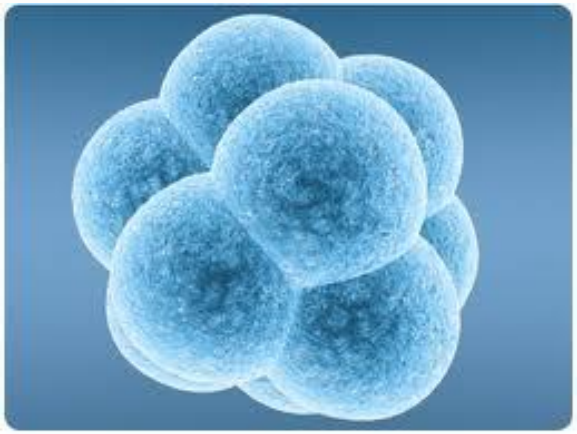
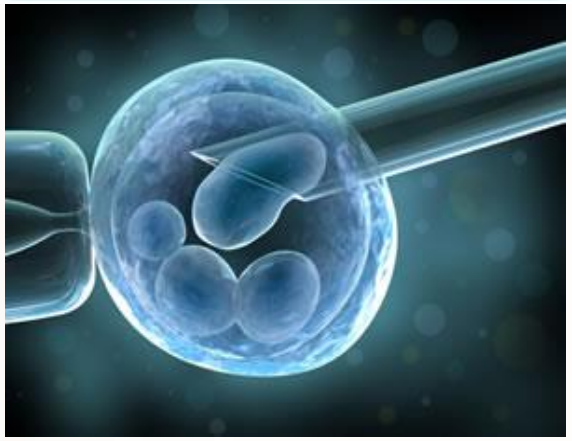
Tek Gen Hastalıklarının Preimplantasyon Genetik Tanısına Yönelik Algoritmalar

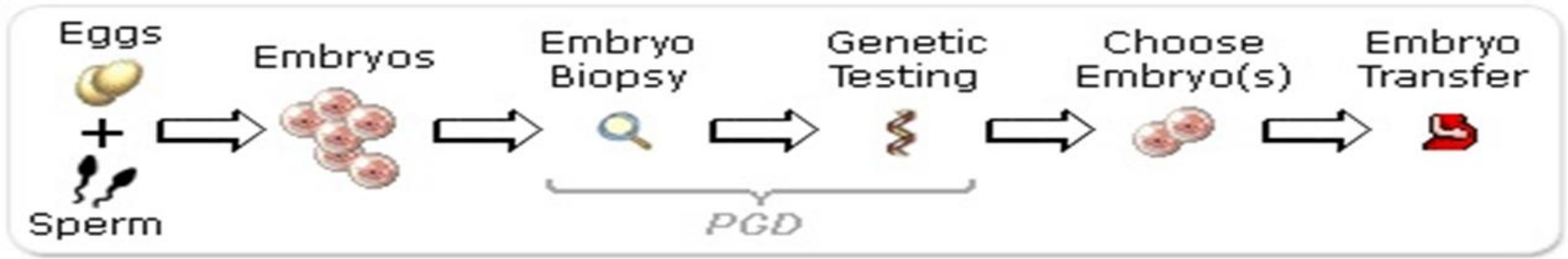
Doç. Dr. Evrim Ünsal

Pre-implantasyon Tanı

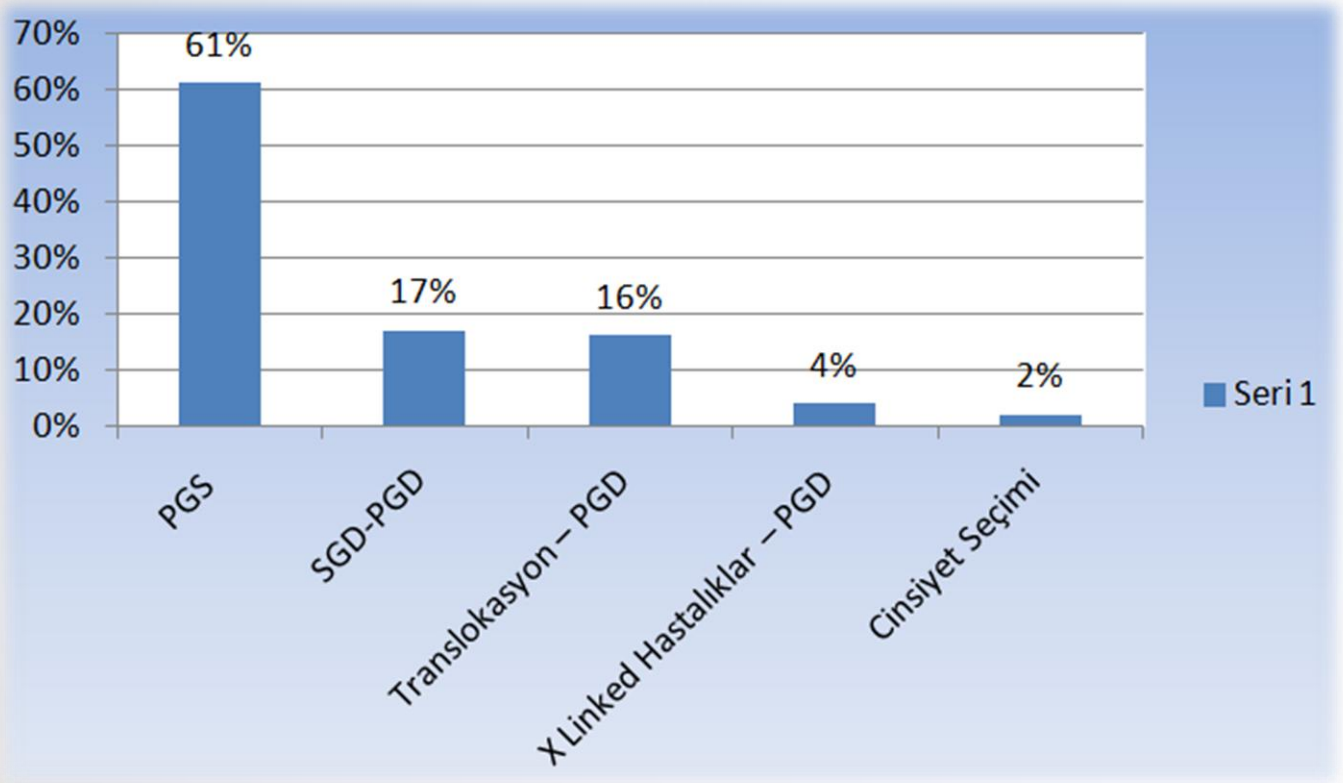
- İlk başarılı PGT 1990 yılında gerçekleştirildi.
- Embriyonik hücrelerin biyopsisini takiben farklı tekniklerle (FISH, PCR, aCGH, NGS), genetik tanısının koyulması ve genetik olarak normal olanların hastaya transfer edilmesi esasına dayalı bir yöntemdir.







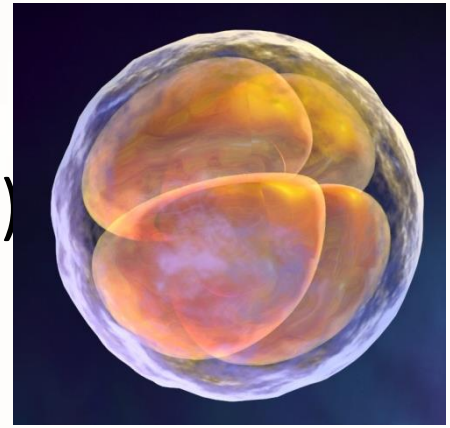
ESHRE PGD Konsorsiyumu (1997–2007) 27,000 siklus



PGT Endikasyonları

- ***Tek gen hastalıkları***

- Bilinen kalıtsal nokta mutasyonlar ya da küçük delesyon / duplikasyonlar.
- Üçlü tekrar hastalıkları(Miyotonik Distrofi)
- Aile haplotiplemesi ile tespit edilebilecek büyük delesyon hastalıkları. (DMD)
- İleri yaşta ortaya çıkan hastalıklar(Huntington)
- HLA tiplemesi
- Mitokondriyal Hastalıklar



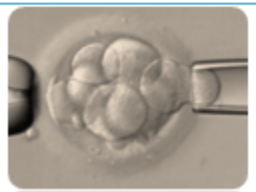
TEK GEN HASTALIĞI TAŞIYICILARINDA PGT

1. Hasta çocuğun doğumu
2. Genetik hastalığın klinik tanısının koyulması
3. Klinik tanının moleküler yöntemlerle doğrulanması

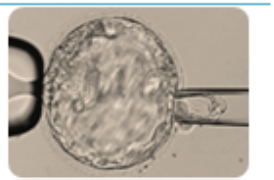
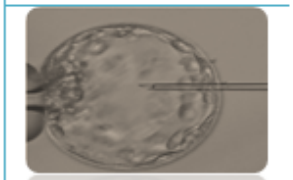
PGD İŞ AKIŞI

1. Raporlanmış mutasyonun doğrulanması
2. PGT Dizaynı (Aileye özgü)
3. PGT Set Up (Gelinen aşamayı bildiren ara rapor)
4. IVF tedavisi
5. Embriyo Biyopsisi

BİYOPSİ YÖNTEMLERİ

HÜCRE TİPİ	AVANTAJLARI	DEZAVANTAJLARI
1. POLAR BODY 	• Embriyo gelişimini etkilemez • Genetik test için geniş zaman tanır • Maternal orijin için iyi bir kaynaktır • Legal ve etik sınırlamalardan etkilenmez	• Daha çok tanısal • Paternal/mayoz2/post zigotik bilgi yok • İleri test gerekebilir (3/5. gün)
2. POLAR BODY 	• Embriyo gelişimini etkilemez • Genetik test için geniş zaman tanır • Maternal orijin için iyi bir kaynaktır • Legal ve etik sınırlamalardan etkilenmez • Mayoz 2 hatalarını gösterir	• Paternal/post zigotik bilgi yok • Tanı için pahalı bir yöntem • İleri test gerekebilir (3/5. gün)
BLASTOMER 	• Biyopsi hasarı yüksek • Yaygın kromozomal mozaisizm (%55-73) • Genetik test için zaman tanır. • Her endikasyonda kullanılabilir.	• Mozaisizm • (Alel Drop Out) ADO oranı yüksek • İmplantasyon oranı düşebilir • Self correction sebepli hatalı değerlendirme iltimali

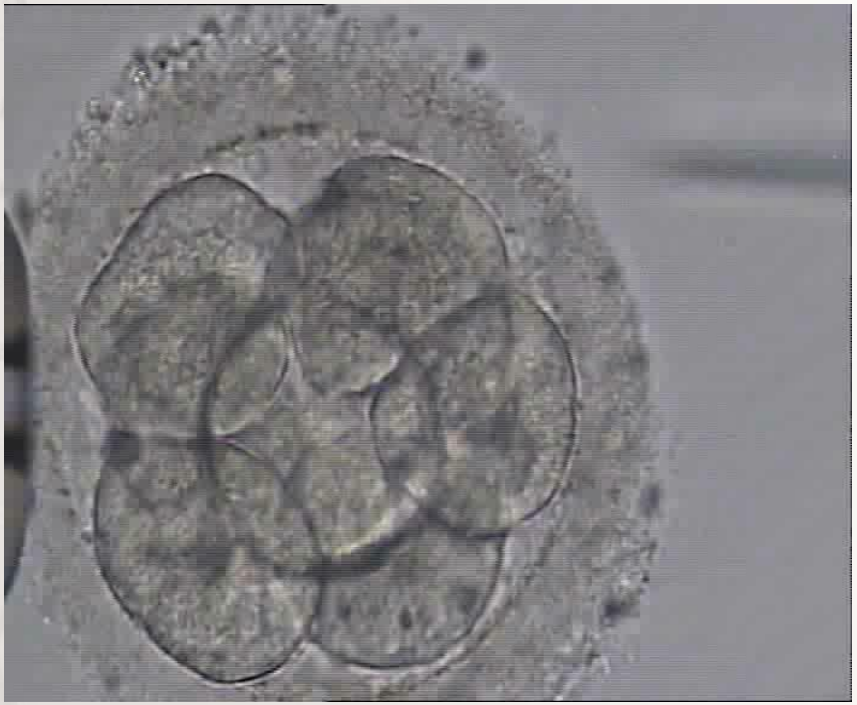
BİYOPSİ YÖNTEMLERİ

HÜCRE TİPİ	AVANTAJLARI	DEZAVANTAJLARI
TROFEKTODERM 	• 3-6 hücrenin ortak bilgisi (güvenirliliği yüksek) • Düşük mozaisizm • Hasta başı düşük maliyet • Her endikasyonda kullanılabilir. • ADO oranı düşüktür.	• Vitrifikasyon gerektirir • Manipulasyon becerisi gerektirirf • Blastokist kültürü gerektirir
BLASTOSÖL SIVISI 	• Hücre hasarlanması diğer yöntemlere nazaran oldukça azdır. • Serbest DNA'nın izolasyon yöntemleri geliştikçe bu teknik umut verici olacaktır.	• Çalışmalar henüz çok sınırlıdır • Rutine girmiş bir yöntem değildir • Boş kültür medyumlarında da DNA parçaları bulunmuştur • Elde edilen DNA embriyonun bütününü yansıtmayabilecektir • Bu DNA'nın dejenere ya da anormal hücrelerden salındığından intakt hücreler kadar iyi sonuç vermeyebilir • Rutine girmiş bir yöntem değildir
KÜLTÜR MEDYUMU 	• Non-invaziv bir yöntemdir • Embriyonel biyopsi sonuçları ile tutarlı bulgulara erişilmiştir. • Umut vaad eden bir yöntemdir.	• Çalışmalar henüz çok sınırlıdır • Rutine girmiş bir yöntem değildir. • Elde edilen serbest DNA konsantrasyonu stabil olmayıp izolasyon tekniklerinin geliştirilmesi gerekmektedir.

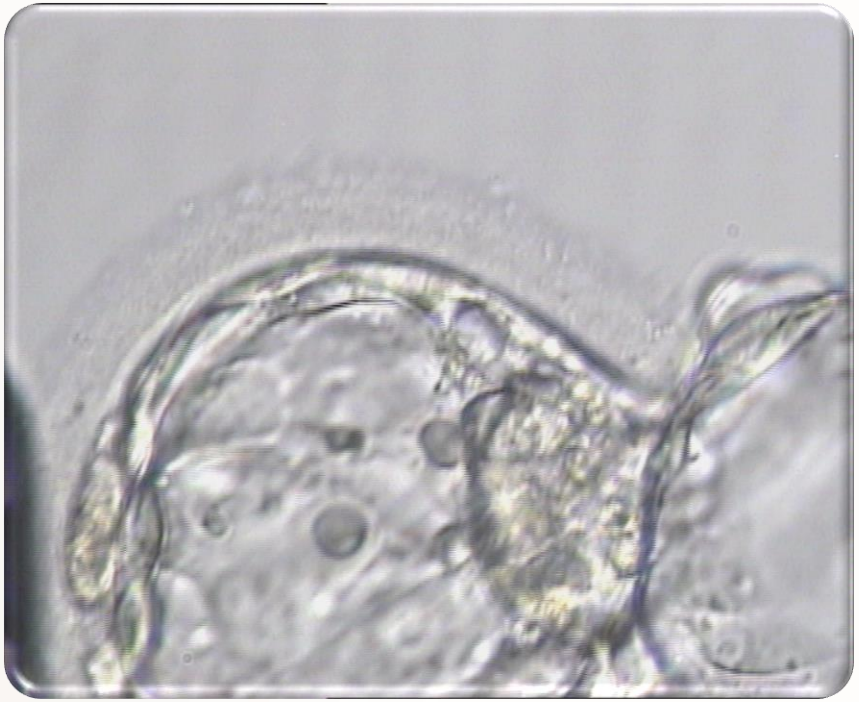
Embriyo Biyopsisi

mikrogen
GENETİK HASTALIKLAR TANI MERKEZİ

Blastomer Biyopsisi



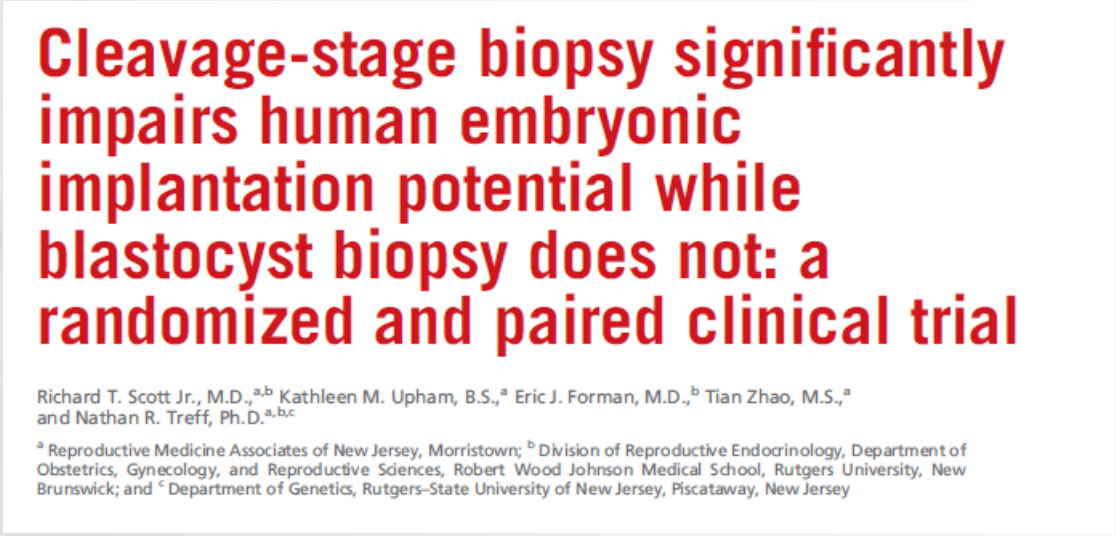
Trofektoderm Biyosisi



3. ve 5. gün Biyopsi Kıyası

	Klivaj (3. GÜN)		Blastokist (5. GÜN)	
	Biyopsi	Yok	Biyopsi	Yok
Implantasyon Oranı	31%	53%	52%	54%
	P<0.05 42% azalma		N.S.	

Scott et al 2013, Fertil &Steril



Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial

Richard T. Scott Jr., M.D.,[a,b] Kathleen M. Upham, B.S.,[a] Eric J. Forman, M.D.,[b] Tian Zhao, M.S.,[a] and Nathan R. Treff, Ph.D.[a,b,c]

[a] Reproductive Medicine Associates of New Jersey, Morristown; [b] Division of Reproductive Endocrinology, Department of Obstetrics, Gynecology, and Reproductive Sciences, Robert Wood Johnson Medical School, Rutgers University, New Brunswick; and [c] Department of Genetics, Rutgers–State University of New Jersey, Piscataway, New Jersey

Embriyo Vitrifikasonu

Biyopsi edilmiş blastokist hücrelerinin vitrifikasyon sonrası

- canlı kazanım başarısı : %94-98'dir.
- İmplantasyon başarısı : %50

Biyopsi edilmiş blastokist hücrelerinin vitrifikasyonu

- Tanı için zaman kazandırır
- Daha fizyolojik bir endometriyum
- Overyan hiperstimülasyon riskini önler

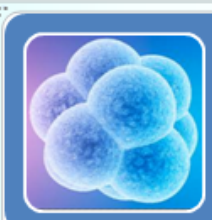
J Assist Reprod Genet
DOI 10.1007/s10815-016-0647-y

ASSISTED REPRODUCTION TECHNOLOGIES

Reproductive outcome is optimized by genomic embryo screening, vitrification, and subsequent transfer into a prepared synchronous endometrium

Jorge Rodriguez-Purata[1] · Joseph Lee[1] · Michael Whitehouse[1] · Marlena Duke[1] · Lawrence Grunfeld[1,2] · Benjamin Sandler[1,2] · Alan Copperman[1,2] · Tanmoy Mukherjee[1,2]

A total of 837 patients underwent 998 cycles and an embryo transfer



Group 1: Fresh only

- A fresh IVF cycle,CCS,and a fresh ET

Group 2: FETonly

- A fresh IVFcycle ,CCS ,no fresh ET, all embryos cryopreserved, FET in the subsequent cycle

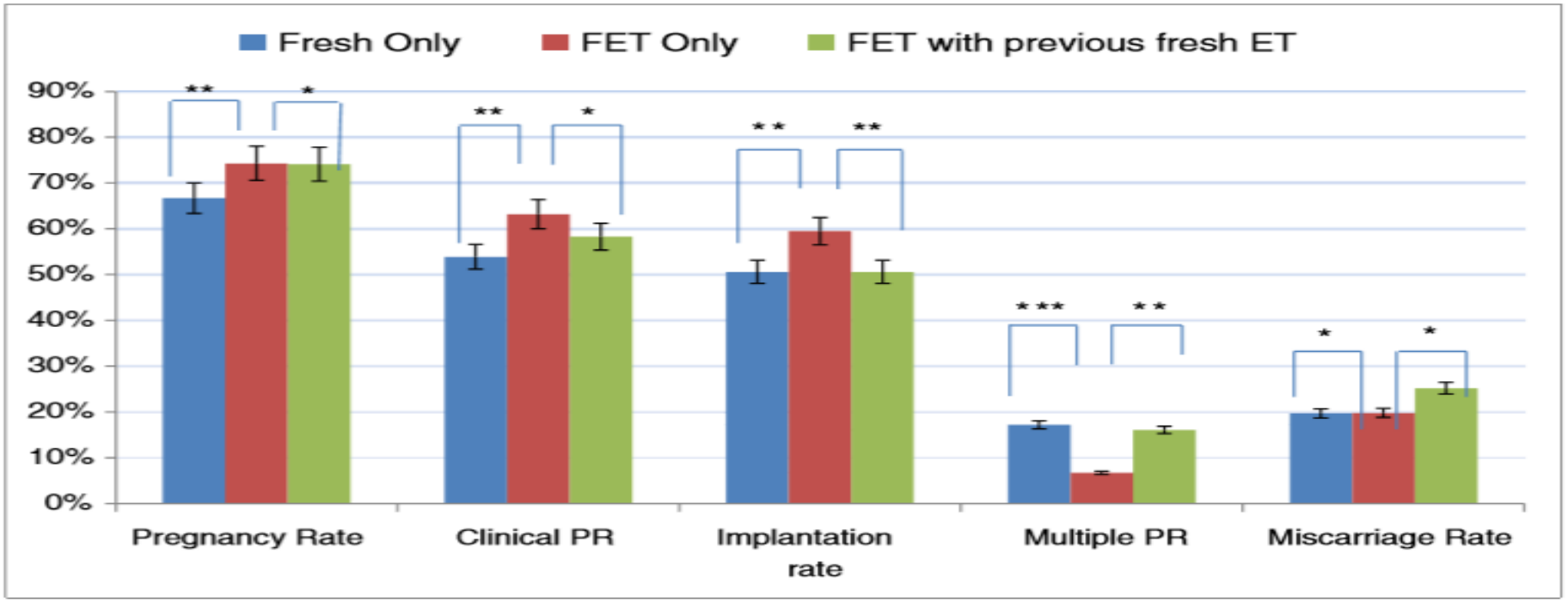
Group 3: FETwith a previous Fresh ET

A fresh IVF cycle, CCS, a fresh ET, remaining embryos cryopreserved, a FET in a subsequent cycle

solely fresh (n=345)

solely frozen (n=514) transfer

139 underwent a fresh ET followed by a FET cycle.



Patients with embryos transferred only in a fresh cycle had statistically lower implantation and live birth rates than those transferred during FET

These findings suggest the transfer of the best available embryo under a synthetically prepared endometrium is more recommended that transferring fresh.

Tek Gen Hastalıkları

- **Multipleks nested PCR ile DNA amplifikasyonu.**
  - Mutasyonu bölgesini çoğaltmak
  - STR marker bölgelerini çoğaltmak
- **Mutasyon Karakterizasyonu**
  - Mutasyon spesifik primerlerle
  - RFLP
  - Dizi Analizi
  - Fragment Analizi

Tek gen hastalıklarının taranmasında embriyonik hücrelerden DNA eldesi

Lizis Tekniği

- Hızlı ve güvenilir
- Testin tekrarına imkan vermemesi
- Multipleks PCR zorunluluğu

Tüm genom amplifikasyonu

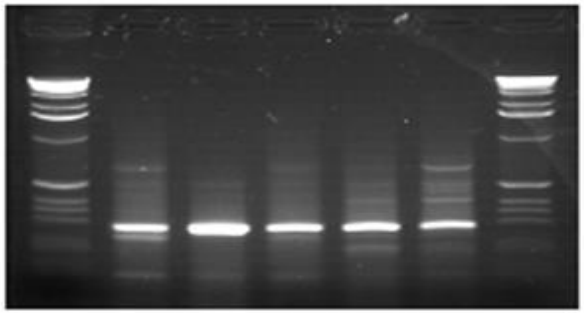
- Birden fazla mutasyon veya hastalığı aynı anda tarama imkanı
- Testi tekrarlayabilme imkanı
- Allel drop out riski
- Optimizasyon zorlukları

Laboratuar İş Akışı

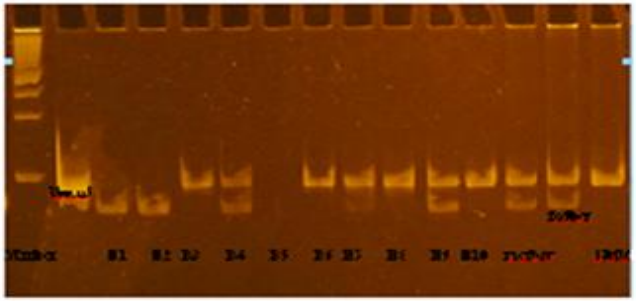
Cell Lysis (45min)

First Round PCR (3,5h)

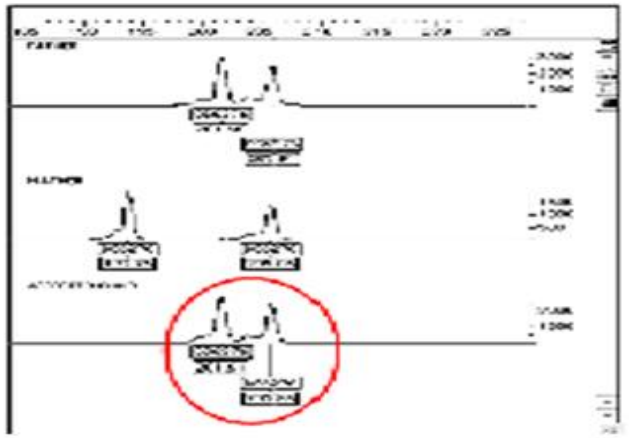
Second Round PCR (1,5h)



PCR Control



RFLP - Mutation Analysis



HLA matched embryo selection with fragment analysis (3-4h)

(STR) markers has been vastly used to improve the accuracy

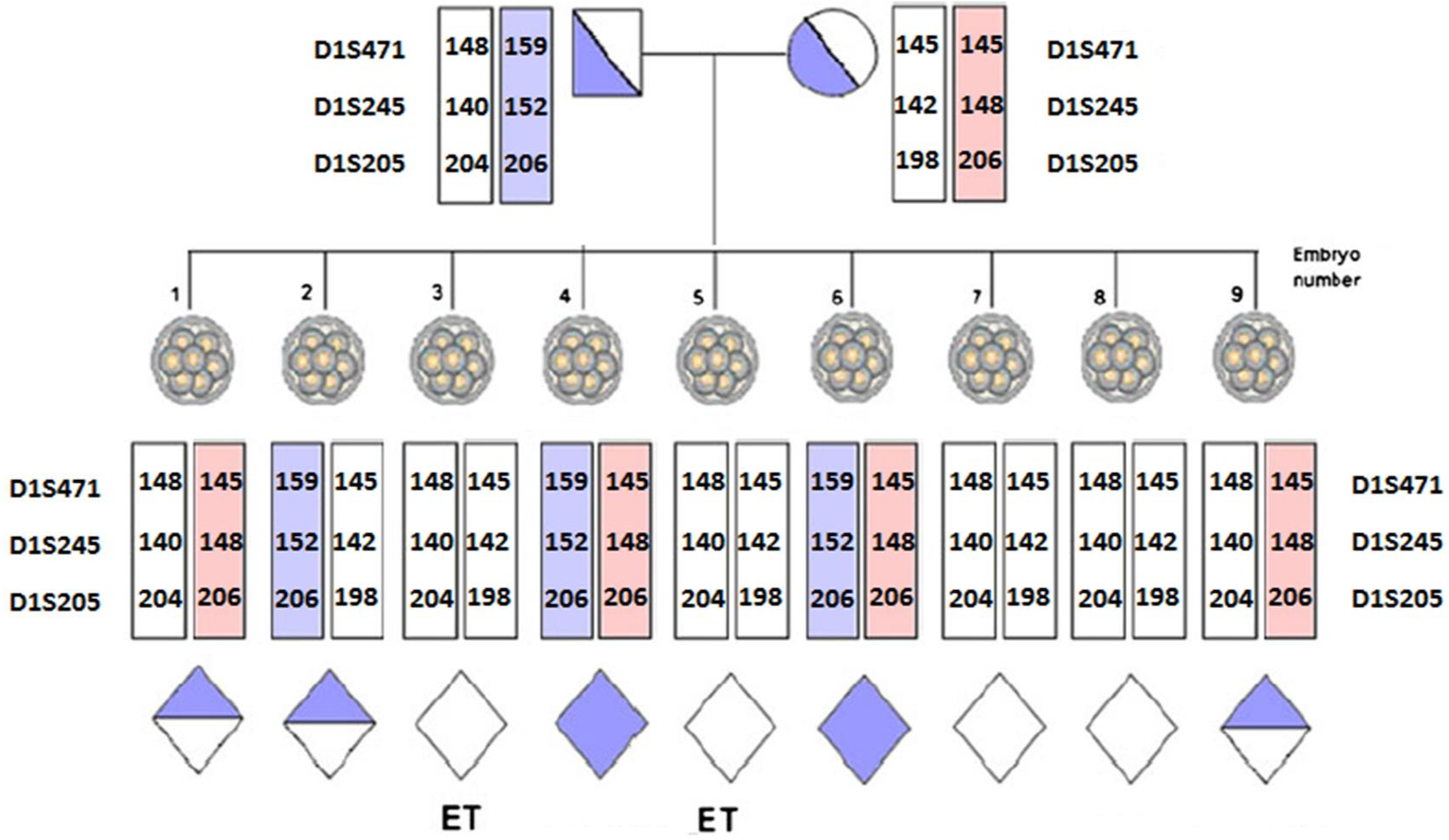
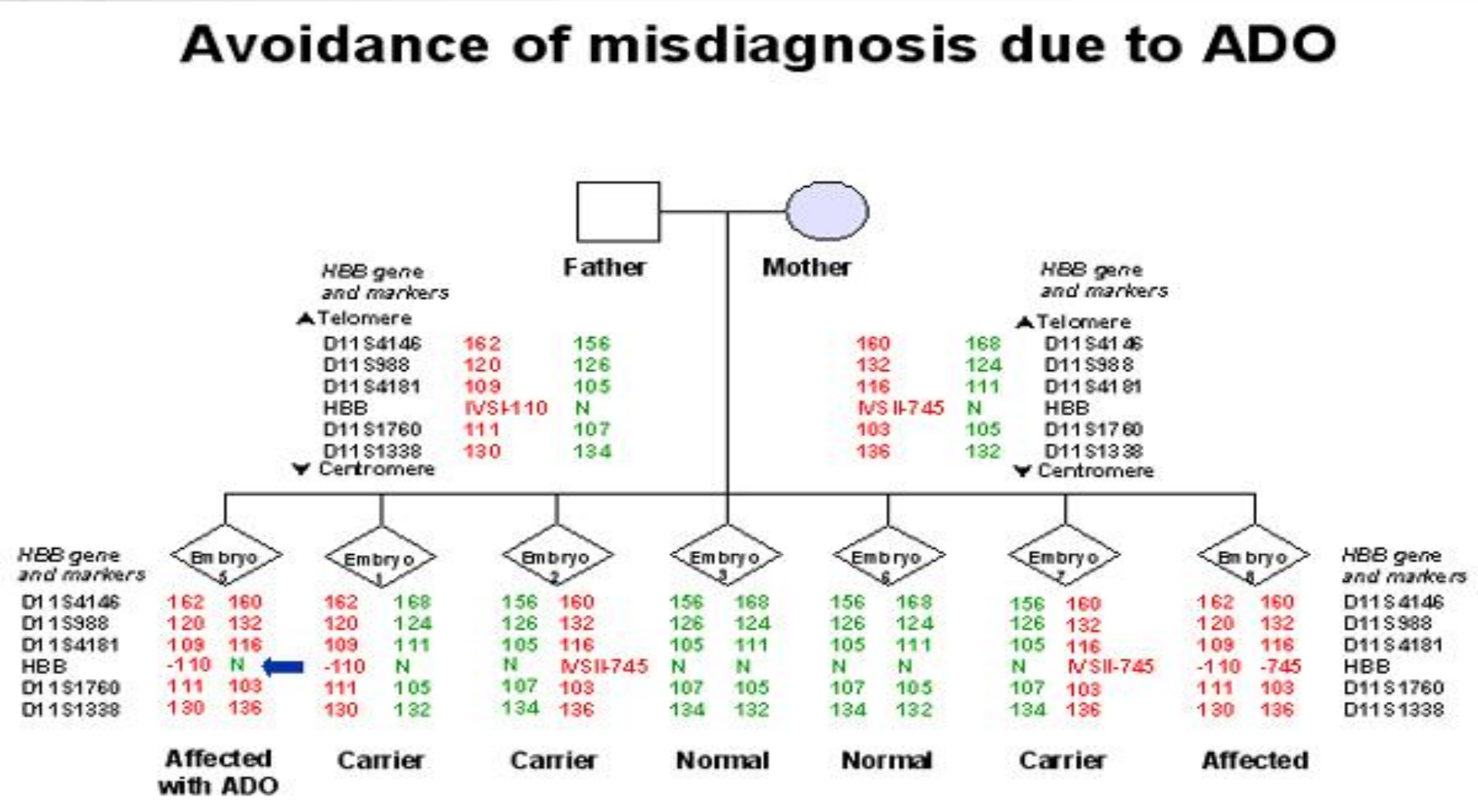


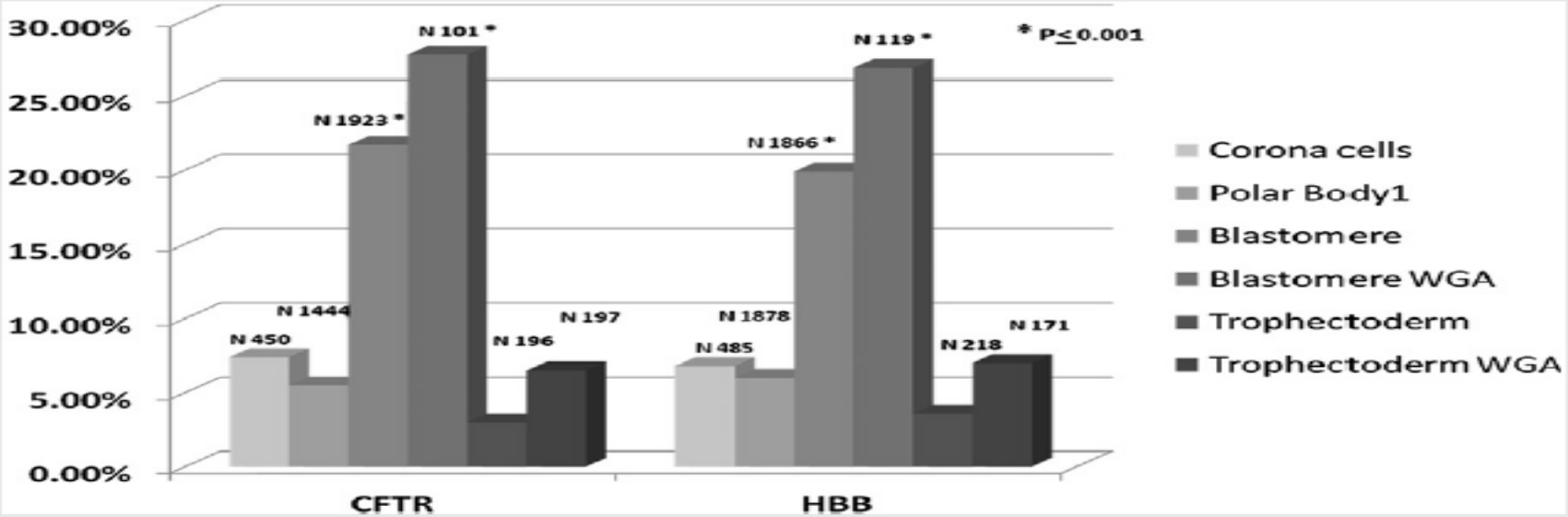
Fig. 1 Pedigree displaying haplotypes of the family members and embryos generated in the PGD cycle

Allele Drop Out (ADO) Markerların Önemi



- Allele Drop Out (ADO)

FIGURE 1



Allele dropout rates in different types of cells heterozygous for the CFTR gene and beta-globin gene mutations, with or without WGA (see description in the text). In both mutations, a significantly higher ADO rate is seen after WGA. This rate is much lower in blastocyst samples than in blastomere samples. HBB = beta-globin gene mutation.

Rechitsky. Combined PGD with 24-chromosome aneuploidy testing. Fertil Steril 2015.

- ADO oranı tüm genom amplifikasyonunda(WGA) ve özellikle blastomere örneklerinde artıyor !

CONTROVERSY: PREIMPLANTATION GENETIC DIAGNOSIS

FERTILITY AND STERILITY®
VOL. 82, NO. 2, AUGUST 2004

Published by Elsevier Inc.
Printed on acid-free paper in U.S.A.

Over a decade of experience with preimplantation genetic diagnosis: a multicenter report

Yury Verlinsky, Ph.D.,[a] Jacques Cohen, Ph.D.,[b] Santiago Munne, Ph.D.,[b] Luca Gianaroli, M.D.,[c] Joe Leigh Simpson, M.D.,[d] Anna Pia Ferraretti, M.D.,[c] and Anver Kuliev, M.D., Ph.D.[a]

1990-2002 arası PGT				
Açıklama	**Anöploidi**	**Tek Gen Hastalıkları**	**Translokasyonlar**	**Toplam**
PGT siklusları	3747	532	469	4748
Embriyo Transferi	3099	466	356	3921
Klinik Gebelik	722	142	123	987
Klinik Gebelik %	23,3	30,5	34,6	25,2
Doğan Bebekler	564	108	82	754

Tablo 1 : PGT konsorsiyumu verilerine göre 1990-2002 yılları arasında yapılan PGT sikluslarına ait detaylar tabloda sunulmuştur.

Human Reproduction Update, Vol.18, No.3 pp. 234–247, 2012
Advanced Access publication on February 16, 2012 doi:10.1093/humupd/dmr052

human reproduction update

The ESHRE PGD Consortium: 10 years of data collection

J.C. Harper[1,2,*], L. Wilton[3], J. Traeger-Synodinos[4], V. Goossens[5], C. Moutou[6], S.B. SenGupta[1], T. Pehlivan Budak[7], P. Renwick[8], M. De Rycke[9], J.P.M. Geraedts[10], and G. Harton[11]

Table I Ten years of PGD Consortium data.

	Cycles to OR	No. embryos biopsied	No. embryos transferred (mean/ET)	Embryo transfer procedures	Clinical pregnancy rate (per OR and per ET)
Single genes	4733	27 980	7035 (1.9)	3727	22% per OR 29% per ET
Structural chromosome abnormalities	4253	27 068	4775 (1.7)	2731	17% per OR 26% per ET
Sexing X-linked	1167	7317	1598 (1.8)	880	19% per OR 26% per ET
Aneuploidy	16 806	90 404	21543 (1.8)	12071	19% per OR 27% per ET
Social sexing	671	4285	993 (2.0)	492	21% per OR 29% per ET

OR, oocyte retrieval; ET, embryo transfer procedure.

First systematic experience of preimplantation genetic diagnosis for single-gene disorders, and/or preimplantation human leukocyte antigen typing, combined with 24-chromosome aneuploidy testing

Svetlana Rechitsky, Ph.D., Tatiana Pakhalchuk, M.S., Geraldine San Ramos, M.S., Adam Goodman, B.S., Zev Zlatopolsky, M.S., and Anver Kuliev, M.D., Ph.D.

Reproductive Genetics Institute, Northbrook, Illinois

TABLE 2

Outcome of PGD with and without 24-chromosome aneuploidy testing in overall experience of 2,401 PGD cycles for SGD and HLA typing.

Test type	No. of patients	No. of cycles	ET	No. of embryos transferred	Pregnancy	SAB	Birth	Babies
SGD	1,120	1,860	1,547	3,014	726	104	622	791
HLA + SGD	79	180	111	177	33	10	23	25
HLA	19	44	30	45	7	1	6	6
Subtotal	1,218	2,084	1,688	3,236	766	115	651	822
			81%	1.9	45.4%	15%	85%	
SGD + aCGH	223	286	196	263	137	8	129	141
HLA + SGD + aCGH	12	26	12	13	5	0	5	5
HLA + aCGH	3	5	4	4	3	0	3	3
Subtotal	238	317	212	280	145	8	137	149
			67%	1.3	68.4%	5.5%	94.5%	
					P≤.0001	P≤.0027		
Total	1,456	2,401	1,900	3,516	911	123	785	969

Note: aCGH = array comparative genomic hybridization; ET = embryo transfer; SAB = spontaneous abortion.

Rechitsky. Combined PGD with 24-chromosome aneuploidy testing. Fertil Steril 2015.

J Genet Counsel (2016) 25:1327–1337
DOI 10.1007/s10897-016-9975-4

ORIGINAL RESEARCH

Preimplantation Genetic Diagnosis (PGD) for Monogenic Disorders: the Value of Concurrent Aneuploidy Screening

Kara N. Goldman[1,2] · Taraneh Nazem[1] · Alan Berkeley[1] · Steven Palter[3] · Jamie A. Grifo[1]

	PGD + 24-chromosome aneuploidy screening (n = 32 patients)	PGD alone (n = 8 patients)	p-value
Mean no. embryos transferred [a]	1.1 ± 0.3	1.9 ± 0.6	.0001
Patients (%) undergoing single ET [b]	87.5 %	25 %	.001
Implantation rate [b]	75 %	53.3 %	.19
Spontaneous abortion rate [b]	20 %	40 %	.56
Multiple gestation rate [b]	12.5 %	12.5 %	1
Live birth rate [b]	59.4 %	37.5 %	1

Data are presented in mean ± standard deviation or percentage (%). Data were analyzed with Student's t-test[a] and Fisher's exact test[b], $p < 0.05$

tek gen hastalıklarının taranması amaçlı PGT uygulmalarına
24-Kromozom anöploidi testinin kombinasyonu

İleri Anne Yaşı

Acil HLA Tiplemesi

Translokasyon Taşıyıcılığı

PGS TECHNOLOGIES

FISH ANAYSIS

- Detects 8-12 chromosome
- Insufficient for euploid embryo selection

ARRAY-CGH

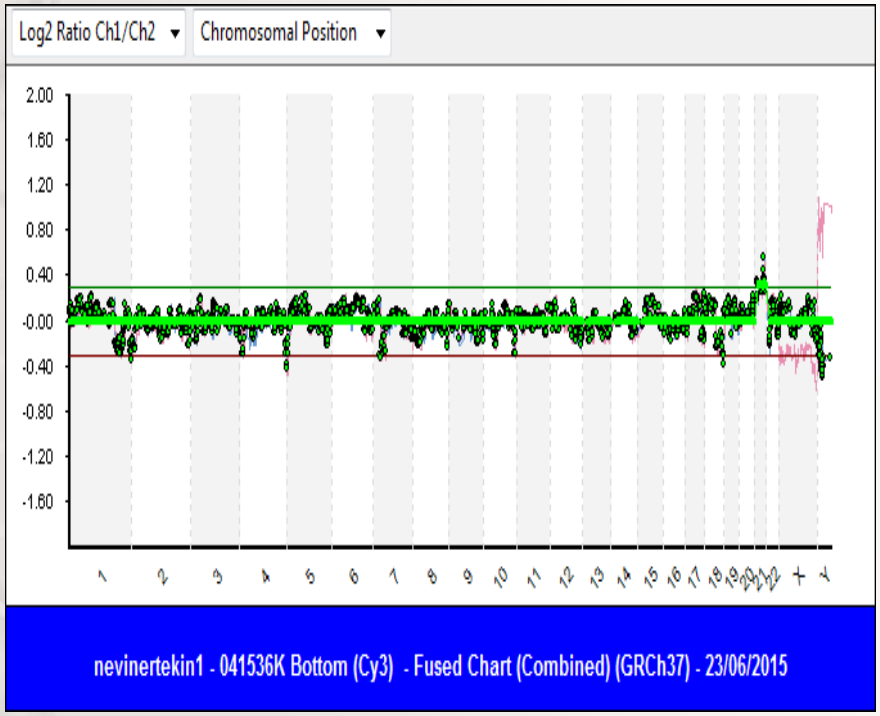
- Detects 24 chromosome
- Improves IVF success

NGS

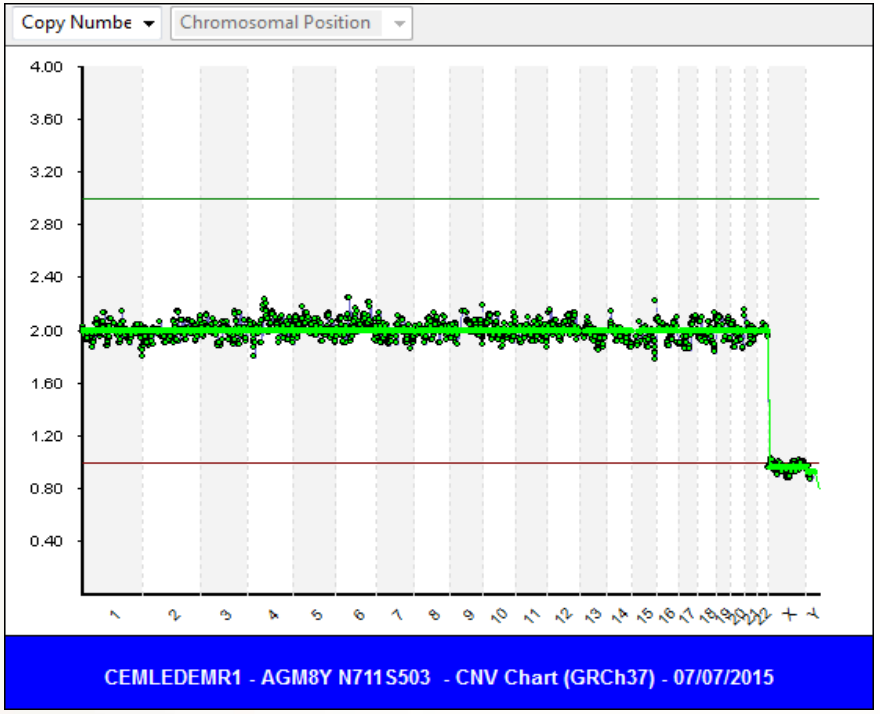
- Novel technology
- Hi fidelity
- High sensitive

Array CGH / NGS Kıyaslaması

Array CGH



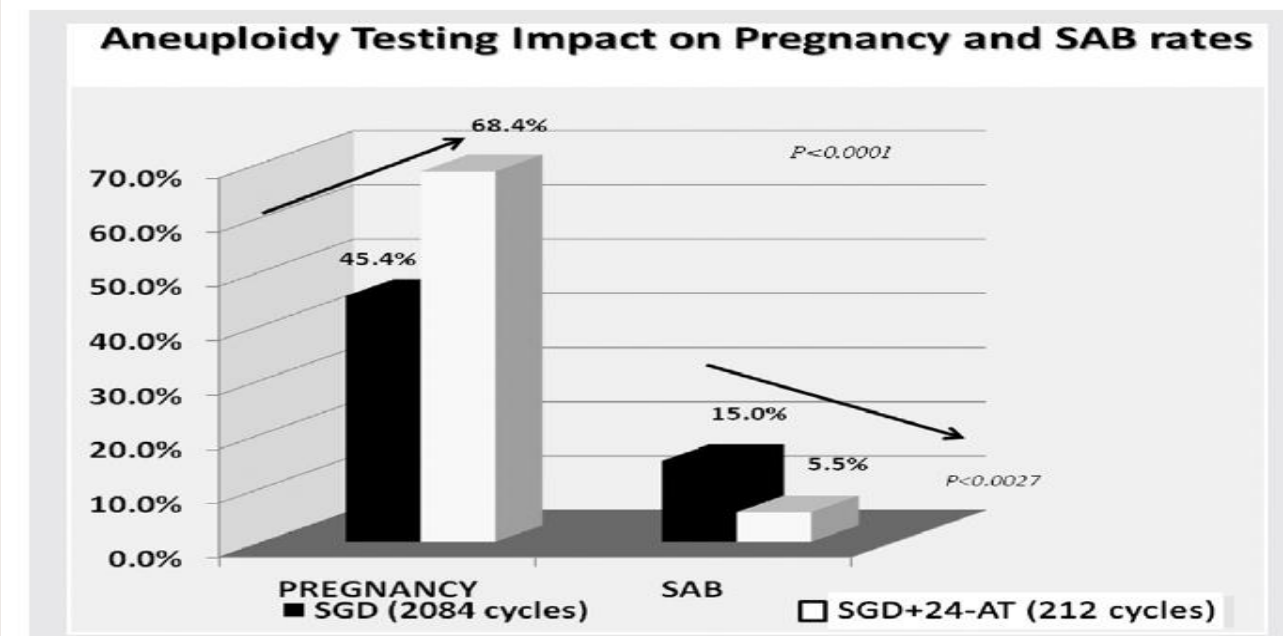
NGS



Tek Gen Hastaları için 24 kromozom taraması gerekli mi ?

Tek gen hastaları genç ve infertilite problemi olmayan bir hasta grubudur

Bu rağmen spontan düşük oranı bu hasta grubunda %15 seviyesinde



Artan implantasyon oranı 68.4 % ve 45.4 %

Azalan spontan düşük 5.5 % vs 15.0 %

J Genet Counsel (2016) 25:1327–1337
DOI 10.1007/s10897-016-9975-4

ORIGINAL RESEARCH

Preimplantation Genetic Diagnosis (PGD) for Monogenic Disorders: the Value of Concurrent Aneuploidy Screening

Kara N. Goldman [1,2] · Taraneh Nazem [1] · Alan Berkeley [1] · Steven Palter [3] · Jamie A. Grifo [1]

Table 4 Outcomes of pre-implantation genetic testing

	PGD + 24-chromosome aneuploidy screening (n = 355 blastocysts)	PGD alone (n = 64 blastocysts)	p-value
SGD-unaffected blastocysts (excluding carriers) [a]	123/355 = 34.6 %	19/64	
SGD-unaffected blastocysts (including carriers) [a]	173/355 = 48.7 %	35/64	
SGD-affected [a]	132/355 = 37.0 %	21/64	
Blastocysts with SGD result	313/355 = 88.1 %	56/64	
Aneuploid blastocysts	177/355 = 49.9 %		
Euploid blastocysts	169/355 = 47.6 %		
SGD-unaffected and aneuploid	58/355 = 16.3 %		
SGD-unaffected and euploid (eligible for transfer)	73/355 = 20.6 %		
Eligible for transfer based on all testing performed[a] *including SGD-negative and SGD-carriers	91/355 = 25.6 %	35/64 = 54.7 %	.001
Patients with ≥1 SGD-unaffected but aneuploid blastocyst	25/47 = 53.2 %		

Data are presented in percentage (%). [a] Data were analyzed with Fisher's exact test where appropriate, $p < 0.05$

J Genet Counsel (2016) 25:1327–1337
DOI 10.1007/s10897-016-9975-4

ORIGINAL RESEARCH

Preimplantation Genetic Diagnosis (PGD) for Monogenic Disorders: the Value of Concurrent Aneuploidy Screening

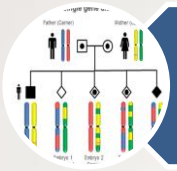
Kara N. Goldman [1,2] · Taraneh Nazem [1] · Alan Berkeley [1] · Steven Palter [3] · Jamie A. Grifo [1]

Table 6 Outcomes of frozen embryo transfer

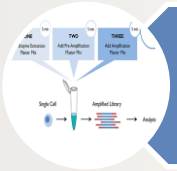
	PGD + 24-chromosome aneuploidy screening ($n = 32$ patients)	PGD alone ($n = 8$ patients)	p-value
Mean no. embryos transferred [a]	1.1 ± 0.3	1.9 ± 0.6	.0001
Patients (%) undergoing single ET [b]	87.5 %	25 %	.001
Implantation rate [b]	75 %	53.3 %	.19
Spontaneous abortion rate [b]	20 %	40 %	.56
Multiple gestation rate [b]	12.5 %	12.5 %	1
Live birth rate [b]	59.4 %	37.5 %	1

Data are presented in mean ± standard deviation or percentage (%). Data were analyzed with Student's t-test[a] and Fisher's exact test[b], $p < 0.05$

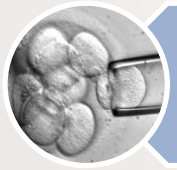
Tek Gen + 24 kromozom tarama uygulamaları



Karyomapping



WGA ile PGT uygulaması



3. Günde çift biyopsi alınması

3. Ve 4. günde ardışık biyopsi planlanması

Trofektoderm hücrelerinin iki parça halinde alınması

Karyomapping

Karyomapping - Diagnostic Laboratory Process

Whole Genome Amplification of samples using SureMDA (2.5 hrs)

Kit = 96 reactions

↓

Process DNAs - Infinium HumanKaryomap-12 DNA analysis kit (20 hrs)

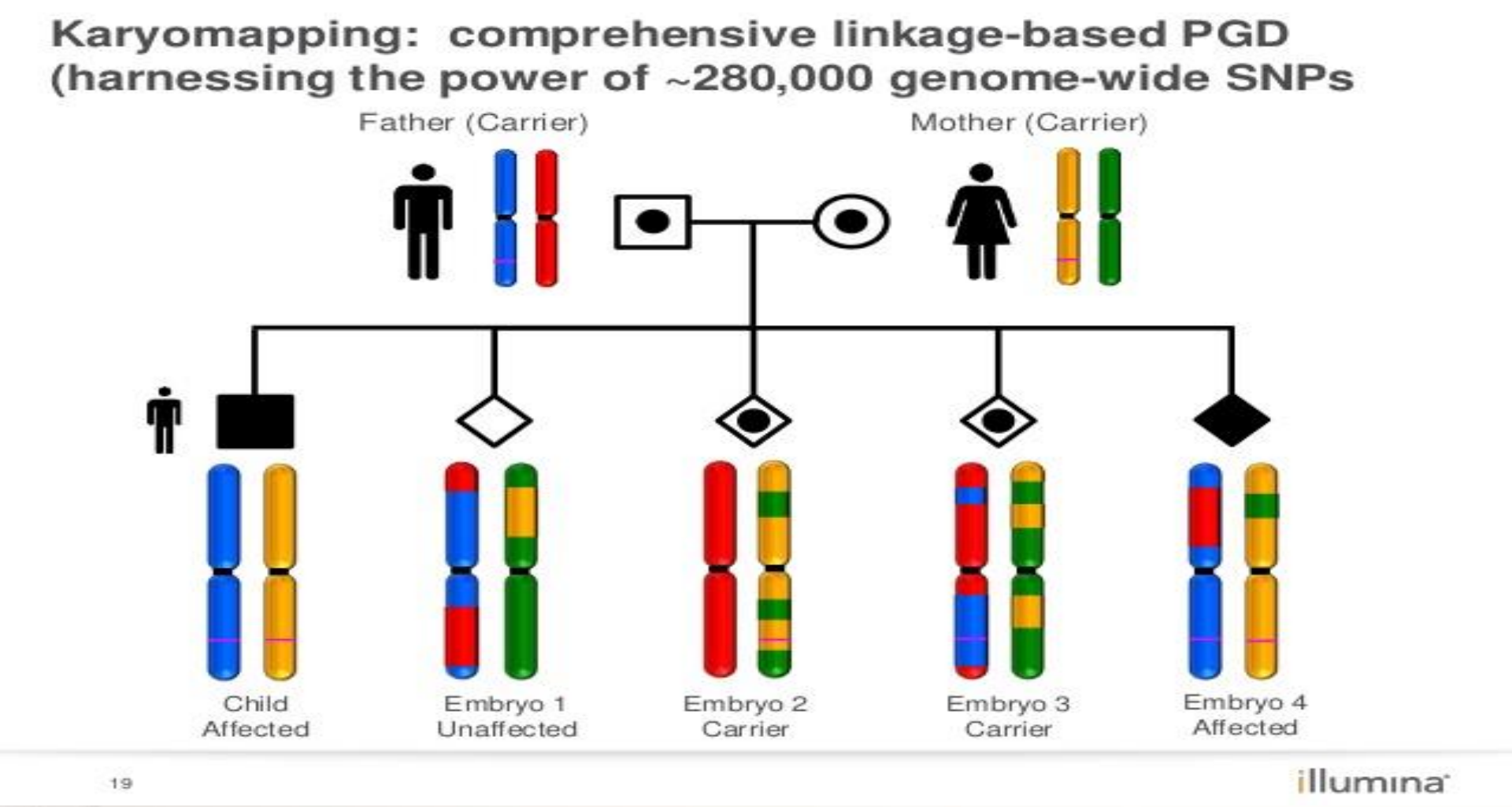
Kit = 24 samples (12 per run)

↓

Scan using iScan (0.5 hr)

↓

Import scan data in to BlueFuse multi v4.0 (karyomapping module), Analyse results, Report (~1 hr)



- Bugüne kadar STR markerleri ile PGT uygulaması altın standart olarak kullanıldı
- Her hasta(lık) için bu testlerin optimize edilmesi gereği

Karyomapping Limitasyonları

- Henüz çok yeni ve tek hasta olduğunda data sorunlu
- Akraba evliliğnde data analizi sıkıntılı***
- Denovo mutasyonlar
- Ek cihaz gerekliliği
- Yaygın hastalıklar için gereksiz ek masraf

*** Akraba evliliği Türkiye PGD talebinin en yaygın sebebi

*** Bu ailelerde STR markerleri ortaklık ve homozigotlaşma gösteriyor. (PGS-IS 2015).

Journal of Genetics and Genomics

Journal homepage: www.journals.elsevier.com/journal-of-genetics-and-genomics/

Original research

Clinical applications of MARSALA for preimplantation genetic diagnosis of spinal muscular atrophy

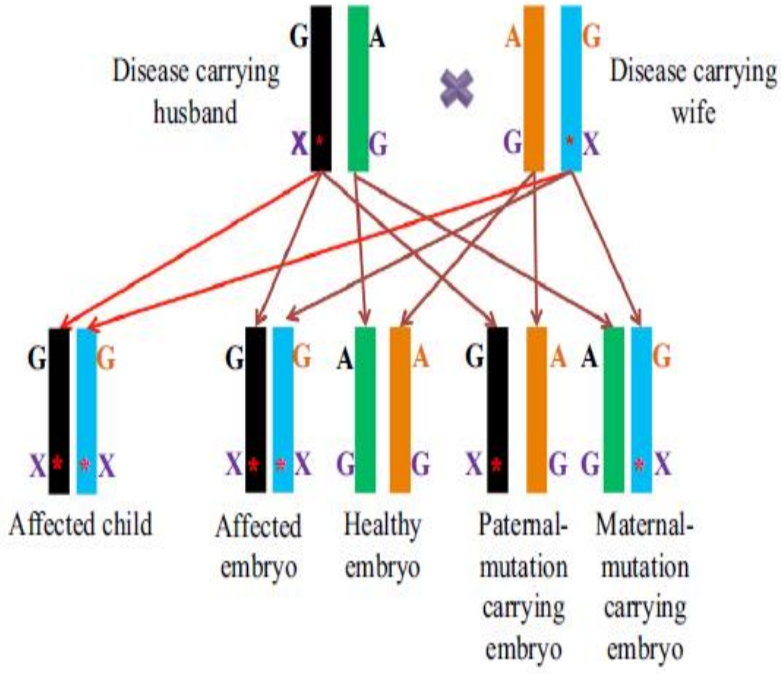
Yixin Ren [a, b], Xu Zhi [a, b], Xiaohui Zhu [a, b], Jin Huang [a, b], Ying Lian [a, b], Rong Li [a, b],

Mutated allele revealed by sequencing with aneuploidy and linkage analyses

- hereditary multiple exostoses
- X-linked hypohidrotic ectodermal dysplasia (Yan et al., 2015).

YÖNTEM

- *NGS ile direkt mutasyon analizi*
- *CNV setleri kullanarak düşük çözünürlüklü NGS ile24 kromozom taraması*
- *SNP'lerle linkage analizi*



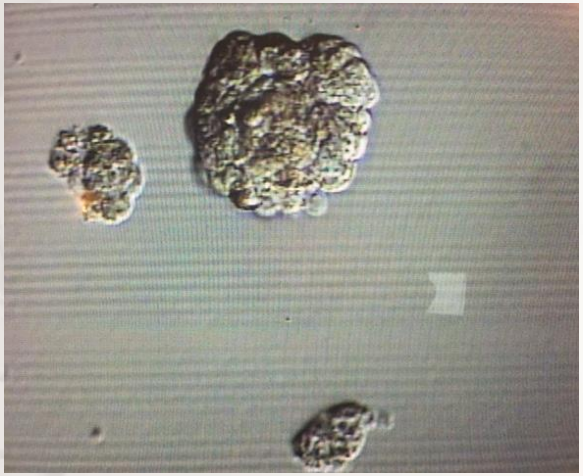
Concurrent PGD for Single Gene Disorders and Aneuploidy on Single Cells

- Two blastomere biopsy on day 3
- Simultaneous biopsy on day 3 and on day 4
- Splitting trophectoderm cells into two pieces

Simultaneous biopsy on day 3 and on day 4

Emb #	MUTATION	MUTATION MARKERS				SINGLE GENE RESULTS	TRANSLOCATION RESULTS
	p.Val9Ala	D11S1883	D11S4191	D11S4076	D11S4205		
1	N/N	144/150	252/231	290/280	300/302	NORMAL	NORMAL
2	p.Val9Ala/ N	142/150	248/231	288/280	300/302	HETEROZYGOUS	AFFECTED EMBRYO. Translocation screening was not performed.
5	p.Val9Ala/ N	142/144	248/231	288/280	300/300	HETEROZYGOUS	AFFECTED EMBRYO. Translocation screening was not performed.
BABA	p.Val9Ala/ N	142/144	248/252	288/290	300/302	HETEROZYGOUS	
ANNE	N/N	144/150	231/231	280/280	300/302	NORMAL	
HASTA ÇOCUK	p.Val9Ala/ N	142/144	248/231	288/280	300/300	HETEROZYGOUS	

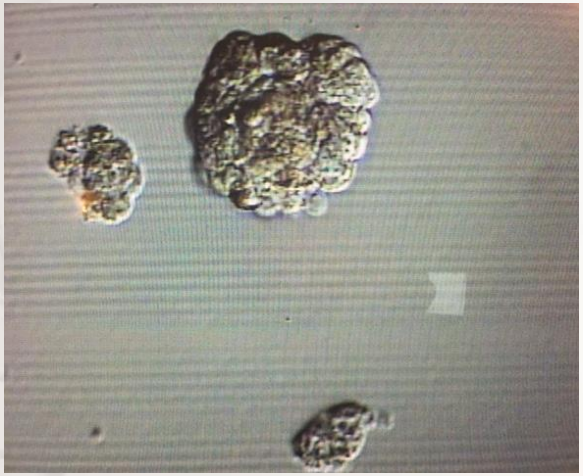
Splitting trofectoderm cells into two pieces



Emb #	Single gene disorder result	24 Chromosome screening results	EMBRYO TRANSFER	Suggestions
1	NORMAL	NORMAL	YES	
2	HETEROZYGOUS	NOT TESTED	NO	
3	NORMAL	NORMAL	YES	
4	HETEROZYGOUS	NOT TESTED	NO	
5	NORMAL	MONOZOMI 14 MONOZOMI 21	NO	
8	HETEROZYGOUS	NOT TESTED	NO	
9	NORMAL	NORMAL	YES	
10	HETEROZYGOUS	NOT TESTED	NO	

Emb #	MUTATION MARKERS					RESULT
	D17S221S	D17S91S	4A	9A	D17S261	
1	204/198	246/258	120/114	108/119	160/158	NORMAL
2	196/198	246/258	124/114	104/119	160/158	HETEROZYGOUS
3	204/198	246/258	120/114	108/119	160/158	NORMAL
4	196/198	246/258	124/114	104/119	160/158	HETEROZYGOUS
5	204/202	246/246	120/120	108/108	160/160	NORMAL
8	196/198	246/258	124/114	104/119	160/158	HETEROZYGOUS
9	204/202	246/246	120/120	108/108	160/160	NORMAL
10	196/202	246/246	124/120	104/108	160/160	HETEROZYGOUS
FATHER	196/204	246/246	124/120	104/108	160/160	HETEROZYGOUS
MOTHER	198/202	246/258	114/120	108/119	158/160	NORMAL
BROTHER OF MALE PARTNER	196/206	246/258	124/114	104/108	160/162	HETEROZYGOUS

Splitting trophectoderm cells into two pieces



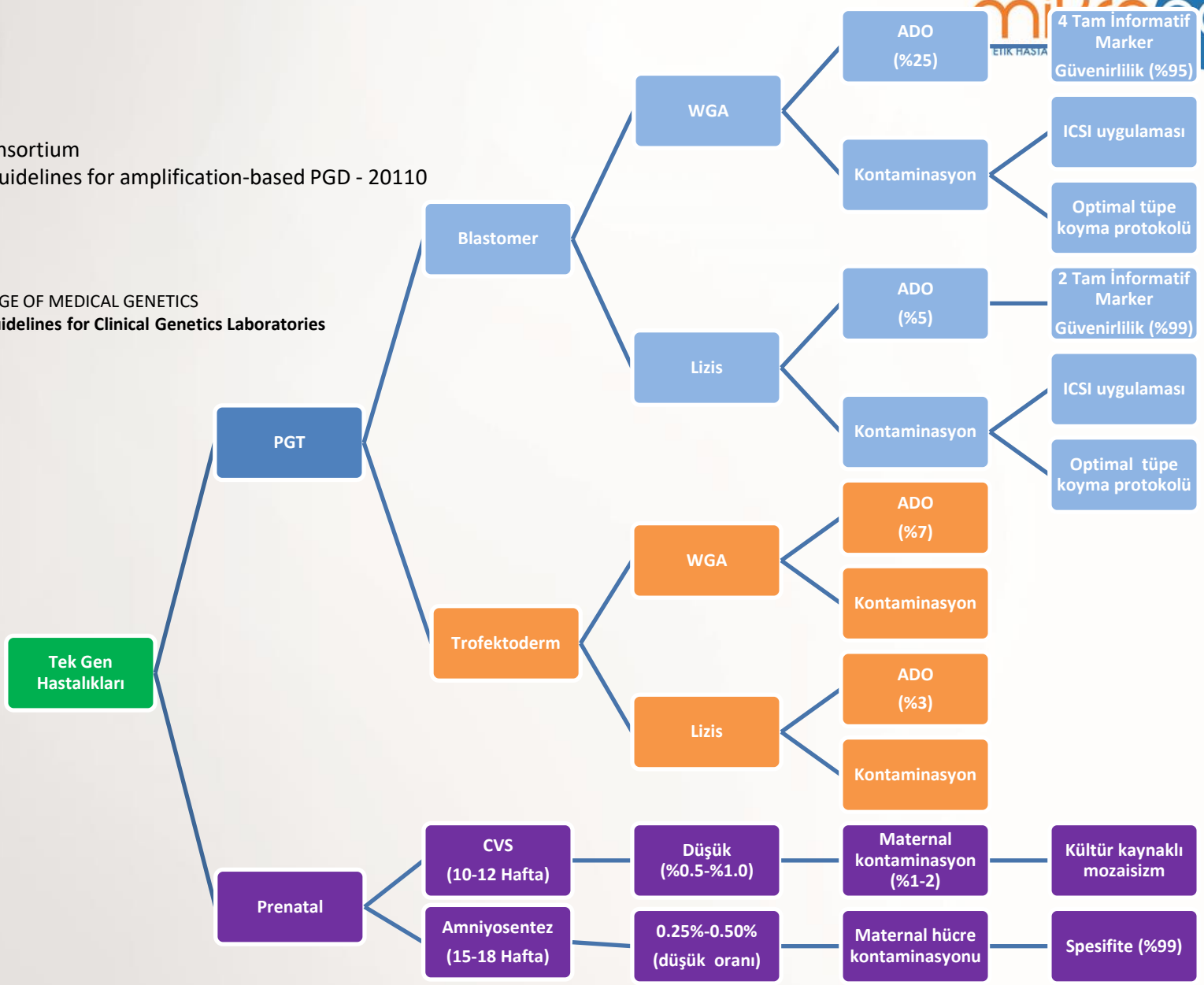
Emb #	SICKLE CELL RESULTS	24 CHROMOSOME RESULTS
1	HETEROZYGOUS	MONOSOMY 15, TRIZOMY 19
2	HETEROZYGOUS	NORMAL
3	NORMAL	TRIZOMY 21, MONOSOMY 4
4	NORMAL	PARTIAL TRIZOMY 19
5	HETEROZYGOUS	MONOSOMY I 22
6	NORMAL	MONOSOMY 9, TRIZOMY 20
7	MUTANT	NOT TESTED

Emb #	HBS	MARKER D11S2362	MARKER D11S4181	MARKER D11S1760	MARKER D11S1338	ORAK HÜCRE SONUÇLARI
1	N/ HBS	216/212	108/112	82/86	141/144	HETEROZYGOUS
2	N/ HBS	216/212	108/112	82/86	141/144	HETEROZYGOUS
3	N/N	216/219	108/108	82/86	141/137	NORMAL
4	N/N	216/219	108/108	82/86	141/137	NORMAL
5	HBS /N	212/219	112/108	86/86	144/137	HETEROZYGOUS
6	N/N	216/219	108/108	82/86	141/137	NORMAL
7	HBS/ HBS	212/212	112/112	86/86	144/144	MUTANT
FATHER	HBS /N	212/216	112/108	86/82	144/141	HETEROZYGOUS
MOTHER	N/ HBS	219/212	108/112	86/86	137/144	HETEROZYGOUS

Tek Gen Hastalıklarının Taranmasında Genetik Algoritmalar

ESHRE PGD consortium
best practice guidelines for amplification-based PGD - 20110

AMERICAN COLLEGE OF MEDICAL GENETICS
Standards and Guidelines for Clinical Genetics Laboratories
2006 Edition.



WHOLE EXOM SEQUENCING ENABLES PGD FOR COMPLICATED CASES WITHOUT MOLECULAR DIAGNOSIS

mikrogen
GENETİK HASTALIKLAR TANI MERKEZİ

PEDİGRİ ANALİZİ:

Your ref.: **112167**
DOB (dd.mm.yyyy): **29.01.2011**
Sample collection date (dd.mm.yyyy): **20.05.2015**
Order received (dd.mm.yyyy): **27.05.2015**

Sex: **female**
Patient no.: **1075725**
Sample type: **DNA**
Order no.: **62240463**

Whole Exome Sequencing (WES) for all available family members (index patient and parents).

Clinical information: The index is a 4 years old girl born to consanguineous parents and diagnosed with epileptic seizures, lactic acidemia, delayed motor milestones, delayed speech, development regression, epilepsy generalized, mental retardation, microcephaly, muscle weakness, dysmorphic features, growth retardation, elevated transaminases. Family history: there is a similarly affected female sibling (5.5 years old). There are other affected members in the family.

Clinically relevant variants with significant phenotype overlapping with your patient

Gene (transcript)	Nucleotide (protein)	Zygosity	Described by	In silico parameters*	MAF**	Variant classification***	Disorder (OMIM#, inheritance)
OCLN NM_001205254.1	c.173_194del (p.Trp58Phefs*10)	Homozygous	-	NA	Not reported	Likely pathogenic	Band-like calcification with simplified gyration and polymicrogyria (OMIM: 251290)

*: number of applied in silico prediction programs that are pathogenic, benign, or not applicable/not conclusive (NA/NC) as well as if the variant is conserved (both GERP++ and PhyloP have positive values) or not. **: minor allele frequency (MAF) of Exome Aggregation Consortium database (ExAC), Exome Sequencing Project (ESP), or 1000Genome project (1000G)). ***: variant classification based on CentoMD® and ACMG recommendations (see additional information below for details on the classification). Further information can be found in the interpretation, disclaimer and methods section.

A genetic diagnosis of band-like calcification with simplified gyration and polymicrogyria is very likely.

PGD for 3 Mutations

Emb	MENKES HASTALIĞI SONUÇLARI	JOUBERT (CEP20) HASTALIĞI SONUÇLARI	JOUBERT (CSPP1) HASTALIĞI SONUÇLARI	EMBRİYO TRANSFERİ
1	HETEROZİGOT	HETEROZİGOT	NORMAL	HAYIR
2	NORMAL	HETEROZİGOT	NORMAL	EVET
3	NORMAL	NORMAL	NORMAL	EVET
4	NORMAL	HETEROZİGOT	HETEROZİGOT	EVET
5	NORMAL	NORMAL	MUTANT	HAYIR
6	NORMAL	NORMAL	MUTANT	HAYIR
7	NORMAL	NORMAL	NORMAL	EVET
8	NORMAL	HETEROZİGOT	MUTANT	HAYIR

Emb	MENKES (ATP7A) MUTASYON VE MARKERLARI					JOUBERT (CEP290) MUTASYON VE MARKERLARI				JOUBERT (CSPP1) MUTASYON VE MARKERLARI			
	p.R1455Q	DXS6800	DXS1225	DXS7131	DXS1197	p.R2112Q	D12S1593	D12S819	D12S1598	p.P1148S	D8S1775	D8S553	D8S1840
1	N/ p.R1455Q	256/244	196/202	381/381	248/250	p.R2112Q /N	95/93	342/346	168/174	N/N	155/155	248/252	234/225
2	N/N	256/263	196/206	381/377	248/250	p.R2112Q /N	95/93	342/346	168/174	N/N	155/155	248/252	234/225
3	N	263	206	377	248	N/N	91/93	339/346	174/174	N/N	155/155	248/252	234/225
4	N	263	206	377	248	N/ p.R2112Q	91/95	339/342	174/168	p.P1148S/N	153/155	250/252	234/225
5	N/N	256/263	196/206	381/377	248/250	N/N	91/93	339/346	174/174	p.P1148S / p.P1148S	153/153	250/250	234/234
6	N/N	256/263	196/206	381/377	248/250	N/N	91/93	339/346	174/174	p.P1148S / p.P1148S	153/153	250/250	234/234
7	N	263	206	377	248	N/N	91/93	339/346	174/174	N/N	155/155	248/252	234/225
8	N	263	206	377	248	p.R2112Q /N	95/93	342/346	168/174	p.P1148S / p.P1148S	153/153	250/250	234/234
BABA	N	256	196	381	250	p.R2112Q/ N	95/91	342/339	168/174	p.P1148S/N	153/155	250/248	234/234
ANNE	N/ p.R1455Q	263/244	206/202	377/381	248/250	N/ p.R2112Q	93/95	346/342	174/168	N/ p.P1148S	155/153	252/250	225/234
SAĞLIKLI ÇOCUK	N	263	206	377	248	N/ p.R2112Q	91/95	339/342	174/168	p.P1148S/N	153/155	250/252	234/225

Preimplantation Diagnosis for Single Gene Disorders

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[1] Department of Obstetrics and Gynecology, Stanford University School of Medicine, Stanford, California

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Semin Reprod Med 2014;32:107–113

- Temelde tek gen hastalarının infertilite infertilite problemleri olmadığından IVF prosedürüne ihtiyaçları yoktur.
- Yaşam boyu hastalık maliyeti düşünüldüğünde IVF + PGT'den doğan hasta başı maliyet sosyal bedelin dğşşrğlmesi anlamına gelmektedir.
- **Cost – benefit analysis:**
  - 40 yaşa kadar PGD'nin naturel konseptusa üstünlüğü vardır
  - Hasta çocuğun yaşam boyu maliyetine net üstünlüğü vardır.

- Türkiyede Talasemi için taşıyıcı sıklığı yaklaşık olarak %2,1 (1.400.000 taşıyıcı birey) olarak bildirilmiştir.
- Yaklaşık olarak 13.000 hasta bireyin bulunduğu bilinmektedir.
- Dünya sağlık örgütünün verilerine göre Dünyada Talasemi ve anormal hemoglobin taşıyıcı sıklığı % 4,5' tir (yaklaşık 270 000 000 taşıyıcı).
- Bir Talasemi hastasının ***yıllık tedavi maliyeti 45 bin lirayı*** bulmaktadır.
- Bir Talasemi hastasının devletimize tedavi maliyeti ***1 milyon 500 bin lirayı*** geçmektedir.

mikrogen
GENETİK HASTALIKLAR TANI MERKEZİ

Laboratuvar Direktörü
Prof. Dr. Volkan Baltacı

Laboratuvar Sorumlu Hekim
MD. PhD. Leyla Özer

Laboratuvar Yöneticisi
Yard. Doç. Dr. Evrim Ünsal

Laboratuvar Sorumlusu
Yard. Doç. Dr. Süleyman Aktuna

İdari İşler Sorumlusu
Sönmez Hasırcı

Zehra Çatal
M. Büşra Köse
Murat Satılmış
Gülhan Seyfi
Berkay Çetinkaya
Gülten Mutluer

Sitogenetik Laboratuvar Sorumlusu
Mehmet Muhtar Şen

Adem Öğütveren
Burak Kasal
Burcu Düzgün
Mediha Kasal
Safiye Böyük
MSc. Zeynep Öncü
Elif Tunç Yaylalı
Yasemin Yılmaz

Moleküler Genetik Laboratuvar Sorumlusu
Yard. Doç. Dr. Süleyman Aktuna

Doç. Dr. Türker Duman
İrfan Atmaca
PhD. Merve Aydın
MSc. Ilgın Gizem Bedir
MSc. Gökçen Kolsal
Nurullah Gökyurt
MSc. Golchin Memari
Ecem Mercan
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Kurumsal İlişkiler Sorumlusu
Orhan Doğan

İbrahim Alpavut
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Cem Sevindik
Barış Demirer
Neşe Aytemiz
Seda Alçın