V. ÜREME TIBBI VE CERRAHİSİ DERNEĞİ KONGRESİ
28 - 31 Ekim 2015
Cornelia Diamond Resort
Belek, Antalya

Türkiye'de PGS Güncel Durum

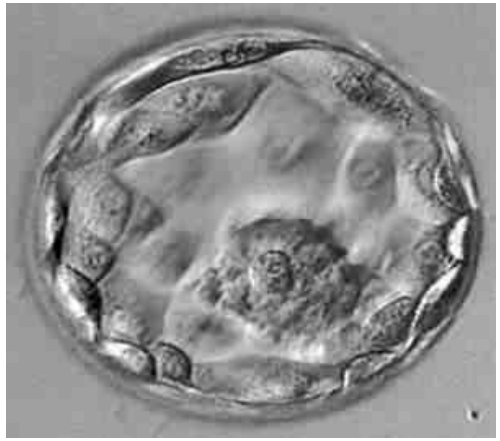
Prof. Dr. Muhterem BAHÇE

MBGENLAB GENETİK HASTALIKLAR TANI MERKEZİ

PGS GEREKLİ Mİ?

- TIME LAPSE

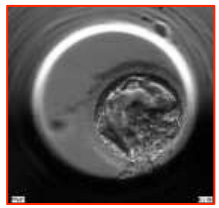
- D5 TRANSFER



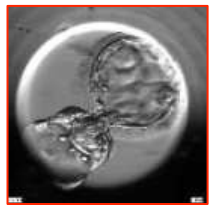
Time-lapse ile morfoloji anöploidi ile kısmi korelasyon gösteriyor

- Hızlı ve yavaş gelişen blastosistler benzer anöploidi oranlarına sahip(2)
- 2.günde, anöploid embriyoların %30'u öploid embriyolar ile aynı gelişimi gösteriyor(3)
- Blastula oluşumu anöploid embriyolarda gecikiyor(1,4), ancak hala iyi gelişen blastosistlerin %50'sinde anöploidi mevcut(1)

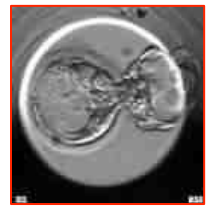
Euploid



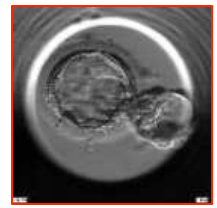
Euploid



Complex abn.



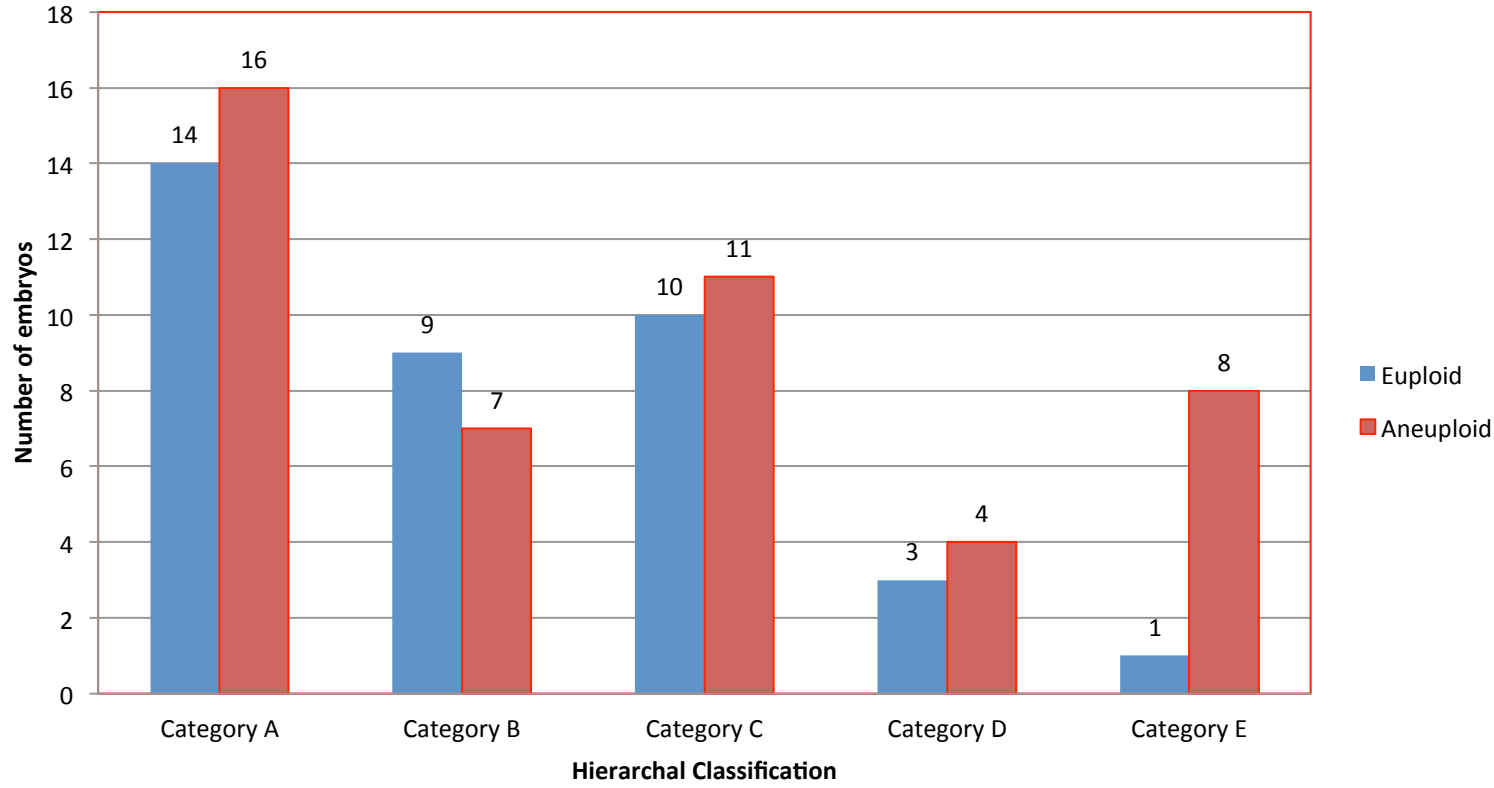
Trisomy 21,22



(1) Melzer et al (in press) (2) Stevens et al. (2012), (3) Chavez et al. (2012), (4) Campbell et al. (2013)

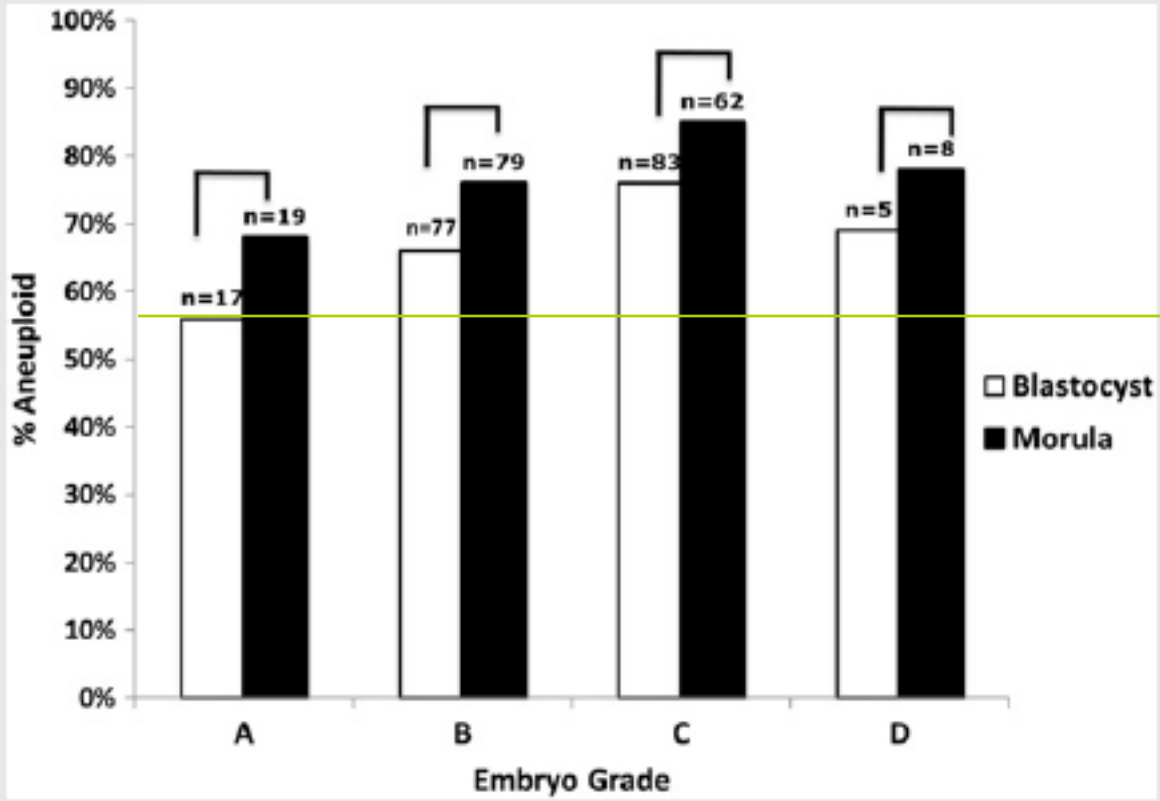
Time-lapse ve anöploidi: Melzer ve ark.ları (2013)

- Only timing of compaction was correlated by aneuploidy
- Only gross morphological abnormalities (group E) were more likely aneuploid.



Melzer et al. (2013) Reprod Biomed Online, in press

5.gün embriyolarında morfoloji kromozomal anomaliler için uygun belirteç midir?



55% of best morphology blastocysts were aneuploid

Comparison of morphologic stage, grade, and aneuploidy rates in day 5 embryos. Logistic regression model was used for the statistical analysis. Embryo number, maternal age, and stimulation protocol type were all controlled for in the calculations. $P<.05$ between morphologic stages at each grade.

Kroener. Aneuploidy and timing of embryonic progression. Fertil Steril 2012.

Reproductive BioMedicine Online (2014) 28, 485–491

www.sciencedirect.com
www.rbmonline.com

ARTICLE

Blastocyst culture selects for euploid embryos: comparison of blastomere and trophectoderm biopsies

Alexis Adler *, Hsaio-Ling Lee, David H McCulloh, Esmeralda Ampeloquio, Melicia Clarke-Williams, Brooke Hodes Wertz, James Grifo

New York University Fertility Center, New York University Langone School of Medicine, 660 1st Ave., 5th Floor, New York, NY 10016, United States

Preimplantation genetic diagnosis and screening improves the chances of achieving a viable pregnancy, not only free of undesired single-gene defects but also aneuploidy. In addition, improvements in vitrification provide an efficient means of preserving embryos (blastocysts). By combining trophectoderm biopsy with recent improvements in vitrification methods, only those embryos that have proved themselves viable and potentially more competent are tested. Using array comparative genomic hybridization (aCGH) to assess all 24 chromosomes, aneuploidy rates were compared between day-3 blastomere biopsy and day-5 trophectoderm biopsy. Of those 1603 embryos, 31% were euploid, 62% were aneuploid and 7% not analysable. **A significantly larger proportion of embryos were euploid on day-5 biopsy (42%) compared with day-3 biopsy (24%, P < 0.0001). The number of euploid embryos per patient was not significantly different.**

J Assist Reprod Genet (2013) 30:1081–1090
DOI 10.1007/s10815-013-0048-4

EMBRYO BIOLOGY

Could time-lapse embryo imaging reduce the need for biopsy and PGS?

Jason E. Swain

Conclusion: With continued effort, the combination of multiple morphologic endpoint assessments and developmental timings and refinement of modeling systems may improve the predictive ability to determine embryonic aneuploidy. This may help select a subset of embryos that are less likely to carry chromosomal abnormalities and improve assisted reproductive outcomes. ***However, embryo biopsy, followed by preimplantation genetic screening/ comprehensive chromosomal screening still remains the most reliable method to assess chromosomal complement of preimplantation embryos.***

PGS ANALİZ YÖNTEMLERİ

- FISH (8?)
- FRAGMAN ANALİZİ (1)
- ACGH (5)
- Snp aCGH (-)
- QUANTITATIVE REAL TIME PCR (qPCR) (-)
- NEXT GENERATION SEQUENCING (2)

3. Gün biyopsisi ve FISH analizinde karşıt veriler

Pozitif etki

Gianaroli et al. 1999
Munné et al 1999
Gianaroli et al 2001a
Gianaroli et al. 2001b
Munné et al. 2003
Gianaroli et al. 2004
Munné et al. 2005
Munné et al 2006
Verlinsky et al. 2005
Colls et al. 2007
Garrisi et al. 2009
Rubio et al. 2009
Rubio et al. 2013 *

Etki yok

Werlin et al. 2003 *
Staessen et al. 2004 *
Platteau et al. 2005 *
Jansen et al. 2008 *
Mersereau et al. 2008 *
Schoolcraft et al. 2009 *

Negatif etki

Mastenbroek et al. 2007 *
Hardarson et al. 2008 *

*** CRT**

FISH

- 5 KROMOZOM
- 9 KROMOZOM
- 12 KROMOZOM

Analiz edilen kromozom sayısı

24-kromozom testleri (CGH, aCGH, etc) FISH-12 ye göre % 50 daha fazla anomali yakalıyor ancak sadece % 20 daha fazla embriyo anormal tanı alıyor.

Colls et al. (2009) Reprod Biomed Online 19:532-538

aCGH d3+d5

Toplam embriyo	: 631
Normal	: 206
Anormal	: 425
Fish	: 307 (%72.23)
aCGH	: 118 (%27.76)

aCGH D3/D5

	D5	D3
Toplam embriyo	: 209	422
Normal	: 72 (%34.44)	126 (%29.85)
Anormal	: 137 (%65.55)	296 (%70.14)
Fish	: 100 (%72.99)	212 (%71.62)
aCGH	: 37 (%27.00)	84 (%28.37)

3.Gün ve blastosist biyopsisinin karşılaştırılması

	cleavage stage		blastocyst	
	biopsy	not	biopsy	not
Implantation rate	**31%**	**53%**	**52%**	**54%**
	P<0.05		N.S.	

…but biopsy is an operator-dependent procedure and its effect may vary

Scott et al. (2013) Fertil Steril

Yang et al. Molecular Cytogenetics (2015) 8:49
DOI 10.1186/s13039-015-0140-9

MOLECULAR CYTOGENETICS

RESEARCH Open Access

Preimplantation genetic screening of blastocysts by multiplex qPCR followed by fresh embryo transfer: validation and verification

Yu-Shih Yang[1†], Shun-Ping Chang[2†], Hsin-Fu Chen[1,3†], Gwo-Chin Ma[2,4†], Wen-Hsiang Lin[2], Chi-Fang Lin[1], Feng-Po Tsai[5], Cheng-Hsuan Wu[6], Horng-Der Tsai[6], Tsung-Hsien Lee[1,7] and Ming Chen[1,2,6,8*]

PGS was conducted by qPCR with selectively amplified markers to detect common aneuploidies (***chromosomes 13, 18, 21, X, and Y***)

Curr Opin Obstet Gynecol. 2015 Jun;27(3):201-5. doi: 10.1097/GCO.0000000000000167.

24-chromosome PCR for aneuploidy screening.

Werner MD[1], Scott RT Jr, Treff NR.

RECENT FINDINGS:

The rigorous preclinical validation of quantitative real-time (q)PCR-based CCS involved an initial validation on cell lines, followed by a blinded evaluation on embryos. Comparison with alternative platforms and a prospective randomized clinical trial demonstrate superior precision and improved sustained implantation and delivery rates. Preclinical validation of targeted PCR-based next-generation sequencing (NGS) has also demonstrated consistency in positive controls, ***equivalent accuracy to commonly used techniques, high resolution, increased throughput, the simultaneous detection of single gene disorders and triploidy, and the potential to decrease costs.*** Prospective randomized controlled trials are ongoing to validate this technique for clinical use.

Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles

Francesco Fiorentino[1,*], Sara Bono[1], Anil Biricik[1], Andrea Nuccitelli[1], Ettore Cotroneo[1], Giuliano Cottone[1], Felix Kokocinski[2], Claude-Edouard Michel[2], Maria Giulia Minasi[3] and Ermanno Greco[3]

Array comparative genomic hybridization (array-CGH) has been demonstrated to be an accurate PGS method and has become the *de facto* ***gold standard***, but new techniques, such as NGS, continue to emerge.

LIMITATION, REASON FOR CAUTION Although clinical results reported high pregnancy outcomes following transfer of screened embryos, ***further data and broad-based clinical application are required to better define the role of NGS in PGS. Before recommending widespread application, a randomized controlled trial confirming its clinical effectiveness is advisable.***

MAIN RESULTS AND THE ROLE OF CHANCE A total of 192 blastocysts were blindly evaluated with the NGS-based protocol. ***Paired comparison between NGS and array-CGH from individual embryos showed concordant results in 191/192 (99.5%) of the blastocysts tested.*** In total 4608 chromosomes were assessed, 211 (4.6%) of which carried a copy number imbalance. NGS specific for aneuploidy calling (consistency of chromosome copy number assignment) was 99.98% (4333/4334; 95% confidence interval [95% CI]: 99.87–100) with a sensitivity of 100% (211/211, 95 CI: 99.25–100).

Zheng et al. Molecular Cytogenetics (2015) 8:38
DOI 10.1186/s13039-015-0143-6

MOLECULAR CYTOGENETICS

METHODOLOGY Open Access

Application of next-generation sequencing for 24-chromosome aneuploidy screening of human preimplantation embryos

Haiyan Zheng[1], Hua Jin[2], Lian Liu[2], Jianqiao Liu[1*] and Wei-Hua Wang[3*]

Results: In the present study, 43 human trophectoderm (TE) biopsy samples and 5 cytogenetically characterized cell lines (Coriell Cell Repositories) were tested. The same whole genome amplified product of each sample was blindly assessed with Veriseq NGS and Agilent aCGH to identify the aneuploidy status. The result showed that the NGS identified all abnormalities identified in aCGH including the numeral chromosomal abnormalities (again or loss) in the embryo samples and the structural (partial deletion and duplication) in the Coriell cell lines. ***Both technologies can identify a segmental imbalance as small as 1.8 Mb in size***. Among the 41 TE samples with abnormal karyotypes in this study, eight (19.5 %) samples presented as multiple chromosome abnormalities. The abnormalities occurred to almost all chromosomes, except chromosome 6, 7, 17 and Y chromosome.

Conclusions: ***Given its reliability and high level of consistency with an established aCGH methodology***, NGS has demonstrated a robust high-throughput methodology ready for extensive clinical application in reproductive medicine, with potential advantages of reduced costs and enhanced precision. Then, a randomized controlled clinical trial confirming its clinical effectiveness is advisable to obtain a larger sequencing dataset and more evidence for the extensive use of NGS-based PGS.,,

TROFOEKTODERM BİOPSİSİ VE 24 KROMOZOM ANALİZİ

- Toplam siklus : 88
- Toplam embriyo :209
- Normal : 72 (%34.44)
- Anormal : 137 (%65.55)
- FISH : 100 (%72.99)
- aCGH : 37 (%27.00)
- sET : 17
- Gebelik : 12
- Devam eden gebelik : 10

Greco et al. SpringerPlus (2015) 4:22
DOI 10.1186/s40064-015-0788-y

SpringerPlus
a SpringerOpen Journal

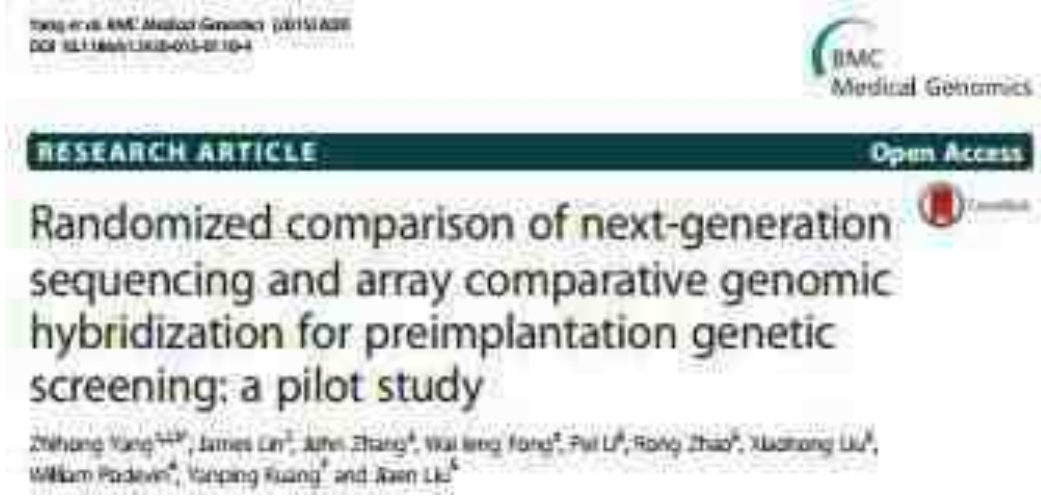
CASE STUDY Open Access

Successful implantation and live birth of a healthy boy after triple biopsy and double vitrification of oocyte-embryo-blastocyst

Ermanno Greco[1*], Anil Biricik[2], Rocio P Cotarelo[1], Elisabetta Iammarone[1], Patrizia Rubino[1], Jan Tesarik[3], Francesco Fiorentino[2] and Maria Giulia Minasi[1]

Case description: An infertile couple, with family history of β-thalassemia, searched for IVF procedure and PGD. First polar bodies biopsy with subsequent vitrification was uninformative due to meiotic crossing-over, so oocytes were inseminated after warming. Two embryos were obtained and blastomere biopsy was performed on day 3 with inconclusive results on their genetic status. Their culture resulted in one expanded blastocyst stage on day 7 that underwent trophectoderm biopsy and vitrification. This embryo showed to be normal. It was then warmed and transferred in an artificial cycle.

This is the first case report of a live birth obtained from a ***three step biopsy*** and ***double vitrification*** procedures of a blastocyst.



BMC Medical Genomics

RESEARCH ARTICLE Open Access

Randomized comparison of next-generation sequencing and array comparative genomic hybridization for preimplantation genetic screening: a pilot study

Zhihong Yang[1,2*], James Lin[3], Jufen Zhang[4], Wai leng Fong[4], Pei Li[4], Rong Zhao[4], Xiaohong Liu[4], William Podsiadly[4], Yanping Kuang[4] and Jiaen Liu[5]

Conclusion: ***In this randomized pilot study, we have demonstrated that NGS detects all types of aneuploidies of human blastocysts accurately and provides an extremely high level of 24-chromosome diagnosis consistency with aCGH.*** Moreover, NGS screening identifies euploid blastocysts for transfer and results in similarly high ongoing pregnancy and implantation rates for IVF-PGS patients compared to aCGH screening. A multi-center randomized clinical trial with a larger sample is planned to define the role of NGS in assisted reproductive medicine.

PGS AMAÇLI GENETİK ANALİZ YÖNTEMLERİNİN KULLANIMI

MERKEZ	FISH	QPCR	REAL TIME PCR	ACGH	SNP ACGH	NEXT GEN
A	+(%66.0)	-	-	+(%34.0)	-	-
B	+(%44.5)	-	-	+(%55.5)	-	+**
C	+(%70.0)	-	-	+(%30.0)*	-	+(%30.0)*
D	-	+(%30.0)	-	+(%70.0)	-	-
E	+	-	-	+	-	-
F	+	-	-	-	-	-
G	+	-	-	-	-	-

Pros and cons of different preimplantation aneuploidy testing platforms

Method	Cost	Speed	Segmental detection	Mosaic detection
aCGH	$$	+++	+++	+++
qPCR	$$	++++	+	+
NGS	$	+	++++	++++
SNP array	$$$	++	++	++

Wells D

Gleicher *et al. Reproductive Biology and Endocrinology* 2014, **12**:22
http://www.rbej.com/content/12/1/22

RB&E REPRODUCTIVE BIOLOGY AND ENDOCRINOLOGY

REVIEW Open Access

Preimplantation genetic screening (PGS) still in search of a clinical application: a systematic review

Norbert Gleicher[1,2*], Vitaly A Kushnir[1] and David H Barad[1,2]

Whether PGS#2 improves IVF outcomes can, therefore, not be determined. Reassessments of data, alleged to support the efficacy of PGS#2, indeed, suggest the opposite. Like with PGS#1, the introduction of **PGS#2 into unrestricted IVF practice again appears premature, and threatens to repeat the PGS#1 experience**, when thousands of women experienced reductions in IVF pregnancy chances, while expecting improvements. PGS#2 is an unproven and still experimental procedure, which, until evidence suggests otherwise, should only be offered under study conditions, and with appropriate informed consents.