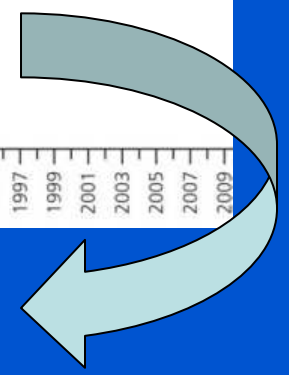
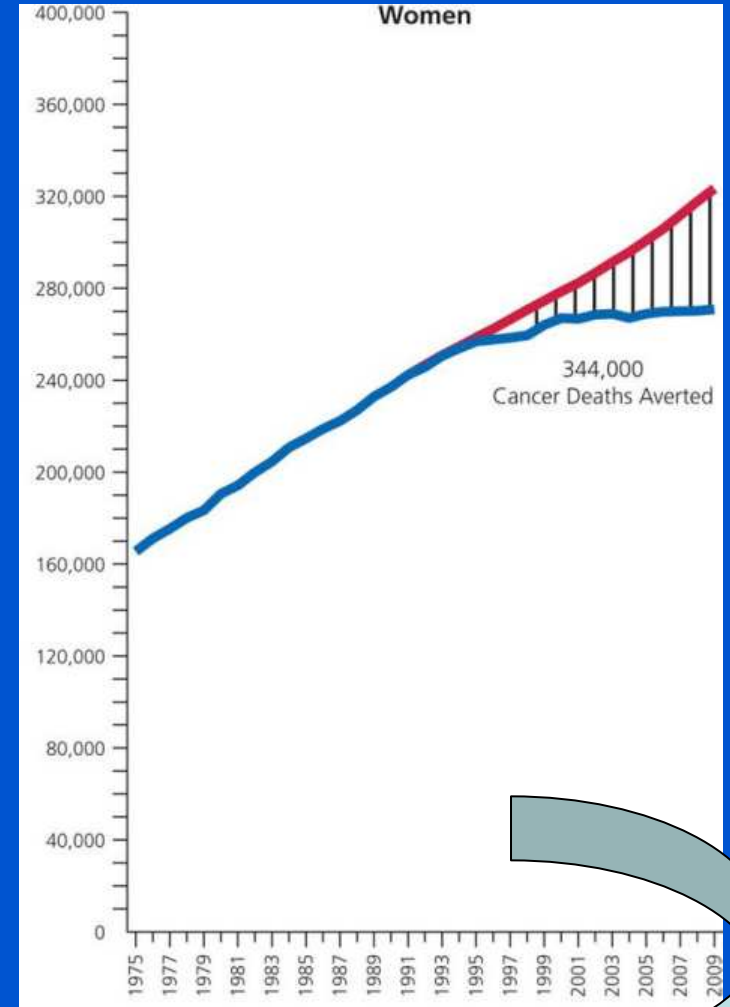
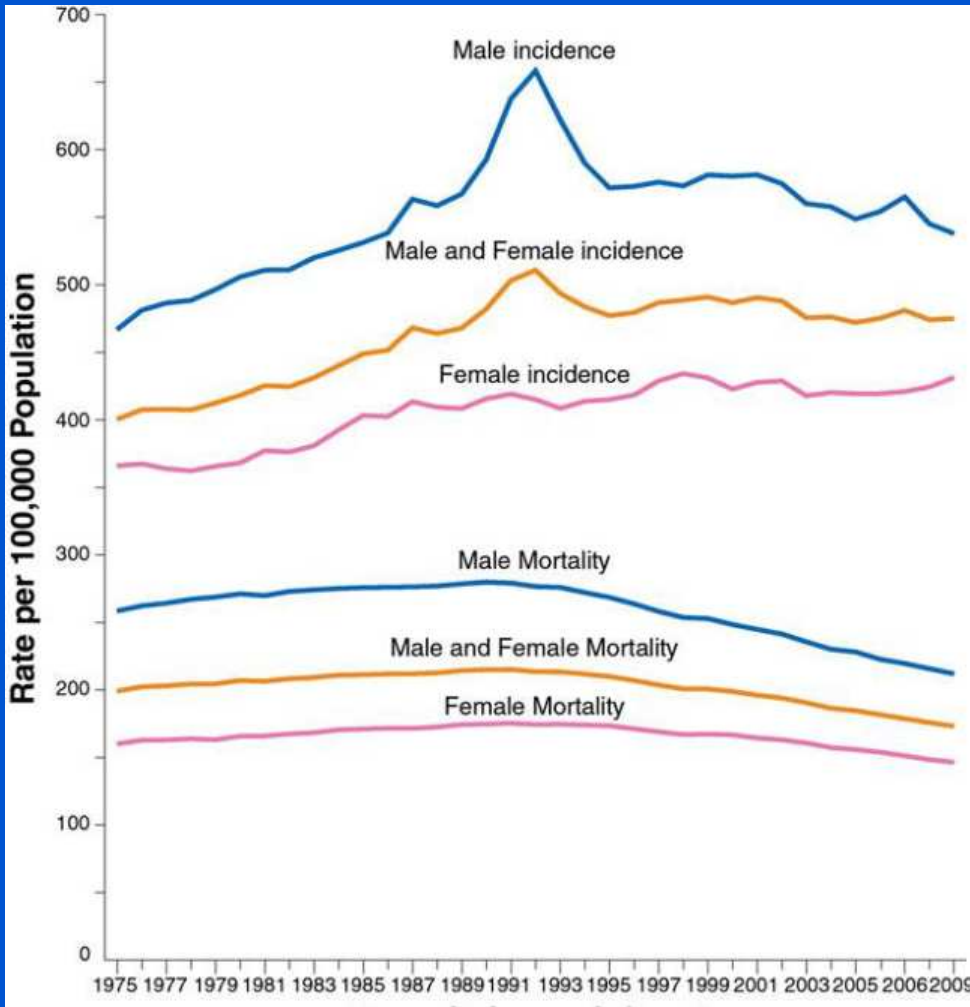




Ovaryan Doku Kriyoprezervasyonu

Murat Sönmezer

Ankara Üniversitesi Tıp Fakültesi



Uzun dönem tedavi ile ilişkili komplikasyonlar

Kanser insidansı

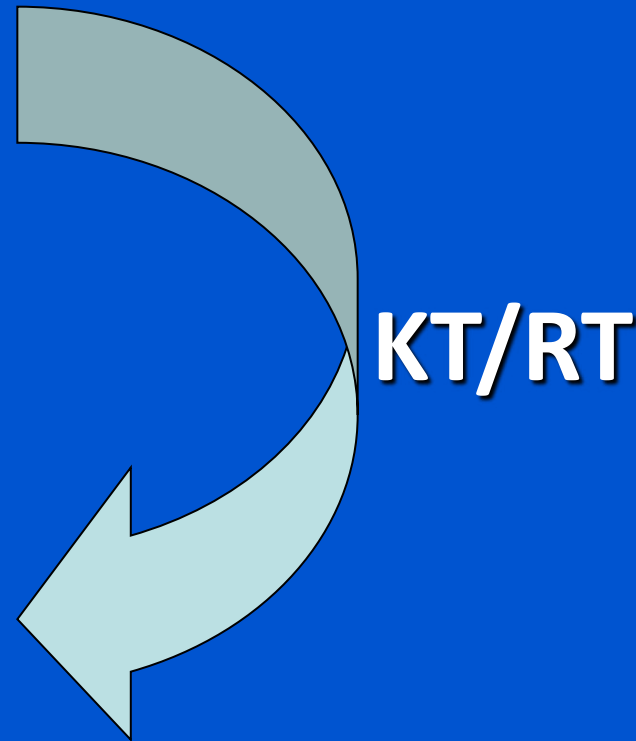
0-40 $\Rightarrow 1/50$

40-50 $\Rightarrow 1/11$

Yaşam boyu $\Rightarrow 1/3$

POF $\Rightarrow \%1 (<40)$

$\Rightarrow \%0.1 (<30)$



Kemoterapi ajanları – gonadal hasar

Yüksek risk

- «Cyclophosphamide»
- Cholorambucil
- Melphalan/Busulfan
- Nitrogen mustard
- L-phenylalanine mustard
- Procarbazine/dacarbazine

Orta risk

- Cisplatin / paclitaxel
- Vinblastine
- Adriamycin
- Etoposide
- Carmustine

Düşük risk/risk yok

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

Yeni ajanlar

- Irinotecan
- Imatinib

Radyoterapi – gonadal hasar

- LDL 50 \Rightarrow 2 Gy

Howell, Shalet 1998, Wallace 2003

Endikasyonlar

- Çocukluk kanserleri
 - Hodgkin/non-Hodgkin lymphoma
 - Leukemias ...
- Erişkin kanserleri
- Turner Sendromu
- Otoimmün ve hematolojik hastalıklar
- Benign ovaryan lezyonlar
- Pelvik RT
- HSCT (malignant, genetik, hematolojik, otoimmün hastalıklar)
- Profilaktik ooporektomi

Fertilite Prezervasyonu Seçenekler

- Embriyo kriyoprezervasyonu
- Matür/immatür oosit kriyoprezervasyonu
- In vitro maturasyon
- Overyan transpozisyon
- Donor oosit uygulaması

- **Overyan doku kriyoprezervasyonu**
- Xenografting
- GnRHa tedavisi
- Antiapoptotik ajanlar \Rightarrow SP1P

Ovarian kriyo. - oosit/embriyo kriyo.

Overyan kriyo.

Oosit /embriyo kriyo.

Avantaj

- KT gecikmez
- Prepubertal dönemde tek seçenek
- Endokrin fonksiyonlar korunur

- Teknik standard
- Yüksek başarı
- Yaygın kullanım

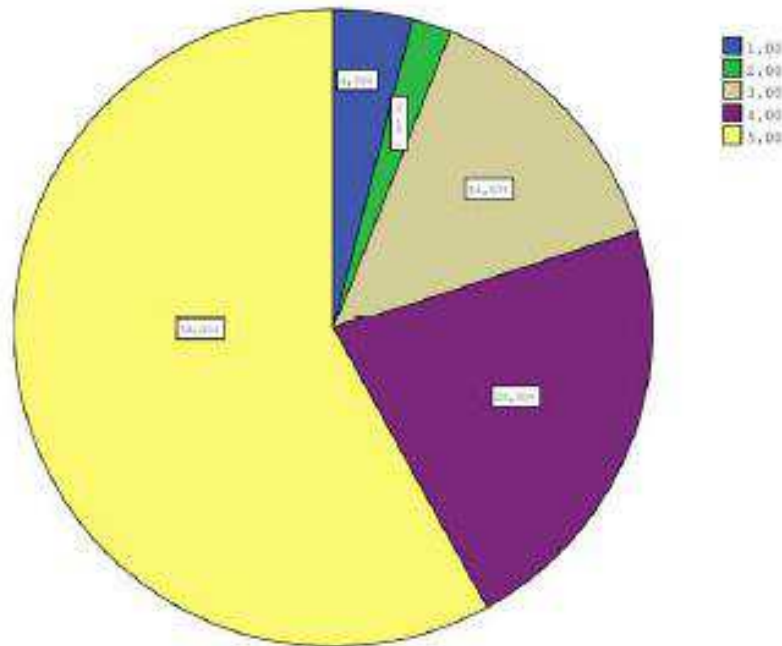
Dezavantaj

- L/S gerekli
- ~30 gebelik
- Kanser yayılımı?

- 2-3 hafta gerekli
- Sınırlı sayıda oosit/embriyo
- Çocuklarda zor

Hastalık ile mücadele ve yaşam kalitesi üzerindeki etkisi

n=86



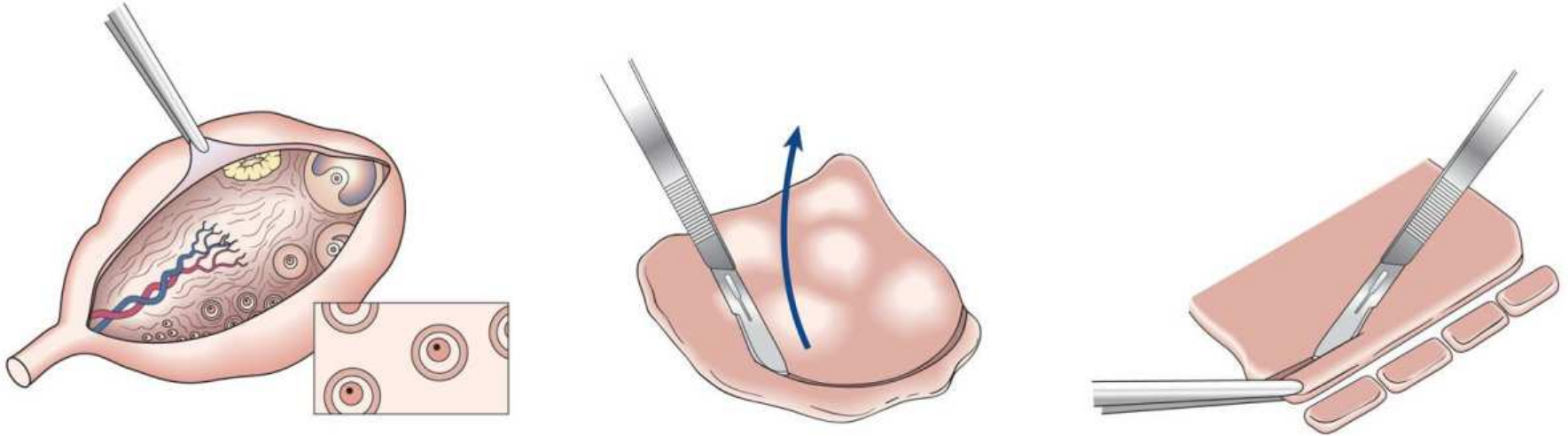
Graph 4: Motivational morale contribution of ovarian cryopreservation to patients' struggle with a malignancy. (1-5, 1; no effect, 5; maximum boosting morale)

Neden over dokusu kriyo?

Ovaryan korteks \Rightarrow «%95 primordial folikül»

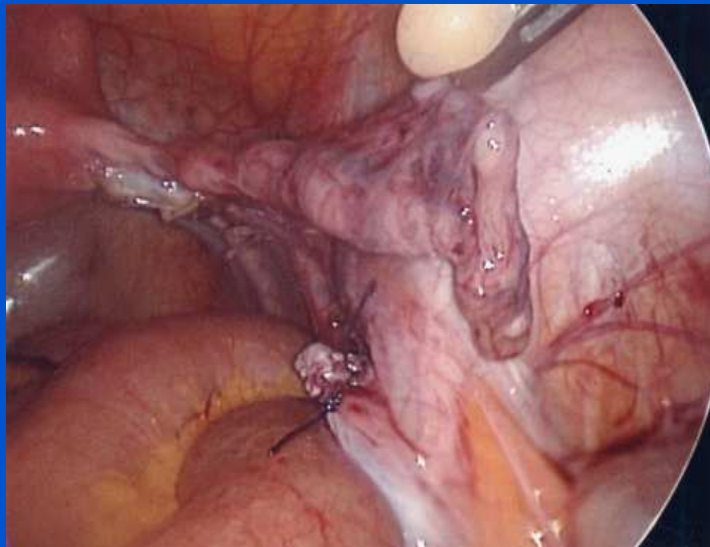
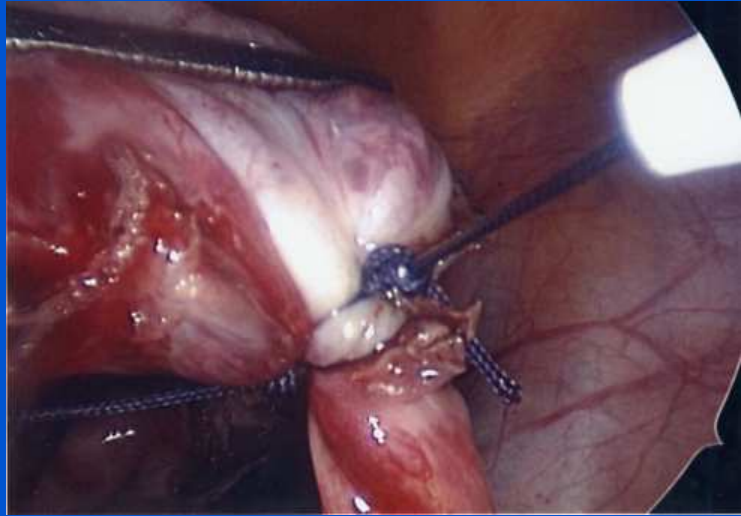
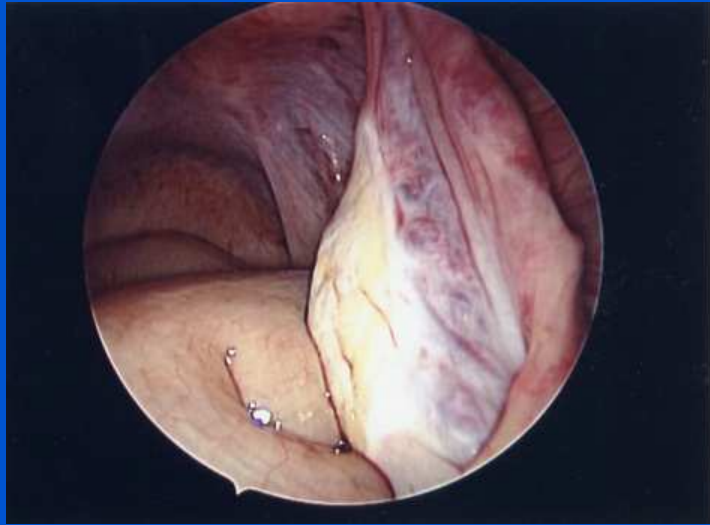
- Arest foliküller / I mayoz, profaz, diploten
- \uparrow yüzey/hacim
- Zona pellusida yok
- Fertilite prezervasyonu / endokrin fonksiyonlar

Overyan kortikal dondurma



- 5x5x1 mm
- 3x5x1 mm

Overyan doku eldesi



Over tutulum riski

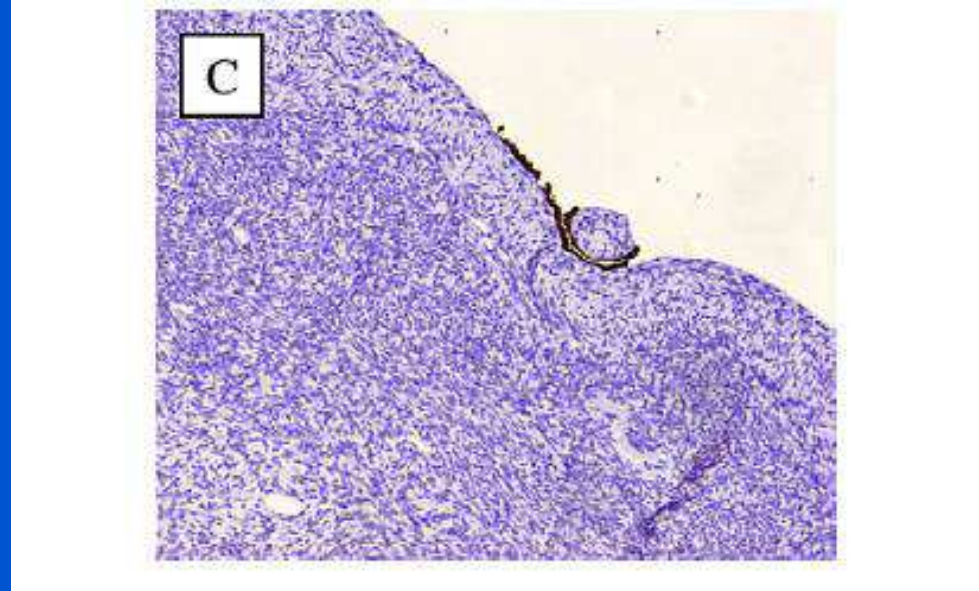
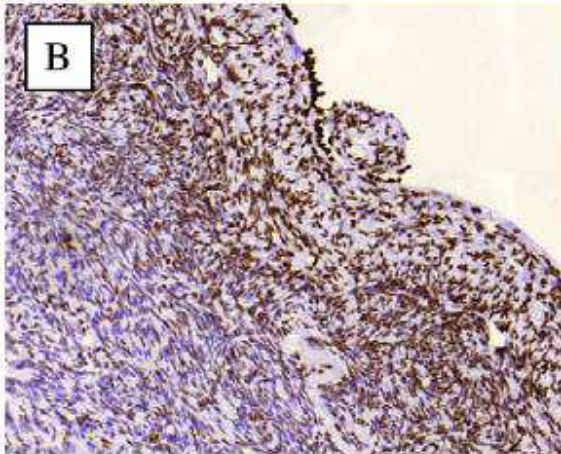
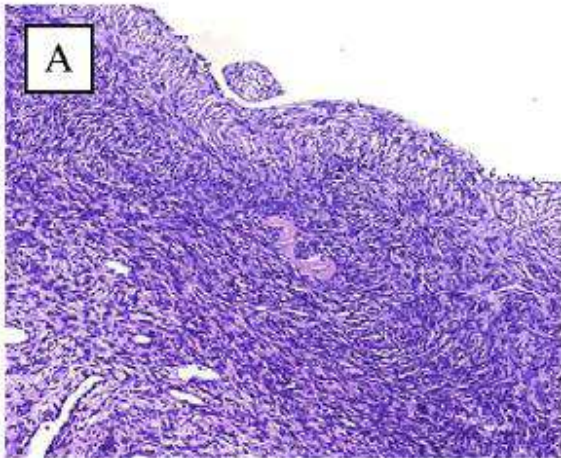
Low risk of ovarian involvement	Squamous cell carcinoma of the cervix Ewing's sarcoma Breast cancer Stage I-III Infiltrative ductal Wilms' tumor Non-Hodgkin's lymphoma Hodgkin's lymphoma Osteogenic sarcoma Non-genital rhabdomyosarcoma
Moderate risk of ovarian involvement	Breast cancer Stage IV Infiltrative lobular Colon cancer (including tumors of rectum and appendix) Adeno/adenosquamous carcinoma of the cervix Upper gastrointestinal system malignancies
Cancers with high risk of ovarian involvement	Leukemia Burkitt lymphoma Neuroblastoma Genital rhabdomyosarcoma

*Sonmezer&Oktay
Cell and Tissue Res, 2005*

Erken evre meme CA

Overyan metastaz

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, $\times 10$.



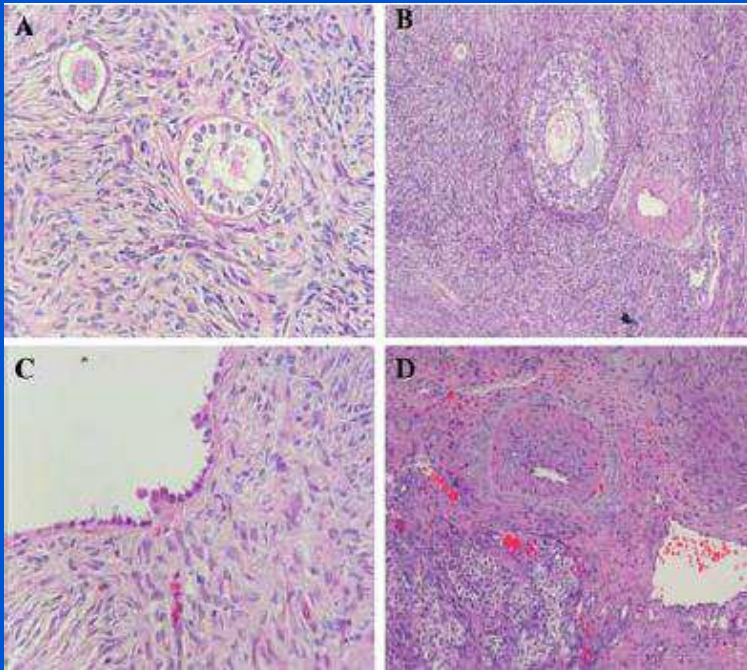
Overyan metastaz : 0/51

Rosendahl, Fertil Steril, 2011

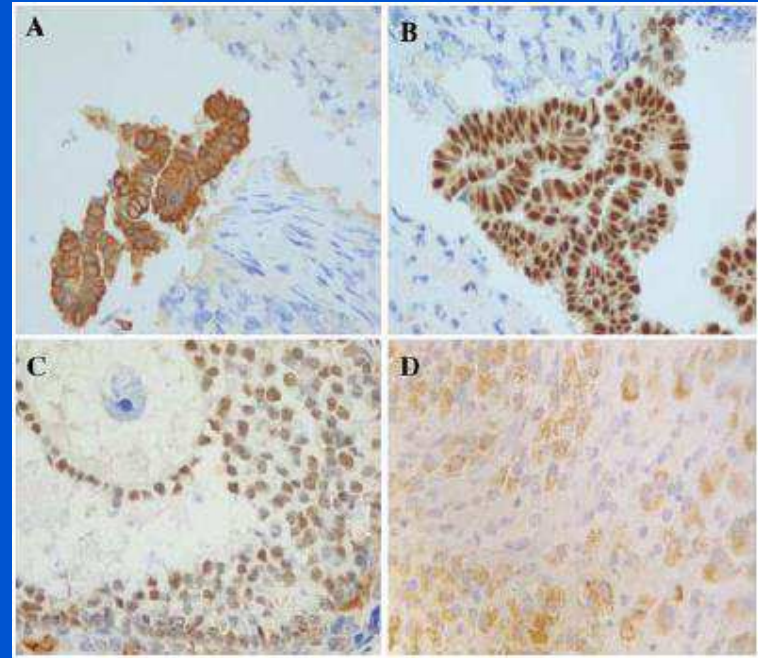
Erken evre meme CA

Overyan metastaz (histoloji & IHC)

IHC



Standard histoloji



Overyan tutulum: overyan kriyo - 0/63 hasta (100 kortikal parça)

Fertility prezervasyonu BRCA1 (+) meme CA

- Olası negatif etki – gösterilmemiş
- Overyan kanser birlikteliği
- BRCA germline mutasyon eliminasyonu – PGD
- Azalmış over rezervi !! Defektif DNA tamir mekanizmaları

Non-jinekolojik kanserler

Overyan metastaz - 150 hasta

- Kolon 30%
- Mide 16%
- Appendiks 13%
- Meme 13%
- Pankreas 12%
- Bilier trakt 15%
- KC 4%

Hodgkin lenfoma

Overyan metastaz

- Lenfoma - 26 hasta
- Standart histoloji ve IHC ile met yok

Lösemi / overyan metastaz

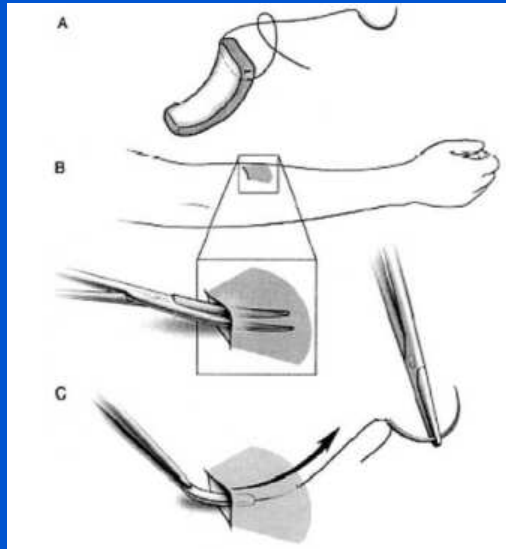
Patient no.	Age at OTC, y	Pathology	Gonadotoxic chemotherapy before OTC*	Molecular markers	Ovarian tissue PCR	Macroscopic evaluation of grafts	Microscopic evaluation of grafts	Microscopic evaluation of mouse livers
1	31	CML	0	BCR-ABL	Positive	Normal	Normal	Normal
2	17	CML	0	BCR-ABL	Negative	Normal	Normal	Normal
3	30	CML	0	BCR-ABL	Positive	Normal	Normal	Normal
4	19	CML	0	BCR-ABL	Negative	Normal	Normal	Normal
5	32	CML	0	BCR-ABL	Negative	Normal	Normal	Normal
6	19	CML	0	BCR-ABL	Negative	Normal	Normal	Normal
7	15	ALL	1, 8	BCR-ABL	Positive	Normal	Normal	Normal
8	21	ALL	0	E2A-PBX1	Positive	2 leukemic ovarian masses	Leukemic invasion	Normal
9	27	ALL	(2, 3, 4, 5, 6) ×2	Ig and TCR- γ	Negative	Normal	Normal	Normal
10	20	ALL	1, 2, 3, 4, 7, 8	Ig and TCR- γ	Positive	Normal	Normal	Normal
11	12	ALL	0	Ig	Positive	Normal	Normal	Normal
12	15	ALL	1, 2	None	NA	Diffuse leukemic masses	Leukemic invasion	Positive
13	15	ALL	0	Ig	Positive	1 leukemic ovarian mass	Leukemic invasion	Normal
14	14	ALL	0	Ig	Positive	Diffuse leukemic masses	Leukemic invasion	Positive
15	16	ALL	1, 2	Ig	Negative	Normal	Normal	Normal
16	5	ALL	1, 2	Ig and TCR- γ	Negative	Normal	Normal	Normal
17	3	ALL	1, 2	None	NA	Normal	Normal	Normal
18	11	ALL	1, 2	TCR- γ	Positive	Normal	Leukemic invasion	Normal

Transplantasyon teknikleri

Orthotopik



Heterotopik



Xenograft



Neovaskülarizasyon iskemisi

- Dondurma tekniğinin folikül kaybı üzerindeki etkisi az
- Revaskülarizasyon sırasında folikül kaybı
 - VEGF yararsız
 - Fazla kanlanan bölgelere tx ile kayıpta azalma yok

Neovaskülarizasyon iskemisi riskini azaltmak mümkün mü?

- Antiapoptotik ajan kullanımı «S1P»
- Overyan implantları plazmadan zengin trombositlerle muamele
- 24-saat soğutma - 5° C - kriyo öncesi

*Soleimani , PlusOne , 2011
Callejo, J Ovarian Res, 2013
Isachenko , Clin Lab, 2012*

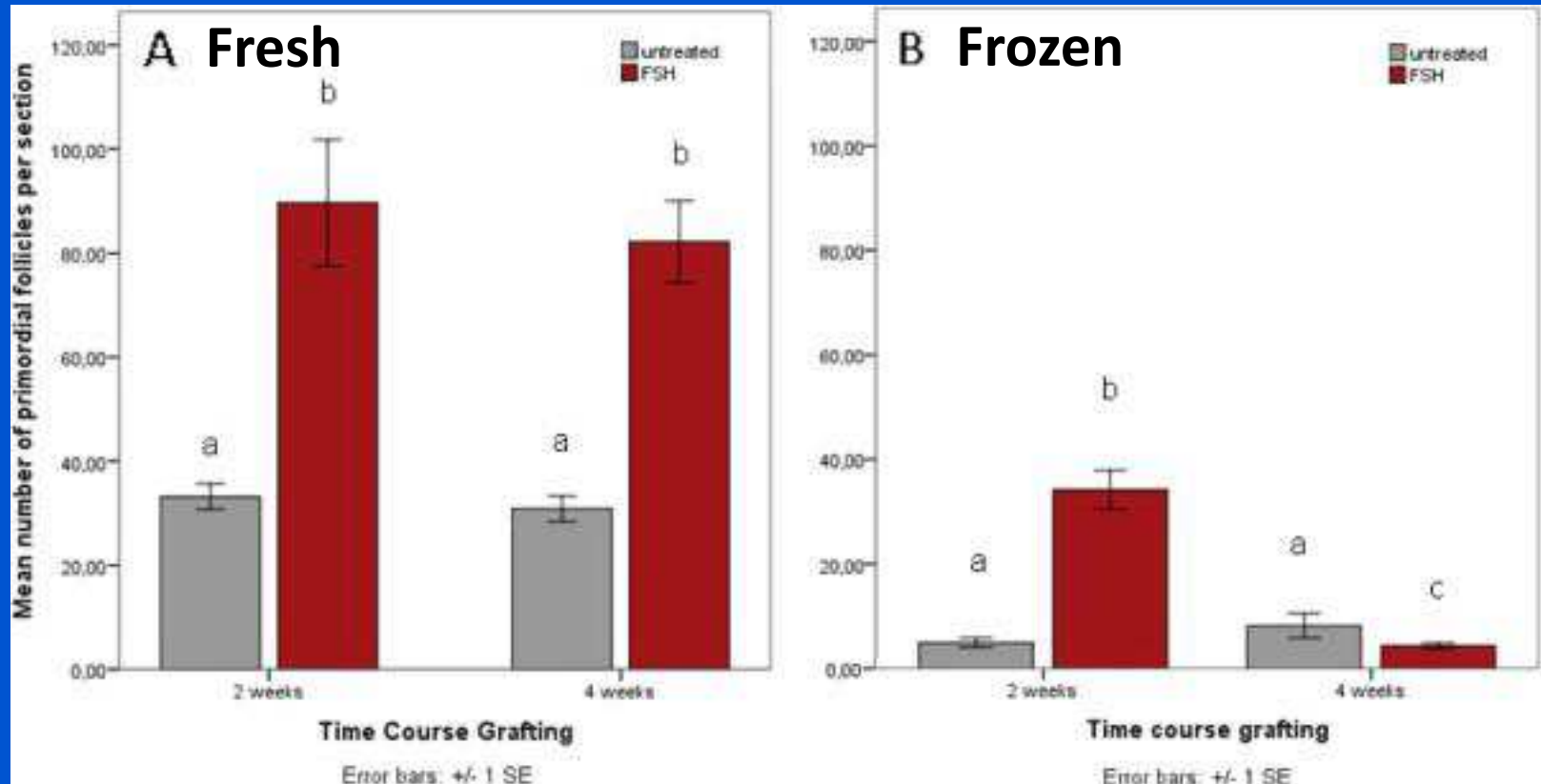
Transplantasyon anında GnRH-analog kullanımı

- «GnRH-a» \Rightarrow belirgin folikül kaybı
- Ooforektomi \Rightarrow artmış folikül sağkalımı?
- Kısa dönem gonadotropin kullanımı ile artmış folikül sağkalımı

Oktaç Fertil Steril, 2000

Maltaris, Reproduction, 2007 ;Maltaris, Fertil Steril, 2007

FSH kullanımı



Tüm Over Kriyo

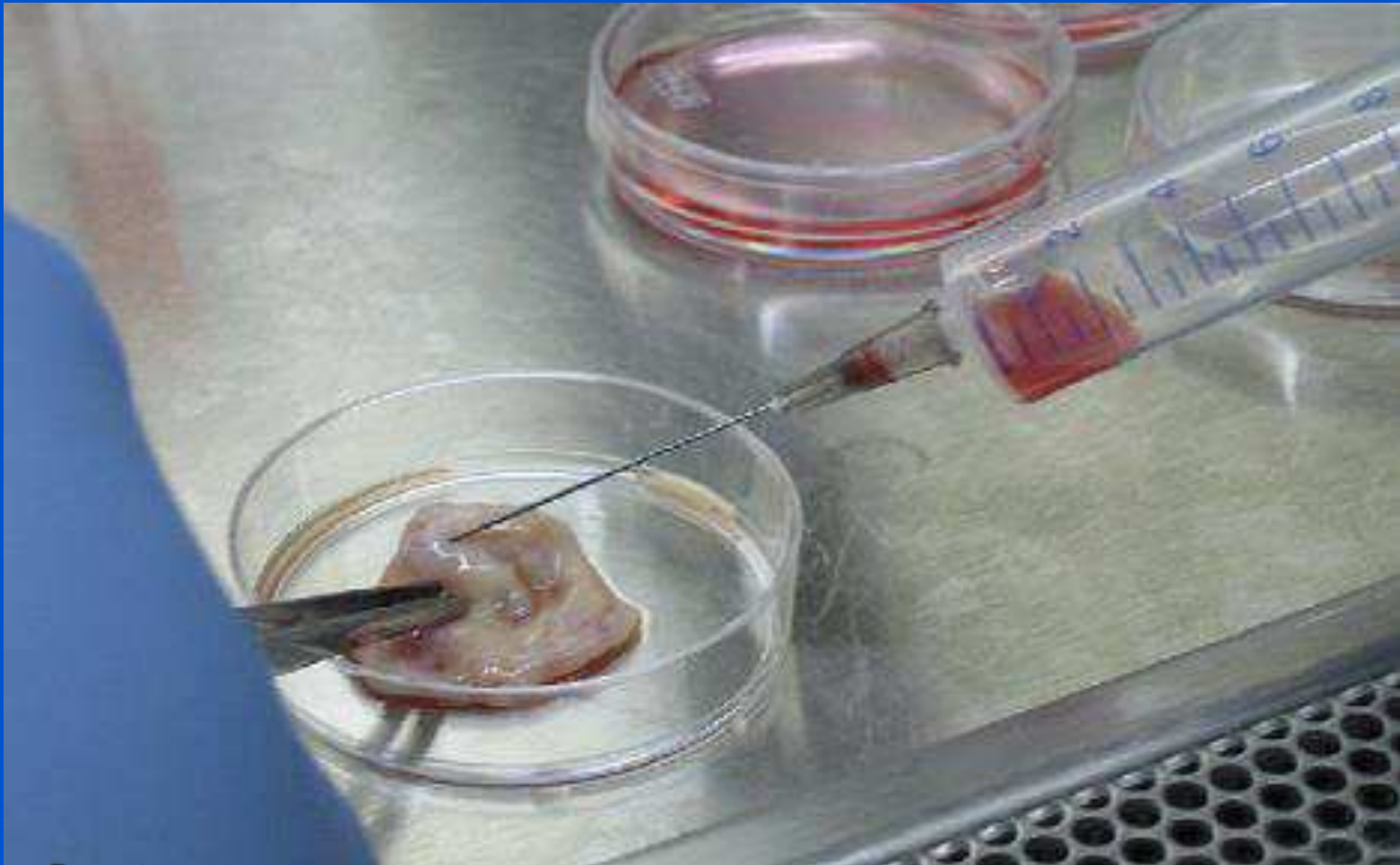
- Vasküler pedikül ile birlikte kriyoprezervasyon
 - Fare ve koyunlarda gebelik (+)
 - İnsan overi oldukça kompleks
 - Vasküler anastomozda tromboz riski
 - Martinez-Madrid \Rightarrow %75 folikül sağkalımı

Bedaiwy, Fertil Steril, 2003

Imhof, Fertil Steril, 2006

Martinez-Madrid, Fertil Steril 2007

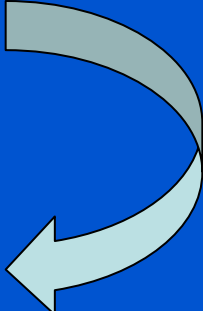
Ovaryan Doku Kriyo + IVM



Ovaryan Doku Kriyo + IVM

- 57 hasta – ovaryan doku kriyo., yaş 8-35
- Çıkarılan overden antral sıvı aspire ediliyor
- 266 oosit elde ediliyor
 - Dejenere 28
 - GV 200
 - MI 35
 - MII 3
- 24-28 saat IVM – Kriyo öncesi (maturasyon oranı %31)

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

- Çalışma grubu : n=12 hasta
 - Kontrol grubu : n=28 hasta
- 
- Çalışma grubunda overin bir kısmı kriyo için çıkarılıyor

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

Çıkarılan overden oosit eldesi

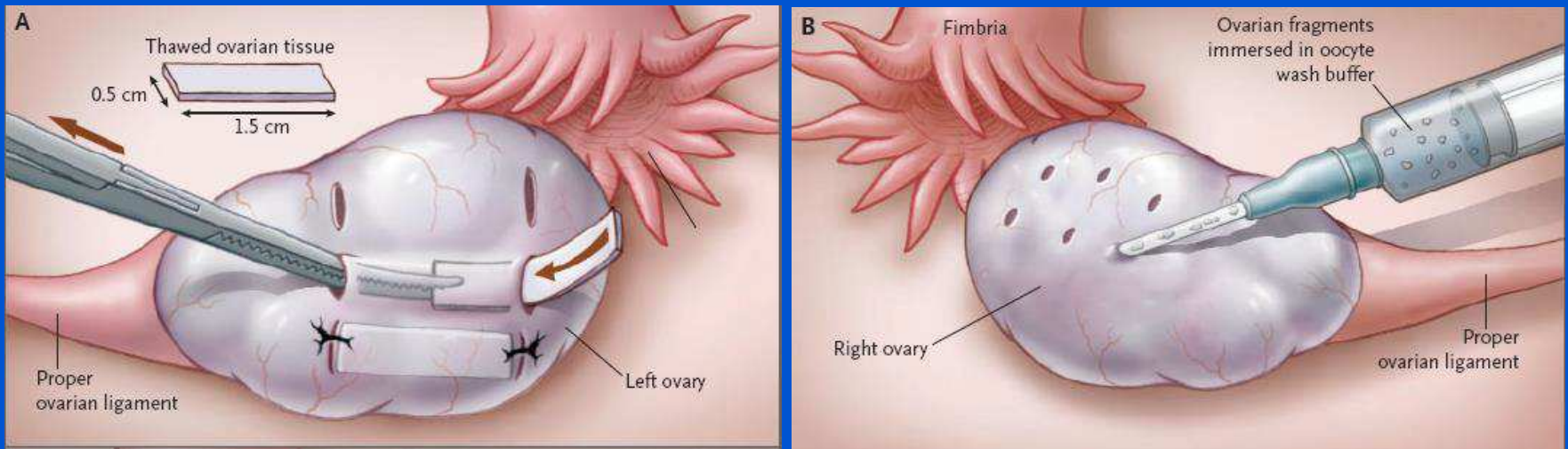
Characteristic of the patient's menstrual cycle	Patients n	Mean age \pm SEM	Mean fragments of ovarian tissue (range)	Oocytes retrieved n (range)	Mean oocytes retrieved /fragment	Mean oocytes retrieved /patients	Stage at collection (%)			IVM rate
							GV	MI	MII	
OC	11	23.1 \pm 1.3	20.1 (10-29)	38 (0-9)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

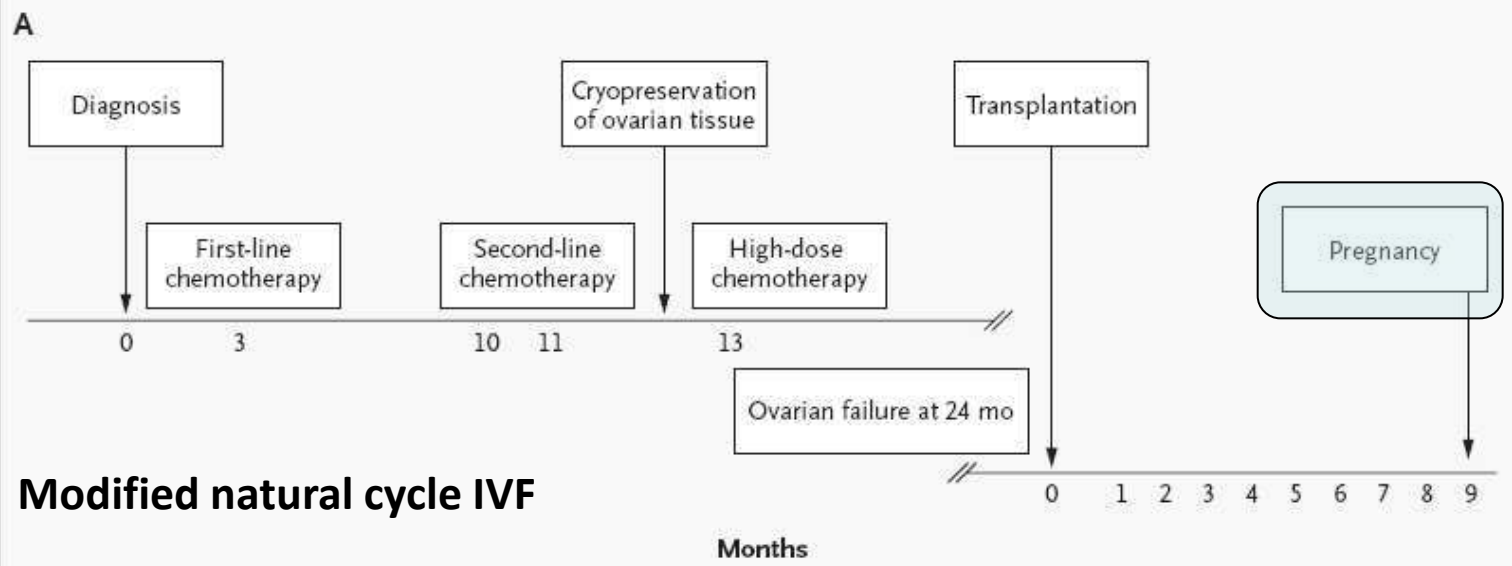
- Mestrüel siklustan ve kontraseptif yöntemden bağımsız olarak oositler elde edilebiliyor

Overyan transplantasyon

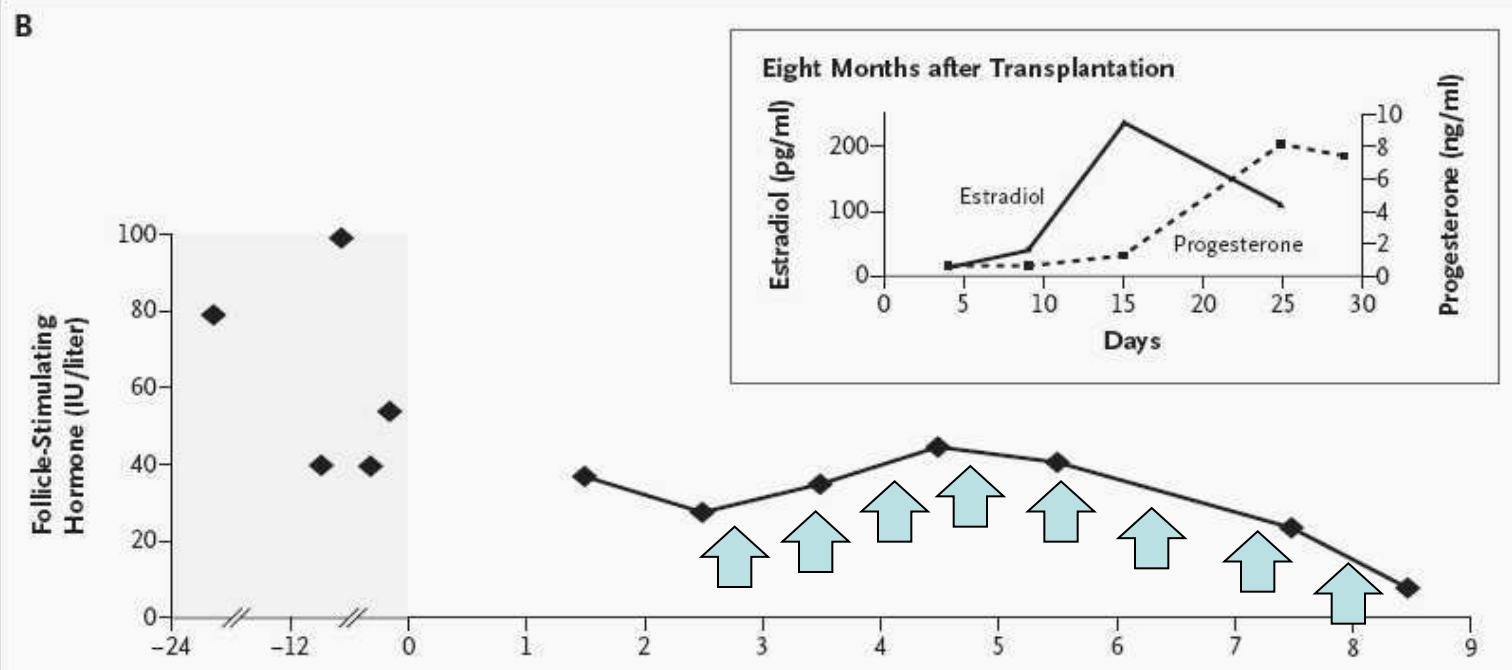
Transplantasyon sonrası ART *Orthotopik bölge*

- Hodgkin's lenfoma, 28-yaş





Modified natural cycle IVF



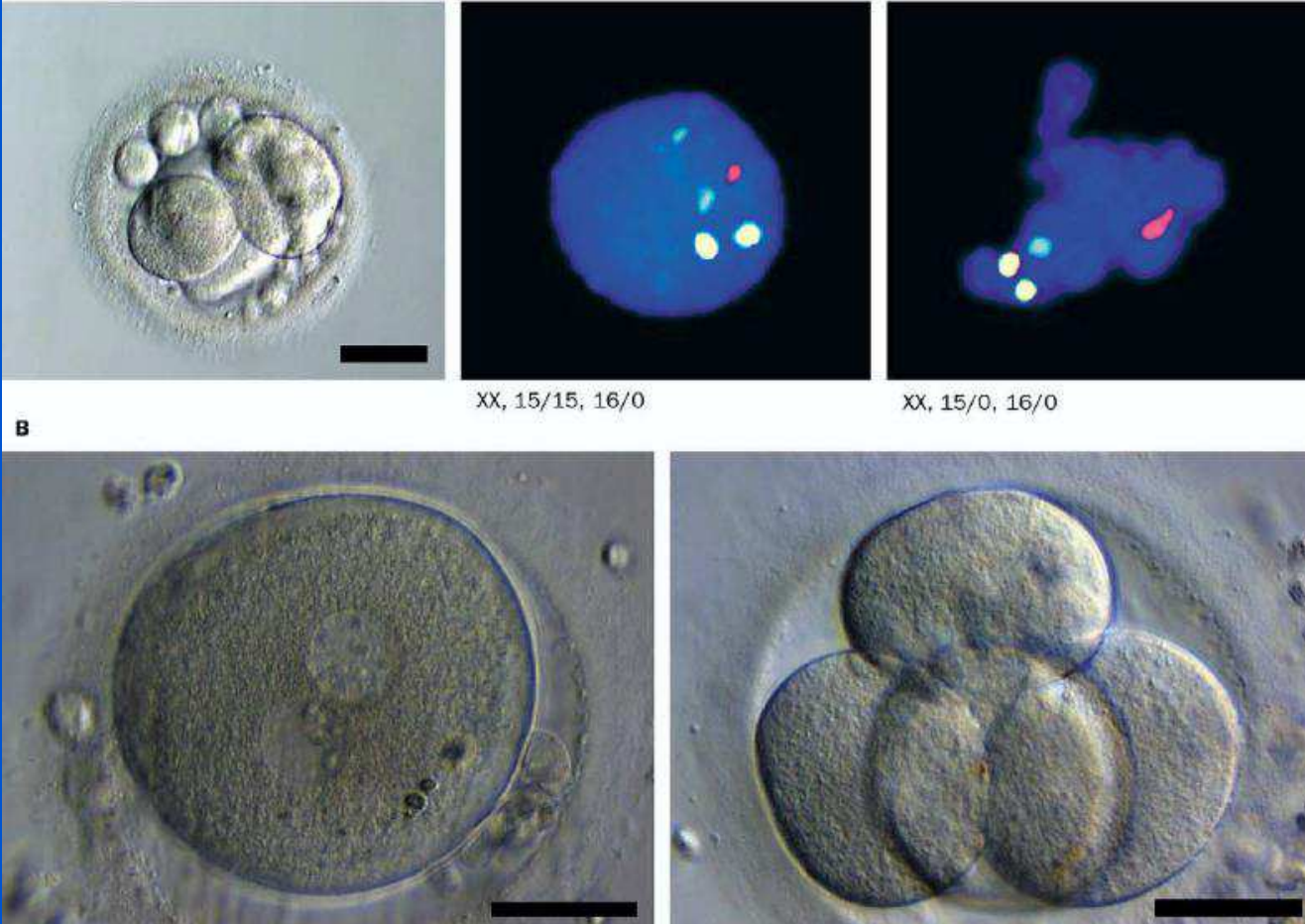
Transplantasyon sonrası ART

Heterotopik bölge

	Stimulation dose (FSH/HMG) and duration*	Peak oestradiol (pmol/L)†	Follicle size (mm)‡	Oocyte
Retrieval				
1	None	888	10.9	GV
3	3675/2175, 13 days	980	14.2	FZ
			10.7	Degenerated
			8.9	GV
			7.3	FZ
4	None	1093	9.1	FZ
			14.5	FZ
5	3225/1725, 11 days	2257	13.1	M-I
			9.5	FZ
			6.4	GV
			12.8	Mature
6	2025/2475, 10 days	1868	10.8	FZ
			8.4	FZ
			11.8	Mature
7	2100/2400, 10 days	921	10	GV
			6.7	Degenerated
			11	Degenerated
8	2025/1500, 10 days	987	9.9	Mature
			9.8	GV
			6.4	GV

Transplantasyon sonrası ART

Heterotopik bölge

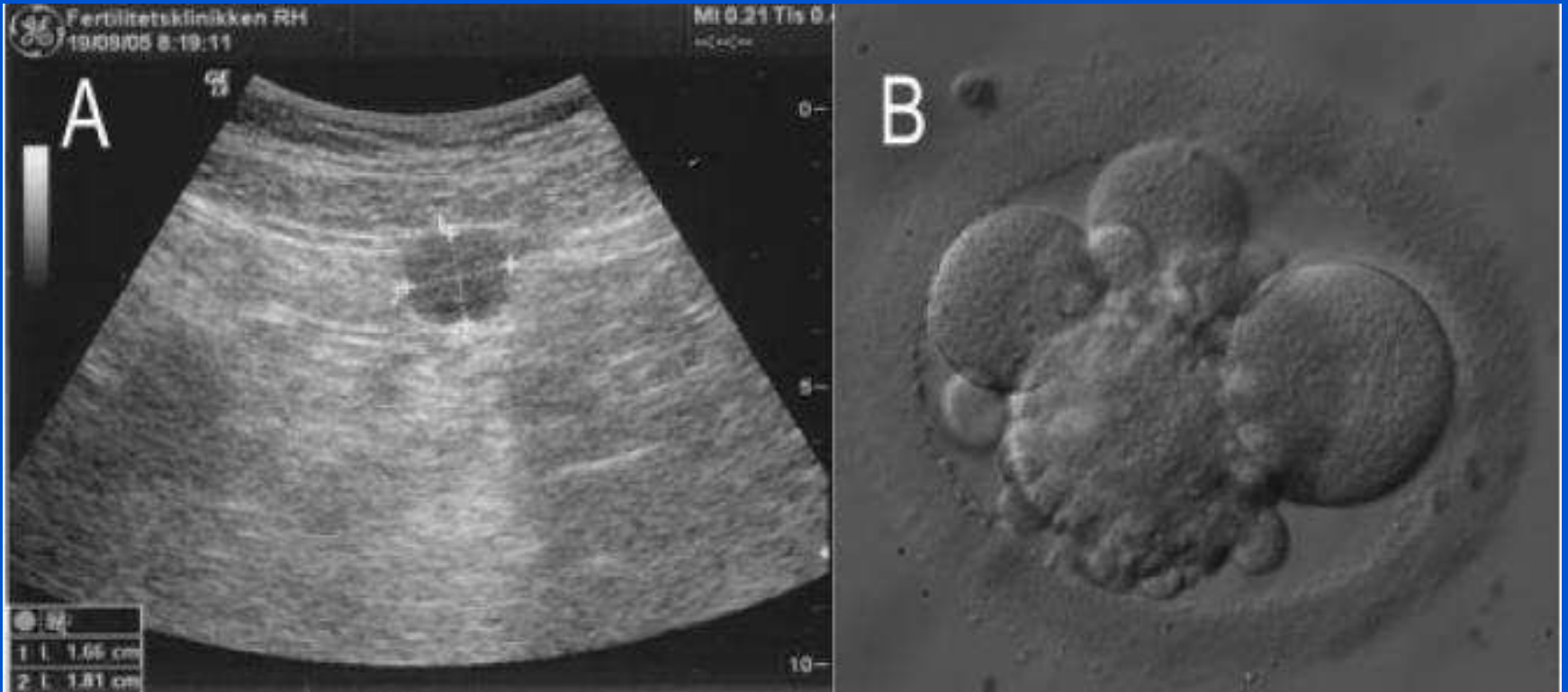


M1 \Rightarrow IVM/MII \Rightarrow ICSI, 4-hücreli embriyo

Oktay, Lancet, 2004

Transplantasyon sonrası ART

Heterotopik bölge



4-hücreli embriyo, frag<%20, BE, BhCG = 22IU/ml

Rosendahl, Hum Reprod, 2006

First reported clinical pregnancy following heterotopic grafting of cryopreserved ovarian tissue in a woman after a bilateral oophorectomy

C.J. Stern^{1,*}, D. Gook¹, L.G. Hale¹, F. Agresta¹, J. Oldham², G. Rozen³, and T. Jobling⁴

¹Reproductive Services, Melbourne IVF and Royal Women's Hospital, Parkville, VIC, Australia ²Women's Ultrasound East Melbourne, East Melbourne, VIC, Australia ³Reproductive Services, Royal Womens Hospital, Parkville, VIC, Australia ⁴Gynaecological Oncology, Monash Health, Moorabbin, VIC, Australia

- 21 yaş, granuloza hücreli tümör
- Bilateral ooforektomi
- Ooforeroktomiyi takiben 7 yıl sonra transplantasyon
- Dominant folikül 16-18mm, peak E2 497pmol/l



Transplantasyon sonrası greft fonksiyonları

- Graft fonksiyon başlaması \Rightarrow 60-244 gün
- Gebelik oranı \Rightarrow %37 (9 gebelik-8/25 hastada (dondurulmuş+taze))
- Over fonksiyonlarının geri dönüş olasılığı taze greftlerde, dondurulmuş greftlere oranla daha fazla

Transplantasyon sonrası gebelik

- 12 hastada ART \Rightarrow 65 oosit/72 hasta
- Gebelik/siklus \Rightarrow 6.9% (5/72)
- Canlı doğum/siklus \Rightarrow 2.8% (2/72)
- Post-transplant overyan fonksiyon 9 ay - 7 yıl
- AMH<1ng/ml (1 hastada spontan gebelik)

Transplant gebelikleri sağlıklı mıdır ?

- Ortotopik transplant sonrası elde edilen 13 hastalık bir vaka serisinde bütün yeni doğanlar sağlıklı olarak bildirilmiştir.

Ovarian Doku Transportu

Live birth after ovarian tissue autotransplantation following overnight transportation before cryopreservation

Ralf Dittrich, Ph.D.,^a Laura Lotz,^a Gudrun Keck, Ph.D.,^b Inge Hoffmann,^a Andreas Mueller, M.D.,^a Matthias W. Beckmann, M.D.,^a Hans van der Ven, M.D.,^c and Markus Montag, Ph.D.^d

^a Department of Obstetrics and Gynecology, Erlangen University Hospital, Erlangen; ^b Department of Obstetrics and Gynecology, Dresden University Hospital, Dresden; ^c Department of Gynecologic Endocrinology and Reproductive Medicine, Bonn University Hospital, Bonn; and ^d Department of Gynecologic Endocrinology and Fertility Disorders, Heidelberg University Hospital, Heidelberg, Germany

Prepubertal hastalar

Case report: Stimulation of puberty in a girl with chemo- and radiation therapy induced ovarian failure by transplantation of a small part of her frozen/thawed ovarian tissue

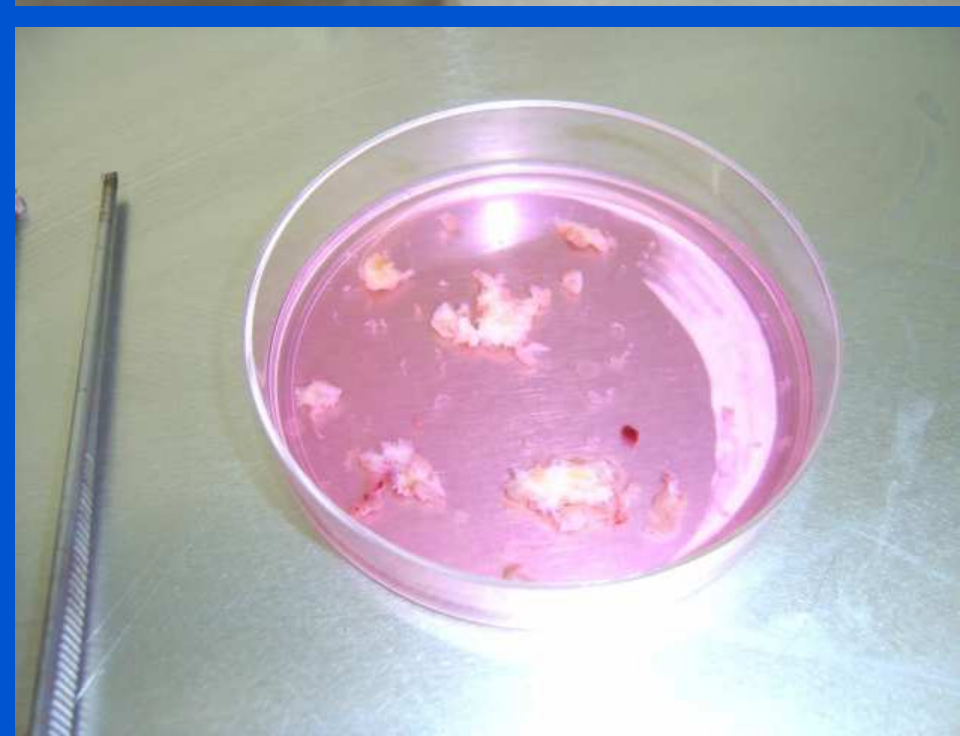
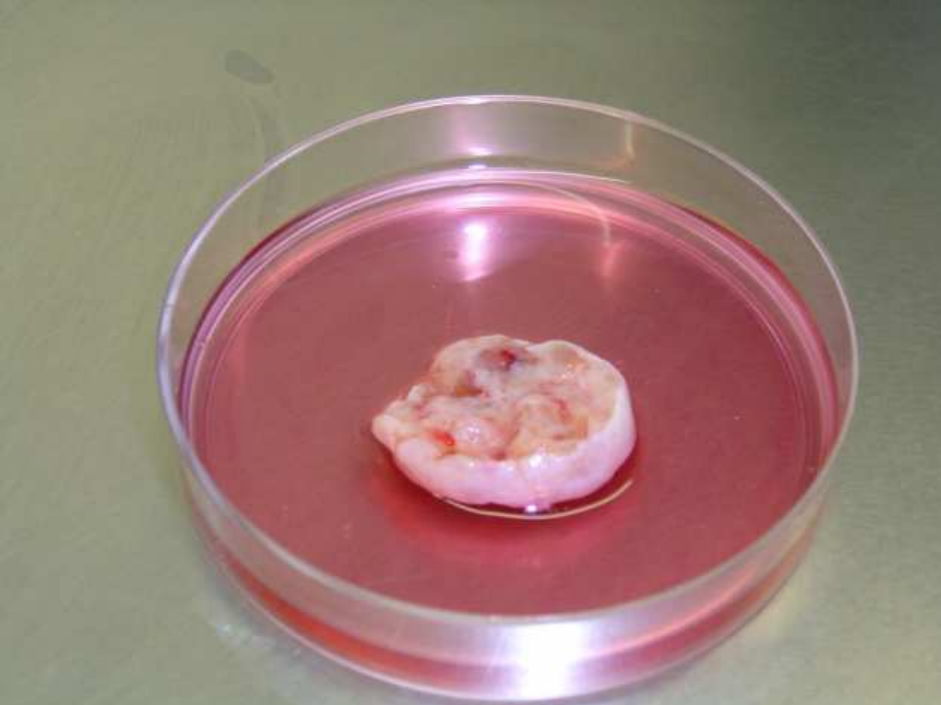
Erik Ernst^a, Mimi Kjærsgaard^b, Niels H. Birkebæk^b, Niels Clausen^b,
Claus Yding Andersen^{c,*}

- 9 yaşında Ewing sarkomu
- Kemo/radyoterapi → POF
- Overyan transplantasyon → puberte stimulasyonu

TÜRKİYE

Mevcut durum

- Kemo/radyoterapi
- Gonadal cerrahi
- DNA finger print analiz
- Yıllık onam
- 5 yıl ?





Ankara Üniversitesi– Transplantasyon

43 yaşında meme CA



Sonmezer,, 2012



Ankara Universitesi - Vakalar

▪ Breast Cancer	26
▪ Leukemia (ex. 1 HSCT)	19
▪ Hodgkin' s lymphoma	13
▪ Bening ovarian cysts	5
▪ Myelodysplastic syndrome (ex GVHD)	3
▪ Thalasemi major (* 1 ex GVHD)	3
▪ Wegener granulamatosi (glomerulonephritis)	3
▪ Borderline ovarian tumors	2
▪ Ewing' s sarcoma	2
▪ Osteosarkom	2
▪ Retroperitoneal sarcoma	2
▪ Turner' s syndrome	2
▪ Cervical adenocancer	1
▪ Burkitt' s lymphoma (ovarian involvement)	1
▪ Fanconi anemia	1
▪ HAIRAN syndrome	1
▪ Pineal gland tumor	1
▪ Total	86

Sonuçlar

- Yaş sınırı ?
- Overyan greftler ne zaman transplantasyon yapılmalı ?
- Graft fonksiyon başlaması \Rightarrow 60-244 gün
- Graft fonksiyon süresi \Rightarrow 8ay-7 yıl
- Bütün gebelikler ortotopik alandan
- Meme kanserinde transplant güvenilir mi? Sessiz tümör hücre aktivasyonu
- 24 saat transport güvenli