anomalisi

Oosit Kriyoprezervasyonu

Slow Freezing





Oosit fizyolojisi ve moleküler Göstergelerin Analizi

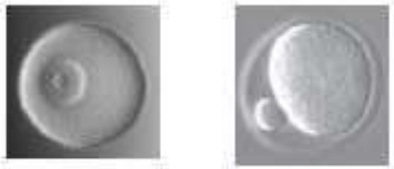
Oosit fizyolojisi ve moleküler Göstergelerin Analizi

60-65% 35-40% 75-80%

Survival rates of human oocytes frozen with the same slow freezing protocol

(Lassalle et al., 1985)

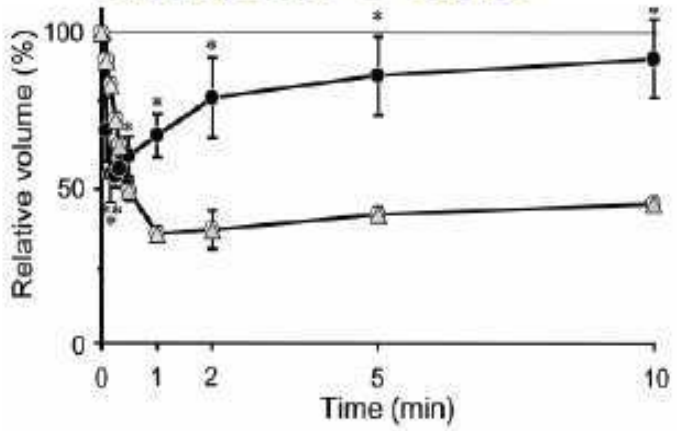
Aquaporin-9, a protein channel that can transport water and other solutes through the plasmalemma is expressed in rat GV-stage **but not** mature oocytes (Ford et al., 2000)



\+ + Permeability

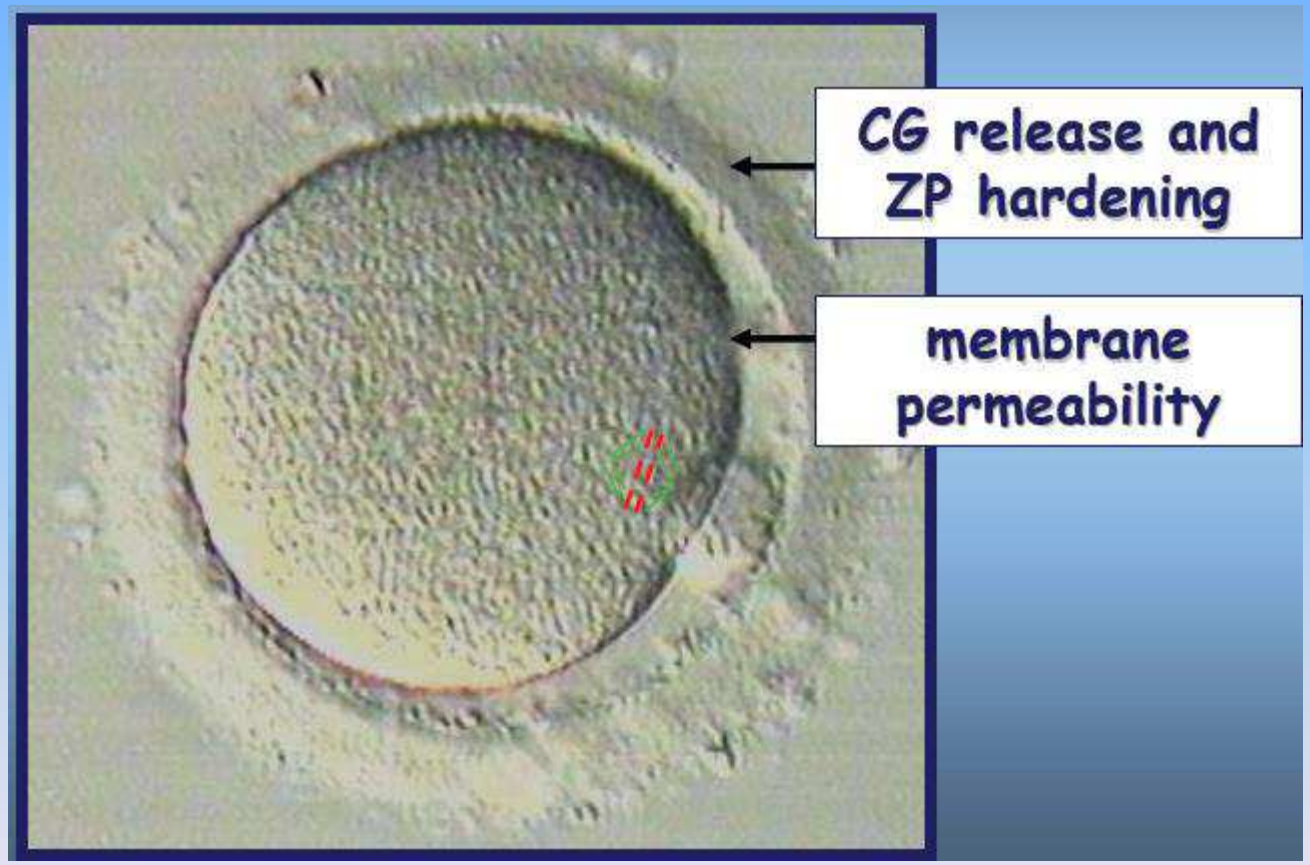
\+ - Aquaporin-9 expression

Osmotic response to glycerol of mouse oocytes injected with Aquaporin-3 cRNA



Edashige et al., 2003

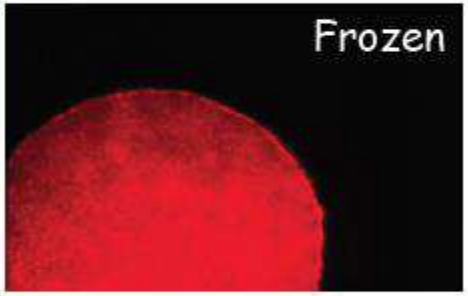
Oosit Fizyolojisinin ve moleküler göstergelerin analizi

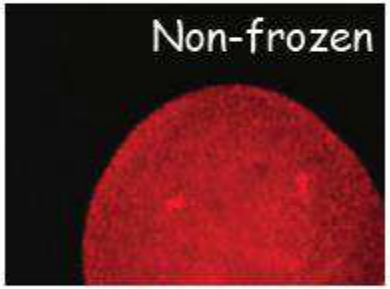


Cortical Granül Salınımı

No evidence of cortical granule discharge in cryopreserved oocytes

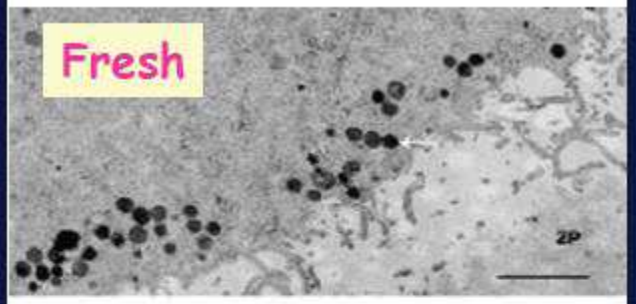
Gook et al., 1993

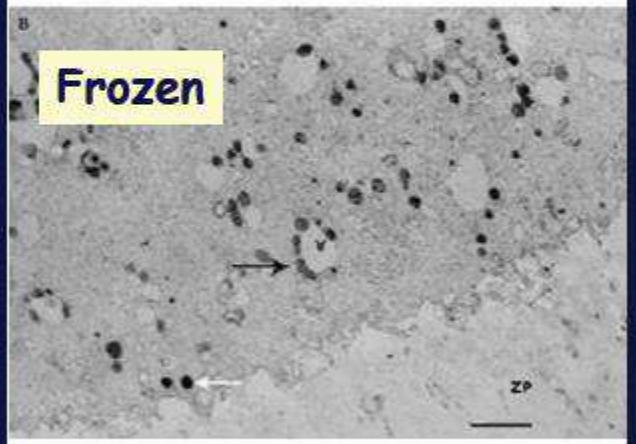




"The immunostaining examination for CG of the frozen–thawed oocytes did not reveal evidence of the premature release of CG."

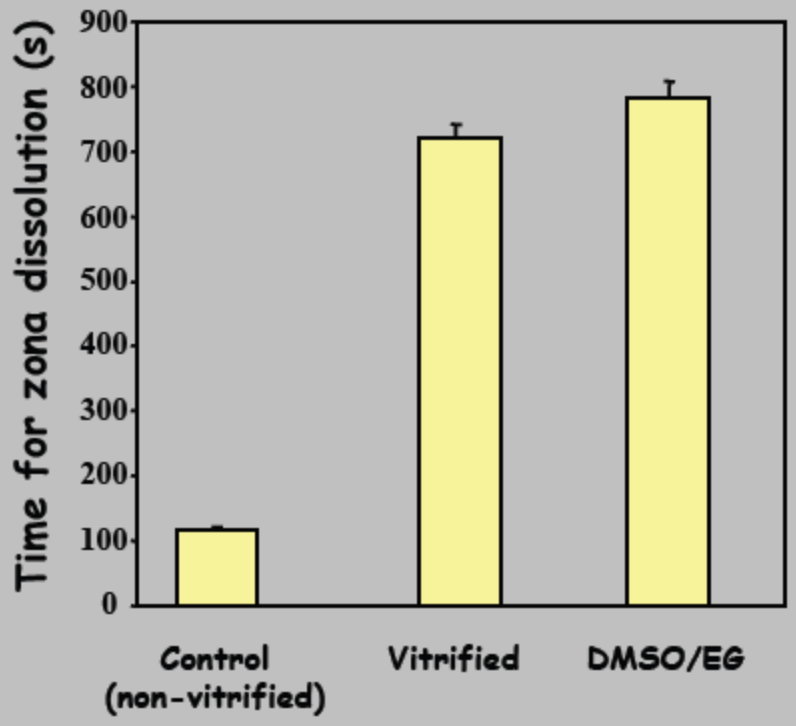
Li et al., 2005





Ghetler et al., 2006

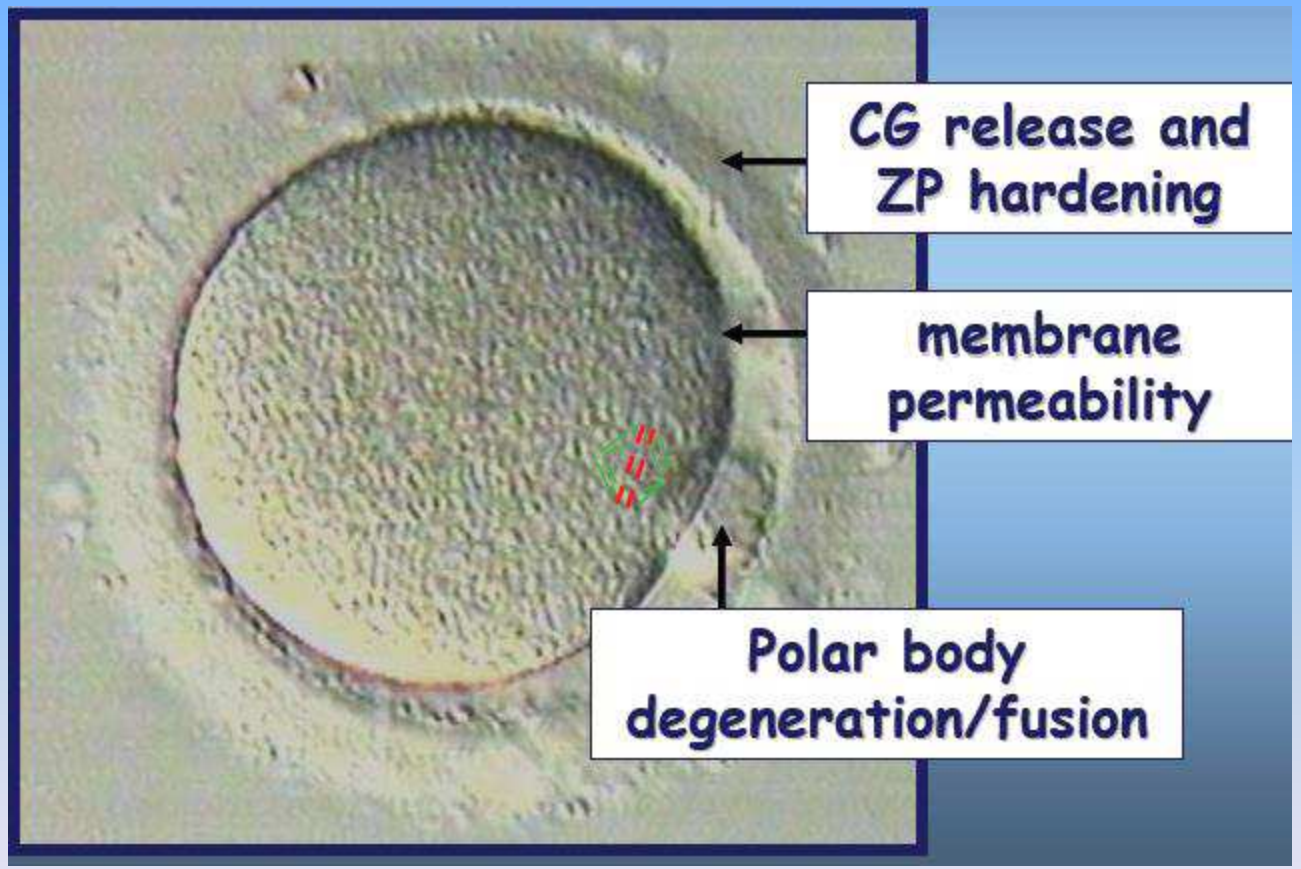
Zona Pellucida Kalınlaşması



Larman et al., 2006

n = greater than 60 oocytes per treatment with 3 replicates

Oosit fizyolojisi ve moleküler Göstergelerin Analizi



Aneuploidy ve Polar Body Tutulması

Early reports on failure of PBII extrusion and increase of aneuploidy in thawed mouse oocytes

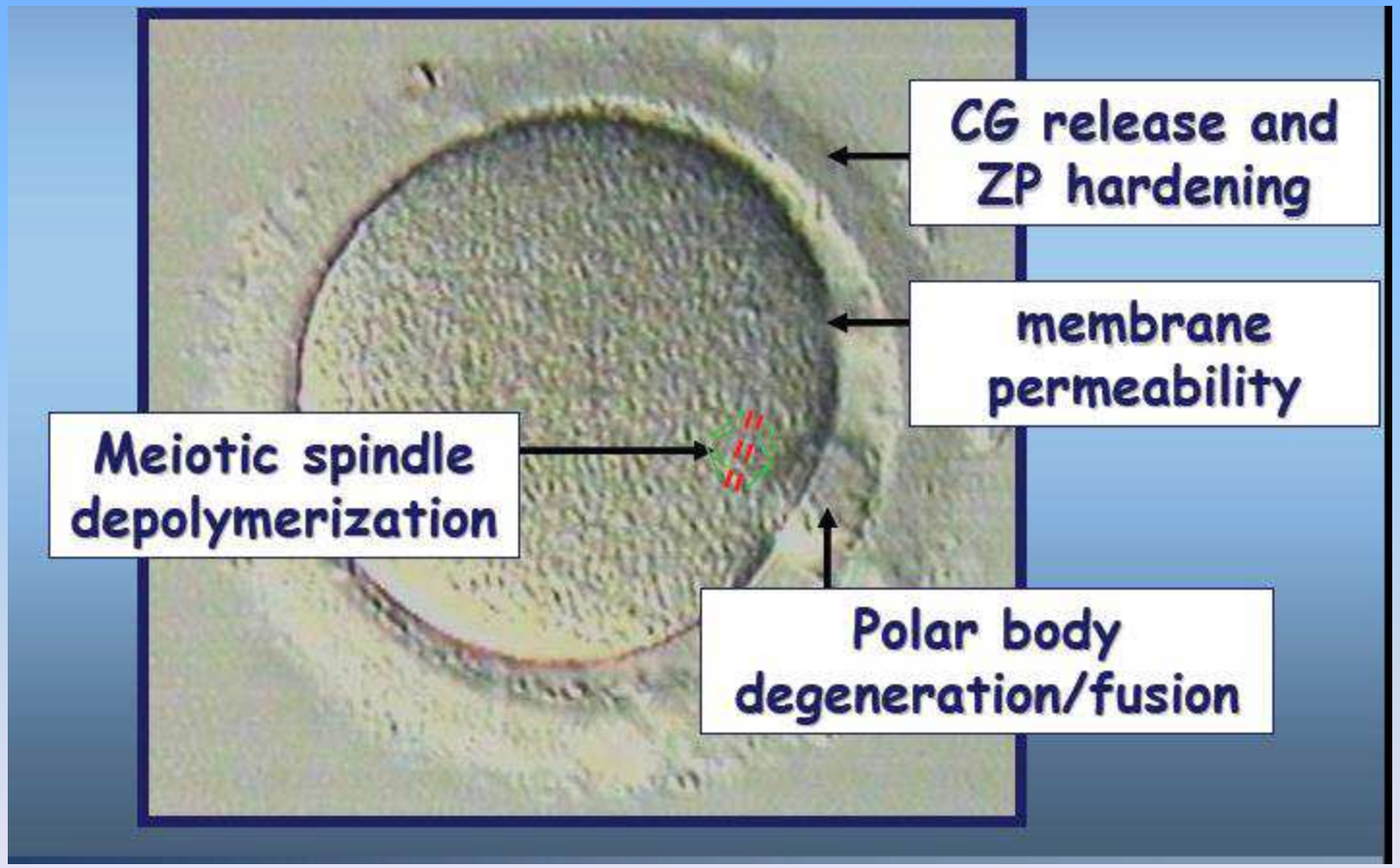
Glenister et al, 1987; Carroll et al., 1989

	No. of Oocytes (%)		
Frozen	Scored	% Aneuploidy	% Retention PB
+	352	6.4	2.6
-	218	8.0	4.4

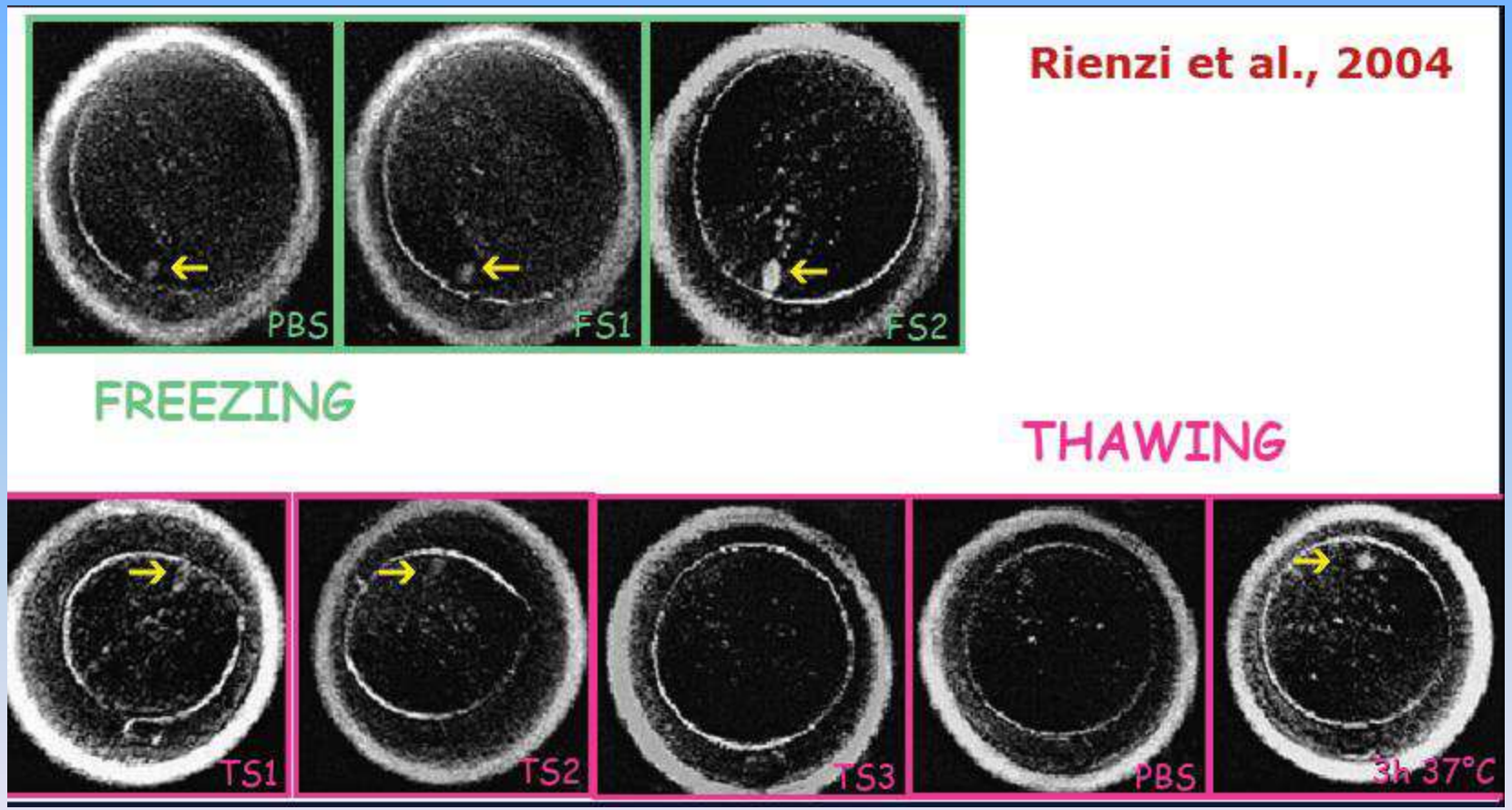
No increase in the rates of aneuploidy/digyny in parthenogenetically activated mouse oocytes after cryopreservation with DMSO/slow freezing

Bos-Mikich and Whittingham, 1995

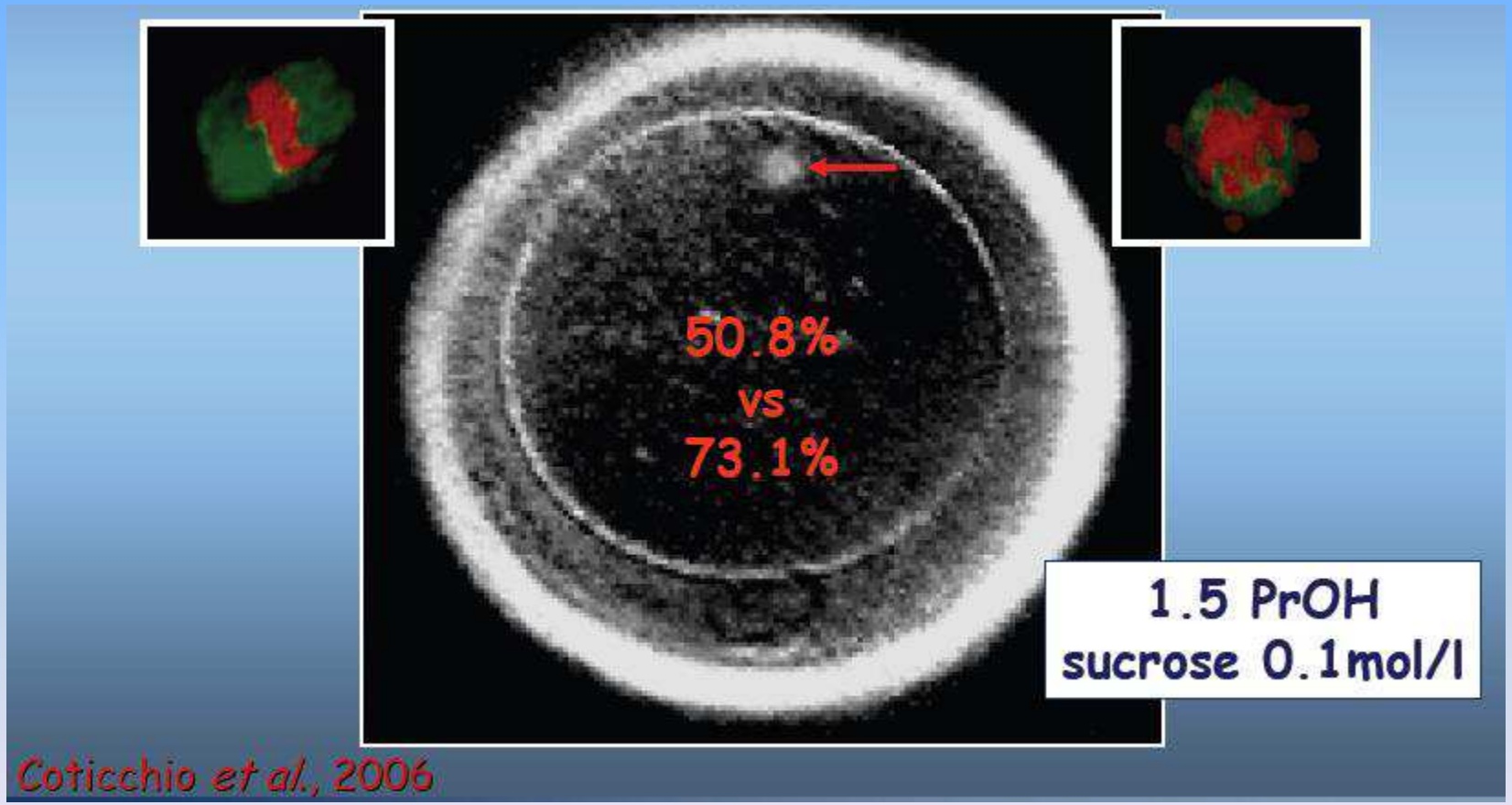
Oosit fizyolojisi ve moleküler Göstergelerin Analizi



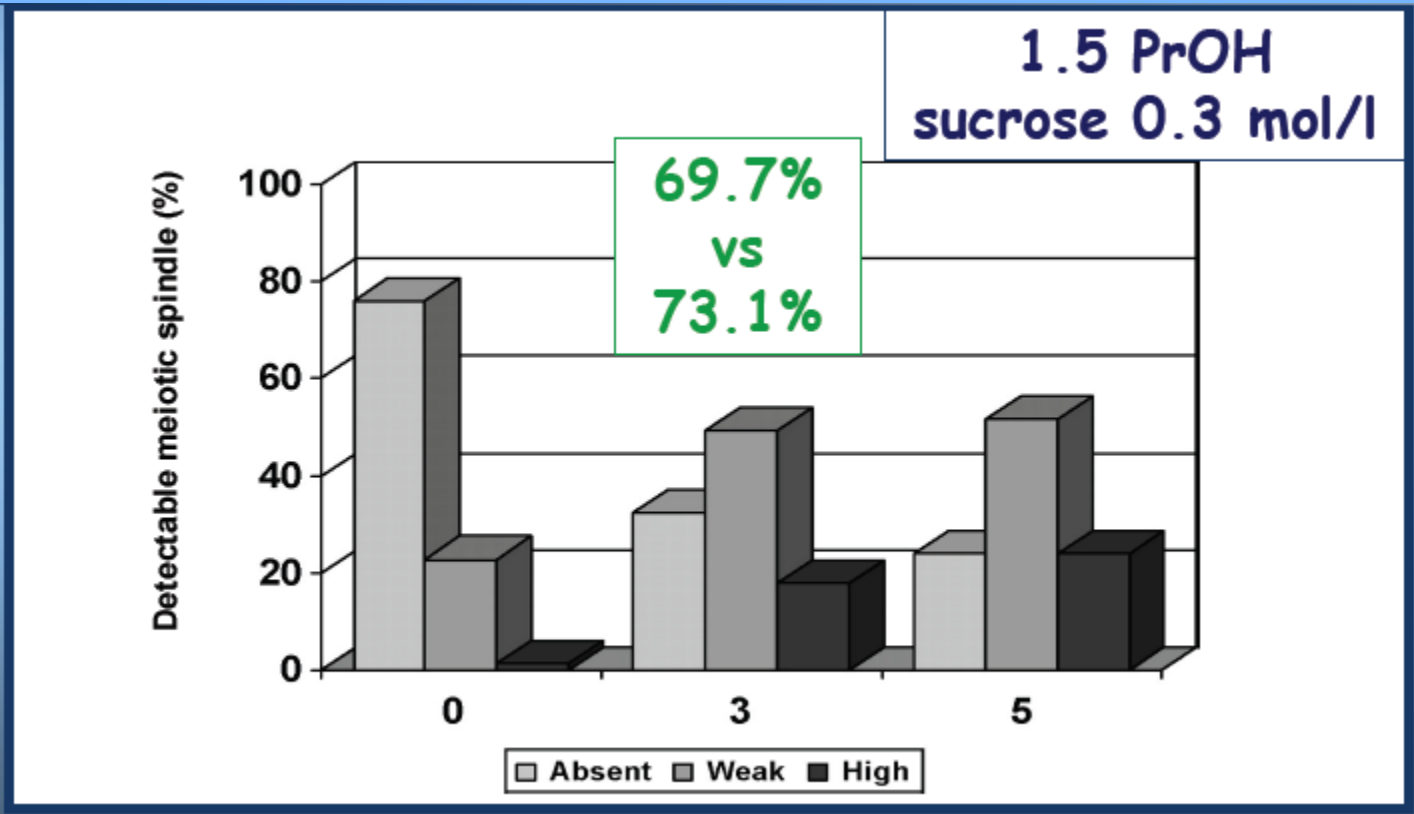
Meiotic spindle analysis during slow freezing



Meiotic spindle analysis during slow freezing



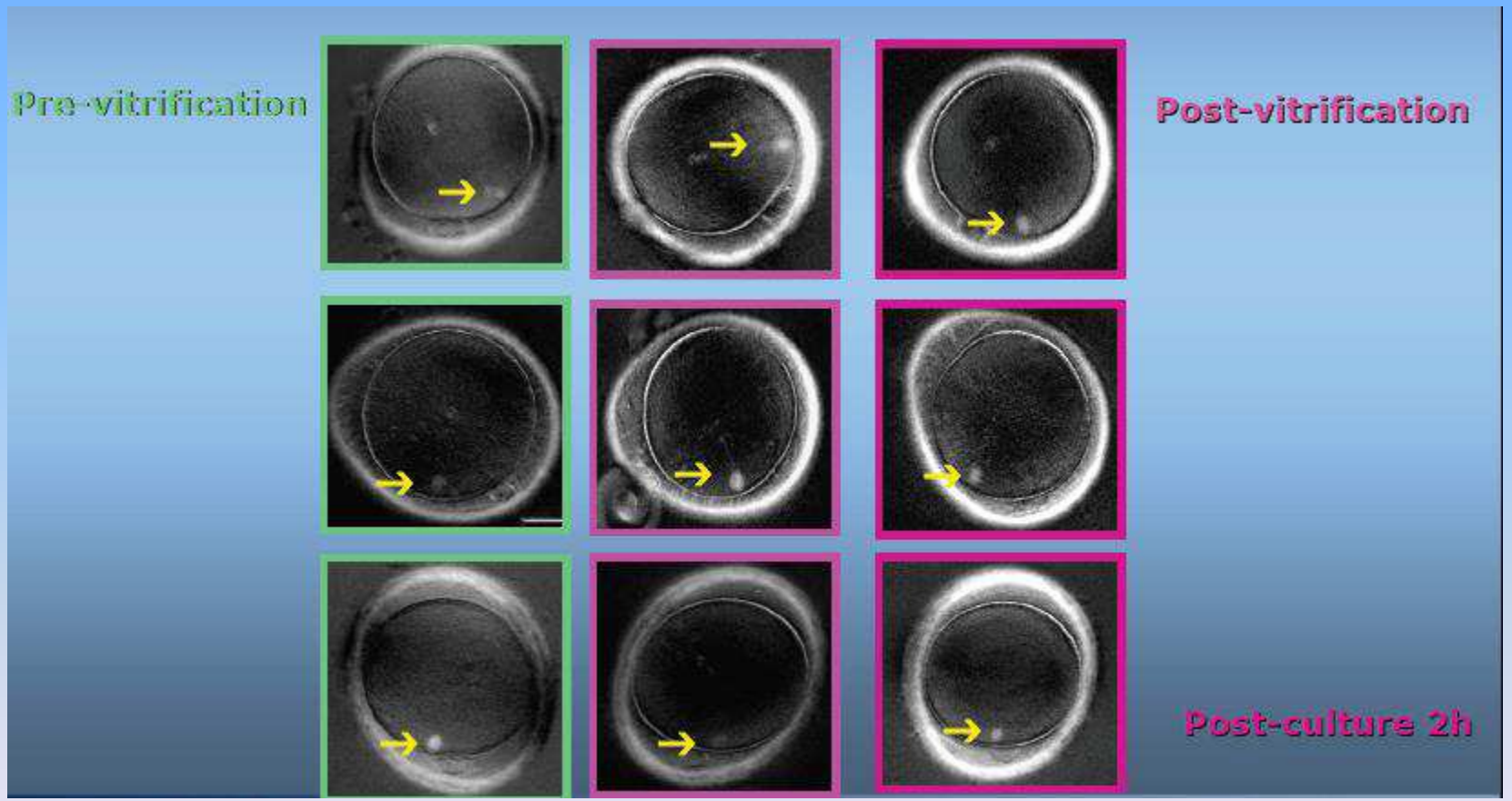
Meiotic spindle analysis during slow freezing



Bianchi *et al.*, Human Reproduction, 2005

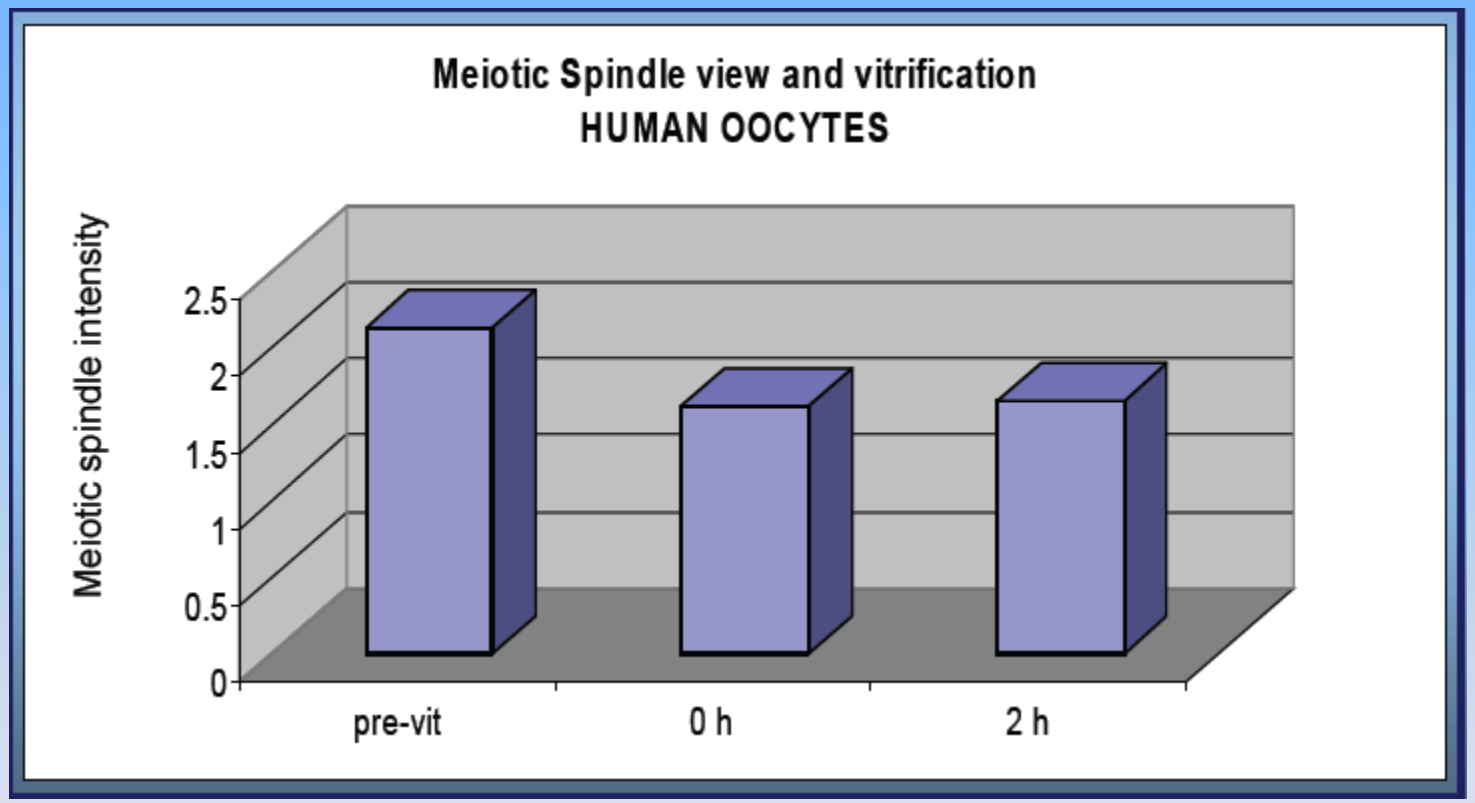
Coticchio *et al.*, 2006

Meiotic spindle analysis during vitrification



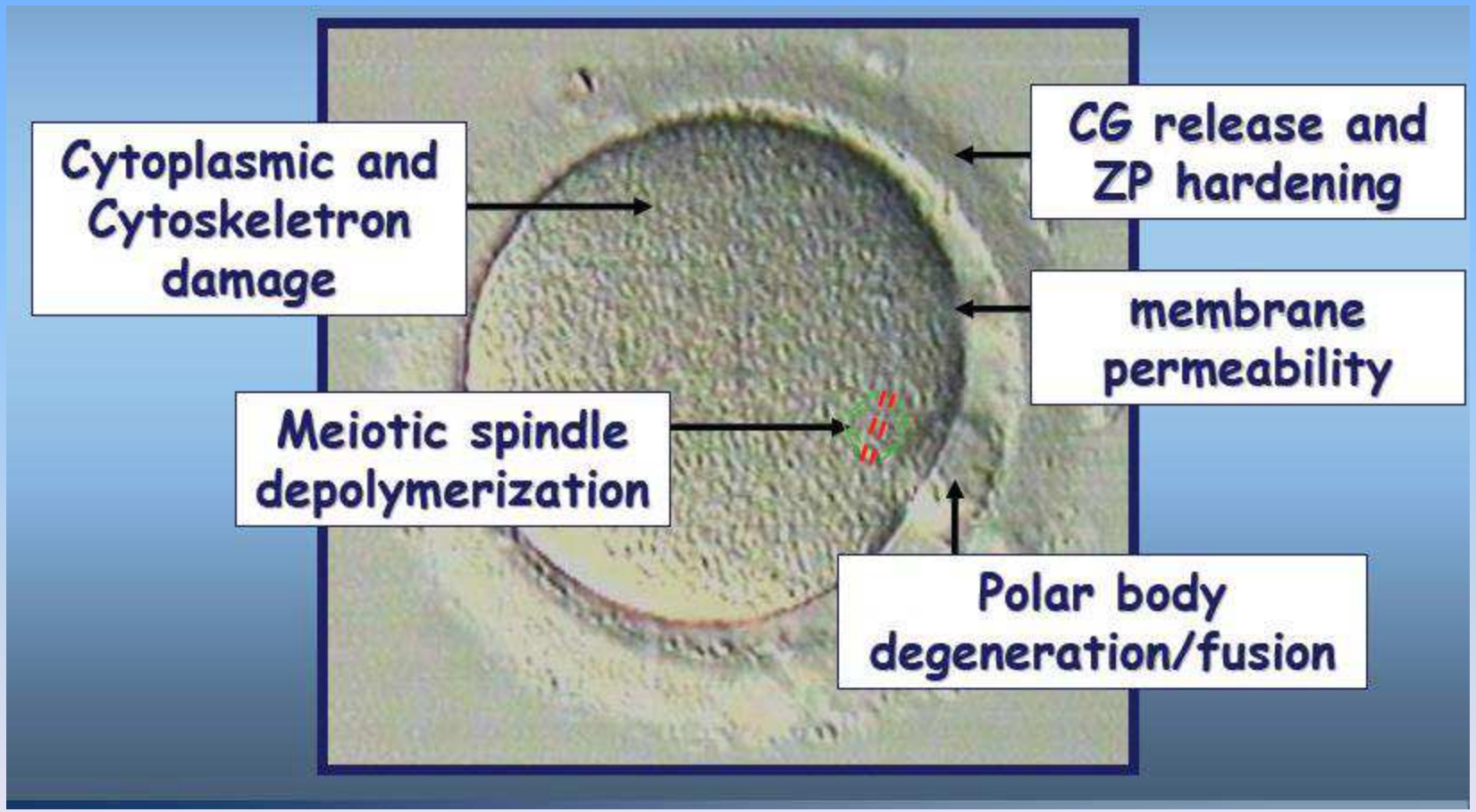
Normal spindle reformasyonu vitrifikasyon sonrası 37^0 de çok daha hızlı gerçekleşmektedir.

Meiotic spindle analysis during vitrification

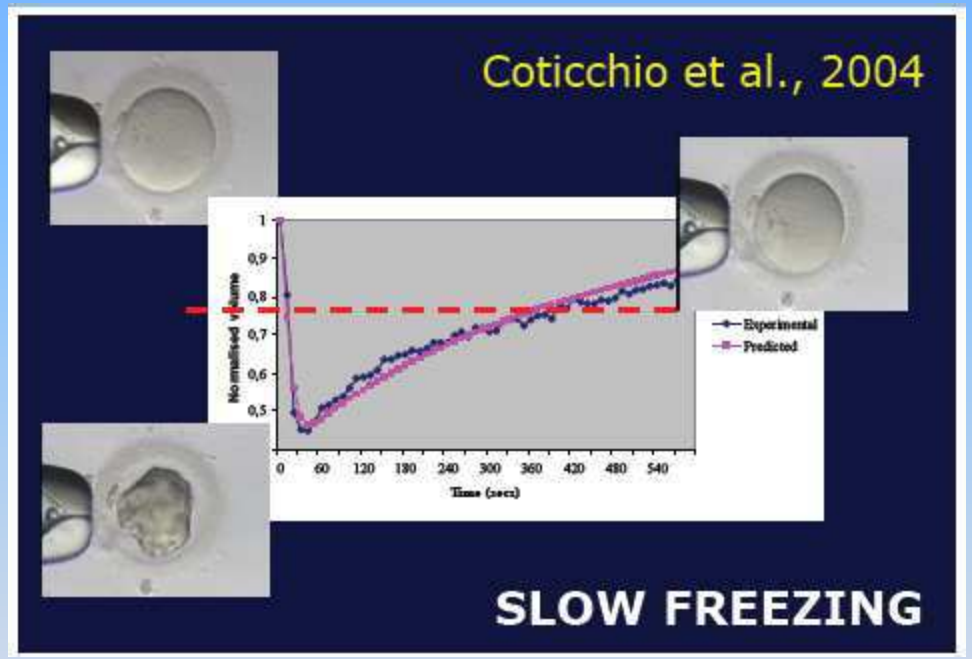


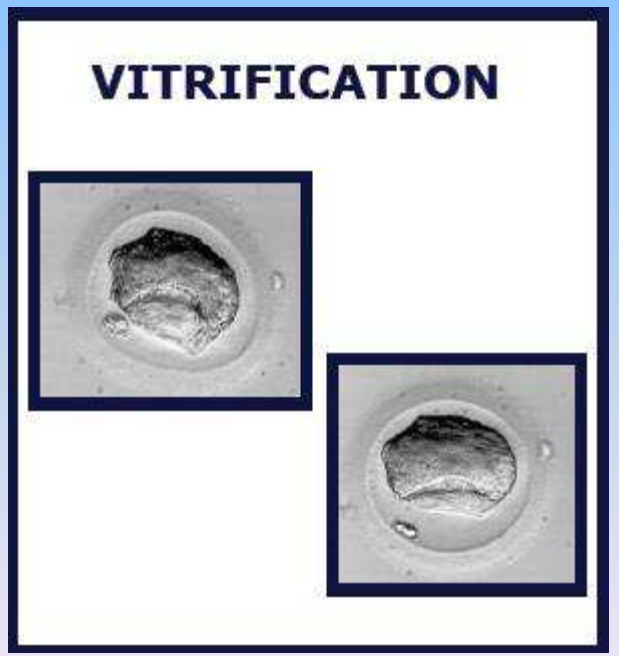
Larman, RBM on line 2007

Oosit fizyolojisi ve moleküler Göstergelerin Analizi



Osmotic toxicity





Osmotic toxicity

OOCYTE OSMOTIC TOLERENCE AND OOLEMMA PERMEABILITY

Temperature of exposure influence shrinking (swelling) patterns

- Oocyte shrinkage tolerance is about 30% of their initial volume

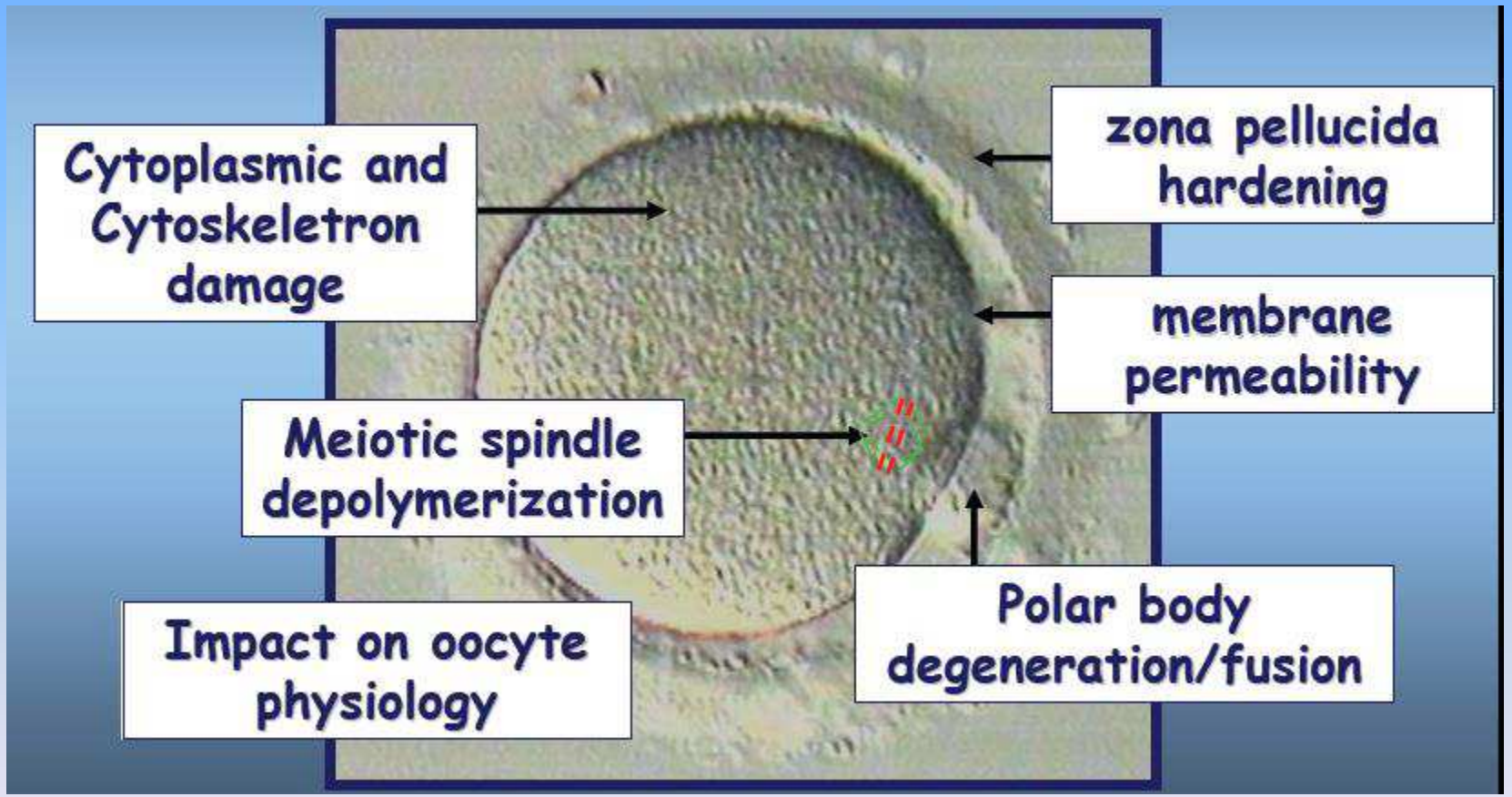
- At 22°C, EG has a lower permeability coefficient relative to DMSO and PG

- The membrane is more selective for EG and DMSO than for PG (mean reflection coefficient Sigma lower for PG)

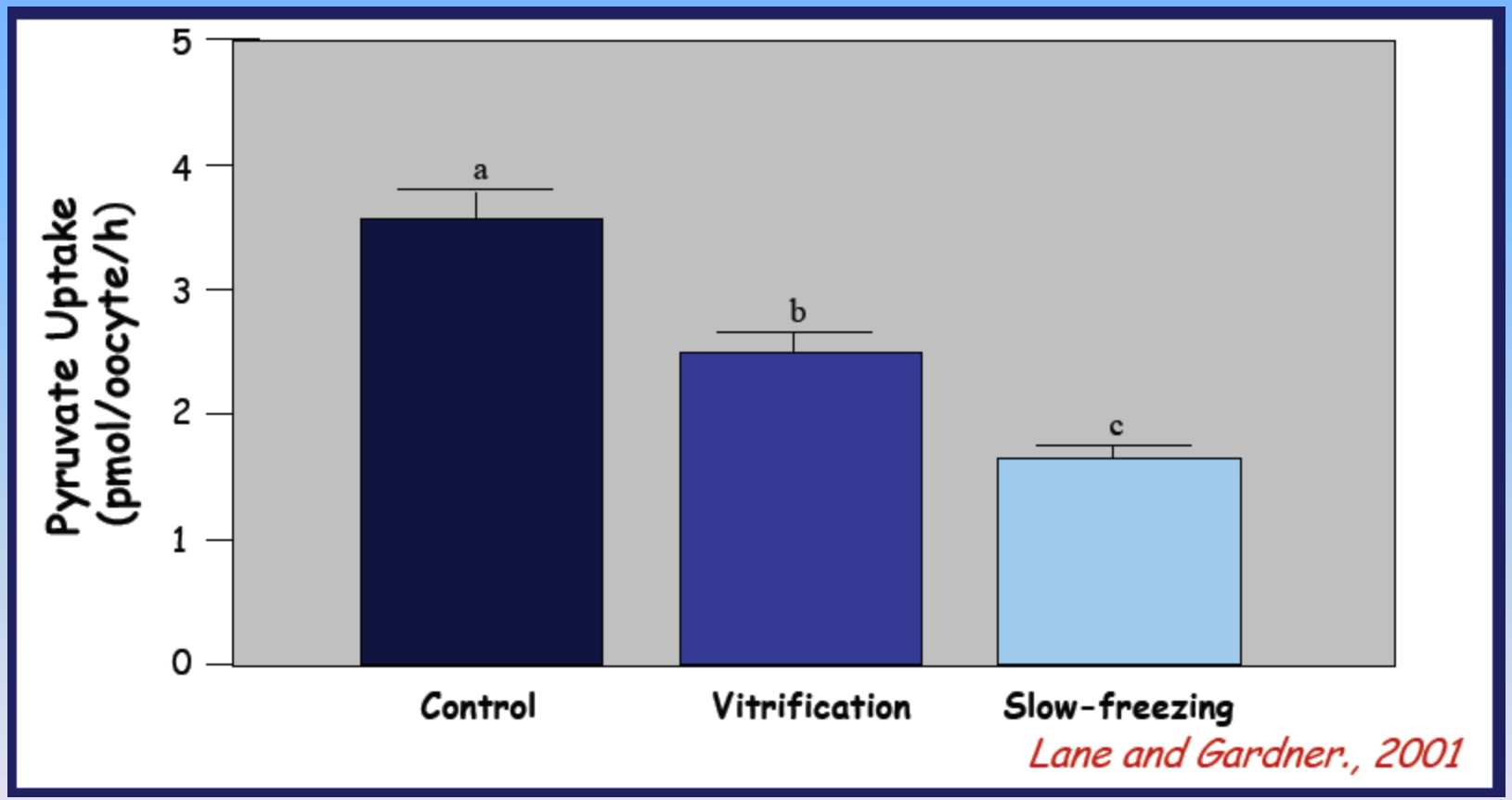
- Permeability coefficients of individual oocytes varied substantially (inherent biological variability)

Van den Abbeel et al., 2007

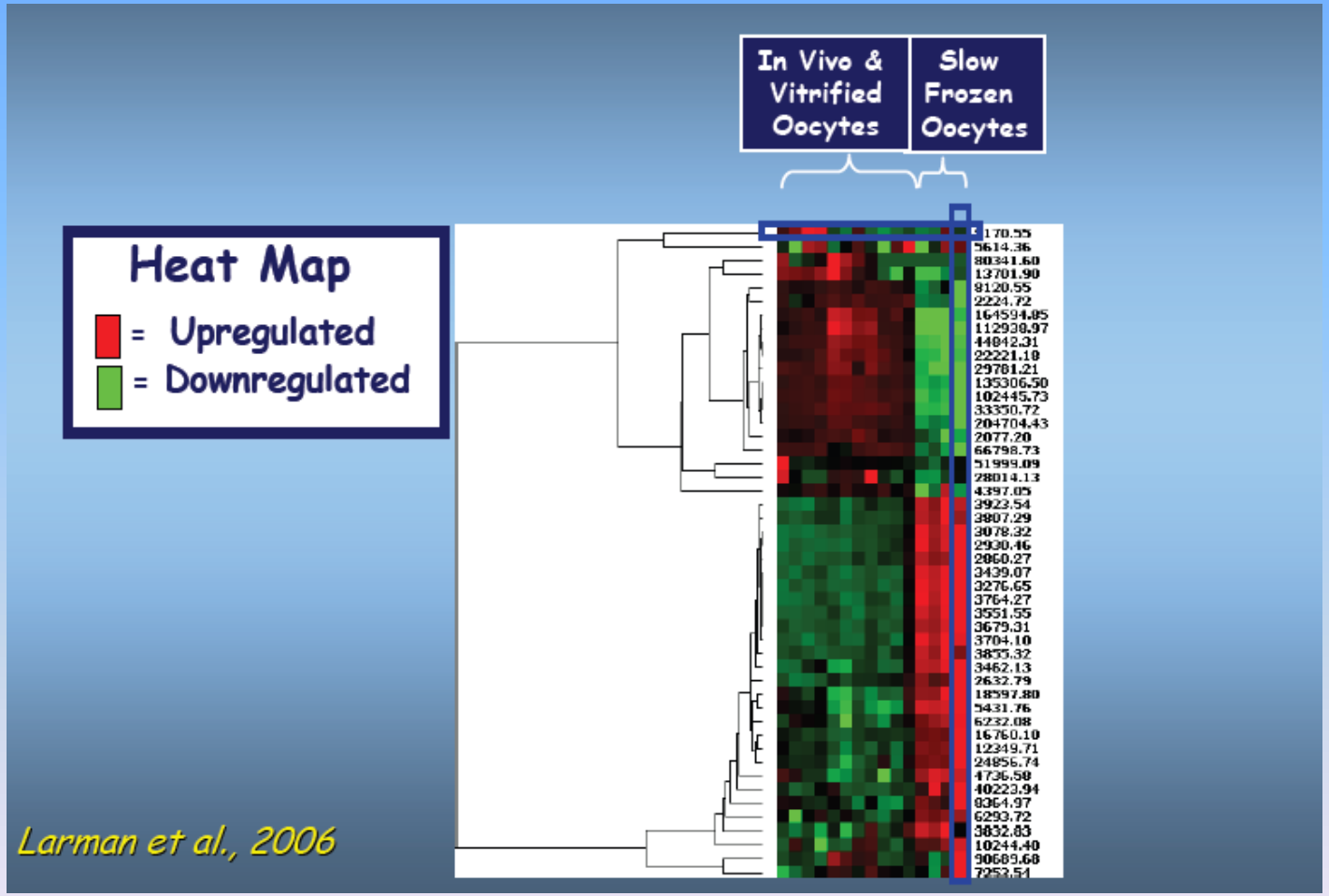
Oosit fizyolojisi ve moleküler Göstergelerin Analizi



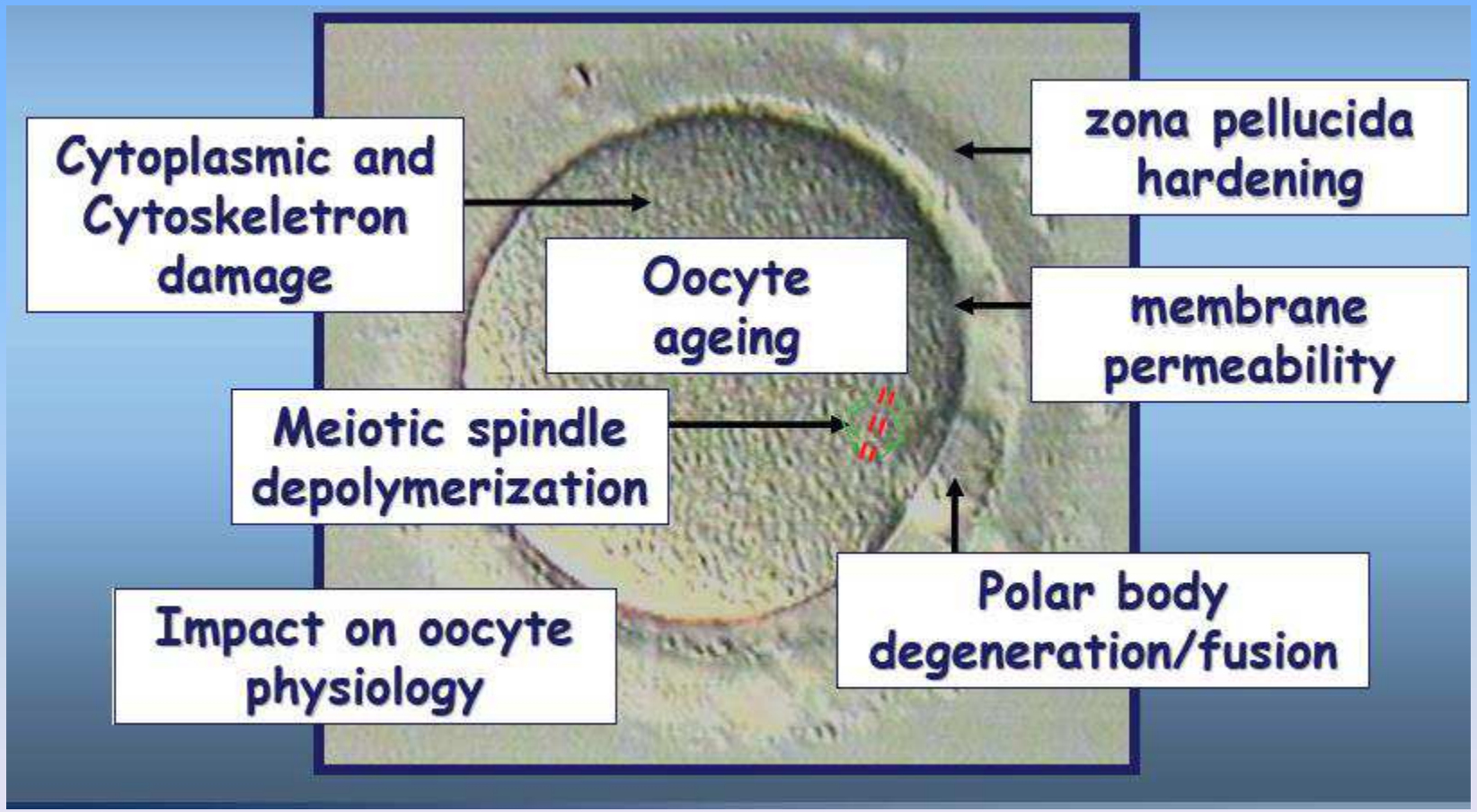
Oocyte metabolism post-cryopreservation



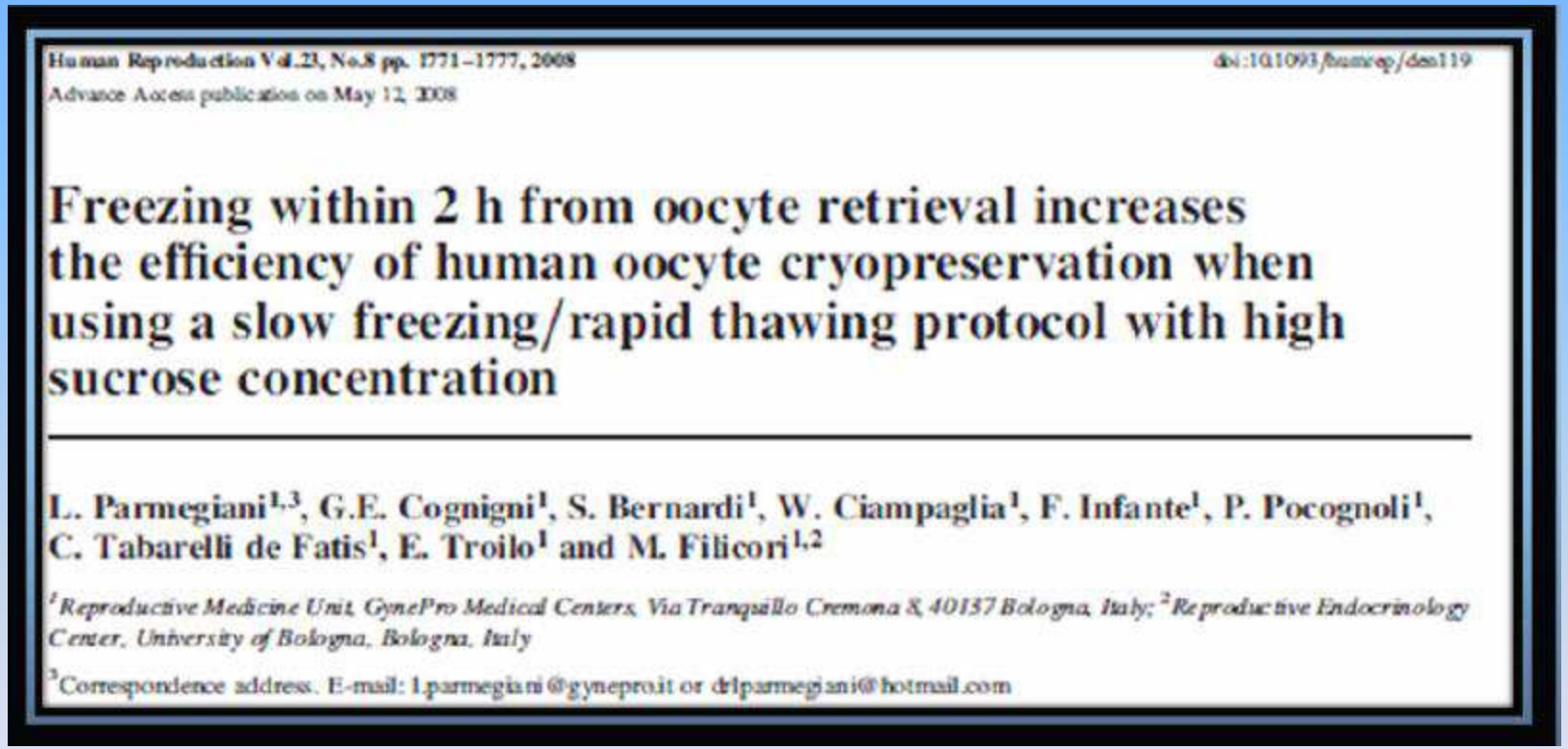
Hierarchical Clustering of Anionic Protein Profile



Oosit fizyolojisi ve moleküler Göstergelerin Analizi



Dondurma Zamanlaması



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Freezing within 2 h from oocyte retrieval increases the efficiency of human oocyte cryopreservation when using a slow freezing/rapid thawing protocol with high sucrose concentration

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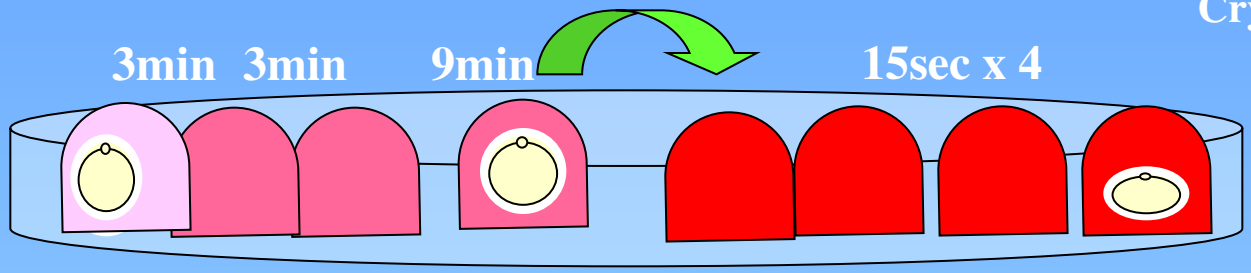
Vitrification

	Equiliblration solution	Vitrification solution
Kuleshowa	10% EG, 40 s; 20% EG, 30 s	40% EG + 0,6 mol/L sucrose, 60 s
Yoon	1.5 mol/L Eg, 2.5 min	5.5 mol/ L GE + 1.0 mol/L sucrose, 20 s
Katayama		15% EG + 15% DMSO + 0.5mol/l sucrose
Kyono	7.5% EG + 7.5% DMSO, 5-20 min	15% EG + 15% DMSO + 0.5mol/l sucrose 45-60 s
Kuwayama	7.5% EG + 7.5% DMSO, 5-20 min	15% EG + 15% DMSO + 0.5mol/l sucrose 45-60 s
Chian	7.5% EG + PROH, 5 min	15% EG + PROH+ 0.5mol/l sucrose 45-60 s
Mukaida	0.625, 1.25, 2.5, 5 and 10% (EG+DMSO), every 2-3 min	20% EG + 20% DMSO + 1% Ficoll + 0.65 mol/L sucrose, 30s 15%EG + 15% DMSO
Sun	7.5% EG + 7.5% DMSO, 3 min	DMSO + 0.5 mol/L sucrose, 30s

Thawing

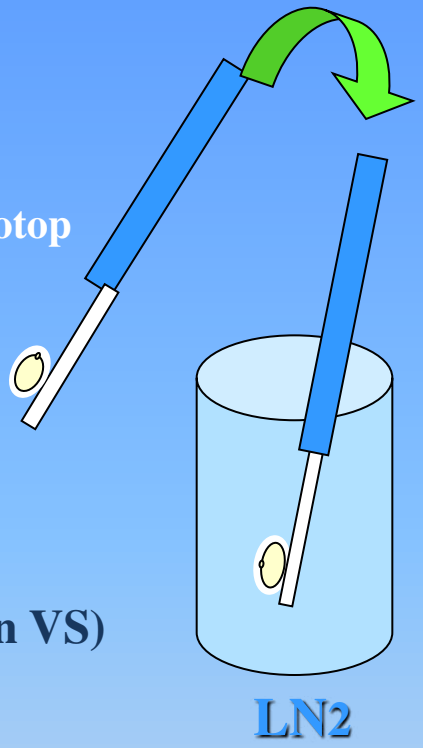
	Thawing Protocol	Culture Medium
Kuleshova	0.4 mol/L sucrose, 2-3 min; 0.25 mol/L sucrose, 2-3 min; 0.125 mol/L sucrose 3-6 min	IVF 50
Yoon	1.0, 0.5, 0.25, 0.125 and 0 mol /L sucrose every 2-3 min	P-1 medium
Katayama		
Kyono	1.0 mol/L sucrose, 1 min; 0.5 mol/L sucrose, 3 min; 0 mol/L sucrose 5 min	Universal IVF medium (Medicult, Denmark)
Kuwayama	1.0 mol/L sucrose, 1 min; 0.5 mol/L sucrose, 3 min; 0 mol/L sucrose 5 min	Modified TCM199
Chian	1.0 mol/L sucrose, 5 min; 0.5 mol/L sucrose, 3 min; 0,25 mol/L sucrose, 3 min, 0 mol/L sucrose	
Muaida	1.0, 0.75, 0.5, 0.25 and 0.125 mol/L sucrose	
Sun	Every 2-3 min 1.0, 0.5 and 0 mol/L sucrose	

Vitrification

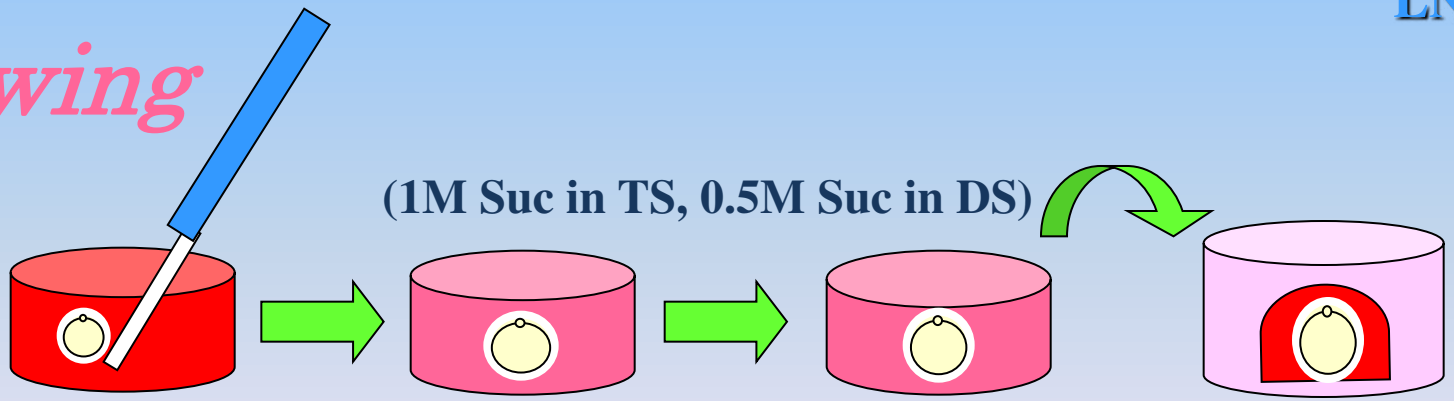


(20μl for each drop, at R.T.)

(7.5%EG,7.5%DMSO in ES)
(15%EG,15%DMSO,0.5M Suc in VS)



Thawing



Oosit Güvenliği

Oocyte cryopreservation poses certainly specific problems:

- The oolemma and not the size of MII oocyte is the key to explain the low survival rates obtained with slow freezing.
- Release of cortical granules (controversial)
- Chemical toxicity from cryoprotectants (type specific)
- Osmotic toxicity
- Meiotic spindle depolymerization (slow freezing)
- Oocyte physiology alteration (metabolism and protein profile) especially true for slow freezing

Oosit Güvenliği

Taxol kullanımı: Hücre iskeleti sabitleyici

Erimiş Nitrojende dondurulma: -210 ^{0} C de dondurma

Sodyum tüketen besiyeri: İmmatür oosit dondurulmasında sodyum yerine Choline kullanılması

SONUÇ

Yükek konsantrasyonda dondurma çözeltileri ve aşırı soğutmayı sağlayan vitrifikasyon teknikleri daha basit ve güvenli bir tekniktir.

Böylelikle ooplasmada buz kristallerinin oluşumu engellenir.

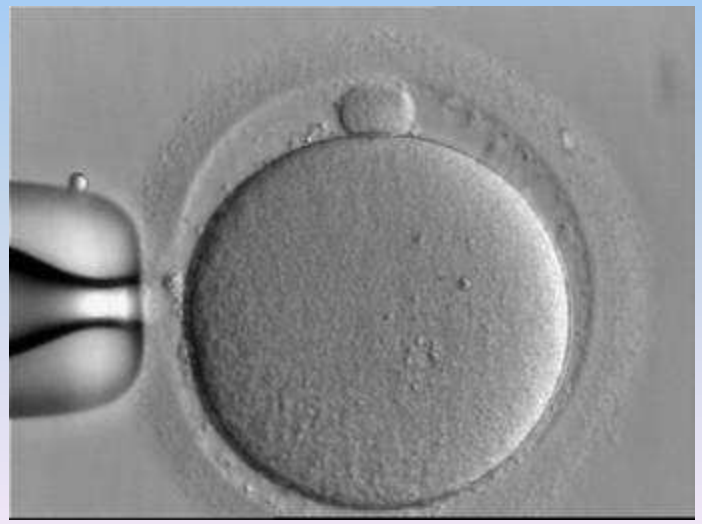
Vitrifikasyon sonrası yüksek canlılık, devam etme oranı, fertilizasyon, klivaj, blastosist oranı Ve normal kromozomal yapılar elde edilmiştir.

Daha yüksek devam eden gebelik ve canlı doğum oranları gözlenmiştir.

SONUÇ

Daha etkili, güvenli ve optimal

sonuçlar için çalışmalar sürdürülmektedir



MUTLU YILLAR…