Where We Are and Where We Are Going: The Future of ART

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TIME
JIMMY CARTER
The Leadership Question
The Test-Tube Baby
Birth Watch in Britain

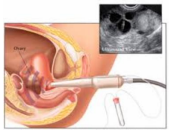

Evening News
Meet Louise, the world's first test-tube arrival
SUPERBABE
Wide-eyed Louise Brown pictured in hospital 18 hours after she was born. Today she's doing well. See Page Three

Milestones in ART: the 80's
Developing the Science & the Tools











1978 1980 1980 1982 1983 1984 1985 1986 1987 1988 1989

1st. IVF baby Steptoe & Edwards

Blastomere biopsy Handyside

CC/hMG Trounson & Leeton

Use GnRHa Fleming

1st US guided aspiration transvesical Lenz & Lakritzen

1st baby cryo embryo Trounson

1st US TV aspiration Wikland

1st Egg donor pregnancy Rosenwaks

Ultrarrapid freezing embryos Trounson

1st MESA baby Silber

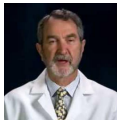
Human embryo biopsy Trounson

hCG for luteal phase, Casper

GIFT Asch

PESA Asch

HTF Quinn

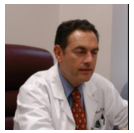


Egg freezing (slow) Chen

SUZI, Laws-King

Milestones in ART: 90's & Beyond Micromanipulation, Drugs, Culture Media & Genetics












1990 1991 1992 1993 1994 1995 1996 1998 2000 2002 2003 2004

PGD sex detection, Handyside

GnRH antagonists, Frydman

ICSI, Palermo

TESE, Silber

r-LH, Loumaye

Aneuploidy testing Cohen

Dolly cloning Wilmut

Blastocyst culture Gardner

1st Successful Ovarian transplant Oktay

Birth after blastocyst biopsy PGD de Boer

Birth after oocyte vitrification Yoon

Birth after orthotopic ovarian transplant Donnez

Assisted Hatching Cohen

FISH PGD Munné

r-FSH Coelingh Bennink

1st pregnancy rFSH +GnRHant Itskovitz Eldor

Single blastocyst transfer Gardner

Milestones in ART: 2005-2015
Freezing, 'Omics', Time-lapse and Massive Sequencing

Metabolomic Profiling → RCT negative

FISH questioned

a-CGH

CCS

RCT's promising

2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015

Whole ovarian transplant (sisters) Silber

Proteomics Ubiquitin

Targeted Metabolomics AAs O2

Time-lapse imaging

Oocyte vitrification no longer experimental

Proteomics Lipocalin I

Vitrification

Genes expressed in CC COXs STAR PTX3

Glucose consumption

Antagonists equal to GnRHa & safer

GnRHa triggering

Tailoring stimulation

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Glucose consumption

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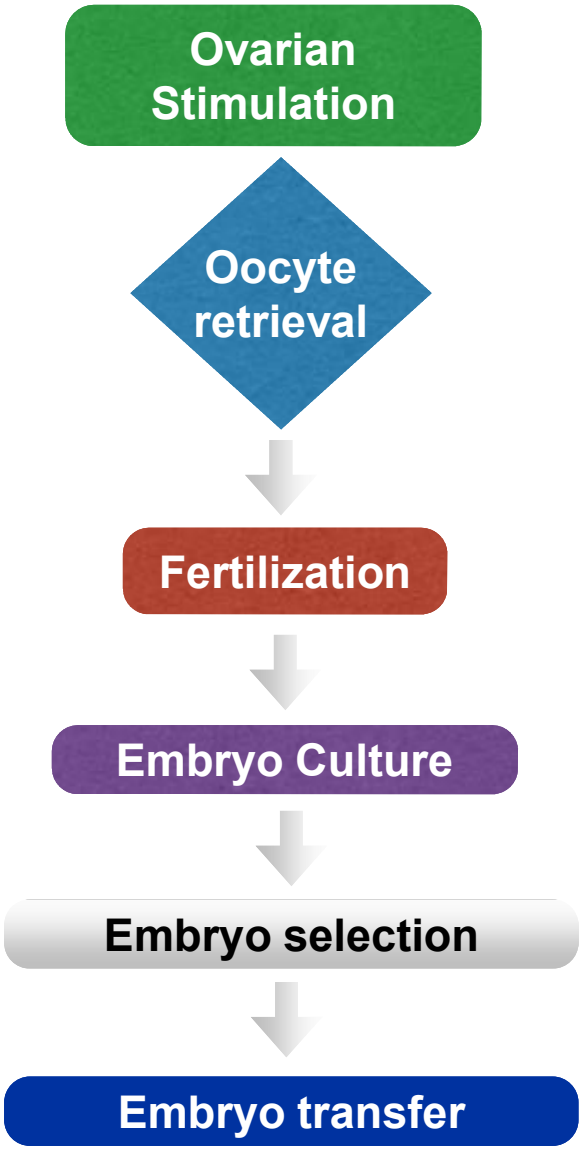
Tailoring stimulation

The goal is a healthy baby born from the transfer of a single, euploid embryo

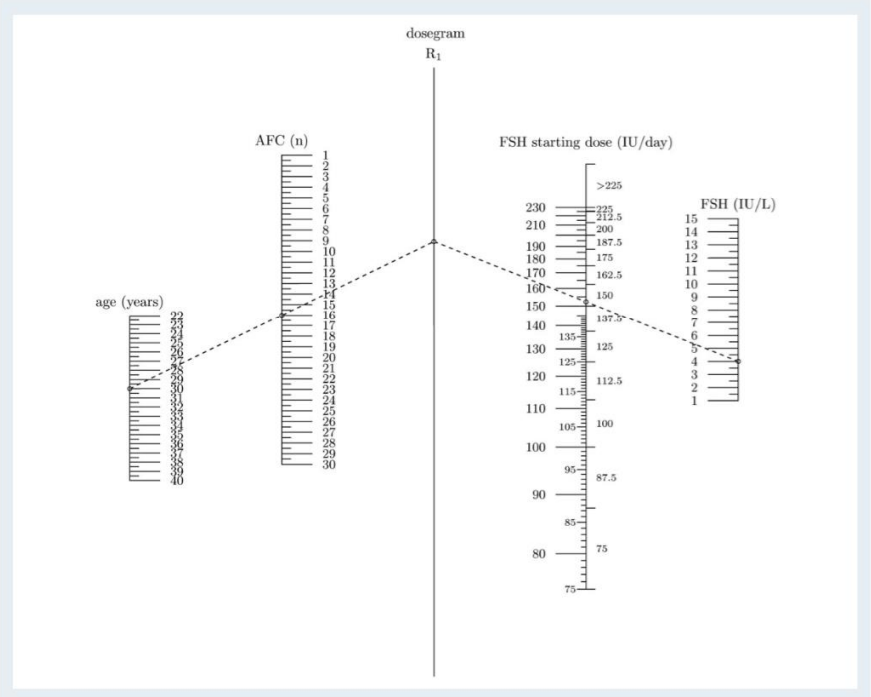
ART is a Multi-step Process

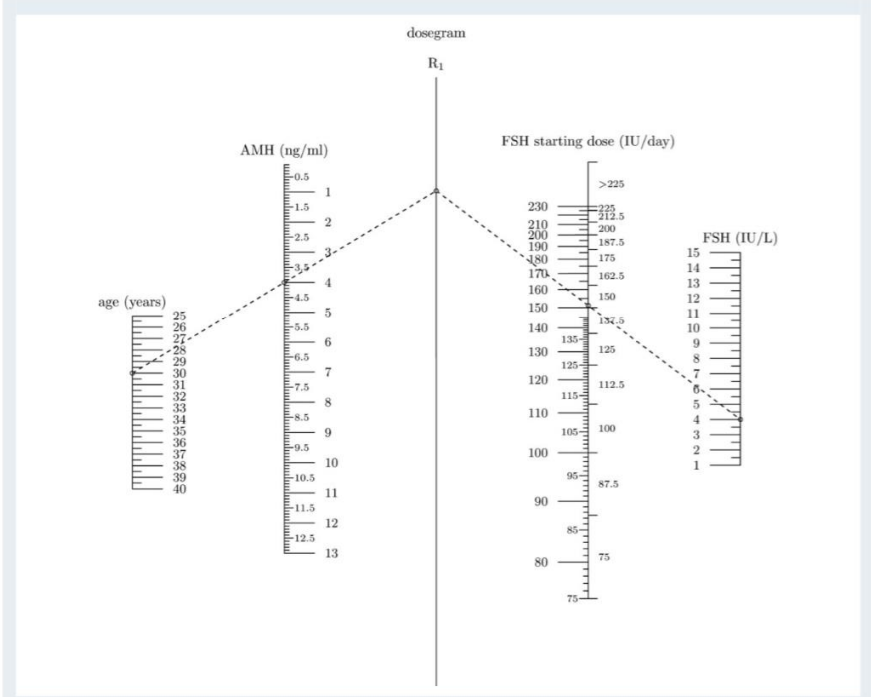


Ovarian Stimulation

- Antagonists vs agonists (Al-Inany, 2011)
  - Safety First!
  - GnRH agonist trigger
- Dosing gonadotropins (La Marca & Sunkara 2011, Yates 2011)
- Poor responders (Bologna criteria, 2011)
  - No recommended protocol before BC
  - Microflare protocols?
  - Corifollitropin alfa?
  - Testosterone pretreatment?

Dosing: The “Dosogram” Based on AFC & AMH





ART Lab Improvements

- Air quality (filtered air, VOC's, positive pressure)
- Culture media (sequential, one-step, transfer)
- Incubators (tri-gas, TLS, automated controls)
- Static Morphologic grading scores + models
- Time lapse systems & morphokinetic grading
- Vitrification
- Metabolomics
- Proteomics
- Genomics

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Time-lapse Current Status

- Promising semi-quantitative and automated tool to monitor embryo development
- Does NOT disturb the embryo in culture
- Could revolutionize workflow in the ART lab
- Improves embryo selection
- Clinical use has yet to be proven

Kaser & Racowsky. *Human Reproduction Update*, Vol.20, No.5 pp. 617ï 631, 2014

Time-lapse Ongoing Trials

Table III Summary of ongoing studies registered with the National Institutes of Health (http://clinicaltrials.gov) using time-lapse monitoring for embryo selection.

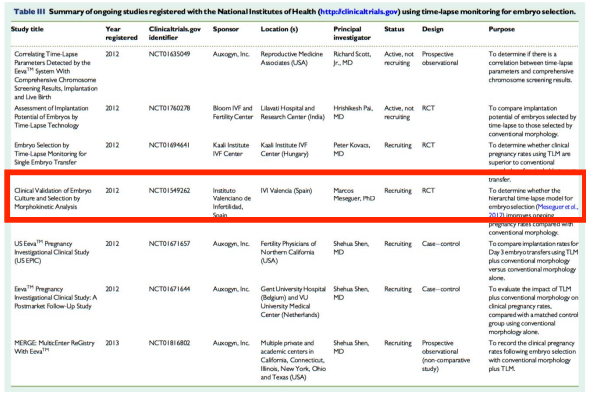
Study title	Year registered	Clinicaltrials.gov identifier	Sponsor	Location (s)	Principal investigator	Status	Design	Purpose	
Correlating Time-Lapse Parameters Detected by the Eeva™ System With Comprehensive Chromosome Screening Results, Implantation and Live Birth	2012	NCT01635049	Auxogyn, Inc.	Reproductive Medicine Associates (USA)	Richard Scott, Jr., MD	Active, not recruiting	Prospective observational	To determine if there is a correlation between time-lapse parameters and comprehensive chromosome screening results.	**Completed Jan 2015 unpublished**
Assessment of Implantation Potential of Embryos by Time-Lapse Technology	2012	NCT01760278	Bloom IVF and Fertility Center	Lilavati Hospital and Research Center (India)	Hrishikesh Pai, MD	Active, not recruiting	RCT	To compare implantation potential of embryos selected by time-lapse to those selected by conventional morphology.	Recruitment status unknown? unpublished
Embryo Selection by Time-Lapse Monitoring for Single Embryo Transfer	2012	NCT01694641	Kaali Institute IVF Center	Kaali Institute IVF Center (Hungary)	Peter Kovacs, MD	Recruiting	RCT	To determine whether clinical pregnancy rates using TLM are superior to conventional morphology for single blastocyst transfer.	Recruitment status unknown? unpublished
Clinical Validation of Embryo Culture and Selection by Morphokinetic Analysis	2012	NCT01549262	Instituto Valenciano de Infertilidad, Spain	IVI Valencia (Spain)	Marcos Meseguer, PhD	Recruiting	RCT	To determine whether the hierarchal time-lapse model for embryo selection (Meseguer *et al.*, 2012) improves ongoing pregnancy rates compared with conventional morphology	**Completed Aug 2014, Published F&S 11/2014**
US Eeva™ Pregnancy Investigational Clinical Study (US EPIC)	2012	NCT01671657	Auxogyn, Inc.	Fertility Physicians of Northern California (USA)	Shehua Shen, MD	Recruiting	Case–control	To compare implantation rates for Day 3 embryo transfers using TLM plus conventional morphology versus conventional morphology alone.	Stopped recruitment, ongoing Oct 2015
Eeva™ Pregnancy Investigational Clinical Study: A Postmarket Follow-Up Study	2012	NCT01671644	Auxogyn, Inc.	Gent University Hospital (Belgium) and VU University Medical Center (Netherlands)	Shehua Shen, MD	Recruiting	Case–control	To evaluate the impact of TLM plus conventional morphology on clinical pregnancy rates, compared with a matched control group using conventional morphology alone.	**Completed Oct 2015, unpublished**
MERGE: MulticEnter ReGistry With Eeva™	2013	NCT01816802	Auxogyn, Inc.	Multiple private and academic centers in California, Connecticut, Illinois, New York, Ohio and Texas (USA)	Shehua Shen, MD	Recruiting	Prospective observational (non-comparative study)	To record the clinical pregnancy rates following embryo selection with conventional morphology plus TLM.	Stopped recruitment, ongoing March 2015

Kaser & Racowsky. *Human Reproduction Update*, Vol.20, No.5 pp. 617ï 631, 2014

Clinical validation of embryo culture and selection by morphokinetic analysis: a randomized, controlled trial of the EmbryoScope

Irene Rubio, Ph.D.,[a] Arancha Galán, Ph.D.,[a] Zaloa Larreategui, Ph.D.,[b] Fernando Ayerdi, Ph.D.,[b] Jose Bellver, M.D.,[a] Javier Herrero, Ph.D.,[a] and Marcos Meseguer, Ph.D.[a]

[a] Instituto Universitario IVI Valencia, University of Valencia, Valencia; and [b] IVI Bilbao, Bilbao, Spain



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RCT comparing Embryoscope (N=438) vs. conventional incubator (N=405)

Equal CPR's, BUT better OPR's (> 10%) and Implantation rates

Fertility & Sterility 2014 102 (5): 1287-1294

TABLE 3

Outcome results per intention to treat, per cycle, per transfers and per embryo transferred.

Outcome	TMS group	Control group	RR	*P* value
All cycles with oocyte retrieval	438	405		
Pregnancy (% of all treated cycles)	61.6 (56.9–66.0)	56.3 (51.4–61.0)	1.09 (0.98–1.23)	.12
Ongoing pregnancy (% of all treated cycles)	51.4 (46.7–56.0)	41.7 (37.0–46.6)	1.23 (1.06–1.43)	.005
All transfers	415	373		
Pregnancy (% of all transfers)	65.3 (60.6–69.7)	61.1 (56.1–65.9)	1.07 (0.95–1.19)	.22
Ongoing pregnancy (% of all transfers)	54.5 (49.6–59.2)	45.3 (40.3–50.4)	1.20 (1.04–1.39)	.01
All pregnant cycles	271	228		
Early pregnancy loss (% of all pregnancies)	16.6 (12.6–21.4)	25.8 (20.6–31.9)	0.64 (0.45–0.91)	.01
All transferred embryos	775	699		
Implantation rate (% of all transferred embryos)	44.9 (41.4–48.4)	37.1 (33.6–40.7)	1.43 (1.05–1.39)	.02

Note: Results shown as proportion with 95% confidence limits in brackets, relative risk (RR) with 95% confidence limits in brackets and the corresponding *P* value (Fisher's exact test). Total number of cycles are also presented in brackets.

Rubio. Clinical validation of EmbryoScope. Fertil Steril 2014.

TABLE 2

Descriptive characteristics of the embryo development and fate in the time-lapse and control groups.

	TMS group (n = 2,638)	Control group (n = 2,427)	*P* value
Embryo fragmentation (%)	7.5 ± 0.1 (7.2–7.9)	6.9 ± 9.4 (6.5–7.1)	.006
No. of blastomeres	6.9 ± 2.3 (6.8–6.9)	6.9 ± 2.7 (6.8–7.0)	NS
Embryo symmetry	1.7 ± 0.5 (1.7–1.7)	1.7 ± 0.5 (1.7–1.7)	NS
Optimal embryos (day 3) (%)	46.2 (1,219) (44.3–48.1)	43.1 (1,046) (41.3–45.1)	.010
Blastocyst rate (%)	52.3 (576) (50.3–54.2)	50.5 (471) (48.5–52.5)	NS
Optimal blastocyst (day 5) (%)	20.9 (230) (19.4–22.4)	16.6 (155) (15.1–18.1)	.001
Transferred embryos	1.86 ± 0.37 (1.8–1.9)	1.86 ± 0.40 (1.8–1.9)	NS
Cryopreserved embryos	3.9 ± 2.2 (3.6–4.1)	3.6 ± 2.2 (3.4–3.9)	NS

Note: Values are mean, and values in brackets are 95% confidence interval or the total number of embryos.

Rubio. Clinical validation of EmbryoScope. Fertil Steril 2014.

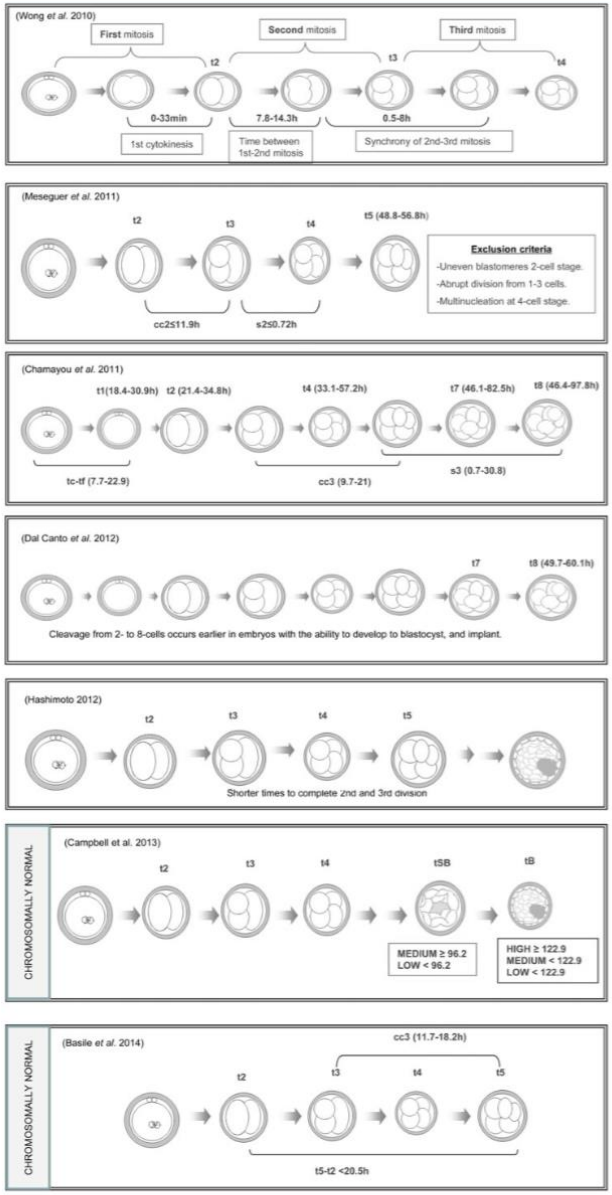


Figure 1 Graphical representation of the published algorithm that, using morphokinetics, suggests a method for embryo selection based on implantation or chromosomal euploidy as the final outcome. Abbreviations are related with timings in hours: t, timing of cleavage from ICSI until the number of cells considered 2,3,4 etc.; cc, cell cycle duration; s, synchrony of the cell cycle. In the figure, the calculations of each of the variables used in the algorithms are described graphically.

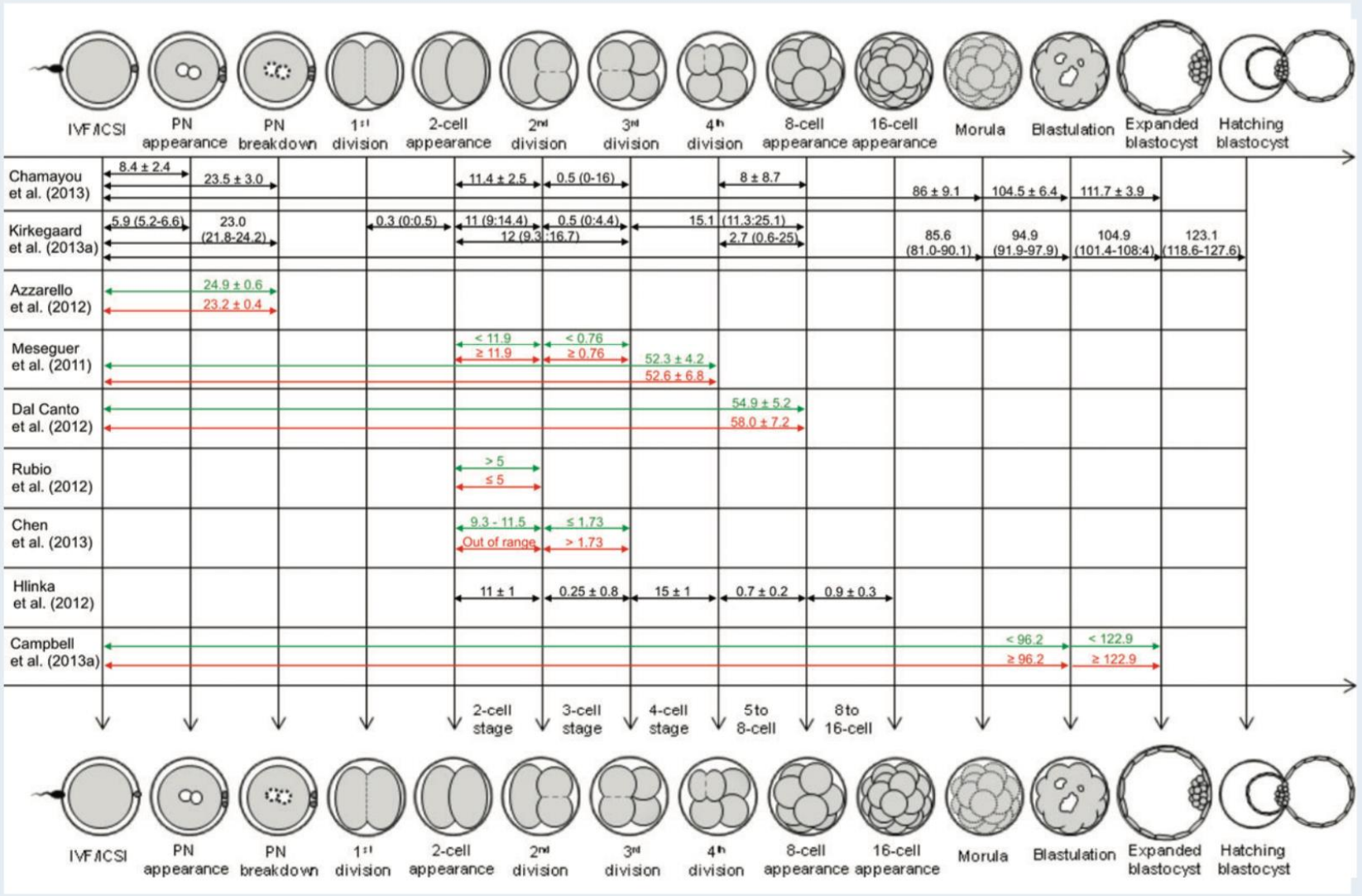


Figure 2 Schematic of preimplantation embryo development with corresponding time-lapse markers from 9 of the 13 studies with time values reported. When there was no significant difference observed between 'implanters' and 'non-implanters', only the value for the implanted embryos is shown (in black). When significant differences were reported, the 'implanter' values are shown in green, and 'non-implanters' are in red. All values are expressed in hours, as mean ± standard deviation or mean (95% confidence interval) for normally distributed variables, and median (minimum:maximum) for non-normally distributed variables. PN, pronuclei. Modified from Chen *et al.* (2013).

Time-lapse Systems

- Promising
- Adds valuable information regarding different checkpoints
- Variability between labs? culture media? ICSI *vs.* IVF
- Embryos undisturbed!
- Need further clinical validation comparing with same incubator
- Efforts are being made to unify nomenclature

Table IV Proposed standardized nomenclature for time-lapse markers.

Proposed nomenclature	Developmental measure	Milestone
t_0	Spermatozoön entry into oocyte (IVF or ICSI)	Time of sperm injection into oocyte (ICSI) or at which sperm head binds to oolemma (IVF)
t_{2pb}	Extrusion of the 2nd polar body	Time that 2nd polar body is first encircled by a complete membrane
t_{2pn}	Appearance of the two pronuclei	Time that two pronuclei are first visualized
$t_{2pn.a}$	Abuttal of the two pronuclei	Time that two pronuclei first remain in contact before onset of dissolution
t_1	Disappearance of the two pronuclei	Time that both pronuclei are no longer visible
t_{cf1}, t_{cf2}, t_{cf3} . . .	Identification of the 1st, 2nd, 3rd etc. cytokinesis furrow	Time at which the 1st, 2nd, 3rd etc. cytokinesis (cleavage) furrow is clearly distinguishable
t_2, t_3, t_4 . . . t_{16}	Formation of 2-cell stage, 3-cell stage, 4-cell stage, etc. through the 16-cell stage	Time at which newly formed cells are completely separated by confluent membranes
t_c	Start of compaction	Time at which membranes of adjacent blastomeres start to become indistinguishable
t_m	Formation of morula	Time at which the membranes of all blastomeres are no longer distinguishable
t_{cav}	Start of cavitation	Time at which a pocket of fluid is first identified between blastomeres
$t_{b.e}$	Formation of early blastocyst	Time at which a single pocket of fluid (the blastocoelic cavity) first occupies less than half the volume of the embryo
$t_{b.xg}$	Formation of expanding blastocyst	Time at which the blastocoelic cavity first occupies more than half the volume of the embryo
$t_{b.f}$	Formation of full blastocyst	Time at which the blastocoelic cavity first occupies the entire volume of the embryo
$t_{b.xd}$	Formation of expanded blastocyst formation	Time at which the embryo first becomes fully expanded
$t_{b.hg}$	Formation of hatching blastocyst	Time at which the trophectoderm starts to herniate through the zona pellucida
$t_{b.hd}$	Formation of hatched blastocyst	Time at which the blastocyst completes escapement from the zona pellucida
$t_{b.c1}$, $t_{b.c2}$, $t_{b.c3}$ . . .	Identification of blastocyst contractions	Time at which the 1st, 2nd, 3rd etc. contraction of the blastocyst occurs (i.e. time of maximum shrinkage during one contraction event)
t_i	Time interval	The time required for the embryo to reach a more advanced stage from a specified earlier stage*
d	Duration	This is a special case of the more general term, time interval, and indicates the time passed between two *consecutive* developmental stages*

* Note that to describe any time interval or duration, a user is required to define both the start and stop times. This standardization allows the annotation of any given measure of interest by using the generalized formula, $t_i = t_y - t_x$, where y is a more advanced developmental stage, and x is a defined referent that is always an earlier developmental stage. For example, the time from ICSI to hatching blastocyst is represented by $t_{b.hg} - t_0$, the duration of the first cytokinesis is $t_2 - t_{cf1}$ and the duration of the 3-cell stage is $t_4 - t_3$.

Kaser & Racowsky *Human Reproduction Update* 2014

Aneuploidy in Reproduction

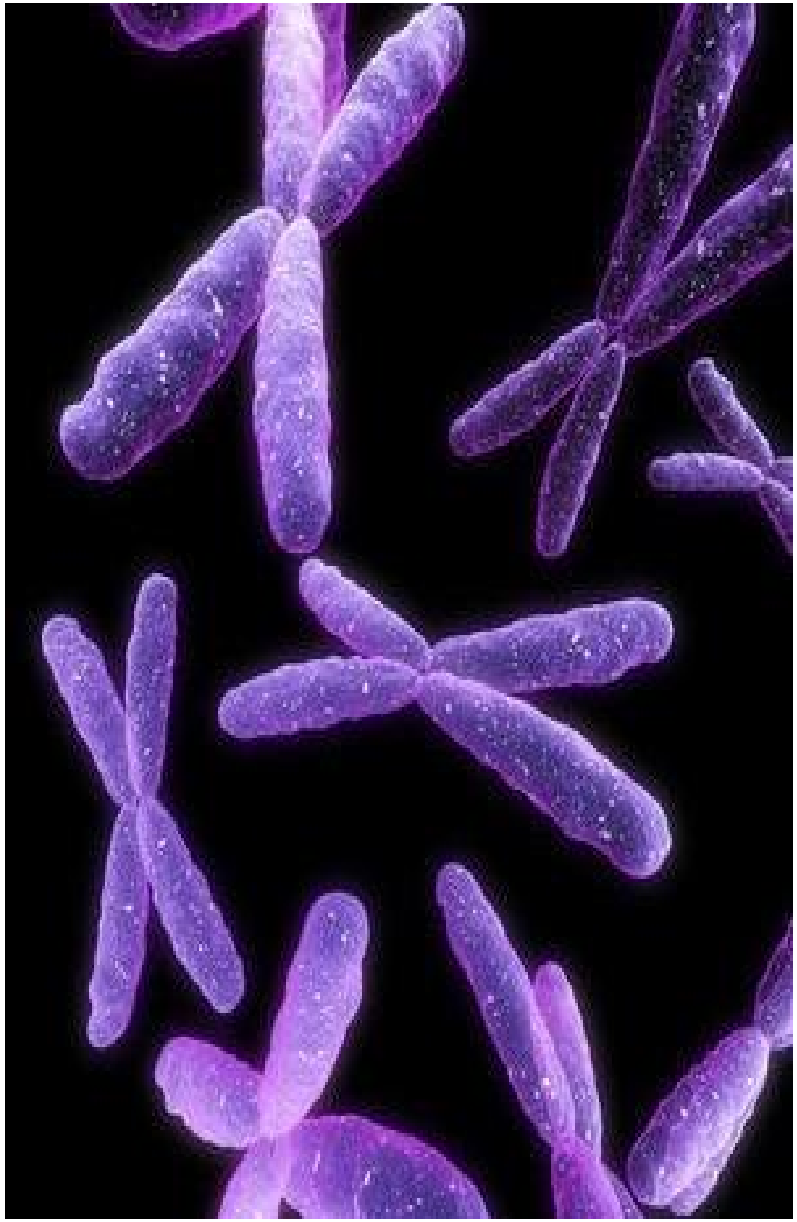
Aneuploidy is extremely common in the early embryo

Trisomy and monosomy are present in 10% to > 50% of pregnancies, related to maternal age

Recurrent implantation failure

Recurrent miscarriage

Sperm chromosomal aneuploidies are responsible for male factor infertility

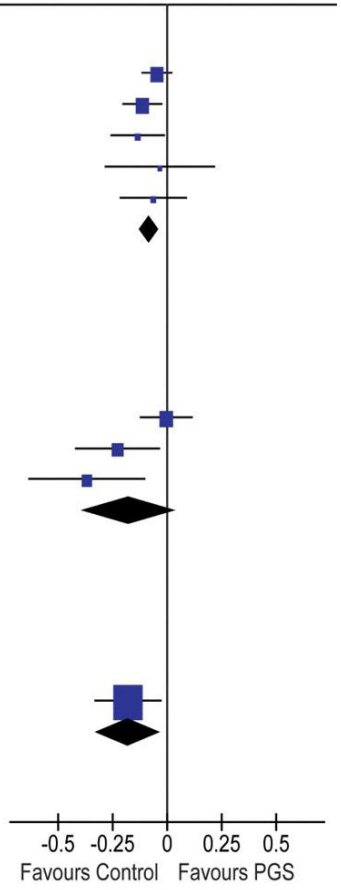


Aneuploidy Screening

Global Fertility Academy

The Effect of PGS on the Live Birth Rate Per Patient

Study or Subgroup	PGS Events	PGS Total	Control Events	Control Total	Weight	Risk Difference
Indication Advanced Maternal Age						**M-H, Fixed, 95% CI**
Staessen 2004	21	199	29	190	36.6%	-0.05 [-0.11, 0.02]
Mastenbroek 2007	49	206	71	202	38.4%	-0.11 [-0.20, -0.03]
Hardarson 2008*	3	56	10	53	10.3%	-0.14 [-0.26, -0.01]
Schoolcraft 2008	16	32	16	30	5.8%	-0.03 [-0.28, 0.22]
Debrock 2009	6	44	10	50	8.8%	-0.06 [-0.21, 0.09]
Subtotal (95% CI)		**537**		**525**	**100.0%**	**-0.08 [-0.13, -0.03]**
Total events	95 (18%)		136 (26%)			
Heterogeneity: $Chi^2 = 2.51$, df = 4 (P = 0.64); $I^2 = 0\%$						
Test for overall effect: Z = 3.38 (P = 0.0007)						
Indication Good Prognosis Patient						**M-H, Random, 95% CI**
Staessen 2008*	37	120	37	120	39.7%	0.00 [-0.12, 0.12]
Jansen 2008*	20	55	27	46	33.3%	-0.22 [-0.41, -0.03]
Meyer 2009*	6	23	15	24	26.9%	-0.36 [-0.63, -0.10]
Subtotal (95% CI)		**198**		**190**	**100.0%**	**-0.17 [-0.39, 0.04]**
Total events	63 (32%)		79 (42%)			
Heterogeneity: $Tau^2 = 0.03$; $Chi^2 = 8.27$, df = 2 (P = 0.02); $I^2 = 76\%$						
Test for overall effect: Z = 1.56 (P = 0.12)						
Indication Repeated Implantation Failure						**M-H, Fixed, 95% CI**
Blockeel 2008	15	72	26	67	100.0%	-0.18 [-0.33, -0.03]
Subtotal (95% CI)		**72**		**67**	**100.0%**	**-0.18 [-0.33, -0.03]**
Total events	15 (21%)		26 (39%)			
Heterogeneity: Not applicable						
Test for overall effect: Z = 2.35 (P = 0.02)						



* Trial was terminated prematurely.
CI = confidence interval; M-H = Mantel-Haenszel method.

S. Mastenbroek et al. *Human Reproduction Update.* 2011;17:454-466

human reproduction update

PGS with FISH - Pitfalls & Limitations

- Technique itself (8-12 chromosomes analyzed)
- Operator experience
- D3 embryo
- No. of cells biopsied
- Mosaicism
- Low sensitivity

Comprehensive Chromosome Screening

Platforms

DNA amplification
DNA labeling
Fluorescence detection
Bioinformatics

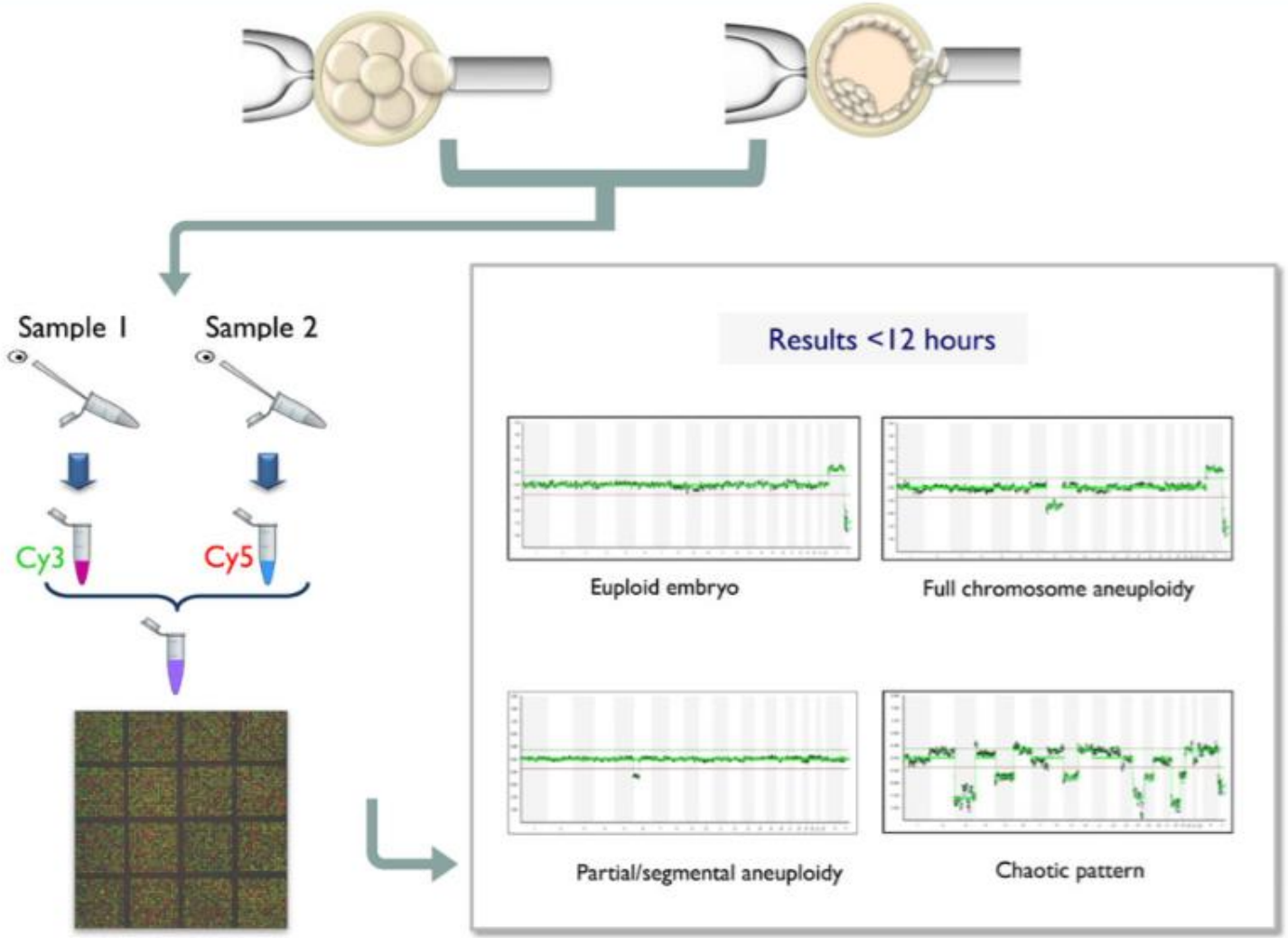
2% non informative
Aneuploidy screening
Reciprocal & Robertsonian Translocations

DNA amplification (WGA)
Compares signals at each position
Characterize recombination sites
Uniparental disomy
Parental origin of aneuploidy
Balanced translocations

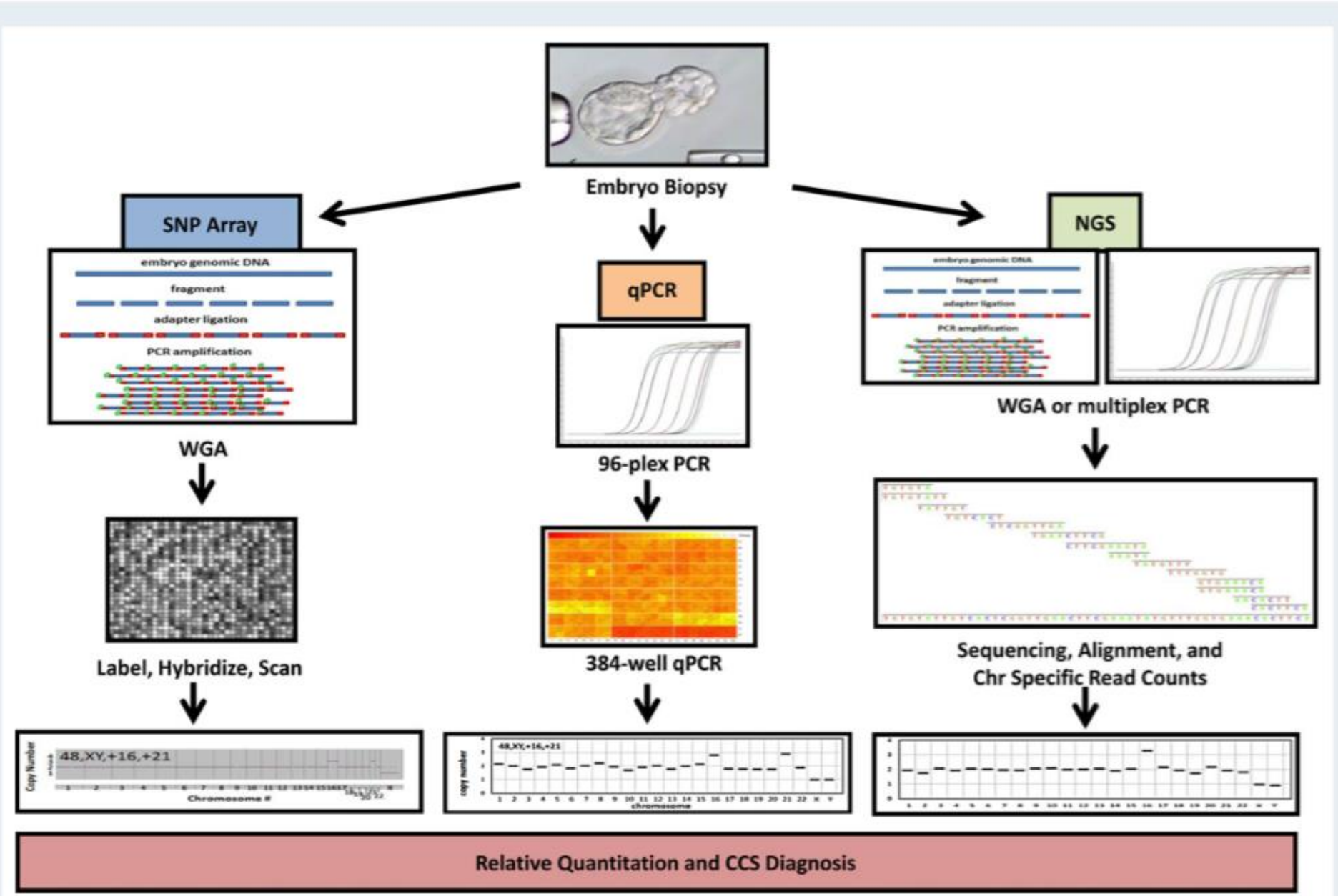
DNA amplification (WGA or multiplex PCR)
Massive parallel sequencing
Read counts and compares to normal samples
Quicker
Cost-effective

Mosaicism (10-15%)

CCS - aCGH


Sample 1
Sample 2
Cy3
Cy5
Results <12 hours
Euploid embryo
Full chromosome aneuploidy
Partial/segmental aneuploidy
Chaotic pattern

CCS - SNP, QPCR & NGS



RCT - Done & Ongoing

Table II RCTs using comprehensive chromosome screening.

Authors	Female age (years)	Intervention	Eligibility	No. of cycles	% Abnormal embryos	Ongoing PR/cycle or delivery rates	MR
Yang *et al.*, *Mol Cytogenet* 2012	<35	SET after blastocyst biopsy versus blastocyst transfer (Array CGH)	Young good prognosis, IVF patients, first cycle, no prior miscarriage	55 PGS 48 control	44.9%	69.1 PGS versus 41.7 ($P = 0.0009$)	2.6 PGS versus 9.1 (NS)
Forman *et al.*, *Fertil Steril* 2013 NCT01408433	<43	SET after blastocyst biopsy versus DET of unscreened blastocysts (qPCR)	All indications ≥2 blastocyst for biopsy	89 PGS 86 control	31%	60.7 PGS versus 65.1 (NS)	11.5 PGS versus 20.0 (NS)
Scott *et al.*, 2013a, b NCT01219283	21–42	Blastocyst biopsy versus blastocyst transfer	All indications ≤1 failed IVF	72 PGS 83 control	28.6%	84.7 PGS versus 67.5 ($P = 0.01$)	–
Schoolcraft *et al.*, ASRM 2012	>35	Fresh blastocyst transfer versus frozen blastocyst biopsy (SNP microarray)	AMA	47 PGS 41 control	–	74.5 PGS versus 53.7 ($P < 0.05$)	–
Rubio *et al.*, ESHRE 2014 NCT01571076	38–41	D3 biopsy with blastocyst transfer versus blastocyst transfer (Array CGH)	AMA <2 miscarriages <2 IVF failures	75 PGS 86 control	77.9%	42.7 PGS versus 25.6 ($P = 0.0294$)	3.3 PGS versus 43.6 ($P < 0.0001$)
ESHRE Study for Oocyte Euploidy (ESTEEM) NCT01532284	36–41	Polar body biopsy (Array CGH)	AMA Recruiting				
Yilun Siu and Shangai Ji Ai Genetics & IVF Institute NCT02223221	18–35	Blastocyst biopsy versus blastocyst transfer (Array CGH)	RPL ≥3 miscarriages Recruiting				
Rubio IVI NCT01571076	<38	D3 biopsy with blastocyst transfer versus blastocyst transfer (Array CGH)	Severe male factor <2 million sperm/ml Recruiting				
Munne Reprogenetics NCT01946945	22–42	Blastocyst biopsy versus blastocyst transfer (NGS)	All indications Recruiting				
Scott RMANJ NCT02032264	18–42	DET blastocyst biopsy (NGS)	≤1 prior failed IVF Recruiting				

CGH, comparative genomic hybridization; SNP, single nucleotide polymorphism; SET, single-embryo transfer; qPCR, quantitative PCR; NGS, next-generation sequencing; PGS, preimplantation genetic screening; AMA, advanced maternal age; RPL, recurrent pregnancy loss; MR, miscarriage rate.

- Increases in CPR & IR
- Reduction in Abortion
- Reduction in TTP

RCT - Done & Ongoing

Table II RCTs using comprehensive chromosome screening.

Authors	Female age (years)	Intervention	Eligibility	No. of cycles	% Abnormal embryos	Ongoing PR/cycle or delivery rates	MR
Yang *et al.*, *Mol Cytogenet* 2012	<35	SET after blastocyst biopsy versus blastocyst transfer (Array CGH)	Young good prognosis, IVF patients, first cycle, no prior miscarriage	55 PGS 48 control	44.9%	69.1 PGS versus 41.7 ($P = 0.0009$)	2.6 PGS versus 9.1 (NS)
Forman *et al.*, *Fertil Steril* 2013 NCT01408433	<43	SET after blastocyst biopsy versus DET of unscreened blastocysts (qPCR)	All indications ≥2 blastocyst for biopsy	89 PGS 86 control	31%	60.7 PGS versus 65.1 (NS)	11.5 PGS versus 20.0 (NS)
Scott *et al.*, 2013a, b NCT01219283	21–42	Blastocyst biopsy versus blastocyst transfer	All indications ≤1 failed IVF	72 PGS 83 control	28.6%	84.7 PGS versus 67.5 ($P = 0.01$)	–
Schoolcraft *et al.*, ASRM 2012	>35	Fresh blastocyst transfer versus frozen blastocyst biopsy (SNP microarray)	AMA	47 PGS 41 control	–	74.5 PGS versus 53.7 ($P < 0.05$)	–
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ESHRE Study for Oocyte Euploidy (ESTEEM) NCT01532284	36–41	Polar body biopsy (Array CGH)	AMA Recruiting				
Yilun Siu and Shangai Ji Ai Genetics & IVF Institute NCT02223221	18–35	Blastocyst biopsy versus blastocyst transfer (Array CGH)	RPL ≥3 miscarriages Recruiting				
Rubio IVI NCT01571076	<38	D3 biopsy with blastocyst transfer versus blastocyst transfer (Array CGH)	Severe male factor <2 million sperm/ml Recruiting				
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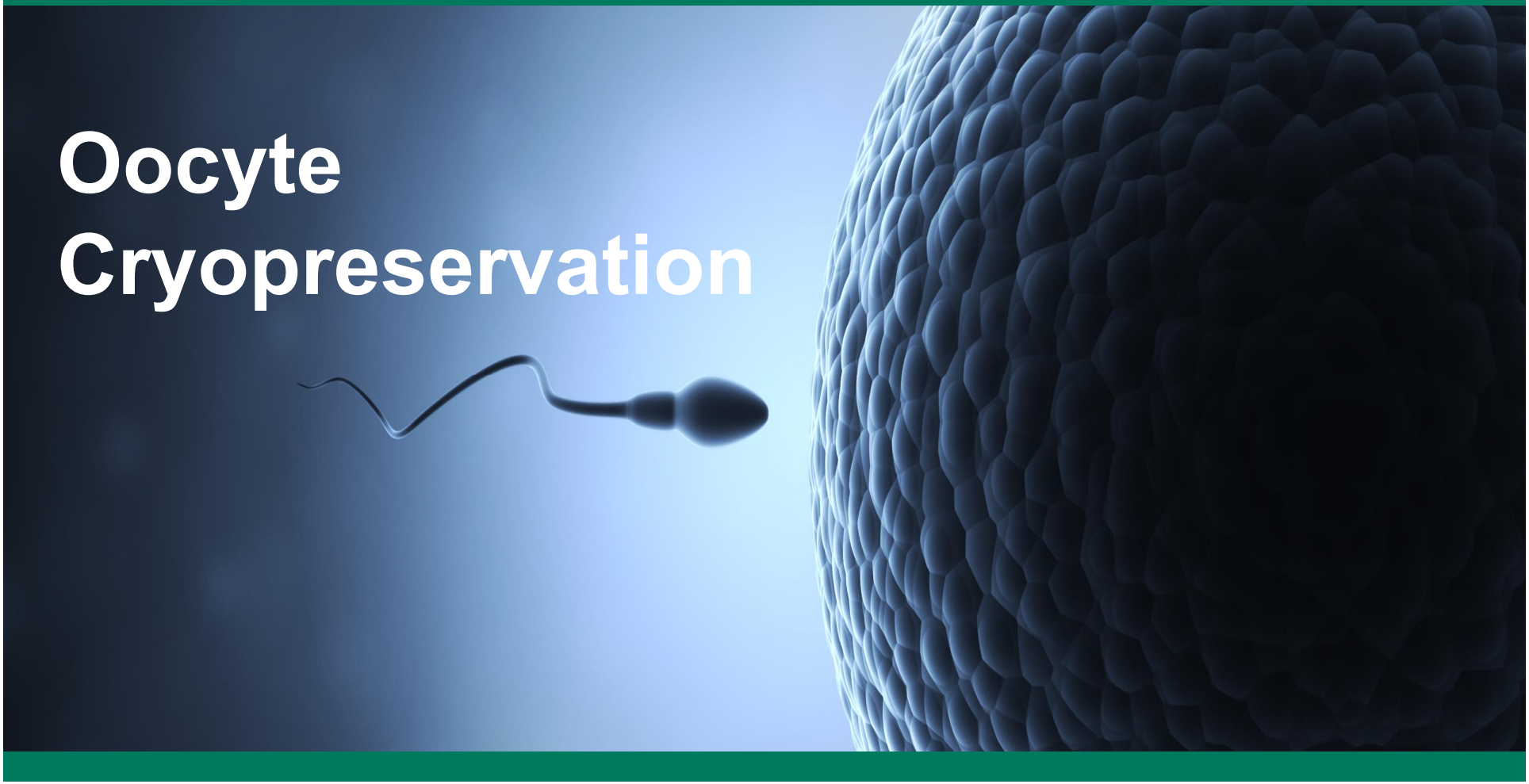
CGH, comparative genomic hybridization; SNP, single nucleotide polymorphism; SET, single-embryo transfer; qPCR, quantitative PCR; NGS, next-generation sequencing; PGS, preimplantation genetic screening; AMA, advanced maternal age; RPL, recurrent pregnancy loss; MR, miscarriage rate.

Oocyte Cryopreservation



Global Fertility Academy

RBM Online - Vol 11. No 3. 2005 300-308 Reproductive BioMedicine Online; www.rbmonline.com/Article/1827 on web 15 July 2005

Article

Highly efficient vitrification method for cryopreservation of human oocytes

Masashige Kuwayama (PhD) is currently the Scientific Director of Kato Ladies' Clinic (Tokyo, Japan), the world's largest IVF unit. In 1986, he began work in the field of embryology with Dr Hanada. They developed assisted reproduction techniques (IVM, IVF, vitrification, embryo culture, ES cell) and established a bovine embryo mass production system as the leader of a National Project in Japan in 1990. He obtained the first calves after oocyte vitrification, IVF, in-vitro culture and blastocyst transfer in 1992. He moved to human IVF in 1999, developed the Cryotop vitrification method for human oocytes and established the first human oocyte bank in 2001. The first babies following oocyte vitrification in USA and Japan were obtained by his group using the Cryotop method. He is also interested in rejuvenescence of old defective oocytes, and obtained the first calf from old infertile cattle with germinal vesicle transfer in 2002.

Dr Masashige Kuwayama

Masashige Kuwayama[1,4], Gábor Vajta[2], Osamu Kato[1], Stanley P Leibo[3]
[1]Kato Ladies' Clinic, Tokyo 160–0023, Japan; [2]Population Genetics and Embryology, Department of Genetics and Biotechnology, Danish Institute of Agricultural Sciences, Research Centre Foulum, DK-8830 Tjele, Denmark; [3]Audubon Centre for Research of Endangered Species, Department of Biological Sciences, University of New Orleans, New Orleans, LA 70131, USA

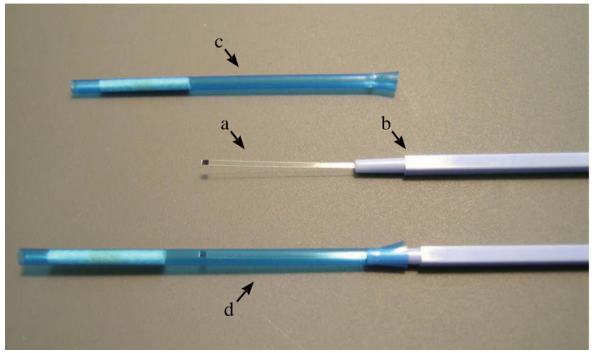


Table 4. In-vivo development of the vitrified human oocytes after embryo transfer on day 2 and day 5. ET = embryo transfer.

Day of ET (no. embryos/ET)	*No. of ET (no. embryos)*	*No. (%) of pregnancies*	*No. of deliveries*[a]	*No. of ongoing pregnancies*
2 (2)	1 (2)	1 (100)	0	0
2 (3)	17 (51)	6 (35.3)	4	1
5 (1)	11 (11)	5 (45.5)	3	2
Total	29 (64)	12 (41.3)	7	3

[a]Take-home babies.

RCTs on Oocyte Cryopreservation

Table 1

Randomized controlled trials with clinical outcomes on oocyte cryopreservation

Author	Study design	Mean age at freezing	Target population	Method	Number of patients	Number of oocytes	Mean embryos	Day transfer	SR (%)	FR (%)	IR (%)	CP/T (%)	LB/T (%)
Nondonor – slow freezing versus vitrification													
Smith *et al.* United States [18]	Randomization appropriate for comparing both embryological and clinical outcome	31 ± 1	Infertile patients who failed in the fresh cycle and had >9 supernumerary oocytes	SF	30	238	3.2	3	67	67	11.5	21.1[b,c]	NA
		32 ± 1		VF	48	349	3.1	3	81	77	13.7	38.3[b,c]	NA
Nondonor – fresh versus vitrified oocytes													
Rienzi *et al.*[a] Italy [19]	Randomization appropriate for comparing embryological not for clinical outcome	35.5 ± 4.8	IVF patients <43 years old with >6 MII oocytes at retrieval	VF	40	124	2.3	2	96.8	79.2	20.4	38.5	30.8 (OPR)
				Fresh	40	120	2.5		NA	83.3	21.7	43.2	38.8 (OPR)
Parmegiani *et al.*[a] Italy [20]	Randomization appropriate for comparing embryological not for clinical outcome	35.0 ± 0.8	IVF patients <42 years old with >5 MII oocytes at retrieval	VF	31	168	2.5	2–3	89.9	84.9	17.1	35.5	22.6
				Fresh	31	NA	2.6		NA	88.3	NA	13.3	0
Forman *et al.* United States [21▪▪]	Randomization appropriate for comparing clinical outcome	29.9 ± 2.3	IVF patients <35 years old with >8 MII oocytes undergoing their first IVF cycle	VF	44 (26 paired transfers)	294	NA	5–6	81.6	77.9[b]	NA	NA	53.9 (OPR)
				Fresh	44 (26 paired transfers)	294	NA		NA	90.5[b]	NA	NA	57.7 (OPR)
Donor – fresh versus vitrified oocytes													
Cobo *et al.* Spain [22]	Randomization appropriate for comparing embryological not for clinical outcome because embryo recipients are not randomized in this study	26.7 ± 3.6	Oocyte donors	VF	30	231	3.8	3	96.7	76.3	40.8	65.2	47.8 (OPR)
				Fresh	30	219	3.9		NA	82.2	100	100	100 (OPR)
Cobo *et al.* Spain [15]	Randomization appropriate for comparing clinical outcome	26.7 ± 3.9	Oocyte donors	VF	295	3286	1.7	3	92.5	74.2	39.9	55.4	49.1
		26.6 ± 3.8		Fresh	289	3185	1.7		NA	73.3	40.9	55.6	48.3

CP/T, clinical pregnancy/transfer; FR, fertilization rate; IR, implantation rate; LB/T, live birth/transfer; SF, slow-freezing; SR, survival rate; VF, vitrification.

[a] sibling oocytes from the same patients were randomized.
[b] significantly different.
[c] CP/thaw cycle.

Cil AP, Seli E. *Curr Opin Obstet Gynecol*. 2013

ICSI for All?

Advantages

- Standardization & task organization in ART labs
- Uniformity (variability, checkpoints in time-lapse)
- “Mastering" the technique for personnel training in other invasive procedures (blastomere & trophectoderm biopsy, assisted hatching, fragment removal, cytoplasmic transfer, etc.)

Disadvantages

- Overlapping tasks overwhelming
- Burden to human resources
- Security? (physiological barriers bypassed)
- Follow up in high risk population confusing
- Cost-efficacy?
- No evidence of benefit in CPR, IR or LBR

IVF ICSI Modern Trends

Human Reproduction, Vol.28, No.5 pp. 1375–1390, 2013
Advanced Access publication on February 26, 2013 doi:10.1093/humrep/det036

human reproduction ORIGINAL ARTICLE *Reproductive epidemiology*

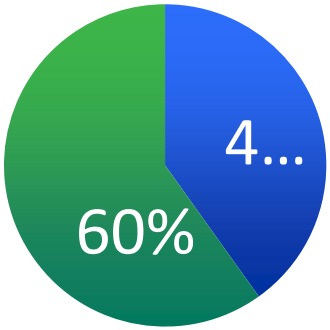
International Committee for Monitoring Assisted Reproductive Technologies (ICMART) world report: assisted reproductive technology 2004†

E.A. Sullivan[1,*], F. Zegers-Hochschild[2], R. Mansour[3], O. Ishihara[4], J. de Mouzon[5], K.G. Nygren[6], and G.D. Adamson[7]

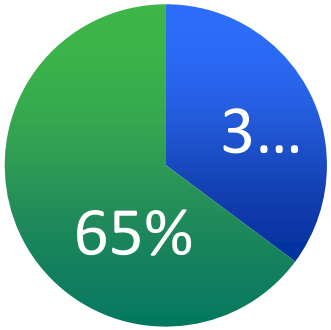
N = 479.141 cycles

Asia

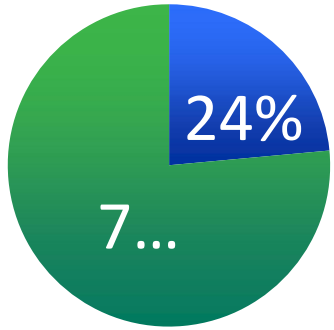
Europe



North America



Latin America



REDLARA 2012

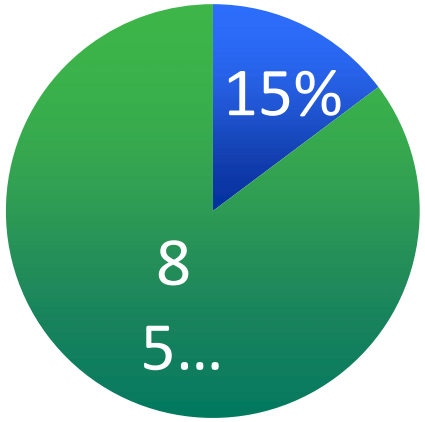


Table 1. Assisted Reproduction technology procedures and access in 2012

Country	Number of clinics	Assisted reproductive techniques							Access (*****)
		IVF/ICSI initiated cycles (*)	IVF (**)	ICSI (**)	FET(***)	OD	FP(****)	Total	
Argentina	25	6,461	504	5,515	3,027	1,543	429	11,031	1,193
Bolivia	1	215	148	62	14	8	923	237	96
Brazil	57	16,030	1,070	13,937	4,252	1,170	0	21,452	447
Chile	8	1,563	131	1,321	549	197	48	2,309	595
Colombia	11	977	293	622	262	247	13	1,486	139
Ecuador	6	608	216	324	165	154	107	927	254
Guatemala	1	100	38	62	7	17	0	124	37
Mexico	27	3,345	1,222	2,017	1,046	1,140	114	5,531	196
Nicaragua	1	91	46	41	0	9	0	100	67
Panama	1	245	7	192	86	33	9	364	452
Peru	6	1,264	298	875	430	547	114	2,241	308
Dominican R.	2	80	42	35	5	26	0	111	48
Uruguay	2	293	20	233	77	46	2	416	585
Venezuela	7	585	369	184	153	259	5	997	148
Total	155	31,857	4,404	25,420	10,073	5,396	1,764	47,326	367.0

(*) initiated cycles; (**) oocyte pick ups; (***) includes the transfer of own and donated oocytes; (****) initiated fertility preservation cycles; (*****) number of cycles/million of women 15-45 years

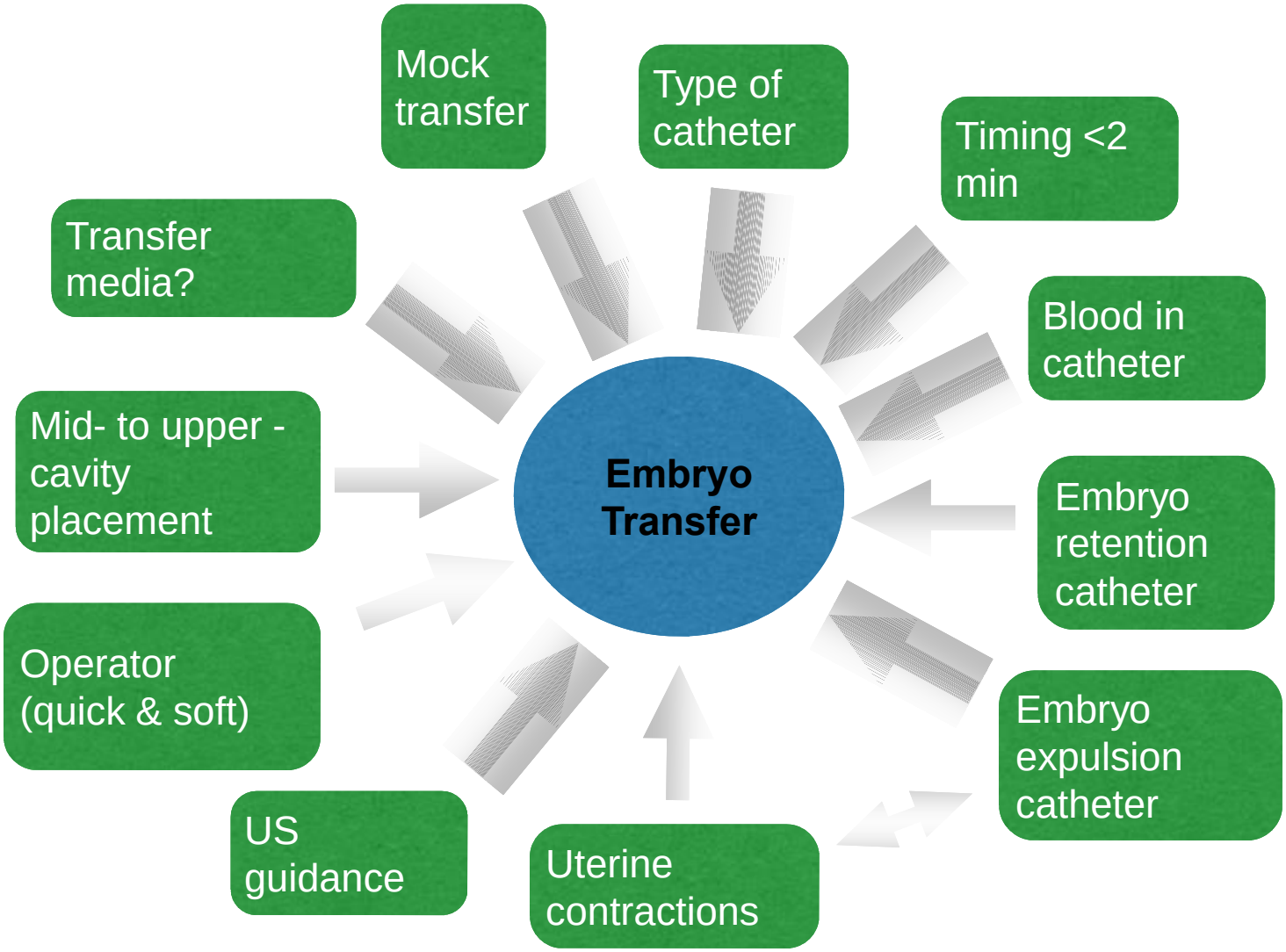
ICSI has become a tool

ICSI Past & New Indications

- Severe Oligoasthenozoospermia
- Use of testicular or epididymal sperm
- Antisperm antibodies
- Repeated IVF failure
- Use of cryopreserved sperm
- Ejaculatory dysfunction
- PGD for monogenic diseases
- PGS with CCS for aneuploidy screening
- Time-lapse?
- Cryopreserved oocytes
- Poor responders (just in case…)

Pregna Medicina Reproductiva 2012-2015 Unselected Population			
Procedure	**IVF**	**ICSI**	**p**
N	1319	1388	
# oocytes	9567 (7.25)	11548 (8.3)	
M2 oocytes	7796 (81.5%)	8301 (71.9%)	
M2 used	7633	8124	
FR	5389/7633 (70.6%)	5093/8124 (62.7%)	<0.0001
# transfers	968	990	0.24
# embryos transferred	1.79	1.79	NS
+ bhCG	379/968 (39.2%)	344/990 (34.7%)	0.04
Clinical Pregnancy	312/968 (32.2%)	291/990 (29.4%)	0.18
Implantation	347/1732 (20%)	340/1777 (19.1%)	0.52
0% fert (>1 M2)	97/1319 (7.3%)	132/1388 (9.5%)	0.04
0% fert (>3 M2)	10/1319 (0.7%)	28/1388 (2.01%)	0.005

Embryo Transfer: A Critical Step



Single Embryo Transfer

Number of embryos for transfer following in vitro fertilisation or intra-cytoplasmic sperm injection (Review)

Pandian Z, Marjoribanks J, Ozturk O, Serour G, Bhattacharya S

- Pregnancy rate is lower sET vs. DET
- Cumulative PR (2 fresh or 1 fresh + 1 FET) similar
- Multiple pregnancies are significantly reduced

Freeze-all Strategies

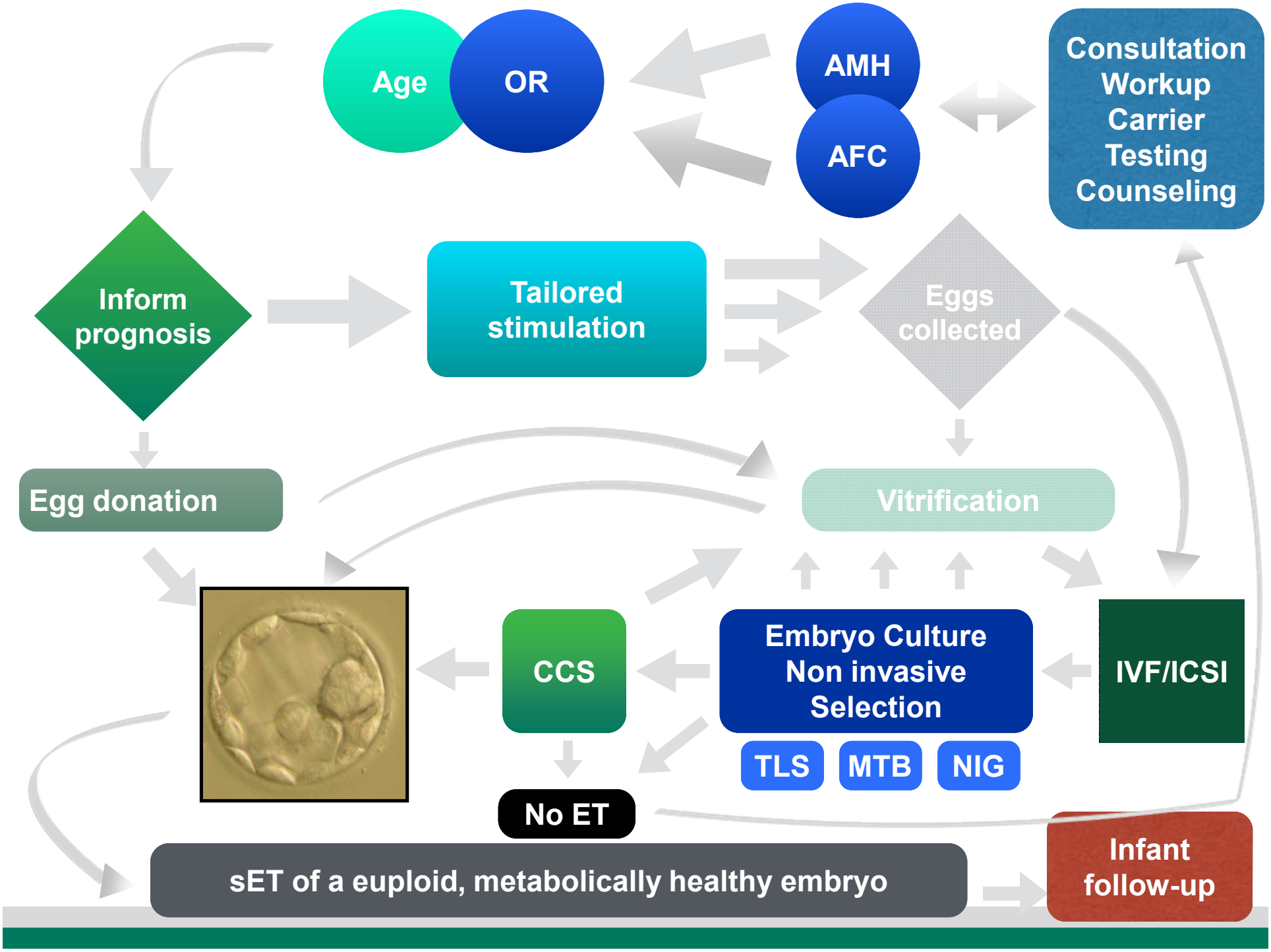
- More “physiological” endometrium
- OHSS
- Progesterone rises
- PGS
- Could improve PR: data still not convincing, need more N

Neonatal and Infant Follow Up

- The incidence of major birth defects, in newborns born after ICSI treatments is 3-4%, in the same range as in the general population. Bonduelle 2002; Palermo 2000; Van Steirteghem 1998
- Hansen in 2002 found an incidence of major birth defects of 9% (almost double than in the general population) in newborns after ICSI, but the same risk in newborns after conventional IVF cycles
- Structural autosomic anomalies (0,36%) and de-novo sex chromosome anomalies (0,83%) are slightly but significantly elevated in newborns after ICSI, but NOT in IVF. te Velde 1998; Van Steirteghem 2002

Neonatal and Infant Follow Up

- There is also an increase, compared to the general population, in birth defects in boys born after ICSI treatments, probably inherited through the paternal pathway. Van Steirteghem 2002
- Davies, in 2012 found an increase in birth defects of newborns from assisted conception cycles (8.3% vs. 5,8% in the general population), RR 1.26 for IVF and 1.77 for ICSI, but after adjusting for parental factors, de RRs were 1.07 y 1.57


Age
OR
AMH
AFC
Consultation
Workup
Carrier
Testing
Counseling
Inform prognosis
Tailored stimulation
Eggs collected
Egg donation
Vitrification
CCS
Embryo Culture
Non invasive
Selection
IVF/ICSI
TLS
MTB
NIG
No ET
sET of a euploid, metabolically healthy embryo
Infant follow-up

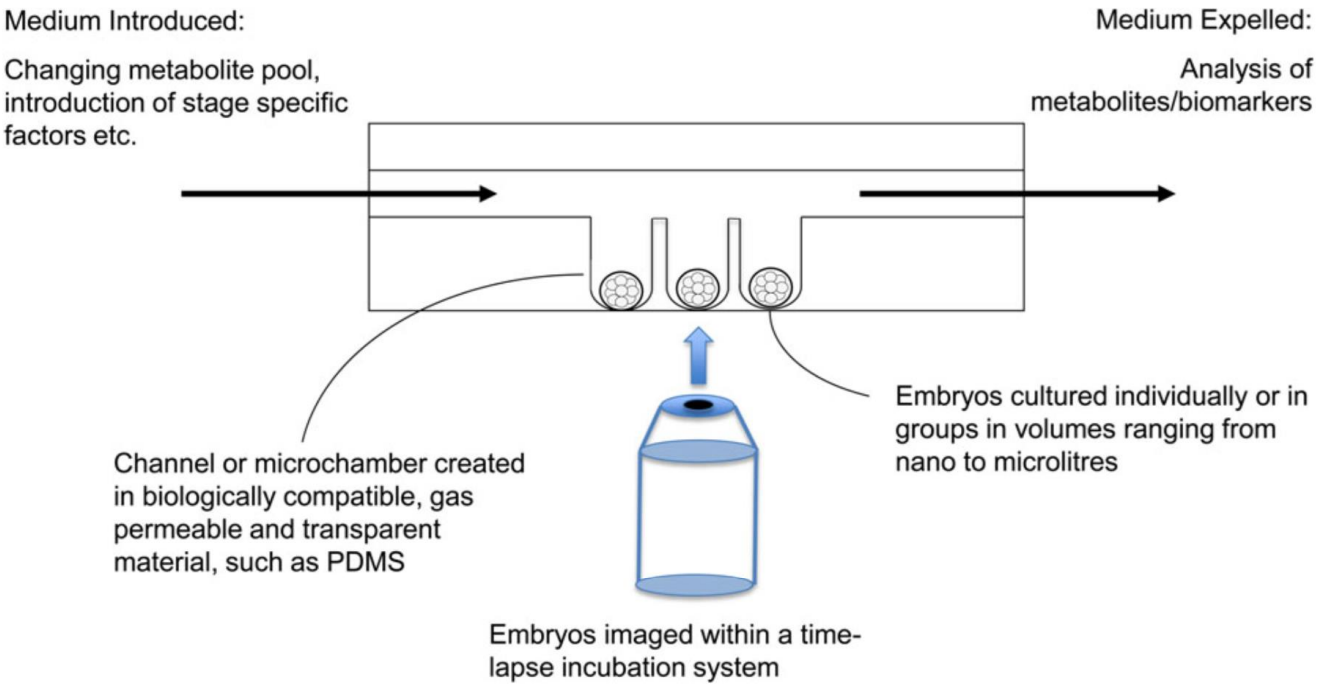


Figure 6 Schematic of an embryo culture system for perfusion culture and analysis of biomarkers. Culture media are continuously passed over the embryo(s). The composition of the culture media can be changed according to the specific requirements of each stage of embryonic development. Toxins, such as ammonium, are not able to build up and impair embryo development, while more labile components of the culture system are not denatured. Samples of culture media can be removed for biomarker analysis. Adapted from Gardner (1994).

Diagnosis of human preimplantation embryo viability

David K. Gardner[1,*], Marcos Meseguer[2], Carmen Rubio[3], and Nathan R. Terrify.
Human Reproduction Update, Vol.21, No.6 pp. 727ï 747, 2015

Embryo Culture
Non invasive
Selection

TLS **MTB** **NIG**

Where We Are and Where We Are Going: The Future of ART

Dr. Marcos Horton
Co-Director and Founder
Pregna Medicina Reproductiva
Past-President, Argentinian Society
for Reproductive Medicine
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Global Fertility Academy