Milestones in assisted reproduction and related technologies

Part 3: Preimplantation genetic diagnosis, testing and therapeutics

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Abstract
The present effort is intended to document the milestones and historical events that contributed to the development of Preimplantation Genetic Diagnosis (PGD) or Testing (PGT) and its application in human assisted reproduction technology (ART). The authors endeavored to record in chart form (Chart 7) every single milestone, whether grandeur or miniscule in the general topic of PGD/PGT. The search for these historical events was performed by extensive online search. It is now 40 years since PGD/PGT has been performed worldwide in the human embryo. Initially PGD was mainly useful for a relatively small number of genetic anomalies. Within a span of 3 decades technological advances has permitted its extensive application for the detection of a wide array of genetic anomalies in the early human embryo. It is obvious more advances are yet to come.

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Introduction

Since the 1990s, genetic evaluation of the pre-implantation embryo has emerged as the single most reliable procedure for the selection genetically normal embryos. This is basically to avoid the transmission genetic disorders to the next generation and for the selection of euploid embryos in patients with known tendency towards high rate of aneuploidy. The latter is intended and anticipated to increase the chance of pregnancy in affected individuals during therapeutic assisted reproduction treatment. Preimplantation genetic diagnosis (PGD) was first reported in 1990 by Handyside and coworkers (Handyside et al., 1990).
Over the last three decades this technology has evolved significantly. The historical events pertaining to the development of the current state of the art of preimplantation genetic diagnosis or testing (PGD/PGT) programs has been previously described in detail (Chen et al., 2019; Olcay et al.,2023; Simpson et al.2018).

In the present communication the authors have attempted to chronicle the individual milestones that led to the development of the methodologies for the efficacious PGD/PGT of the human embryo as completely as possible in a concise manner in chart-form. The significant contributions in PGD/PGT whether grandeur or miniscule is recorded as accurately as possible, irrespective of its geographical origins. Furthermore this endeavor also attempts to

ameliorate subtle imprecisions which may have created confusion on the origination of research findings and methodologies in PGD/PGT so that the genuine contributions of some are not erroneously attributed to others.

The authors acknowledge this task of chronicling the milestones in the development and acquisition of knowledge in the PGD/PGT technologies will inadvertently leave out some contributions, in particular those not published in the English mainstream scientific literature. In reality, the enormity of this task dictates that a complete narration is unattainable by a small group of workers given the limited resources available to them within the span of a few years of work.

Nevertheless, it is felt this effort has recorded almost all the significant contributions in PGD/PGT. It is anticipated this work will become a useful reference material to the profession and is inspirational for the younger generation.

Material and methods

The authors have chronicled the milestones that led to the development of the PGD/PGT technologies as comprehensively as possible using resources available to them. Online resources utilised were mainly the Google Scholar and the U.S. National Library of Medicine (Pubmed) and individual journals.

Results

The Chart 7 below chronicles the milestones pertaining to the development of the PGD/PGT technologies in their chronological order of development. The authors will endeavour to publish the milestones pertaining to the application of artificial intelligence in ART, in vitro maturation, micromanipulation and embryonic stem cell technology in ART from Charts 8 onwards in the same journal separately subsequently.

Discussion

We have chronicled the milestones in the development of the science and methods of human PGD/PGT as accurately as possible given the resources available to us. We have also tried to present these milestones as fairly as possible without bias in acknowledging and recording important contributions.

Conclusion

In conclusion, the quest for the genetic evaluation of the human embryo that commenced in the 1990's appears to have evolved tremendously and does not appear to be stopping at any time in the near future. Much more undoubtedly is anticipated to be accomplished in this agra of endeavour in the years to come.

Reference

Chen HF, Chen SU, Ma GC, Hsieh ST, Tsai HD, Yang YS, Chen M. Preimplantation genetic diagnosis and screening: Current status and future challenges. J Formos Med Assoc. 2018;117(2):94-100. doi:10.1016/j.jfma.2017.08.006.

Handyside A, Kontogianni EH, Hardy K, Winston RM. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. Nature 1990;344:768e70

Olcay O, Ergun Y, Basar M. Historical Roadmap of Preimplantation Genetic Testing from Past to Present; What is Expected in the Future. J Reprod Med Gynecol Obstet. 2023;8(129):2.

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Chart 7. Milestones in Preimplantation Genetic Diagnosis and Testing

Year	Milestone/Event	Reference
1952	Seidel performed the first embryo biopsy on 2 cell stage rabbit embryo.	Seidel F. Die Entwicklugspotenzen einen isoliertn Blastomere des Zweizellenstadiums im Saugetierei. Natur- wissenschaften 1952; 39: 355–356
1967	Tarkowski and Wróblewska perfomed embryo biopsy on 4-8 cell stage mice embryos.	Tarkowski AK and Wróblewska J. Development of blasto- meres of mouse eggs isolated at the 4- and 8-cell stage. J Embryol Exptl Morphol 1967; 18(1): 155–180.
1967	Edwards biopsied the trophectoderm of rabbit blastocyst and managed to determine the gender by identifying sex chromatin.	Edwards RG and Gardner RL. Sexing of live rabbit blastocysts. Nature 1967; 214(5088): 576–577
1968	Gardner and Edwards noted that the rabbit offspring were of the predicted sex.	Gardner RL and Edwards RG. Control of the sex ratio at full term in the rabbit by transferring sexed blastocyts. Nature 1968; 218(5139): 346–349.
1971	Steptoe and co-workers tried to repeat the work of Edwards and Gardner in the human blastocyst but the experiment failed.	Steptoe PC, Edwards RG and Purdy JM. Human blastocysts grown in culture. Nature 1971; 229(5280): 132–133.
1976	Chromosomal assessment of cow embryos prior to transfer.	Hare WCD., Mitchell D, Betteridge KJ, Eaglesome MD, Randall GCB. Sexing two-week old bovine embryos by chromosomal analysis prior to surgical transfer: preliminary methods and results. Theriogenology. 1976; 5:243–253.
1978	Human spermatozoa successfully karyotyped in penetrated zona-free hamster eggs.	Rudak, E., Jacobs, P. A. & Yanagimachi, R. (1978). Direct analysis of the chromosome constitution of human spermatozoa. Nature, Lond. 1978;274, 911–913.
1980	Attempt to visualize the mouse second polar body Chromosomes but the authors noted their technique has limitations and may not be useful for practical application.	Modlinski J, Mclaren A. A method for visualizing the chromo-somes of the 2nd Polar body of the mouse egg. J Embryol Exp Morphol. 1980;60:93–97
1985	Biologic and morphological development of human blastocyst recovered by uterine lavage were described by Buster and coworkers	Buster JE, Busillo M, Rodi IA, Cohen SW, Hamilton M, Simon JA, Thorneycroft IH and Marshall SR. Biologic and morphological development of donated human ova recovered by non-surgical uterine lavage. American Journal of Obstetrics and Gynecology 1985; 153: 211–217.

1983/1985	**Landmark development** PCR. The idea for the development of the PCR technique was described by Kary Banks Mullis in 1983 while he was at Cetus Corporation, CA, USA, in 1983, which won him the 1993 Nobel Prize. The technique was perfected by his colleagues at Cetus Corp but Mullis procrastinated on writing a paper on the PCR. Frustrated by this delay Saiki et al., 1985 co-authored a paper that described the PCR technique. Mullis left Cetus Corp since he was not given first authorship for the said paper.	K.B. Mullis. Dancing Naked in the Mind Field. Jan 04, 2000. ISBN 9780679774006
1985	Saiki RK, Scharf S, Faloona F, helped make the PCR technique work. They helped develop the polymerase chain reaction (PCR) protocol. Mullis was part of this team but he was fourth author in the paper coa-authored by Randall Saiki.	Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA and Arnheim N. Enzymatic amplification of betaglobulin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 1985; 230(4): 1350–1354
1986	Zona drilling performed to demonstrate only few spermatozoa are required for fertilization following zona drilling in unfertilized mouse oocytes as a means to overcome oligospermia. This technique was later found to be useful for embryo biopsy for removal of blastocmeres for PGD.	Gordon JW, Talansky BE. Assisted fertilization by zona drilling: a mouse model for correction of oligospermia. J Exp Zool. 1986;239(3):347-354.
1986	Wilton and Trounson developed a technique for biopsy of a single cell from mouse embryo without affecting the embryo viability.	Wilton LJ and Trounson AO. Viability of mouse embryos and blastomeres following biopsy of a single cell. In Proceedings of the 18th Annual Conference of the Australian Society for Reproductive Biology, Brisbane, Australia
1987	Penketh wrote a review article on “Prospects for prenatal diagnosis during preimplantation human development”.	Penketh R and McLaren A. Prospects for prenatal diagnosis during preimpl-antation human develop-ment. Baillieres Clin Obstet Gynaecol 1987; 2(5): 421–424.
1987	Nijis Removed one cell from two cell embryo.	Nijs M and Van Steirteghem AC. Assessment of different isolation procedures for blastomeres from two-cell mouse embryos. Human Reproduction 1987; 2(5): 421–424.
1987	Hooper made genetically engineered mice with defect in the HPRT gene in order to use it as a model for PGD.	Hooper M, Hardy K, Handyside A, Hunter S and Monk M. HPRT-deficient (Lesch–Nyhan) mouse embryos derived from germline colonization by

		cultured cells. Nature 1987; 326(6110): 292–295.
1987	Monk biopsied 1 and 2 blastomeres from 8 cell stage mouse embryo for the purpose of genetic diagnosis of hypoxanthine phosphoribosyl transferase. This is the initial marking about the possibility of performing preimplantation diagnosis by biopsy and analysis of a single blastomere.	Monk M, Handyside A, Hardy K and Whittingham D. Preimplantation diagnosis of deficiency for hypoxanthine phosphor-ribosyl transferase in a mouse model for Lesch–Nyhan syndrome. Lancet 1987; 2: 423–425.
1987/ 1990	The first polar biopsy by performed by Verlinsky and colleagues reporting their first clinical case at the 1987 International Congress on IVF in Kyoto. Japan but was only published some years later.	Verlinsky Y, Pergament E, Binor Z et al. Genetic analysis of human embryos prior to implantation: future applications of in-vitro fertilization in treatment and prevention of human genetic diseases. In: Feichtinger W, Kemeter P (eds) Future aspects in human in vitro fertilization. Springer-Verlag, Berlin, pp 262–266, 1987 Verlinsky Y, Ginsberg N, Lifchez A et al. Analysis of the first polar body: preconception genetic diagnosis. Hum Reprod 1990; 5 (7):826–829
1987	High level of chromosomal abnormality in embryos was reported by Plachot.	Plachot M, de Grouchy J, Junca A-M, Mendelbaum J, Turleau C, Couillin P, Cohen J and Salat-Baroux J. From oocyte to embryo: a model, deducted from in vitro fertilization, for natural selection against chromosome abnormalities. Annales de Genetique 1987; 30: 22–32.
1988	Monk et al performed trophectoderm biopsy in mouse blastocyst for the purpose of genetic diagnosis for Lesch–Nyhan disease.	Monk M, Muggleton-Harris A, Rawlings E and Whittingham D. Preimplantation diagnosis of HPRT-deficient male, female, and carrier female mouse embryos by trophectoderm biopsy. Human Reproduction 1988; 3: 377–381.
1988	Sexing of pre-implantation mouse embryos for x-linked diseases.	Monk M, Handyside AH Sexing of pre-implantation mouse embryos by measurement of X-linked gene dosage in a single blastomere. J Reprod Fertil 1988; 82(1):365–368
1988	Marmoset monkey embryos developed normally after trophectoderm biopsy by Summers.	Summers PM, Campbell JM and Miller MW. Normal in vivo development of marmoset monkey embryos after trophectoderm biopsy. Human Reproduction 1988; 3: 389–393.

1988	Edwards Suggested sexing the sperm. Edwards suggested non-invasive analysis of the media. Edwards indicated that biopsy could affect implantation rate. Edwards suggested the use of DNA probe as a reliable technique for single-cell diagnosis to identify the chromosomes of human embryos.	Edwards RG, Hollands P. New advances in human embryology: implications of the preimplantation diagnosis of genetic disease.Human Reprod 1988; 3(4): 549–556.
1988	Development of a very sensitive microassay for detecting femtomolar amounts of reaction products in a single blastomere from a mouse 8-cell embryo	Benson C and Monk M. Microassay for adenosine deaminase, the enzyme lacking in some forms of immuno-deficiency, in mouse preimplantation embryos. Human Reproduction 1988; 3: 1004–1009.
1988	Development of a PCR protocol that can amplify single human cells.	Li A, Gyllenstein UB, Cui X, Saiki RK, Erlich HA and Arnheim N. Amplification and analysis of DNA sequences in single human sperm and diploid cells. Nature 1988; 335: 414–419.
1989	The activity of the HPRT gene activity in unfertilised oocytes and single blastomeres biopsied from human preimplantation embryos were analysed by Braude.	Braude P, Monk M, Pickering S, Cant A and Johnson M. Measurement of HPRT activity in the human unfertilised oocyte and preimplantation embryo. Prenatal Diagnosis 1989; 9: 839–850.
1989	Developing of PCR technique that detect mutations in single cells.	Holding C and Monk M. Diagnosis of beta thalassaemia by DNA amplification in single blastomeres from mouse preimplantation embryos. Lancet 1989; ii: 532–535.
1990	Sexing of human embryo by Y-specific DNA amplication.	Handyside AH, Konto-gianni EH, Hardy K et al. Pregnancies from biop-sied human pre-implantation embryos sexed by Y-specific DNA amplification. Nature 1990; 344(6268): 768–770.
1993	Griffin and coworkers described the use of fluorescence in situ hybridization (FISH) technique in PGD for gender detection.	Griffin DK, Wilton LJ, Handyside AH, Atkinson GHG, Winston RML, Delhanty JDA (1993) Diagnosis of sex in preimplantation embryos by fluorescent in situ hybridisation. BMJ 306:1382.
1990	Genetic analysis of polar bodies.	Monk M and Holding C. Amplification of a beta haemoglobin sequence in individual human oocytes and polar bodies. Lancet 1990; 335: 985–8.
1990	Use of acid Tyrode's solution on day 3 embryos to drill a hole in the zona in order to facilitate aspiration of one or two cells from eight-cell embryos, and allowing the embryos to grow on to day five.	Hardy K, Martin KL, Leese HJ, Winston RML and Handyside AH. Human preimplantation development in vitro is not adversely affected by biopsy at the 8-cell stage. Human Reprod 1990; 5(6): 708–714.

1990	The first use of partial zona dissection PZD and calcium and magnesium free media to facilitate blastomere biopsy.	Grifo JA, Boyle A, Fischer E, Lavy G, DeCherney AH, Ward DC and Sanyal MK. Preembryo biopsy and analysis of blastomeres by in situ hybridization. American Journal of Obstet Gynecol 1990; 163(6 Pt. 1): 2013–2019
1990	Brambati suggested uterine washing to get human blastocyst for the purpose of preimplantation genetic diagnosis.	Brambati B and Tului L. Preimplantation genetic diagnosis: a new, simple uterine washing system. Human Reproduction 1990; 5(4): 448–50
1990	Dokras performed trophectoderm biopsy on human blastocyst.	Dokras A, Sargent IL, Ross C, Gardner RL and Barlow DH. Trophectoderm biopsy in human blastocysts. Human Reproduction 1990; 5(7): 821–825.
1990	**Landmark development** The first human pregnancy after PGD	Handyside AH, Kontogianni EH, Hardy K and Winston RML. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. Nature 1990; 244: 768–770
1990	The first pregnancy after PGD for cystic fibrosis.	Strom CM, Verlinsky Y, Milayeva S, Evsikov S, Cieslak J, Lifchez A, Valle J, Moise J, Ginsberg N and Applebaum M. Preconception genetic diagnosis of cystic fibrosis. Lancet 1990; 336: 306–307
1991	Use of PGD to detect aneuploidy in mouse embryos.	Kola I and Wilton LJ. Preimplantation embryo biopsy: detection of trisomy in a single cell biopsied from a four cell mouse embryo. Molecular Reproduction and Development 1991; 29: 16–21.
1991	The first application of Fluorescent in situ hybridization FISH to human preimplantation embryos with X and Y chromosome specific probes.	Griffin DL, Handyside AF, Penketh RJ, Winston RML and Delhanty JD. Fluores-cent in situ hybridization to inter-phase nuclei of human preimplantation embryos with X and Y chromosome specific probes. Human Reprod 1991; 6(1): 101–105.
1991	Metaphase chromosomes spreads was obtained occasionally from mouse blastomeres by Dyban	Dyban AP Experimental cytogenetics of pre-implantation development. In: Joe Leigh Simpson et al. Verlinsky Y, Kuliev A (eds) Preimplantation Genetics. Plenum Press, New York, pp 15–23. 1991
1992	Simultaneous amplification of X and Y chromosomes in human blastomeres.	Grifo JA, Tang YX, Cohen J, Gilbert F, Sanyal ML and Rosenwaks Z. Pregnancy after embryo biopsy and coamplification of DNA from X and Y chromosomes. Journal of the American Medical Association 1992; 268(6): 727–729.

1992	Pregnancy following embryo biopsy subjected to both X and Y FISH	Grifo JA, Boyle A, Tang YX et al Preimplant-ation genetic diagnosis. In situ hybridization as a tool for analysis. Arch Pathol Lab Med 1992;116(4): 393–397 Grifo JA, Tang YX, Cohen J, Gilbert F, Sanyal MK, Rosenwaks Z. Pregnancy after embryo biopsy and coamplification of DNA from X and Y chromo-somes. JAMA. 1992; 268 (6):727-9. Pregnancy after embryo biopsy and amplification of DNA from X and Y chromo-somes. JAMA. 1992; 268(6):727–729
1992	The use of cell conversion, a technique in which the chromosomes of a polar body were converted into metaphase stage chromosomes to facilitate viewing.	Dyban AP, De Sutter P, Dozortsev D and Verlinsky Y. Visualization of second polar body chromosomes in fertilized and artificially activated mouse oocytes treated with okadaic acid. Journal of Assisted Reproduction Genetics 1992; 9(6): 572–579.
1993	A FISH procedure using fluorochrome (multicolored) was developed by Munne et al., for human application in preimplantation embryos to analyze the five chromosomes most involved in human aneuploidy (X, Y, 18,13 and 21).	Munné S, Lee A, Rosenwaks Z, Grifo J, Cohen J. Fertilization and early embryology: Diag-nosis of major chromosome aneuploid-ies in human preimplant-ation embryos. Human reproduction. 1993;8(12): 2185-2191.
1993	The use of directly labelled FISH probe for sexing of human blastomeres.	Munné S, Weier HUG, Stein J, Grifo J and Cohen J. A fast and efficient method for simultaneous X and Y in situ hybridization of human blastomeres. J Assist Reprod Genet 1993; 10: 82–90.
1993	Whole genome amplification of human blastomere using a novel technique called the Primer Extension Preamplification (PEP).	Xu K, Tang Y, Grifo JA, Rosenwaks Z and Cohen J. Primer extension pream-plification for detection of multiple genetic loci from single human blasto-meres. Human Reprod 1993; 8(12): 2206–2210.
1993	Preimplantation genetic screening (PGS) using 5 FISH chromosomes.	Munné S, Lee A, Rosenwaks Z, Grifo J and Cohen J. Diagnosis of major chromosome aneuploidies in human preimplantation embryos. Human Reproduction 1993b; 8: 2185–2192.
1994	Cell recycling allows performing FISH and PCR on the same blastomere.	Thornhill A, Holding C and Monk M. Recycling the single cell to detect specific chromosomes and to investigate specific gene sequences.

		Human Reproduction 1994; 9(11): 2150–2155.
1994	Development of a new FISH protocol that yields the results in two hours.	Harper JC, Coonen E, Ramaekers FCS, Delhanty JDA, Handyside AH, Winston RML and Hopman AHN. Identifi-cation of the sex of human preimplantation embryos in two hours using an improved spreading method and fluorescent in situ hybridization (FISH) using direct labelled probes. Human Reproduction 1994; 9: 721–724.
1994	Development of a new nucleus spreading techniue that allows the analysis of all the cells in an embryo.	Coonen E, Harper JC, Ramaekers FCS, Delhanty JDA, Hopman AHN, Geraedts JPM and Handyside AH. Presence of chromosomal mosaic-ism in abnormal preimpl-antation embryos detect-ed by fluorescence in situ hybridisation. Hum Genetics. 1994;94:609–615.
1995	Normal pregnancy after PGD for Duchenne Muscular Dystrophy.	Liu J, Lissens W, Van Broeckhoven C, Löfgren A, Camus M, Liebaers I and Van Steirtegham A. Normal pregnancy after preimplantation DNA diagnosis of a dystrophin gene deletion. Prenatal Diagnosis 1995; 15(4): 351–358
1995	PGD for triple repeats disorder.	Daniels R, Kinis T, Serhal P, Monk M. Expression of the myotonin protein kinase gene in preimpl- antation human embryos. Human Mol Genetics 1995; 3: 389–393.
1995	FISH analysis of polar bodies	Verlinsky Y, Cieslak J, Ivakhnenko V et al. Birth of healthy children after preimplantation genetic diagnosis of common aneuploidies by polar body fluorescent in situ hybridization analysis. Fertility and Sterility 1996; 66: 126–129
1996	Detection of the allele drop out (ADO) after PCR of a single blastomere . The ADO is considered as one of the disadvantages of single cell PCR.	Ray PF and Handyside AH. Increasing the denature-ation temperature during the first cycles of amplification reduces allele dropout from single cells for preimplantation genetic diagnosis. Mol Human Reprod 1996; 2(3): 213–18.
1995	The first PGD for translocations.	Conn CM, Harper JC, Winston RML and Delhanty JDA. Preimplan-tation diagnosis of trisomies 13, 14, 18 & 21 in translocation carriers using multicolor fluores-cent in situ hybridization (FISH). Am J Human Genet 1995; 57(4): A277
1996	The first PGD of autosomal dominant single gene.	Harton GL, Tsipouras P, Sisson ME, Starr KM, Mahoney BS, Fugger EF,

		Schulman JD, Kilpatrick MW, Levinson G and Black SH. Preimplantation genetic testing for Marfan syndrome. Mol Human Reprod 1996; 2(9): 713–715
1998	PGD of the inherited cancer familial adenomatous polyposis coli.	Ao A, Wells D, Handyside AH, Winston RML and Delhanty JDA. Preimplant - ation genetic diagnosis of inherited cancer; familial adenomatous polyposis coli. J Assist Reprod Genet 1998; 15: 140–144.
1998	The use of LASER for human embryo biopsy.	Boada M, Carrera M, De La Iglesia C, Sandalinas M, Barri PN and Veiga A. Successful use of a laser for human embryo biopsy in preimplantation gene-tic diagnosis: report of two cases. J Assist Reprod Genet 1998; 15: 302–307.
1998	Application of fluorescent PCR for PGD	Sermon K, De Vos A, Van de Velde H, Seneca S, LissensW, Joris H, Vandervorst M, Van Steirteghem A and LiebaersI. Fluorescent PCR and automated frag- ment analysis for the clinical application of preimplantation genetic diagnosis of myotonic dystrophy (Steinert's disease). Mol Human Reprod 1998; 4(8): 791–796.
1998	The use of spectral karyotyping (SKY) for karyotyping of oocytes and polar body.	Márquez C, Cohen J and Munné S. Chromosome identification on human oocytes and polar bodies by spectral karyotyping. Cytogenet Cell Genet 1998; 81: 254–258.
1998	FISH to identify unbalanced chromosomal translocations	Munné S, Fung J, Cassel MJ, Márquez C, Weier HU. Preimplantation genetic analysis of translocations: case-specific probes for interphase cell analysis. Human Genet. 1998;102: 663-674.
1999	Birth of first unaffected baby after successful PGD for spinal muscular atrophy type I.	Fallon L, Harton GL, Sisson ME, Rodriguez E, Field LK, Fugger EF, Geltinger M, Sun Y, Dorfmann A, Schoener C, Bick D et al. Preimplantation genetic diagnosis for spinal muscular atrophy type I. Neurology 1999;53: 1087 –1090.
1999	The two research groups of Wells and coworkers, and Vouaire and coworkers independently demons-trated the usefulness of Comparative Genomic Hybridi-zation (CGH) technology on human blastomeres for aneuploidies in all chromosomes.	Wells D, Sherlock JK, Handyside AH, Delhanty JDA (1999) Detailed chromosomal and molecular genetic analysis of single cells by whole genome amplification and comparative genomic hybridisation. Nucleic Acids Res 27:1214–1218

		Voullaire L, Wilton L, Slater H, Williamson R (1999) Detec tion of aneuploidy in single cells using comparative genomic hybridization. Prenat Diagnosis Publ Affil With Int Soc Prenat Diagnosis 19:846–851
2000	Successful freezing and thawing of biopsied embryos	Lee M, Munné S. Pregnancy after polar body biopsy and freezing and thawing of embryos. Fertil Steril 2000; 73: 645–647.
2000	The use of comparative genomic hybridisation (CGH) for PGD	Wells D and Delhanty JDA. Comprehensive chromo-somal analysis of human preimplantation embryos using whole genome amplification and single cell comparative genomic hybridization. Mol Human Reprod 2000; 6: 1055–1062
2001	The use of PGD for Human Leukocyte Antigen (HLA) matching.	Verlinsky Y, Rechitsky S, Verlinsky O, Schoolcraft W, Strom C and Kuliev A. Preimplantation diagnosis for Fanconi anemia combined with HLA matching. JAMA. 2001; 285: 1–4
2001	The first PGT case for inherited adult onset cancer was performed by Verlinsky et al.	Verlinsky Y, Rechitsky S, Verlinsky O, Xu K, Schattman G, Masciangelo C, Ginberg N, Strom C, Rosenwaks Z, Kuliev A. Preimplantation diagnosis for p53 tumour suppressor gene mutations. Reprod Biomed Online. 2001 ;2(2):102-105.
2005	McArthur et al. demonstrated feasibility of blastocyst biopsy for PGT-A	McArthur SJ, Leigh D, Marshall JT, de Boer KA, Jansen RP. Pregnancies and live births after trophectoderm biopsy and preimplantation genetic testing of human blastocysts. Fertil Steril. 2005;84(6):1628-36.
2007	Seli et al. performed metabolimic analysis of culture media.	Seli E, Sakkas D, Scott R, Kwok SC, Rosendahl SM and Burns DH. Noninvas- ive metabolomic profiling of embryo culture media using Raman and near-infrared spectroscopy correlates with reprod-uctive potential of emb-ryos in women under-going in vitro fertili-zation. Fertil Steril. 2007;88(5):1350–1357
2007	A controlled trial of three IVF cycles with or without preimplantation genetic screening (PGS) performed in women 31-41 years showed PGS significantly reduced the rates of ongoing pregnancies and live births	Mastenbroek S, Twisk M, van Echten-Arends J, Sikkema Raddatz B, Korevaar JC, Verhoeve HR, Vogel NEA, Arts EGJM, de Vries JWA, Bossuyt PM et al (2007) In vitro fertilization with preimplantation genetic screening. N Engl J Med

	after IVF in women above 35 years of age	357:9–17
2008	Wells and coworkers demonstrated the use of microarray and CGH platforms for the detection of aneuploidy of all 23 chromosome pairs.	Wells D, Alfarawati S, Fragouli E (2008) Use of comprehensive chromo-somal screening for embryo assessment: microarrays and CGH. Mol Hum Reprod 14:703–710
2009	The usefulness of vitrification following trophictoderm biopsy of human blastocysts for comprehensive chromosomal screening (CCS) was demonstrated.	Schlenker T, Stevens J, Rawlins M, McCormick S, Katz-Jaffe MG, Schoolcraft WB. Clinical success with vitrification following trophectoderm biopsy for omprehensive chromosomal screening. Fertility and Sterility. 2009 Sep 1;92(3):S71.
2009-2018	Children conceived after PGD showed developmental neuropsy-chological outcomes that are apparently within normal limits.	Wang F, Xiao M et al (2009) Evaluation of blastomere biopsy using a mouse model indicates the potential high risk of neurodegenerative disorders in the offspring. Mol Cell Proteomics 8:1490–1500 Zhao H-C, Zhao Y, Li M, Yan J, Li L, Li R, Liu P, Yu Y, Qiao J (2013) Aberrant epigenetic modification in murine brain tissues of offspring from preimplantation genetic diagnosis blastomere biopsies. Biol Reprod 89:111–117 Sacks GC, Altarescu G, Guedalia J, Varshaver I, Gilboa T, Levy-Lahad E, Eldar-Geva T (2016) Developmental neuropsychological assessment of 4- to 5-year-old children born following preimplantation genetic diagno-sis (PGD): a pilot study. Child Neuropsychol 22:458–471 Kuiper D, Bennema A, la Bastide-van Gemert S, Seggers J, Schendelaar P, Mastenbroek S, Hoek A, Heineman MJ, Rose boom TJ, Kok JH et al (2018) Developmental outcome of 9-year old children born after PGS: follow-up of a randomized trial. Hum Reprod 33:147–155 54. Yu Y, Wu J, Fan Y, Lv Z, Guo X, Zhao C, Zhou R, Zhang Z,
2010	Karyomapping was demonstrated by the group of Handyside in 2010	Handyside AH, Harton GL, Mariani B, Thornhill AR, Affara

		N, Shaw MA, Griffin DK (2010) Karyomapping: a universal method for genome wide analysis of genetic disease based on mapping crossovers between parental haplotypes. J Med Genet 47:651–658
2011	First births reported after preimplantation genetic diagnosis of some abnormalities using comparative genomic hybridization and microarray analysis.	Alfarawati S, Fragouli E, Colls P, Wells D (2011) First births after preimplantation genetic diagnosis of structural chromo some abnormalities using comparative genomic hybridization and microarray analysis. Hum Reprod 26:1560–1574.
2013	Yin and coworkers demonstrated the successful use of NGS technology for PGT-A	Yin XY, Tan K, Vajta G, Jiang H, Tan YQ, Zhang CL, Chen F, Chen SP, Zhang CS, Pan XY et al (2013) Massively parallel sequencing for chromosomal abnormality testing in trophectoderm cells of human blastocysts. Biol Reprod. https:// doi. org/10. 1095/ BIOLR EPROD. 112. 106211
2013	It became clear that single blastomere biopsy reduced implantation rates by 39% relative to controls in a randomized controlled experiment (RCT)	Scott RT Jr, Upham KM, Forman EJ, Zhao T, Treff NR (2013) Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. Fertil Steril 100:624–630
2013-2016	**Minimally invasive technique for PGT.** A procedure to source for embryonic DNA for Minimally invasive PGT for preimplantation genetic assessment from blastocel fluid was reported. It is an injection and aspiration procedure which avoids serious risks to the embryo (Palini et al., 2013; Perloe et al, 2013; Poli et al, 2013; Gianaroli et al, 2014; Zhang et al., 2016). DNA is present in blastocoele fluid (BF). BF appears to be another source of DNA for PGT with lower risk of contamination and significantly less invasive than biopsy of the embryo.	Palini S., Galluzzi L., De Stefani S., Bianchi M., Wells D., Magnani M., Bulletti C. Genomic DNA in human blastocoele fluid. Reprod. Biomed. Online. 2013;26:603–610 Poli M, Jaroudi S, Sarasa J, Spath K, Child T, Wells D. The blastocoel fluid as a source of DNA for preimplantation genetic diagnosis and screening. Fertil Steril. 2013;100:S37 Perloe M, Welch C, Morton P, Venier W, Wells D, Palini S. Validation of blastocoele fluid aspiration for preimplantation genetic screening using array comparative geno-mic hybridization (aCGH) Fertil Steril. 2013; 100: S208. Gianaroli L, Magli MC, Pomante A, Crivello AM, Cafueri G, Valerio M, Ferraretti AP. Blastocentesis: a source

		of DNA for preimplantation genetic testing. Results from a pilot study. Fertil Steril. 2014;102:1692–1699 Zhang Y, Li N, Wang L, Sun H, Ma M, Wang H, Xu X, Zhang W, Liu Y, Cram DS, Sun B, Yao Y. Molecular analysis of DNA in blastocoele fluid using next-generation sequencing. J Assist Reprod Genet. 2016 May;33(5):637-645
2013	**Minimally invasive technique for assessment of embryo competence and viability** The identification and quantification of embryonic proteins present in extreme low concentrations in the blastocoel was described by Poli et al., 2013. This technique could allow crucial traits of embryo competence to be measured using a proteomic-based strategy, with negligible risk of harm to the living embryo.	Poli M, Ori A, Child T, Jaroudi S, Spath K, Beck M, Wells D. Characterization and quantification of proteins secreted by single human embryos prior to implantation. EMBO Mol Med. 2015;7(11):1465-1479.
2013-2024	Several groups are involved in developing noninvasive methods for the identification of biological markers of embryo competence and viability, such as the use of miRNAs and extracellular vesicles which are released at all stages of embryo development in surrounding media.	McCallie B, Schoolcraft WB, Katz-Jaffe MG (2010) Aberration of blastocyst microRNA expression is associated with human infertility. Fertil Steril 93:2374–2382 121. Capalbo A, Ubaldi FM, Cimadomo D, Noli L, Khalaf Y, Farcomeni A, Ilic D, Rienzi L (2016) MicroRNAs in spent blasto cyst culture medium are derived from trophectoderm cells and can be explored for human embryo reprod-uctive competence assessment. Fertil Steril 105:225–235 122. Rosenbluth EM, Shelton DN, Sparks AET, Devor E, Christenson L, Van Voorhis BJ (2013) MicroRNA expression in the human blastocyst. Fertil Steril 99:855–861 Fang F, Li Z, Yu J, Long Y, Zhao Q, Ding X, Wu L, Shao S, Zhang L, Xiang W (2021) MicroRNAs secreted by human embryos could be potential biomarkers for clinical outcomes of assisted reproductive technology. J Adv Res 31:25–34

		Battaglia R, Palini S, Vento ME, La Ferlita A, Lo Faro MJ, Caroppo E, Borzi P, Falzone L, Barbagallo D, Ragusa M et al (2019) Identification of extracell- ular vesicles and characterization of mirna expression profiles in human blastocoel fluid. Sci Rep 9:84 Vyas P, Balakier H, Librach CL (2019) Ultrastructural identification of CD9 positive extracellular vesicles released from human embryos and transported through the zona pellucida. Syst Biol Reprod Med 65:273–280 Pallinger E, Bognar Z, Bodis J, Csabai T, Farkas N, Godony K, Varnagy A, Buzas E, Szekeres-Bartho J (2017) A simple and rapid flow cytometry-based assay to identify a competent embryo prior to embryo transfer. Sci Rep 7:39927 Veraguas D, Aguilera C, Henriquez C, Velasquez AE, Melo-Baez B, Silva-Ibañez P, Castro FO, Rodriguez-Alvarez L (2021). Evaluat- ion of extracellular vesicles and GDNA from culture medium as a possible indicator of developmental competen- ce in human embryos. Zygote 29:138–149 Wang Y, Li Y, Zhu X, Yang M, Liu Y, Wang N, Long C, Kuo Y, Lian Y, Huang J, Jia J. Concurrent preimplantation genetic testing and competence assessment of human embryos by transcriptome sequencing. Advanced Science. 2024 Aug;11(32):2309817.
2018	The work of Friendenthal and co-workers showed that the compari-son of aCGH versus NGS for PGT-A marginally improved results with NGS and eSET.	Friedenthal J, Maxwell SM, Munné S, Kramer Y, McCulloh DH, McCaffrey C, Grifo JA (2018) Next generation sequencing for preimplantation genetic screening improves pregnan- cy outcomes compared with array comparative genomic hybridization in single thawed euploid embryo transfer cycles. Fertil Steril 109:627–632

2018	Spinella and coworkers showed that the extent of mosaicism influences the success rate of IVF	Spinella F, Fiorentino F, Biricik A, Bono S, Ruberti A, Cotroneo E, Baldi M, Cursio E, Minasi MG, Greco E (2018) Extent of chromosomal mosaicism influences the clinical outcome of in vitro fertilization treatments. Fertil Steril 109:77–83. https:// doi. org/ 10. 1016/J. FERTN STERT. 2017. 09. 025
2019	Backenroth and coworkers developed a 24-PGD detection test for monogenic diseases and aneuploidy.	Backenroth D, Zahdeh F, Kling Y, Peretz A, Rosen T, Kort D, Zeligson S, Dror T, Kirshberg S, Burak E et al (2019) Haploseek: a 24-hour all-in-one method for preimplantation genetic diagnosis (PGD) of monog-enic disease and aneuploidy. Genet Med 21:1390–1399
2020	Rubio et al. reported that the impact of methodology between the “invasive“ PGT-A and the “non-invasive” embryonic cell-free DNA and trophectoderm biopsies on the embryo competence were similar.	Rubio C, Navarro-Sánchez L, Garcia-Pascual CM, Ocali O, Cimadomo D, Venier W, Barroso G, Kopcow L, Bahçeci M, Kulmann MIR et al (2020) Multicenter prospective study of con cordance between embryonic cell-free DNA and trophectoderm biopsies from 1301 human blastocysts. Am J Obstet Gynecol 223:751-e1
2020-2023	The work of Sha et al., and Wang et al., demonstrated the usefulness of scRNA-seq to investigate into factors that might affect the development of 8-cell human embryos.	Sha QQ, Zheng W, Wu YW, Li S, Guo L, Zhang S, Lin G, Ou XH, Fan HY (2020) Dynamics and clinical relevance of maternal MRNA clearance during the oocyte-to-embryo transition in humans. J Nat Commun. https:// doi. org/ 10. 1038/ S41467- 020- 18680-6. Wang W, Zhao M, Zuo H, Zhang J, Liu B, Chen F, Ji P, Liu G, Gao S, Wei S et al (2023) Evaluate the developmental competence of human 8-cell embryos by single-cell RNA sequencing. Reprod Fertil 4(2):e220119.
2013	The embryo culture medium was studied using Raman spectra which allowed a prediction developmental potential of the embryo with an accuracy of 71.5% providing evidence for the use noninvasive tools for PGT-A and avoiding false positive and negative outcomes.	Meng H, Huang S, Diao F, Gao C, Zhang J, Kong L, Gao Y, Jiang C, Qin L, Chen Y, et al. 2023 Rapid and non-invasive diagnostic techniques for embryonic developmental potential: a metabolomic analysis based on raman spectroscopy to identify

		the pregnancy outcomes of IVF-ET. Front Cell Dev Biol, 11
2023	A novel method of non-invasive method for the detection of competence of embryos include the use of computational biology, machine learning, and artificial intelligence.	Barnes J, Brendel M, Gao VR, Rajendran S, Kim J, Li Q, Malm sten JE, Sierra JT, Zisimopoulos P, Sigaras A et al (2023) A non-invasive artificial intelligence approach for the prediction of human blastocyst ploidy: a retrospective model development and validation study. Lancet Digit Heal 5:e28–e40
2025	**Landmark development** The extra copy of chromosome 21 that cause Down syndrome can be removed. The work of Hashizume and coworkers successfully demonstrated the efficient elimination of excess chromosomes using multiple allele-specific targeting. This finding can be used to rescue somatic cells with trisomy eventhough the preferred method is the Non- chromosome-breaking elimination method.	Hashizume R, Wakita S, Sawada H, Takebayashi SI, Kitabatake Y, Miyagawa Y, Hirokawa YS, Imai H, Kurahashi H. Trisomic rescue via allele-specific multiple chromosome cleavage using CRISPR-Cas9 in trisomy 21 cells. PNAS nexus. 2025 Feb;4(2):pgaf022.
2026	The work of Zhang et al. delineates the genetic architecture of oocyte and early embryo competence defects (OECD) in affected paatients. This may provide insights that could assist in the development of diagnostic genetic screenings and provide a reference for standardized subtyping of patients with OECD.	Zhang C, Zheng W, Zhang H, Zhao S, Hu H, Xu X, Wan X, Sun J, Su W, Wang Y, Cui Y. Genetic architecture and phenotypic diversity of oocyte and early embryo competence defects in female infertility. Nature Medicine. 2026 Jan;32(1):72-81.