Preserving Fertility and Ovulation Induction in Breast Cancer

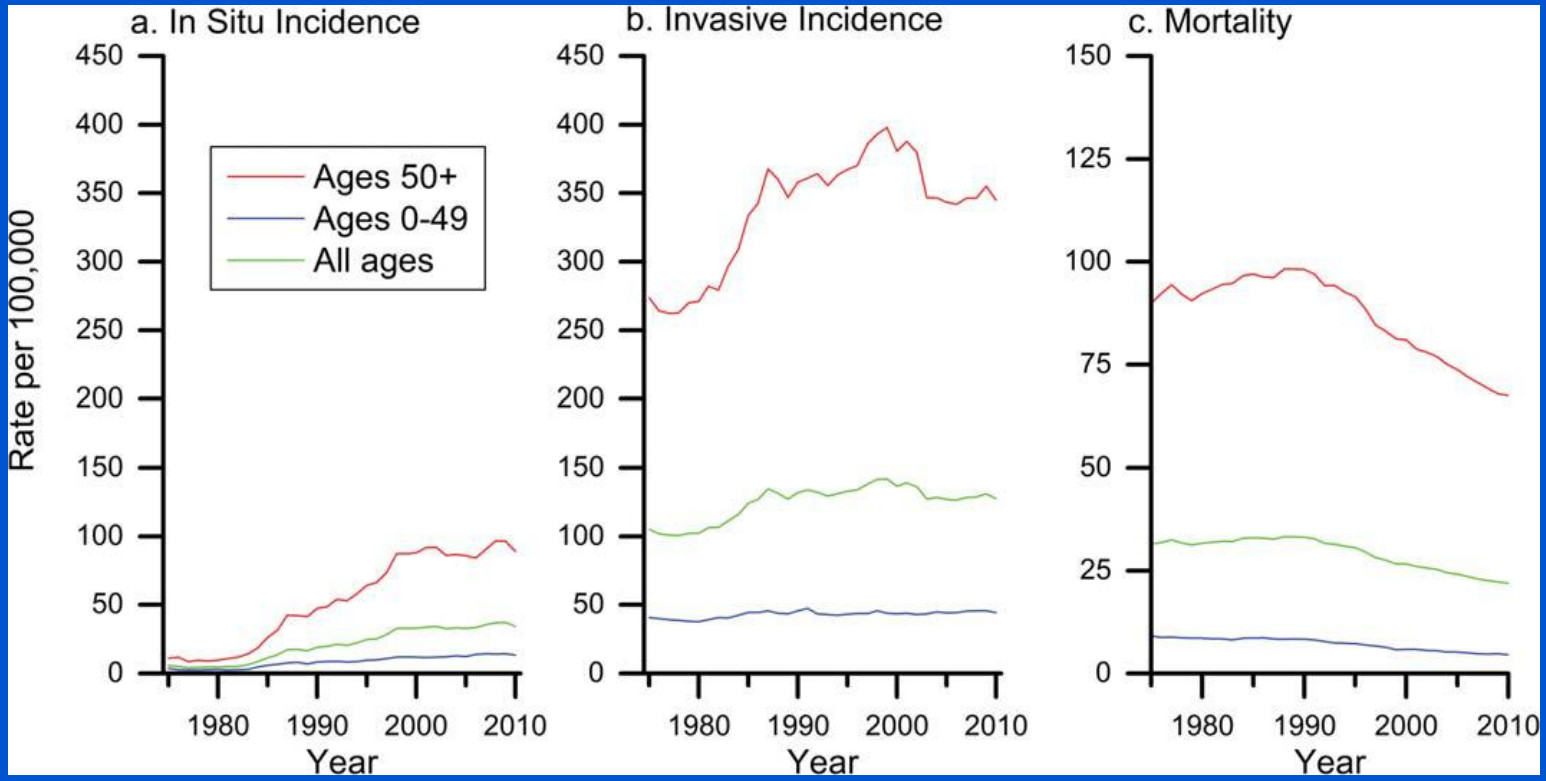
Murat Sönmezer
Ankara University School of Medicine

Breast Cancer

- The most common cancer in women (1/3 all cancers)
- A woman has a 12.3%, or a 1-in- 8, lifetime risk of being diagnosed with breast cancer
- Worldwide, around 1.4 million women are diagnosed with breast cancer annually
- In 2011, 13,110 new cases diagnosed in women of «reproductive age» in the US

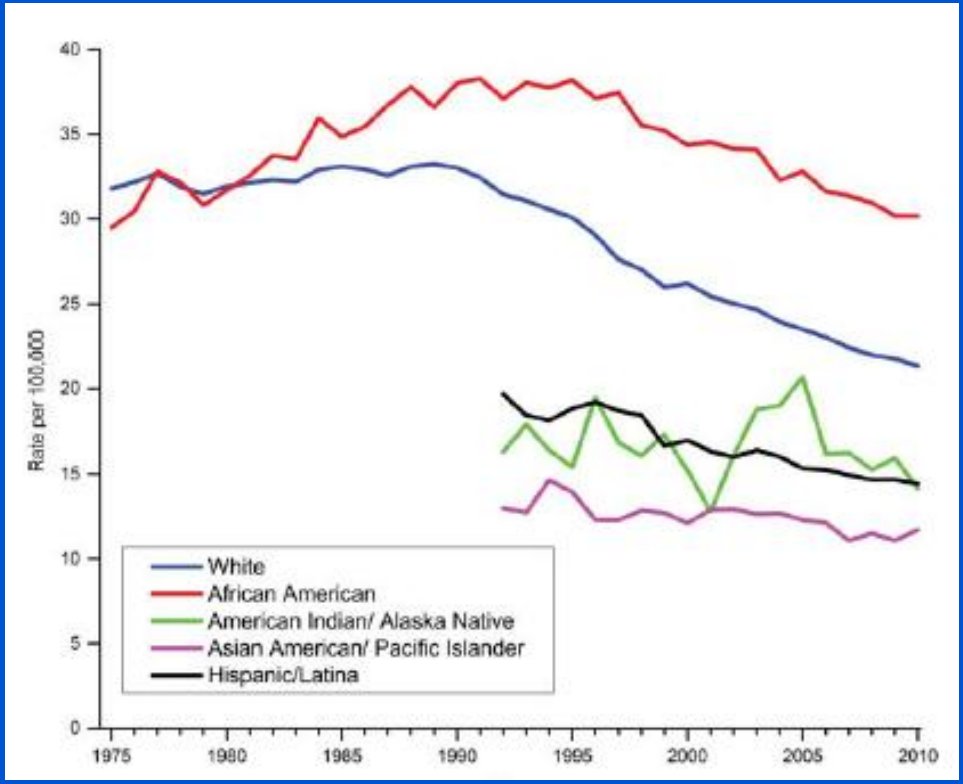
DeSantis, CA Cancer J Clin, 2014
GLOBOCAN, 2008

Breast Cancer
Incidence



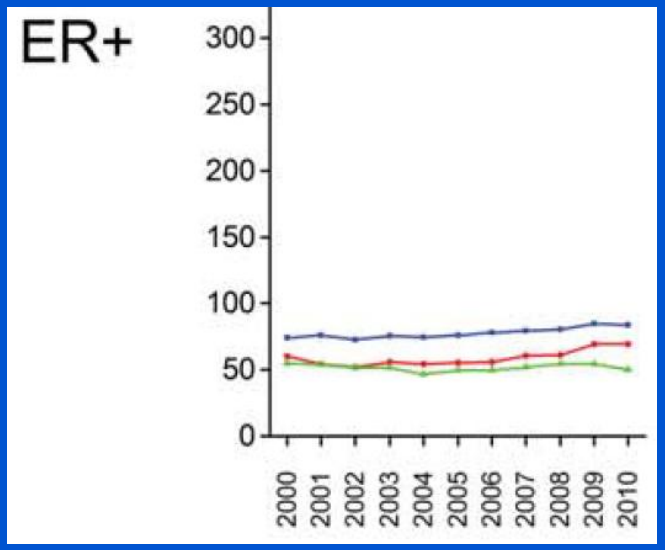
DeSantis, CA Cancer J Clin, 2014

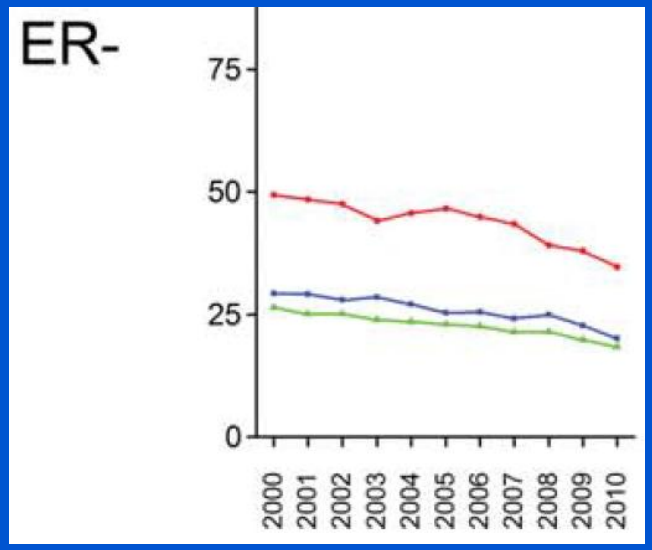
Breast Cancer
Deaths



DeSantis, CA Cancer J Clin, 2014

Breast Cancer
ER status





Incidence rates increased for estrogen receptor-positive breast cancers in the youngest white women

DeSantis, CA Cancer J Clin, 2014

Chemotherapy / Gonadal Damage

High risk agents

- *Cyclophosphamide*
- Cholarambucil
- Melphalan
- Busulfan
- Nitrogen mustard
- Procarbazine

Medium risk agents

- Cisplatinum
- Adriamycin
- Paclitaxel
- Imatinib

Low risk agents

- Methotrexate
- 5-Fluorouracil
- Vincristine
- Actinomycin D
- Bleomycin

New agents / risk ?

- Irinotecan
- Imatinib

Sonmezer&Oktay, Uptodate, 2014

Fertility preservation for breast cancer
Critical points

- Fertility/ovarian function is preserved
- Positive impact on QoL and on coping with the disease
- Safety of COH in ER dependent tumors
- Time constraints

Options for Fertility Preservation

- **Embryo cryopreservation**
- **Mature/immature oocyte cryopreservation**
- In vitro maturation
- Ovarian transposition
- Donor oocyte

- Ovarian tissue cryopreservation
- Xenografting
- GnRHa pretreatment
- Antiapoptotic treatments $\Rightarrow$SP1P

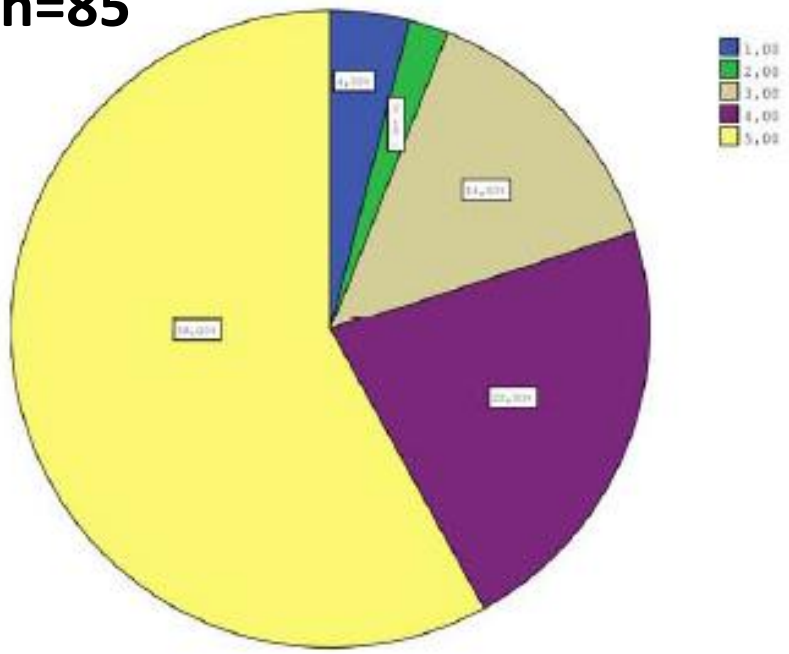
Sonmezer&Oktay, Hum Reprod Update, 2004

Ovarian cryo. vs. oocyte/embryo cryo.

	Ovarian cryo.	Oocyte /embryo cryo.
Advantages	• Chemo. not delayed • Only option for prepubertal girls • Resumption of endocrine functions	• Technique standard • High success rates • Widespread use
Disadvantages	• L/S upfront • Limited number of pregnancies • Cancer reseeding ?	• 2-3 W required • Limited number of oocyte/embryo • Not easily applicable in children

Positive impact on QoL and on coping with the disease

n=85



Graph 4: Motivational morale contribution of ovarian cryopreservation to patients' struggle with a malignancy. (1-5, 1; no effect, 5; maximum boosting morale)

Sonmezer&Ozkavukcu, ASRM, 2013

Letrozole / TMX Stimulation - IVF

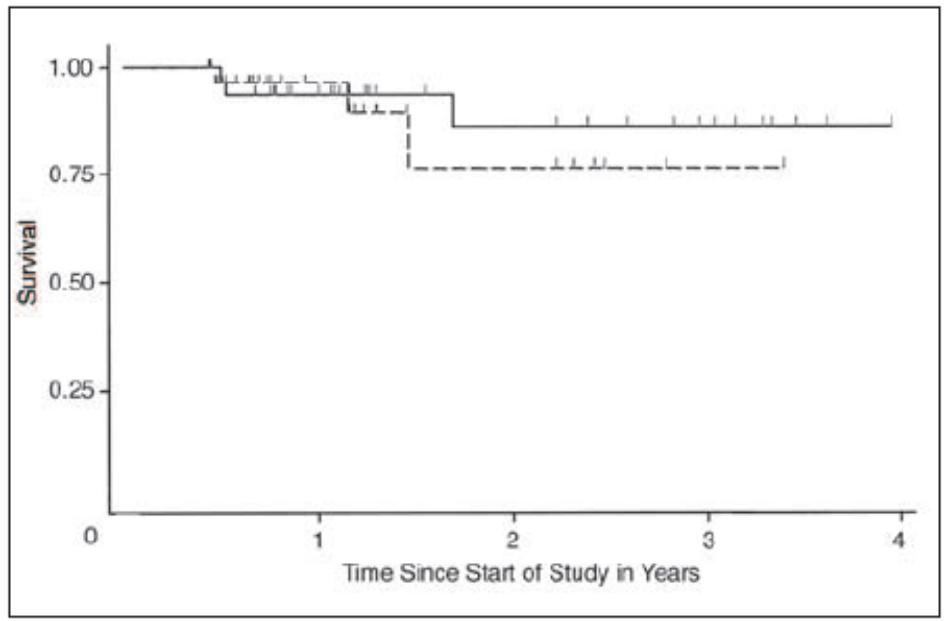
Fertility Preservation in Breast Cancer Patients: A Prospective Controlled Comparison of Ovarian Stimulation With Tamoxifen and Letrozole for Embryo Cryopreservation

Kutluk Oktay, Erkan Buyuk, Natalie Libertella, Munire Akar, and Zev Rosenwaks

- 29 women aged 24-43 years, 33 COH cycles
  - TMX 60mg/d alone → 12 patients, 13 cycles
  - TMX-FSH → 7 patients 9 cycles
  - Letrozole (5mg/d)-FSH → 11 patients 11 cycles

Oktay, JCO, 2005

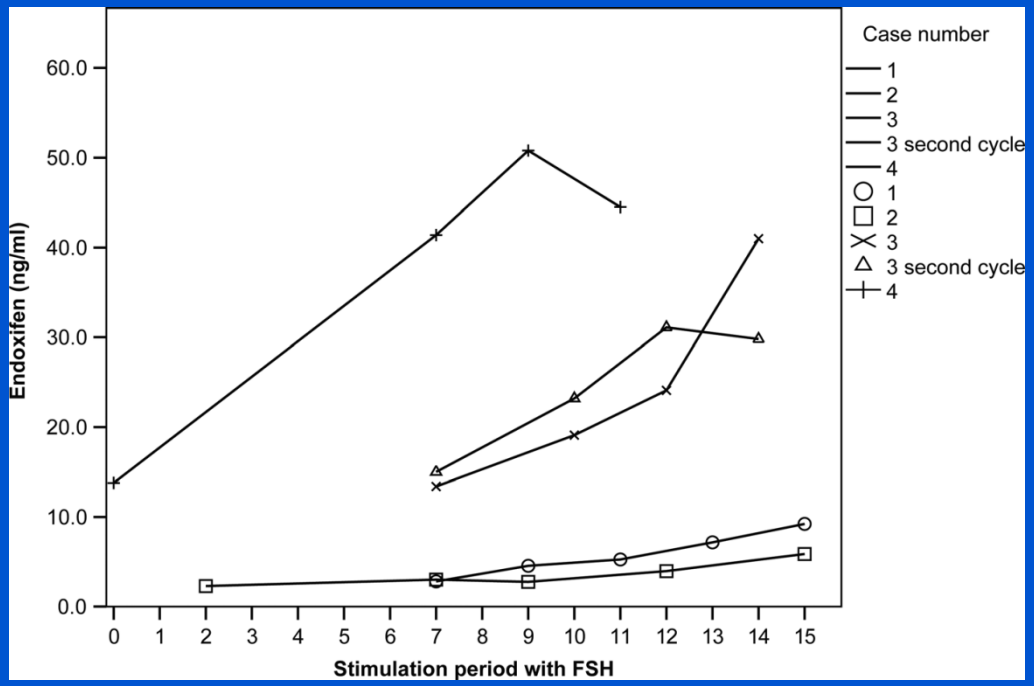
Variable	Mean ± Standard Deviation			P		
	Tam-IVF (a)	TamFSH-IVF (b)	Letrozole-IVF (c)	a *v* b	a *v* c	b *v* c
Age, years	36.6 ± 1.6	38.3 ± 1.9	38.5 ± 1	NS	NS	NS
Baseline FSH, mU/mL	9.4 ± 1.5	9.4 ± 1.5	6.2 ± 1.1	NS	NS	NS
PeakE_2, pg/mL†	419 ± 39	1,182 ± 271	380 ± 57	< .05	> .05	< .05
Total follicles, No.	2 ± 0.3	6 ± 1	7.8 ± 0.9	< .01	< .001	> .05
Follicle > 17 mm, No.	1.2 ± 0.1	2.6 ± 0.4	3.2 ± 0.4	< .05	< .001	> .05
Total oocytes, No.	1.7 ± 0.3	6.9 ± 1.1	12.3 ± 2.5	< .05	< .001	> .05
Mature oocytes, No.	1.5 ± 0.3	5.1 ± 1.1	8.5 ± 1.6	< .05	< .001	> .05
Total embryos, No.	1.3 ± 0.2	3.8 ± 0.8	5.3 ± 0.8	< .05	< .001	> .05



Oktay, JCO, 2005

A prospective case series of women with estrogen receptor-positive breast cancer: levels of tamoxifen metabolites in controlled ovarian stimulation with high-dose tamoxifen

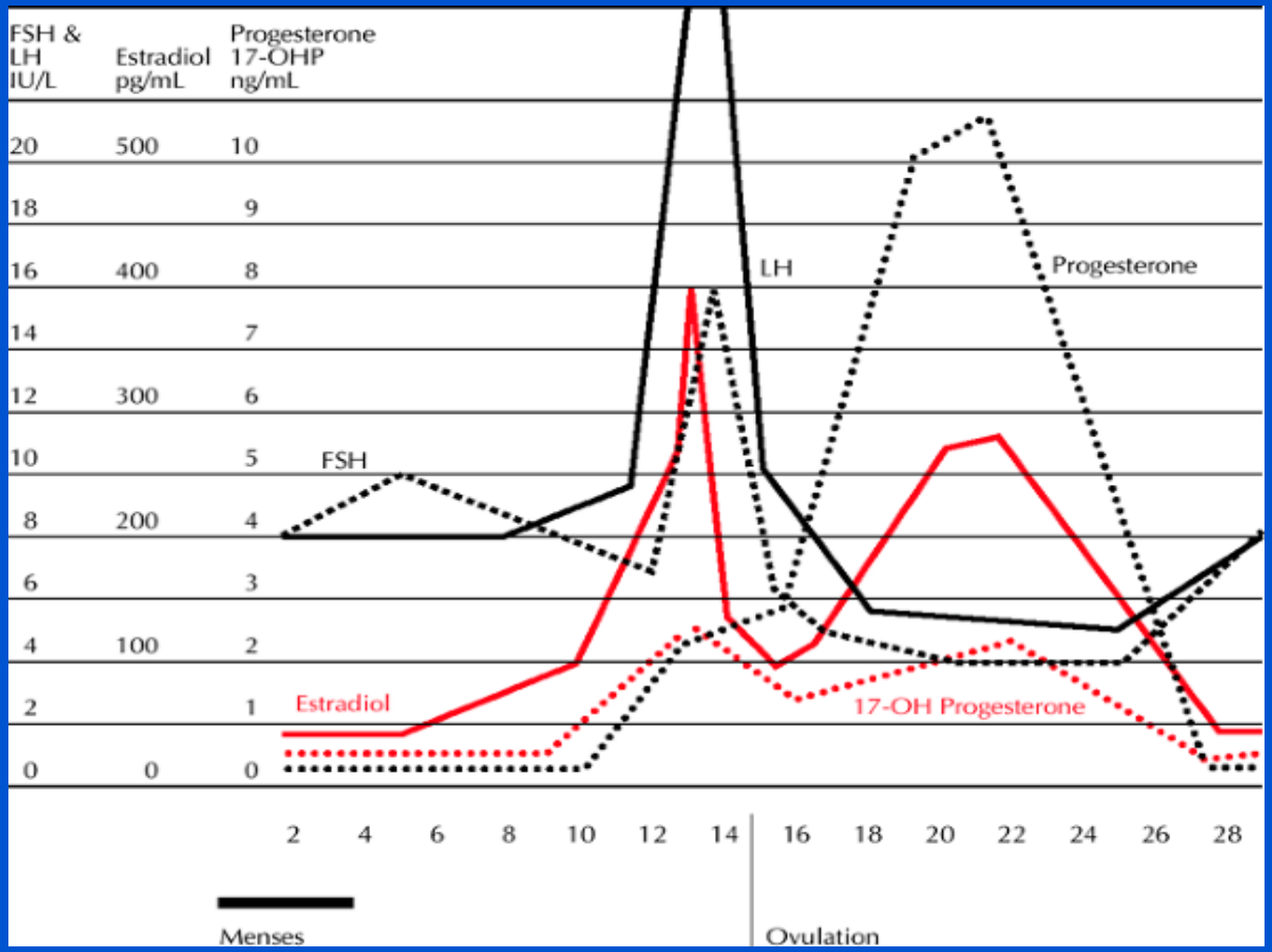
E.M.E. Balkenende[1], T. Dahhan[1,*], S.C. Linn[2], N.G.L. Jager[3], J.H. Beijnen[3], and M. Goddijn[1]



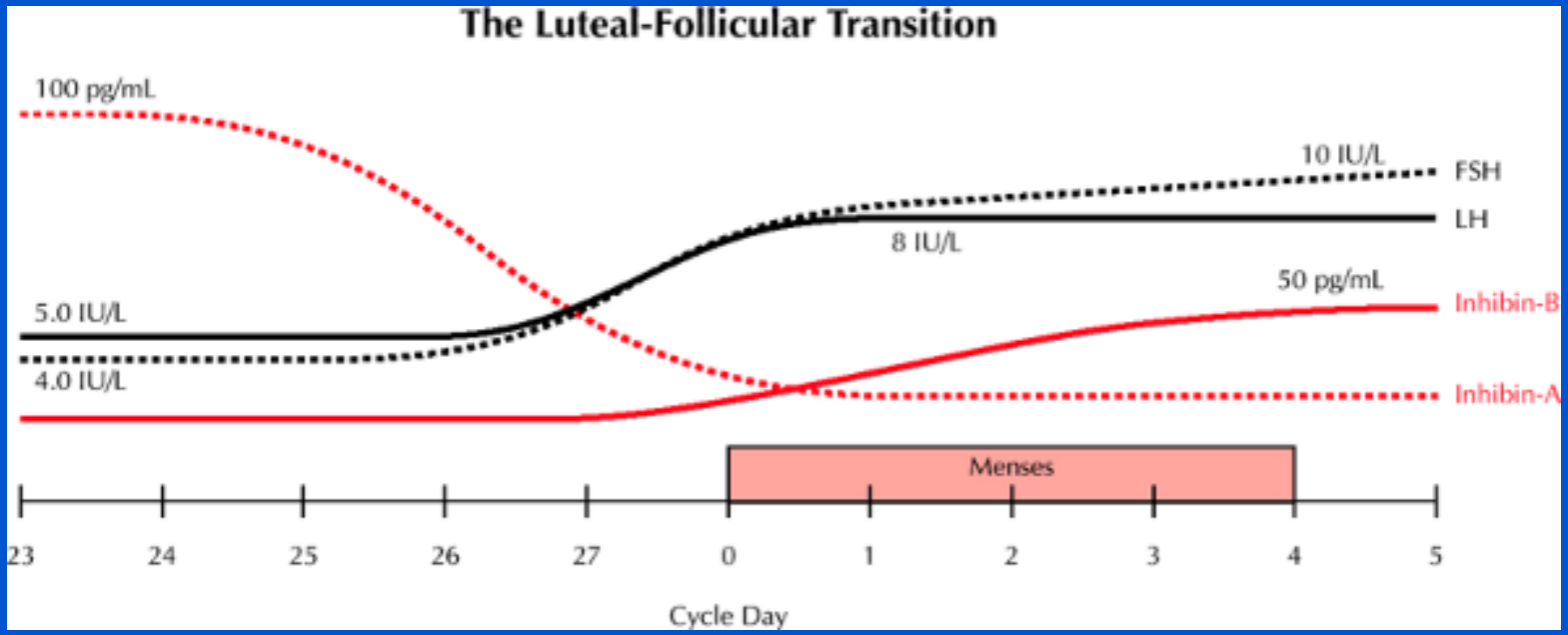
- TMX 60mg/day+FSH
- 4 patients
- 5-11 oocytes vitrified
- Endoxifen >7ng/ml (ER inhibition)

Balkenende, Hum Reprod, 2012

Physiology of Ovarian Follicular Development



Physiology of Ovarian Follicular Development



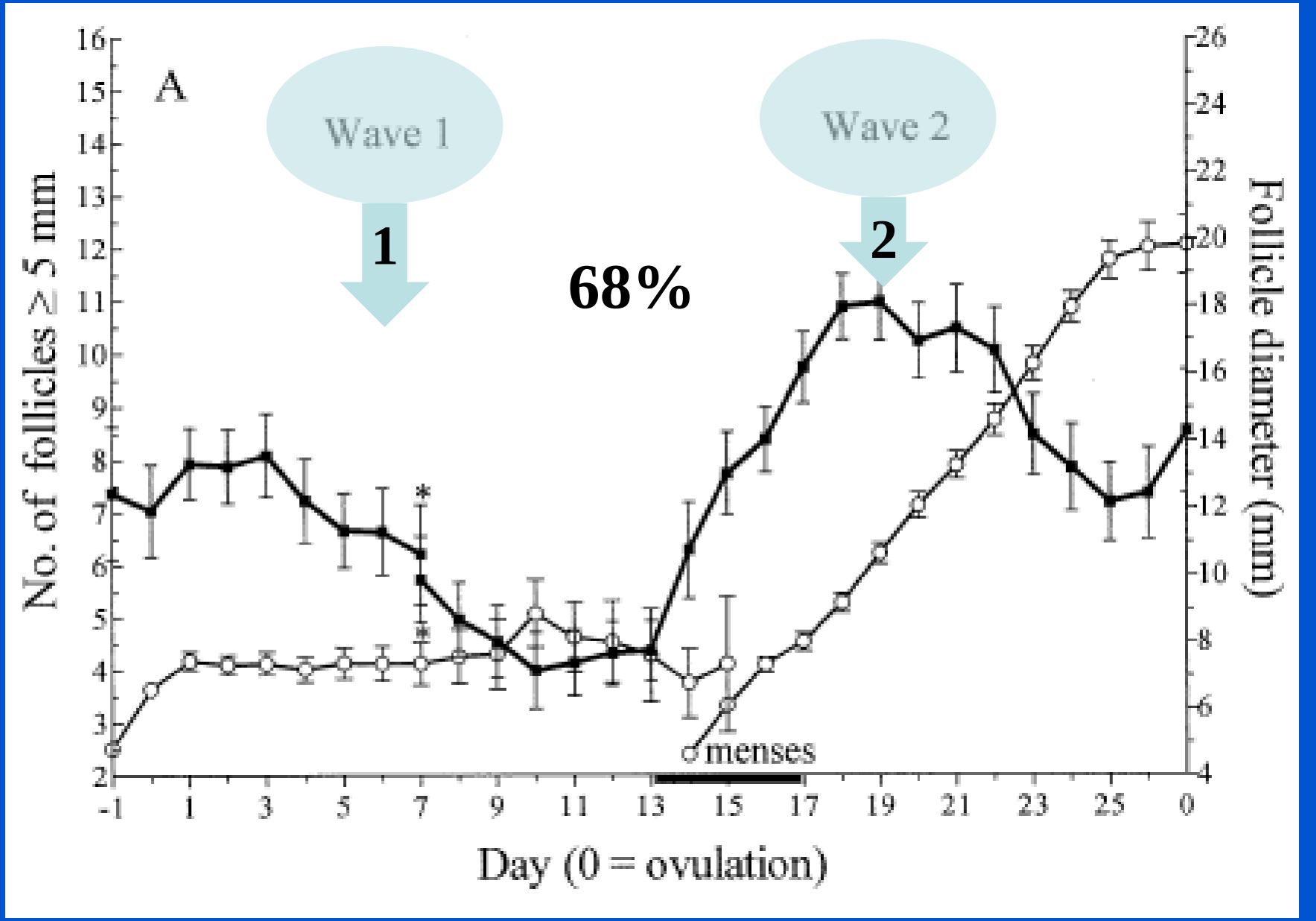
A new model for ovarian follicular development during the human menstrual cycle

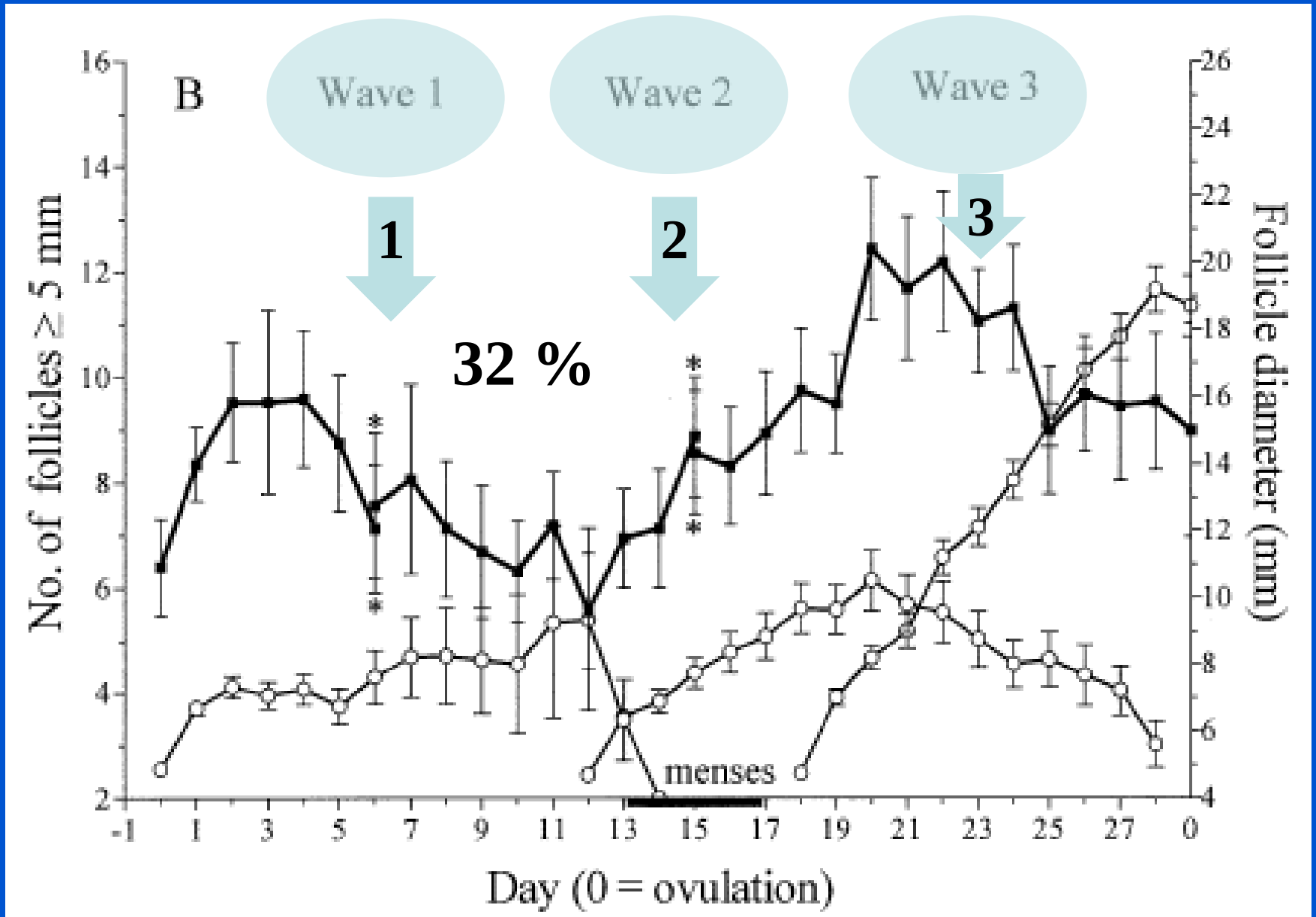
Angela R. Baerwald, B.Sc. Hon.,[a] Gregg P. Adams, D.V.M., M.S., Ph.D.,[b] and Roger A. Pierson, M.S., Ph.D.[a]

University of Saskatchewan, Saskatoon, Saskatchewan, Canada

- Is there only one major follicular wave in a single menstrual cycle ?
- n=50 healthy ovulating women

Barewald, Fertil Steril, 2003


A
Wave 1
Wave 2
1
2
68%
menses
No. of follicles ≥ 5 mm
Follicle diameter (mm)
Day (0 = ovulation)


B
Wave 1
Wave 2
Wave 3
1
2
3
32 %
menses
No. of follicles ≥ 5 mm
Follicle diameter (mm)
Day (0 = ovulation)
16
14
12
10
8
6
4
2
26
24
22
20
18
16
14
12
10
8
6
4
-1
1
3
5
7
9
11
13
15
17
19
21
23
25
27
0

Random-start controlled ovarian hyperstimulation for emergency fertility preservation in letrozole cycles

Murat Sönmezer, M.D.,[a,b] *Ilgın Türkçüoğlu, M.D.,*[c] *Uğur Coşkun, M.D.,*[d] *and Kutluk Oktay, M.D.*[e]

TABLE 1

Baseline characteristics and COH outcome of the patients with breast cancer undergoing emergency fertility preservation.

Characteristic	Case 1	Case 2	Case 3
Age (y)	29	26	26
Stage	I	II	II
Histology	Invasive ductal	Mixed invasive ductal + lobular	Invasive ductal
COH start day	14	11	17
FSH (mIU/mL)	6.2	2.8	4.6
LH (mIU/mL)	5.8	2.8	1.2
E_2 (ng/mL)	62	269	50
P (pg/mL)	1.2	0.4	2.5
Endometrial thickness (mm)	7	6.5	9
Antral follicle count (n)	11	20[a]	20[b]
GnRH antagonist start day	5	1	5
Peak E_2 (pg/mL)	499	988	478
Duration of COH (d)	9	12	9
Oocytes retrieved (n)	9	17	16
Metaphase II, no. (%)	7 (77.7)	10 (58.8)	11 (68.75)
Metaphase I + germinal vesicle, no. (%)	2 (22.3)	7 (41.2)	5 (31.25)
Fertilization rate, no. (%)	7/8 (87.5)	10/12 (83.3)	9/13 (69.2)
Cleavage rate (%)	7/7 (100)	NA	NA
Embryos frozen (n)	7	10	9

Sonmezer, Fertil Steril, 2011

Random Start COH

- Random start n=35
- Conventional start n= 93

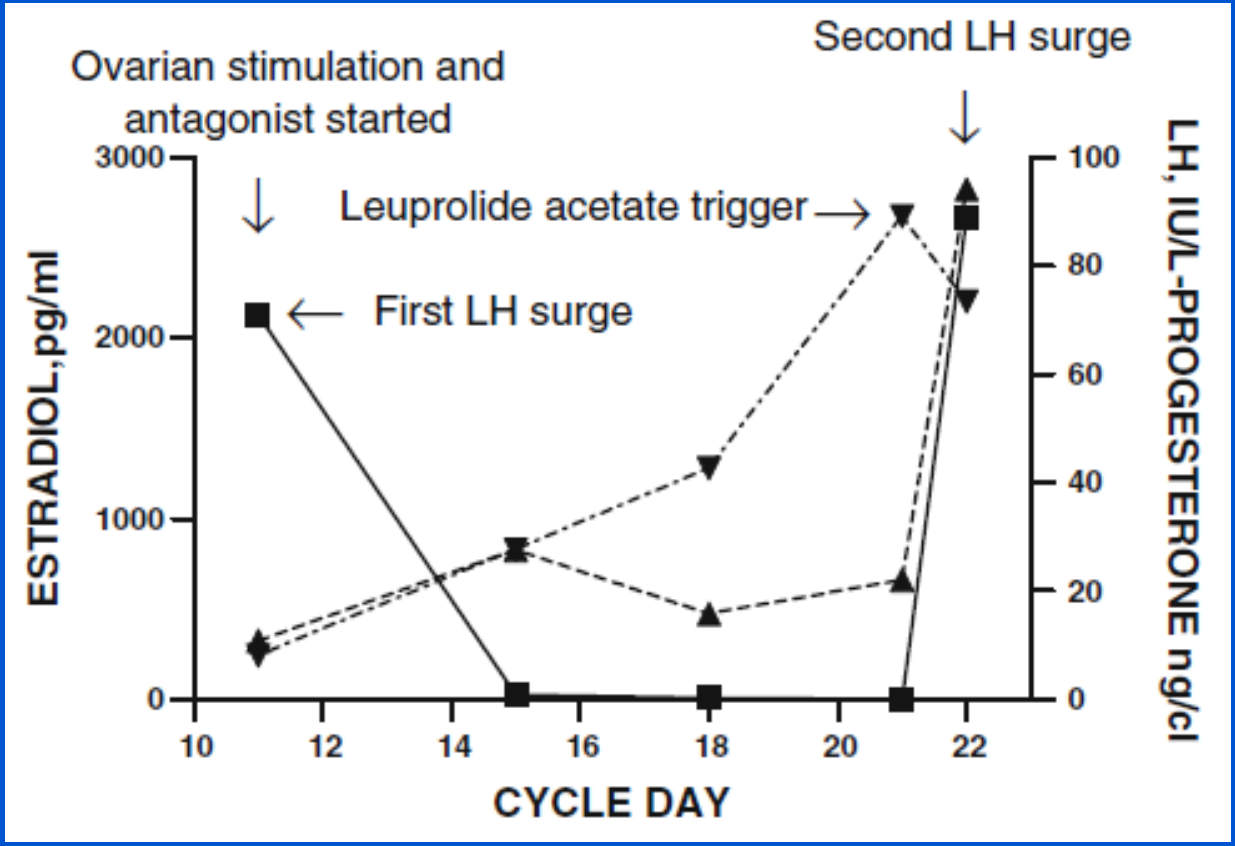
Comparison of outcomes of conventional-and random-start controlled ovarian stimulation cycles.

	Conventional start (n = 88; 103 cycles)	Random start (n = 35; 35 cycles)	*P* value	Late follicular phase start (n = 13; 13 cycles)	Luteal phase start (n = 22; 22 cycles)	*P* value[a]
Antral follicle count (AFC)	13.0 (11.7–14.5)	11.5 (9.6–13.8)	NS	10.5 (7.8–14.2)	12.1 (9.6–15.2)	NS
Days of ovarian stimulation	9.3 (9.0–9.5)	10.9 (10.4–11.5)	< .001	10.5 (9.6–11.4)[b]	11.2 (10.5–12.0)[c]	< .001
Total dose of gonadotropins (IU)[d]	3,404 (3,180–3,628)	4,158 (3,774–4,542)	.001	3,842 (3,213–4,472)	4,344 (3,860–4,827)[e]	.005
Gonadotropin daily dose (IU/d)[d]	361 (345–378)	372 (343–400)	NS	371 (324–418)	373 (337–409)	NS
Follicles ≥ 13 mm	10.5 (9.3–11.9)	11.8 (9.6–14.5)	NS	10.9 (7.8–15.4)	12.3 (9.5–16.0)	NS
Oocytes retrieved	14.4 (12.8–16.2)	14.5 (11.8–17.8)	NS	13.0 (9.3–18.2)	15.5 (11.9–20.1)	NS
Mature oocytes (MII) retrieved	9.7 (8.4–11.2)	9.9 (7.7–12.7)	NS	9.1 (6.0–13.7)	10.3 (7.5–14.2)	NS
MII oocytes/total oocytes ratio	0.66 (0.62–0.71)	0.67 (0.59–0.76)	NS	0.68 (0.56–0.82)	0.67 (0.58–0.78)	NS
Oocytes/AFC ratio	1.09 (0.99–1.19)	1.26 (1.07–1.49)	NS	1.24 (0.95–1.62)	1.28 (1.04–1.57)	NS
Mature oocytes/AFC	0.73 (0.65–0.82)	0.85 (0.70–1.04)	NS	0.84 (0.61–1.17)	0.86 (0.67–1.10)	NS
Fertilization rate after ICSI (2PN/MII)	0.72 (0.65–0.80)	0.87 (0.72–1.00)	NS	0.85 (0.67–1.00)	0.88 (0.70–1.00)	NS

Cakmak, Fertil Steril, 2014

Luteal phase GnRHa trigger in random start fertility preservation cycles

Enis Ozkaya • Gabriel San Roman • Kutluk Oktay



Ozkaya, Oktay, JARG, 2012

hCG vs GnRHa for ovulation trigger
Letrozole + FSH

Characteristic	hCG (n = 47)	GnRHa (n = 27)
Age (years)	35.0 ± 4.3	33.6 ± 4.4
Body mass index (kg/m^2)	23.3 ± 4.2[a]	21.5 ± 2.5[a]
Baseline FSH (mIU/ml)	6.8 ± 2.7	8.2 ± 2.9
FSH stimulation duration (day)	9.6 ± 1.6	9.9 ± 1.6
Total gonadotrophin dose (IU)	2012.8 ± 603.5	1994.4 ± 549.1

Oktay, RBM Online, 2010

Parameter	hCG trigger (n =47)	GnRHa trigger (n= 27)	P-value
Peak estradiol (pg/ml)	**472.6 ± 345.5**	**695.5 ± 539.0**	**0.044**
Endometrial thickness (mm)	8.8 ± 1.8	8.4 ± 2.3	NS
Total oocytes	12.8 ± 7.7	16.4 ± 10.3	NS
Mature oocytes	**7.4 ± 4.9**	**11.9 ± 6.6**	**<0.001**
Oocyte maturation rate (%)	**68.5 ± 23.3**	**77.3 ± 21.1**	**0.049**
Two-pronuclei embryo[a]	**6.3 ± 4.6**	**9.3 ± 5.7**	**0.008**
Fertilization rate (%)	**74.0 ± 24.9**	**84.1 ± 11.1**	**0.027**
Drop in E2 from day 0 to 4 (%)	**79.0 ± 13.4**	**89.5 ± 6.3**	**0.013**
Mild or moderate OHSS (%)	**10 (21.3)**	**1 (3.7)**	**0.047**

Oktay, RBM Online, 2010

Consecutive COH Cycles with Letrozole

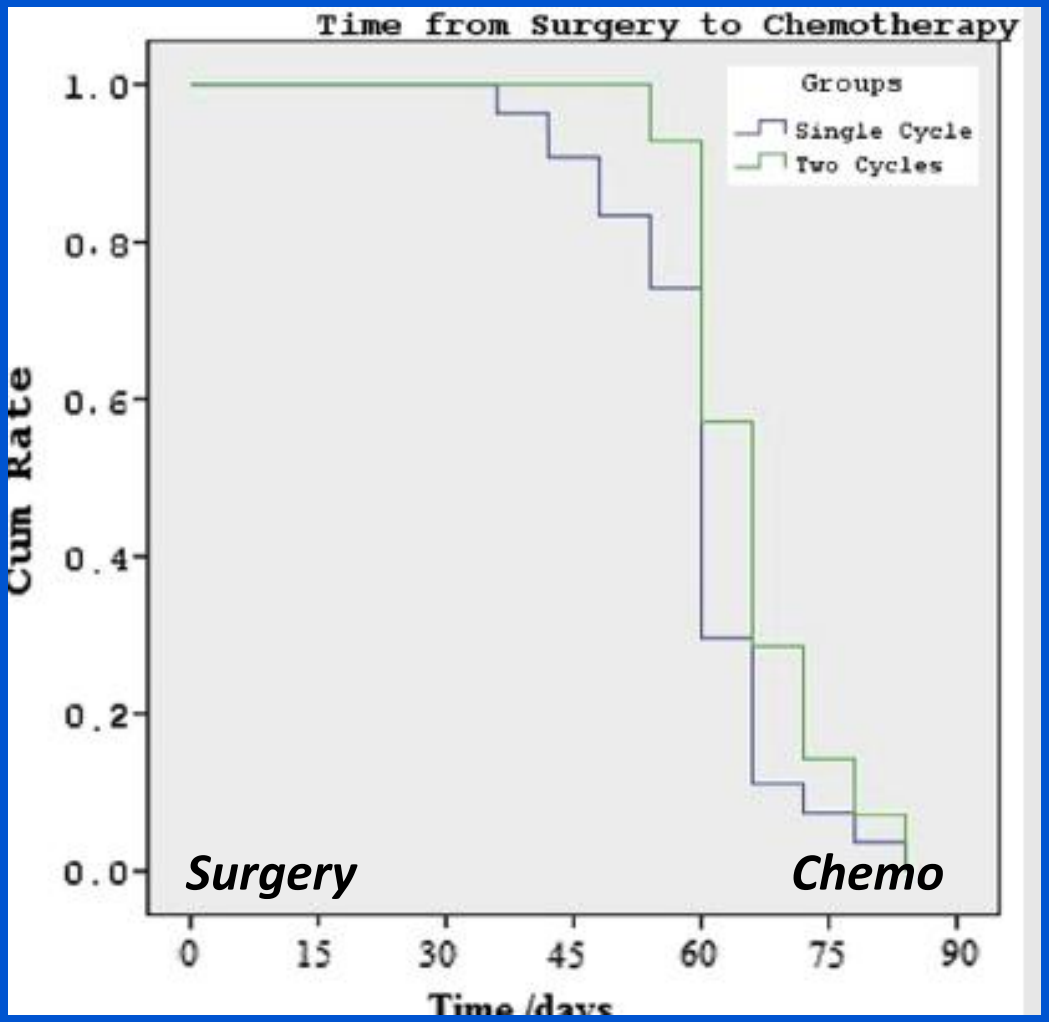
- **Single cycle stimulation n=61**
- **Two cycle stimulation n=17**

Comparison of fertility preservation cycle outcomes of patients with breast cancer after performing two cycles.

Outcome	Single cycle (n = 61)	Two cycles (n = 17)	*P* value
Oocytes (n)	9.1 ± 5.2	16.1 ± 13.2	.008
Mature oocytes (n)	6.2 ± 3.0	10.3 ± 7.7	.004
Inseminated oocytes (n)	6.0 ± 3.9	9.8 ± 5.5	.002
Fertilized oocytes (n)	5.4 ± 2.3	7.4 ± 3.9	.040
Embryos (n)	3.7 ± 3.1	6.4 ± 2.9	.019

Turan, Fertil Steril, 2013

Consecutive COH Cycles with Letrozole



Turan, Fertil Steril, 2013

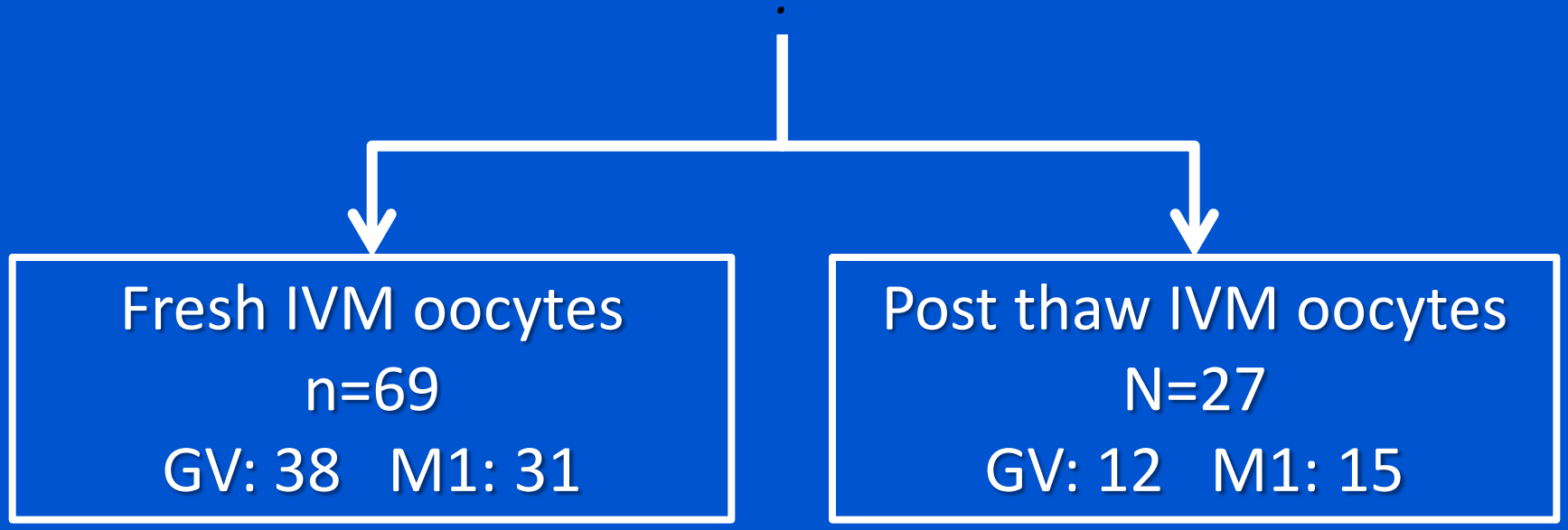
Luteal phase immature oocyte retrieval and IVM

- Single patients aged 21,30, and 40 years seeking fertility preservation before chemo
- 7,5, and 7 immature oocytes retrieved
- Following IVM 5,3, and 5 MII oocytes vitrified

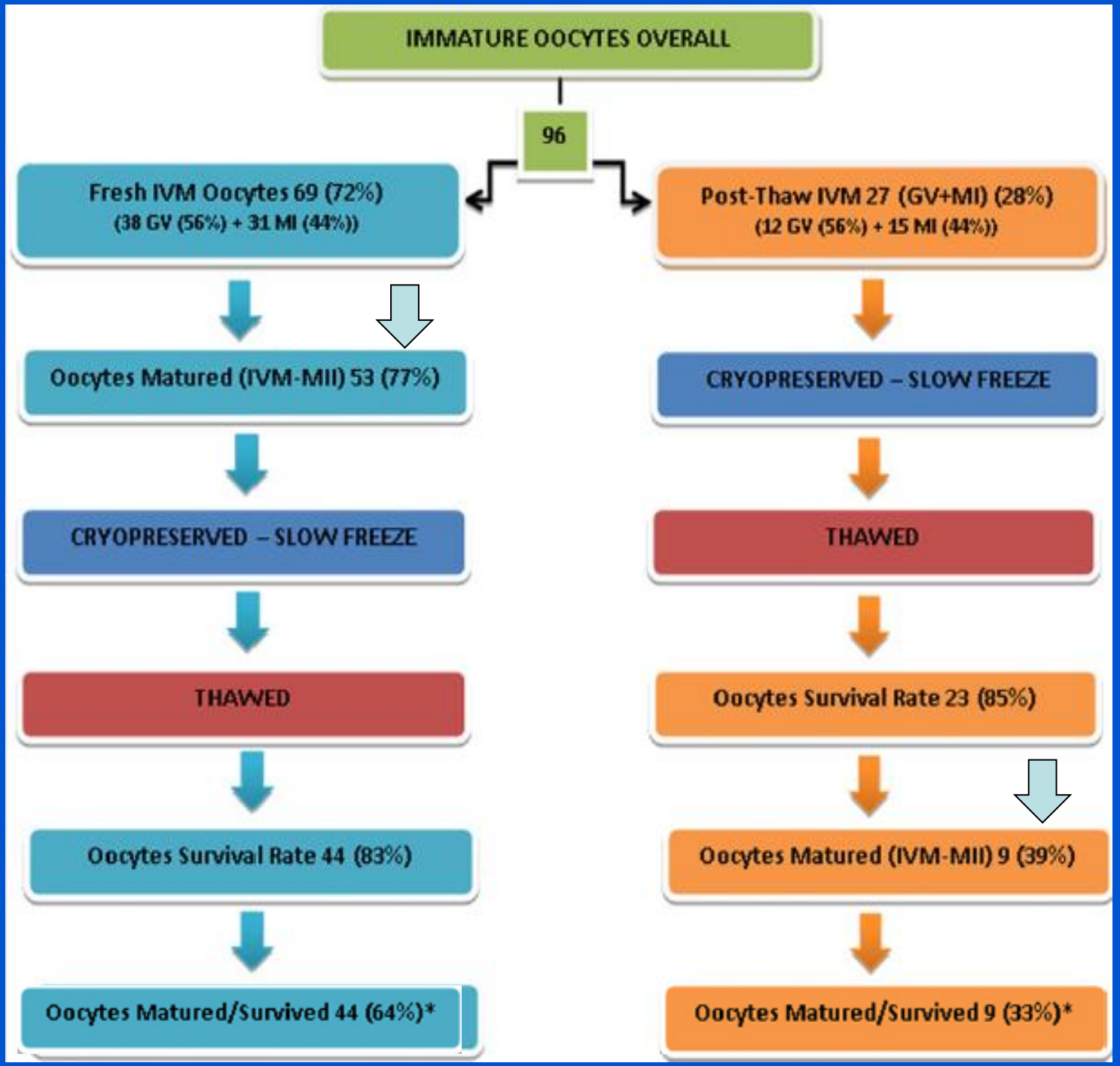
Demirtas, RBM Online, 2008

Immature Oocyte Cryopreservation
Which comes first? IVM vs. Cryopreservation

- Donated oocytes following standard GnRH-ant protocol
  - n=71 patients, 96 oocytes



Lee JA, Fertil Steril. 2013

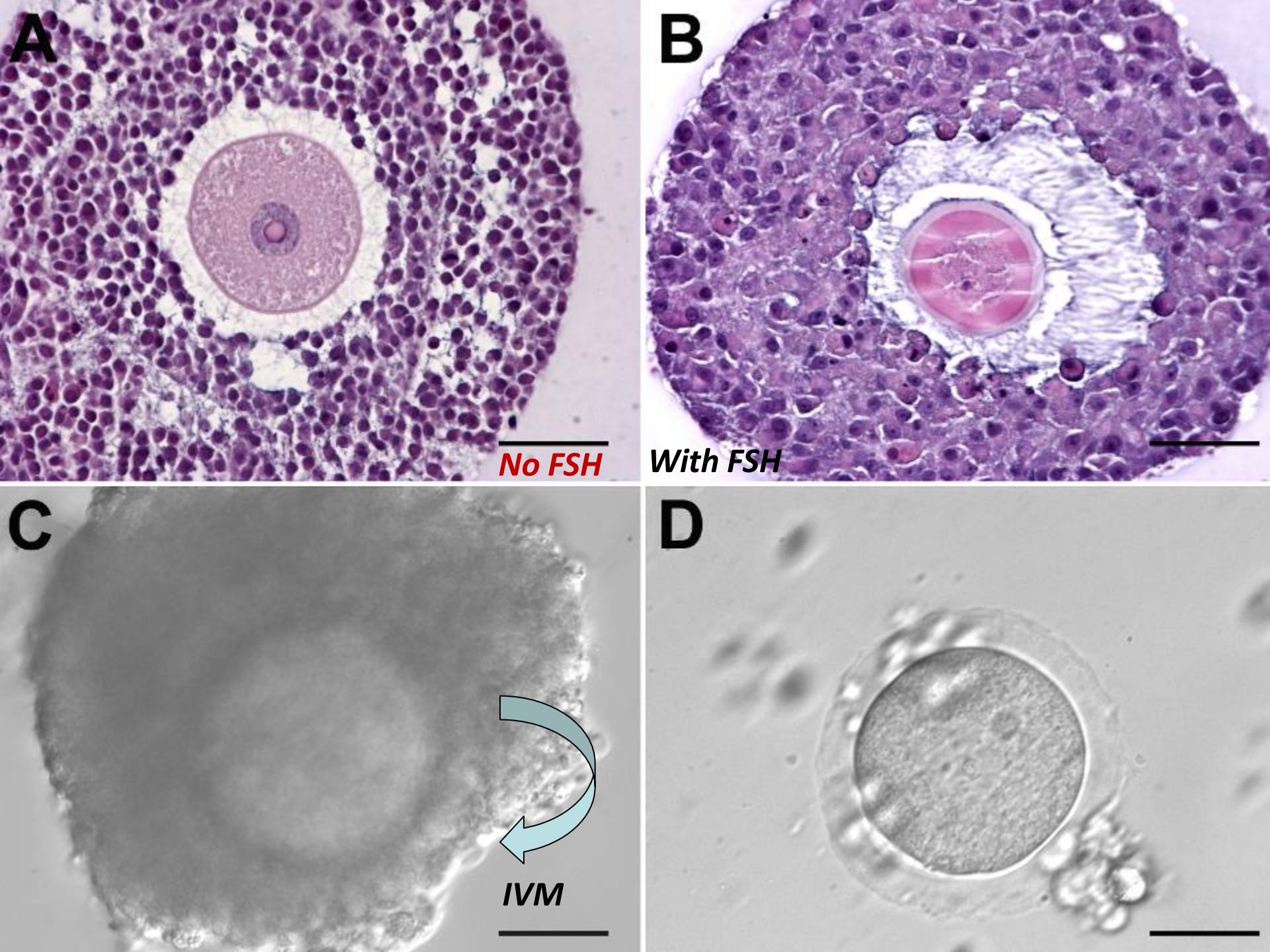

IMMATURE OOCYTES OVERALL
96
Fresh IVM Oocytes 69 (72%)
(38 GV (56%) + 31 MI (44%))
Post-Thaw IVM 27 (GV+MI) (28%)
(12 GV (56%) + 15 MI (44%))
Oocytes Matured (IVM-MII) 53 (77%)
CRYOPRESERVED – SLOW FREEZE
CRYOPRESERVED – SLOW FREEZE
THAWED
THAWED
Oocytes Survival Rate 23 (85%)
Oocytes Survival Rate 44 (83%)
Oocytes Matured (IVM-MII) 9 (39%)
Oocytes Matured/Survived 44 (64%)*
Oocytes Matured/Survived 9 (33%)*

BIOLOGY OF REPRODUCTION 84, 689–697 (2011)
Published online before print 1 December 2010.
DOI 10.1095/biolreprod.110.088674

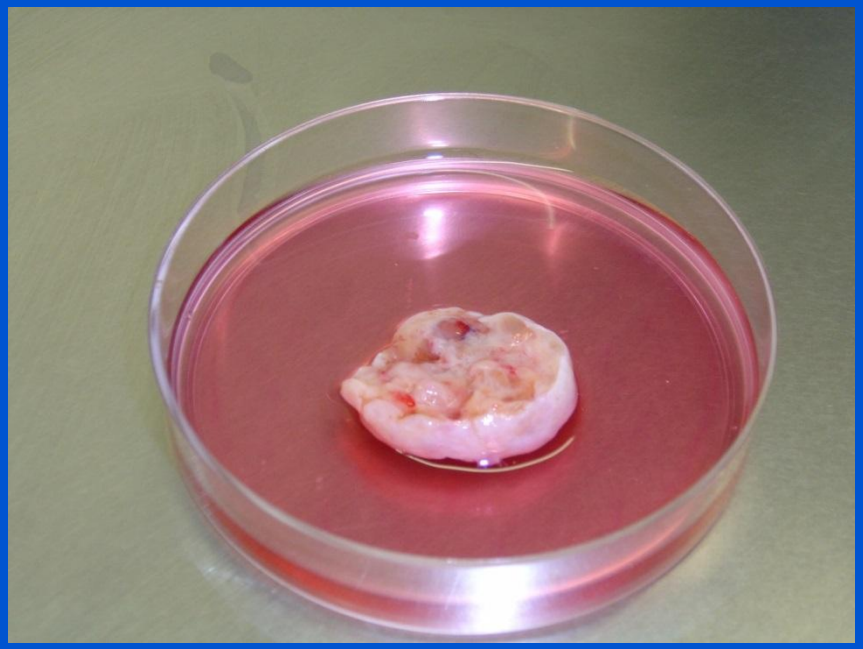
In Vitro Oocyte Maturation and Preantral Follicle Culture from the Luteal-Phase Baboon Ovary Produce Mature Oocytes[1]

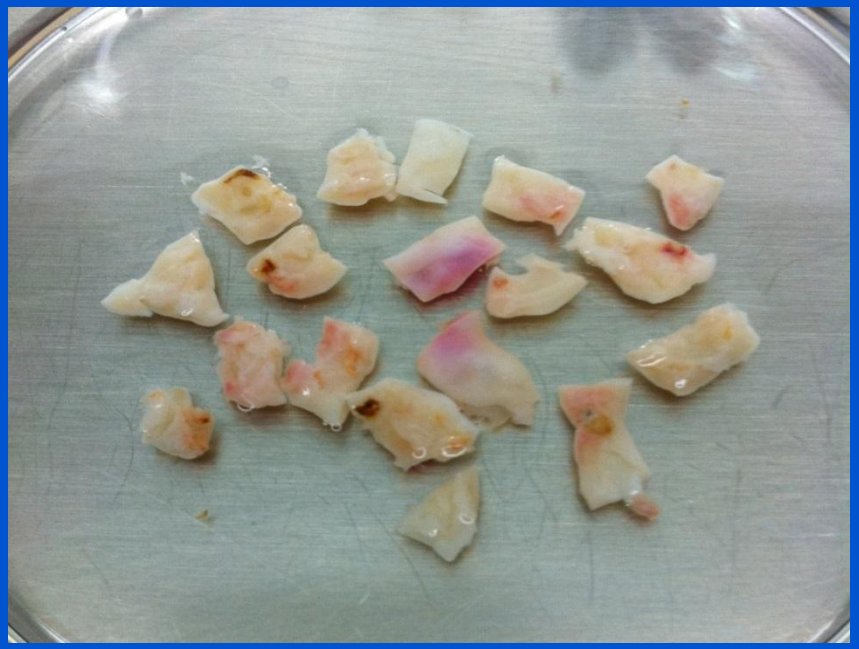
Min Xu,[3,4] Asgerally T. Fazleabas,[5] Ariella Shikanov,[4,6,7] Erin Jackson,[3,4] Susan L. Barrett,[3,4] Jenny Hirshfeld-Cytron,[3,4] Sarah E. Kiesewetter,[3,4] Lonnie D. Shea,[4,6,7] and Teresa K. Woodruff[2,3,4]

- Oocytes from small antral follicle COC with «multiple cumulus layers» (42%) were more likely to resume meiosis and progress to metaphase II (MII) than oocytes with a «single layer of cumulus cells» or less (23% vs. 3%, respectively).
- MII oocytes- ICSI →Morula (25%)


A
No FSH
B
With FSH
C
IVM
D

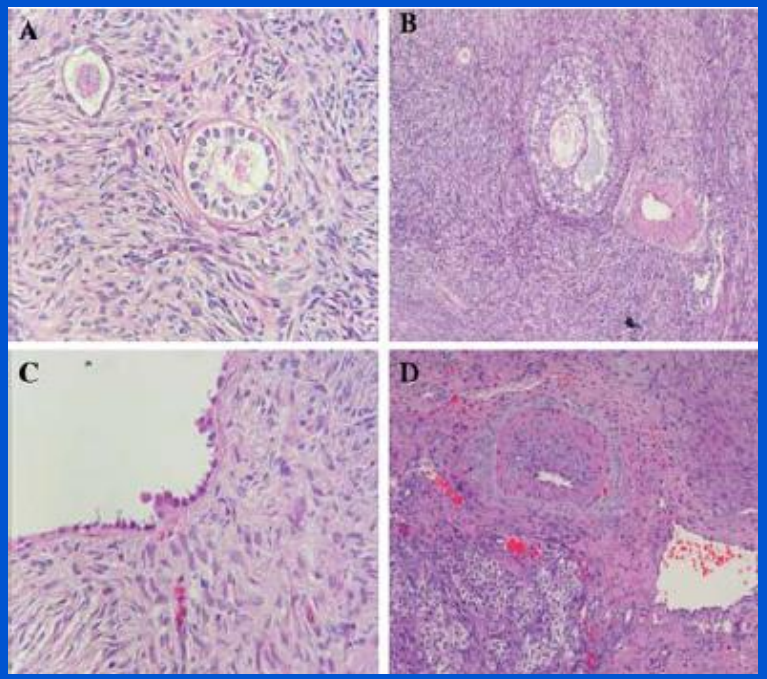
Ovarian Tissue Cryopreservation in Breast Cancer



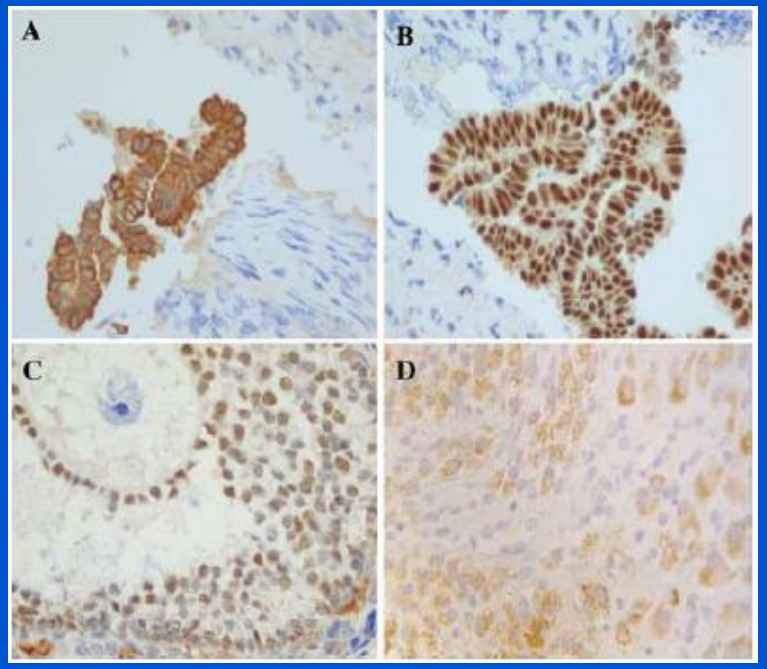


Early stage breast cancer
Ovarian metastasis (histology and IHC)

IHC



Standard histology

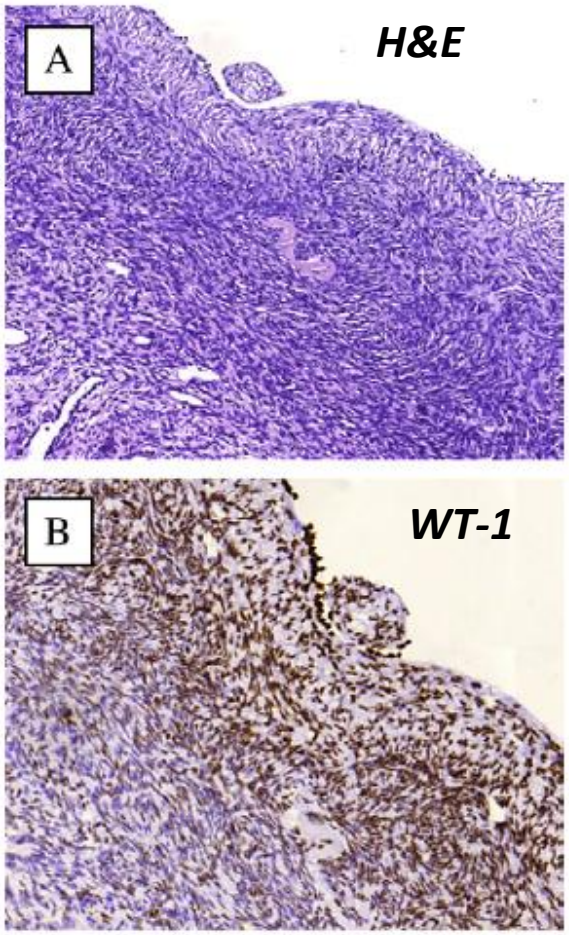


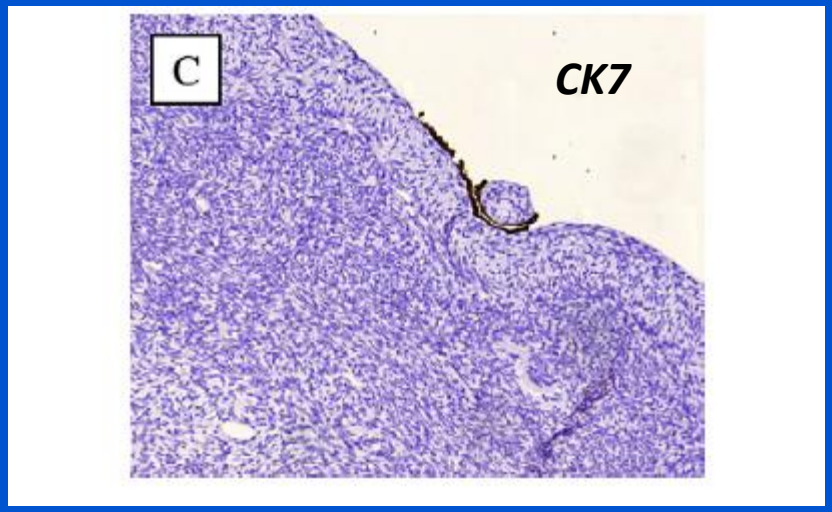
Ovarian involvement: 0/63 patients (100 cortical pieces) undergoing ovarian cryopreservation

Sanches M, Hum Reprod, 2009

Early stage breast cancer
Ovarian metastasis (histology and IHC)

Ovarian cortex from one of the 51 women with breast cancer. Histologic and immunohistochemical analysis shows no signs of metastatic infiltration. (**A**) H&E. (**B**) WT-1. (**C**) CK-7. Magnification, ×10.





Ovarian involvement: 0/51

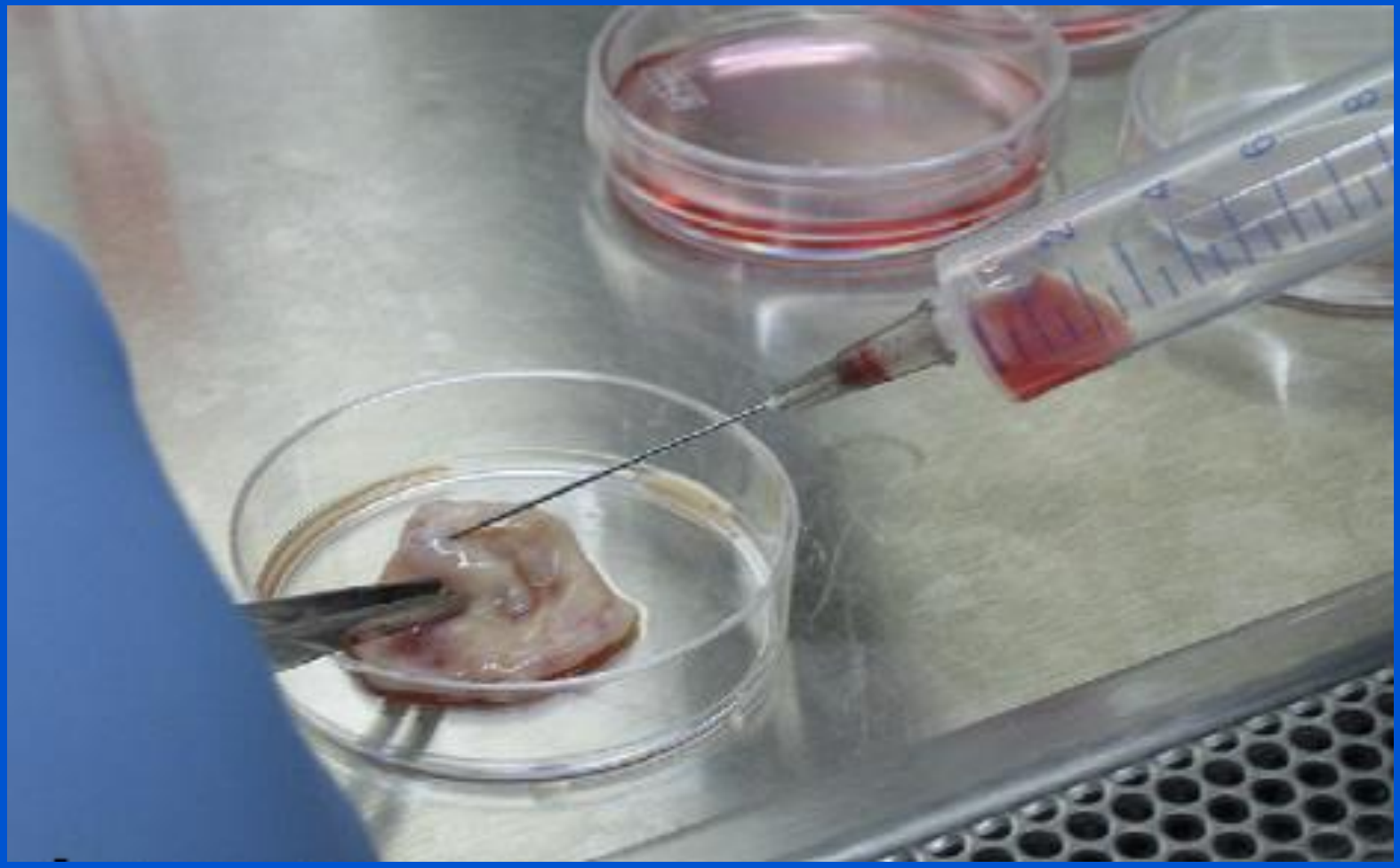
Rosendahl, Fertil Steril, 2011

Non gynecoogic ovarian metastasis
Analysis of 150 cases

- Colon 30%
- Stomach 16%
- Appendix 13%
- Breast 13%
- Pancreas 12%
- Biliary tract 15%
- Liver 4%

Alvarado-Cabrero I, Anal Quant Cytol Histol. 2013

Ovarian Tissue Cryopreservation + IVM



Ovarian tissue cryopreservation and IVM

- 57 patients undergoing ovarian tissue cryo., aged 8-35 years
- Antral fluid was collected from removed ovary
- 266 oocyted retrieved
  - Degenerated 28
  - GV 200
  - MI 35
  - MII 3
- 24-28h IVM before cryopreservation (maturation rate 31%)

Fasano, Reprod Biol Endocrinol. 2011

Combining ovarian tissue cryobanking with retrieval of immature oocytes followed by in vitro maturation and vitrification: an additional strategy of fertility preservation

Jack Y. J. Huang, M.D., Togas Tulandi, M.D., M.H.C.M., Hananel Holzer, M.D., Seang Lin Tan, M.D., M.B.A., and Ri-Cheng Chian, Ph.D.

Department of Obstetrics and Gynecology, McGill University Health Center, McGill University, Montreal, Quebec, Canada

Patient no.	Age	Cancer type	Day of menstrual cycle	Surgical procedure	No. of GV oocytes retrieved from ovarian tissue	No. of MII oocytes following IVM	Maturation rate (%)	No. of MII oocytes vitrified
1	21	Hodgkin lymphoma	2	Ovarian wedge resection	3	3	100	3
2	35	Breast	19	Oophorectomy	1	1	100	1
3	18	Hodgkin lymphoma	5	Ovarian wedge resection	4	2	50	2
4	38	Rectal cancer	14	Ovarian wedge resection and oophoropexy	3	2	67	2

Huang, Fertil Steril, 2008

Improving fertility preservation in cancer: ovarian tissue cryobanking followed by ovarian stimulation can be efficiently combined

- Study group : n=12 patients
- Control group : n=28 patients
- In the study group, half ovarian tissue was removed

Hueber Zeeb, Fertil Steril, 2011

Main characteristics and stimulation outcome of patients with (study group) and without (control group) ovarian biopsy.

Parameter	Study group (n = 12)[a]	Control group (n = 28)
Age of patients (y), mean ± SD	31.1 ± 6.2	27.6 ± 5.0
Days of stimulation, mean ± SD	10.2 ± 2.6	10.6 ± 2.5
Dosage of stimulation (IU), mean ± SD	2527 ± 942	2255 ± 945
Total no. of aspirated oocytes	145	367
Aspirated oocytes per patient, n	12.1	13.1
MII-oocytes/aspirated oocytes, %[b]	65.5	83.8
No. of MII oocytes (processed for ICSI)[c]	44	66
Fertilization rate/MII oocytes, %	75.0	60.6

Subgroup analysis of biopsied and nonbiopsied ovaries in the study group.

Parameter	Oocyte source	
	Biopsied ovaries	Nonbiopsied ovaries
Total no. of aspirated oocytes	70	75
Aspirated oocytes per patient, n[a]	5.8	7.5
MII oocytes/aspirated oocytes, %	70.0	61.3
No. of MII oocytes (processed for ICSI)[b]	25	19
Fertilization rate/MII oocytes, %	80.0	68.4

Hueber Zeeb, Fertil Steril, 2011

Oocyte retrieval from removed ovary

Characteristic of the patient's menstrual cycle	Patients n	Mean age ± SEM	Mean fragments of ovarian tissue (range)	Oocytes retrieved n (range)	Mean oocytes retrieved /fragment	Mean oocytes retrieved /patients	Stage at collection (%)			IVM rate
							GV	MI	MII	
OC	11	23.1 ± 1.3	20.1 (10-29)	38 (0-9)	0.17 ± 0.07[a]	3.4 ± 1.06[a]	71%	29%	0%	42.1%
Natural cycle FP	19	26.3 ± 1.5	21.6 (7-32)	69 (0-15)	0.17 ± 0.06[a]	3.6 ± 1.09[a]	80%	19%	1%	27.9%
Natural cycle LP	16	27.9 ± 1.1	18.1 (12-26)	44 (0-13)	0.15 ± 0.05[a]	2.8 ± 0.83[a]	84%	14%	2%	39.5%
Post-partum	5	31 ± 2.2	26.3 (16-36)	33 (1-12)	0.23 ± 0.12[a]	6.6 ± 1.86	91%	6%	3%	28.1%
Unknown	2	29.5 ± 0.5	20-32	8 (0-8)	0.15 ± 0.26	4 ± 4	100%	0%	0%	12.5%
Prepubertal	4	9.2 ± 1.4	31.7 (17-40)	46 (2-22)	0.36 ± 0.28[b]	11.5 ± 4.27[b]	93%	7%	0%	23.9%
Total	57	26 ± 0.9	21.8 (7-40)	238 (0-22)	0.19	4	84%	14.7%	1.3%	31%

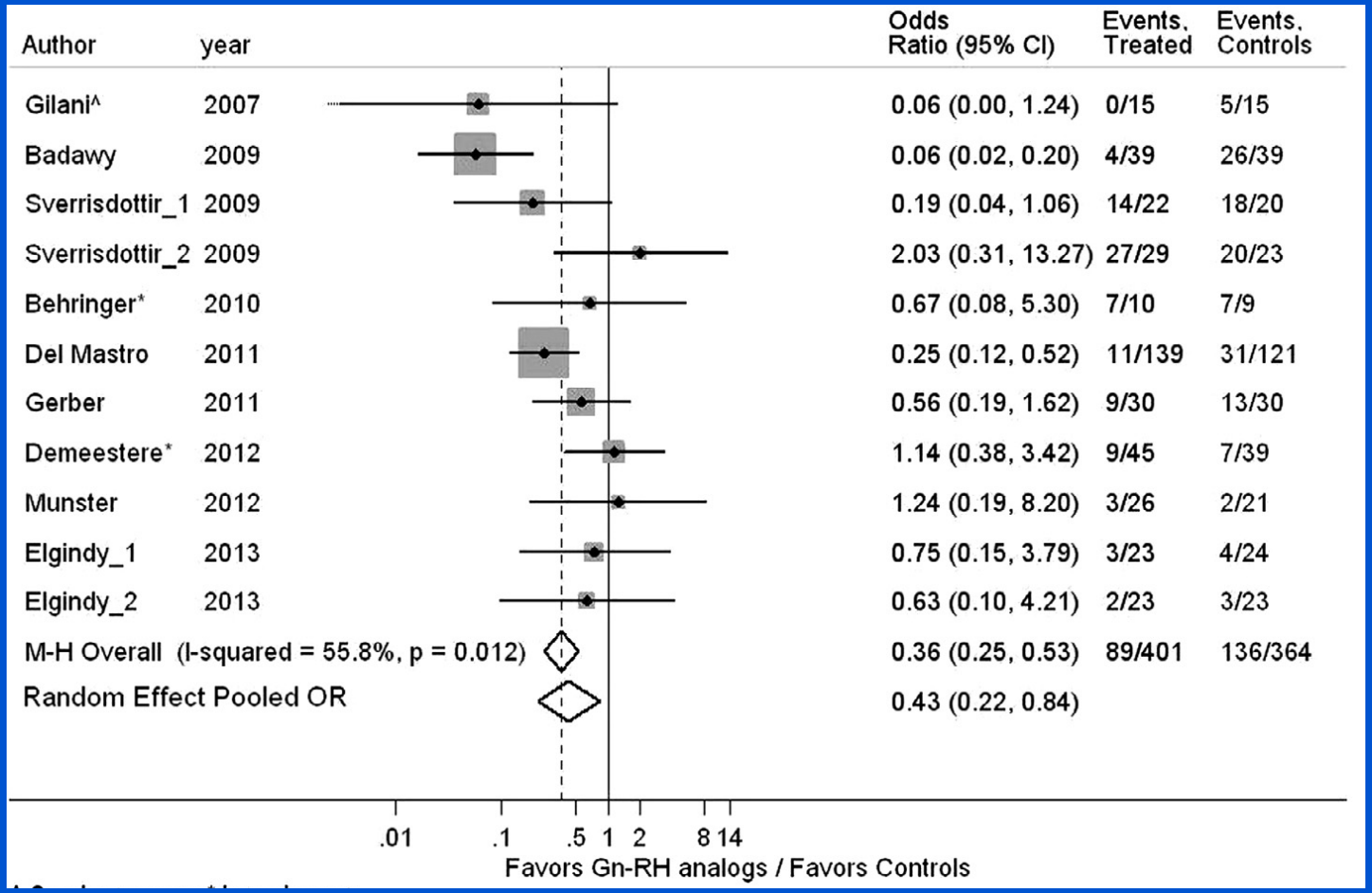
- Oocytes were retrieved regardless of menstrual cycle period or contraception

Fasano, Reprod Biol Endocrinol. 2011

Fertility preservation in BRCA1 positive breast cancer patients

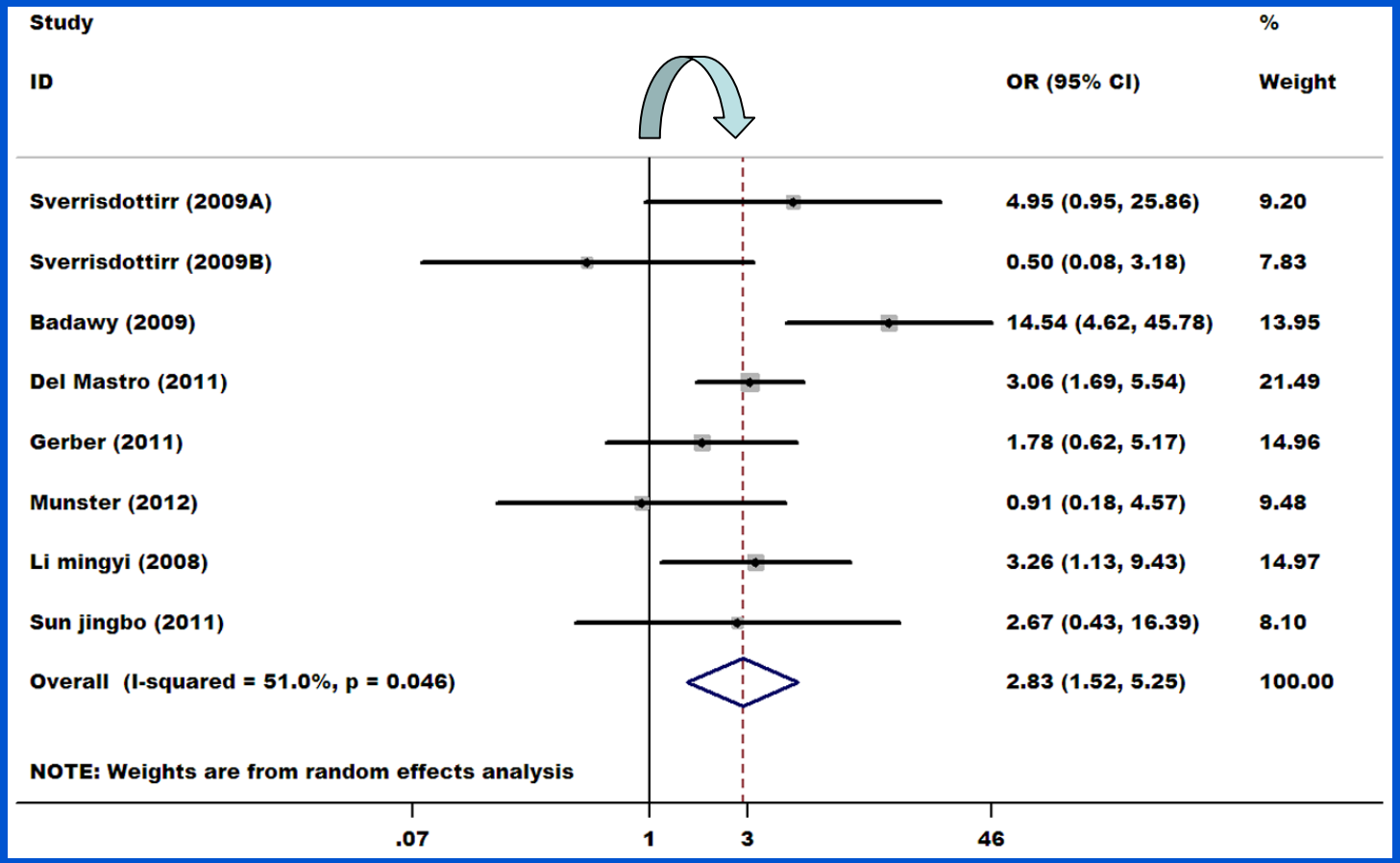
- A possible negative impact of subsequent conception on breast cancer survivors – «not demonstrated»
- Coexistence of an ovarian cancer
- PGD to avoid transmitting BRCA mutations
- Decreased ovarian reserve !! Deficient DNA repair make oocytes more vulnerable

GnRHa Cotreatment during Chemo



Wang C, Plos One 2013

GnRHa Cotreatment during Chemo
Spontaneous menstruation



Del Mastro, Cancer Treat Reviews, 2014

GnRHa Cotreatment during Chemo
Ovarian reserve

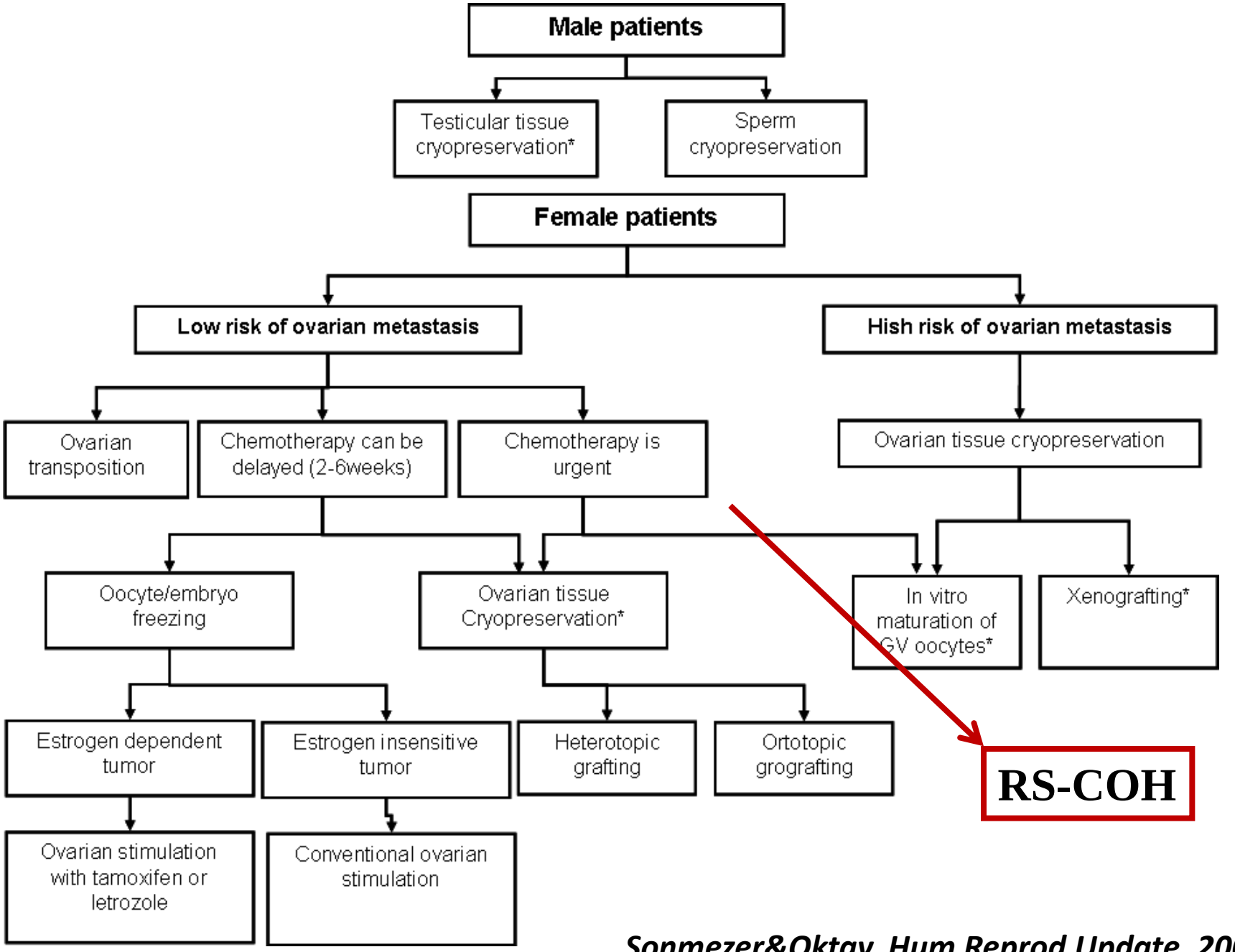
- One trial showed higher anti-Müllerian hormone levels (improved ovarian reserve) after use of a GnRH agonist (1.4 ± 0.35 versus 0.5 ± 0.15 ng/mL in untreated controls)
- After 1 year follow up 20% of patients in each group developed POF, «showing no evidence of ovarian protection»

Demestree, J Clin Oncol, 2013

GnRHa Cotreatment during Chemo Concerns

- Normality of offspring – DNA breaks during chemo (Down Syndrome)
- Increased gonadodoxicity by decreasing the effect of detoxifiying enzymes – GST
- Extrapituitary GnRH receptors
  - Antigonadotropic, antiproliferative, antiapoptotic effects
  - Possible reduction of chemo effect on breast cancer
- Definition of ovarian failure is heterogeneous
- No study showed increased fertility

Sonmezer&Oktay , The Oncologist, 2007



Sonmezer&Oktay, Hum Reprod Update, 2004

Conclusions - I

- Patients should be referred as early as possible to increase the success rate of FP
- In an emergent setting, random start COH can be safely and effectively performed
- Breast cancer recurrence risk seems not increased following Letrozole-IVF

Conclusions - II

- Ovarian tissue cryopreservation appears safe in early stage breast cancer
- Ovarian tissue cryopreservation can be performed along with COH-embryo/oocyte cryopreservation
- The possibility of dormant tumor cell growth should be considered in ER dependent breast cancer following ovarian transplantation
- More data establishing the safety of ovarian suppression in cancer patients and its long-term efficacy in preserving fertility (not just resumption of menses) are needed.