

E Scott Sills *Editor*

Screening the Single Euploid Embryo

Molecular Genetics in Reproductive
Medicine

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E Scott Sills
Center for Advanced Genetics
Reproductive Research Section
Carlsbad, CA, USA

Faculty of Science and Technology
University of Westminster
London, UK

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In Memoriam



Professor Robert P.S. Jansen (1946–2014)

It is with great sadness that we acknowledge the passing of Professor Robert Jansen.

Even while he was not well, Robert continued to guide and advise on research directions, with his contributions to this book being among his final writings. The impact of his scholarly work continues to have a global reach and will be felt for many years.

Robert played a pivotal role in reproductive medicine and the development and progress of assisted conception in Australia over the last three decades. The founder and leader of Sydney IVF (now Genea), he led advances in clinical IVF application as well as the culture and incubation of human embryos. His contributions include the introduction of transvaginal ultrasound techniques in IVF, the development of MINC incubators and culture media which now enjoy global usage, PGD undertaken at the blastocyst stage, and many other groundbreaking developments. In an effort to improve pregnancy outcomes nearly 20 years ago, Robert used his own clinic as a research base to demonstrate that single embryo

transfers were not only a feasibility but also a practical reality, and patient outcomes need not suffer in the process.

A true visionary in his outlook, Robert published in areas as diverse as reproductive surgery, endometriosis, patient advocacy, ethics in reproductive medicine, as well as more applied areas of mitochondria and the aging oocyte, the environment of the early embryo in vivo, and blastocyst biopsy as an appropriate approach to efficient PGD. Holding a personal Chair at the University of Sydney, he actively taught both science and medical students, predominantly at postgraduate level up to and beyond his retirement 3 years ago. Professor Jansen was on the editorial board for two journals and served as a referee for many others.

Along with other founders in the IVF field in Australia, Robert was instrumental in achieving recognition for and developing the Reproductive Endocrinology & Infertility (REI) subspecialty within RANZCOG and was chair of the CREI subspecialty committee for many years.

He coordinated and led one of the largest and most successful educational meetings ever held in Australia, the World Congress of IVF and Human Reproductive Genetics in 1999. Robert was made a life member of FSA in 2012 and was a board member of PGDIS for several years during its early developing stages.

Medical specialist, physician, scientist, researcher, ethicist, advocate, philosopher, author, entrepreneur, wine and food connoisseur, poker player, sailor, surfer, teacher, Porsche enthusiast, art collector, husband, father, and grandfather; the untimely departure of Professor Robert Jansen is a great loss to family, friends, and colleagues throughout the scientific and medical communities.

Sydney, NSW 2000, Australia

Don Leigh, Ph.D.

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About the Editor

E Scott Sills received his undergraduate degree from Vanderbilt and holds a PhD (molecular biology) from London's University of Westminster. Dr. Sills was awarded the MD degree from University of Tennessee in 1992. After a gynecology residency at NYU Downtown Hospital, he completed his subspecialty training in reproductive endocrinology at Cornell University. Having authored more than 100 peer-reviewed publications, Dr. Sills served as editor-in-chief for the *Journal of Experimental and Clinical Assisted Reproduction* from 2004 to 2014. He is board-certified by the American Board of Obstetrics and Gynecology and is a Fellow of the American College of Obstetricians and Gynecologists, American College of Surgeons, Royal College of Physicians (Ireland), and Society of Biology (UK). At present, Dr. Sills is Medical Director at the Center for Advanced Genetics, an in vitro fertilization program based in Carlsbad, CA.

He is registered and/or licensed for medicine in California, New York, and the United Kingdom.

Chapter 1

The Development of PGD

Joy D.A. Delhanty

Introduction

By 1987 early prenatal diagnosis of both chromosomal and single gene defects was possible via chorionic villus sampling, so why was there a perceived need to develop preimplantation genetic diagnosis (PGD)? There were two groups of patients that provided driving forces. Firstly couples known to be at high genetic risk had expressed the fervent wish to be able to start a pregnancy knowing that it would not be affected; many of these couples had experienced the trauma of repeated second trimester terminations of much wanted pregnancies. A second group, for whom PGD would obviously be of great benefit, included couples where the women had been shown from pedigree analysis to be carriers of an X-linked condition for which at the time there was no specific diagnostic test. For this group, the only option was prenatal testing of an established pregnancy to determine the sex. This then led to the termination of all male pregnancies, of which only 50 % were likely to be affected [1]. Various events around this time had made the development of PGD a possible option. In 1983 Trounson and Mohr [2] had shown that it was possible for a normal pregnancy to occur even after the destruction of blastomeres following embryo freezing. This finding indicated that it should be feasible to remove one or two cells from a cleavage stage embryo for diagnosis without compromising its further development. In the UK, Dame Mary Warnock chaired a government committee composed of people from a variety of backgrounds that considered the status of the human embryo before preimplantation with regard to the ethics of research on embryos at this stage of their development. The subsequent report was published in 1984 by the DHSS; it proposed a time limit for research of 14 days after

J.D.A. Delhanty (✉)

UCL Centre for PGD, Institute for Women's Health, University College London,
London WC1E 6HX, UK

e-mail: j.delhanty@ucl.ac.uk

fertilisation, well beyond the time when cells would be removed to allow genetic diagnosis. This paved the way for subsequent government legislation that was in line with the committee's recommendations.

Early Steps in PGD

With the aim of helping couples at risk of an X-linked disorder, the first approach to PGD was in order to sex the embryo. Handyside and colleagues at the Hammersmith Hospital in London reported in 1989 that they had been able to biopsy single cells from 30 embryos and that the expected proportion had developed to blastocysts after 6 days in culture [3]. Furthermore in all the normally fertilised embryos they were able to determine the sex by DNA amplification of a Y-chromosome-specific repetitive sequence. In 15 cases, the sex was confirmed by means of *in situ* hybridisation or Y chromosome fluorescence in metaphases. Shortly this was followed by the report from the same group of pregnancies from embryos sexed by Y-specific DNA amplification [4]. However, this approach proved to be error prone since crucially it relied on a negative result to identify the females. The development of the rapid and reliable technique of fluorescence *in situ* hybridisation (FISH) at the end of the 1980s proved a saviour and was quickly applied to biopsied cells from human embryos with excellent results [5]. The application of FISH for embryo sexing at UCL in London gave reliable diagnostic results but also gave the first indication of the frequency of aneuploidy and chromosomal mosaicism in these embryos created by *in vitro* fertilisation (IVF) from fertile patients [6]. Prior to this, the IVF specialists were looking forward to treating PGD patients who would be fertile, anticipating that IVF would have a much improved success rate compared with that for infertile couples. Simultaneously, FISH was being applied to biopsied cells from cleavage stage embryos by Munne's group in the USA and in 1993 they also reported the diagnosis of major aneuploidies in mosaic and full form [7]. Evidently, embryos created by IVF from couples of proven fertility were also prone to mosaic aneuploidy of an extent that was going to affect viability and implantation rates as well as the accuracy of PGD. So it was that an additional aim was added: as well as using PGD to detect heritable genetic disorders, it could be applied to help improve the success rate of IVF for infertile couples by using FISH to detect aneuploidy—this was the birth of PGS—preimplantation genetic screening.

Meanwhile, in 1992 the Hammersmith group reported the first successful PGD for a single gene disorder: the birth of a normal girl, free of cystic fibrosis, after PGD [8]. Within a few years, FISH was being applied in London to biopsied blastomeres to help couples at risk of passing on an unbalanced form of a reciprocal or Robertsonian translocation [9], and in the USA preconception diagnosis was achieved for maternal carriers by testing the first polar body alone while in Chicago it was tested in combination with karyotyping of the second polar body, also only for maternal carriers [10, 11]. While few would dispute on ethical grounds the application of PGD to avoid single gene defects that affect children, its use to avoid

passing on genes that predispose to late-onset disorders such as adult cancers provoked more controversy.

Nevertheless, the UK Human Fertilisation and Embryology Authority licensed the procedure in the case of the APC gene that causes familial adenomatous polyposis (and inevitable colorectal cancer) when mutated and the first PGD diagnosis for inherited cancer, and of this condition, was carried out in London and reported in 1998 [12].

The Development of Comprehensive Chromosomal Analysis

Initially, from 1999 onwards it was reported that the outcome for infertile couples improved significantly after PGS was applied to their embryos, compared with comparable control groups [13]. However, since FISH is perceived as an easy and reliable technique that any laboratory scientist may apply and achieve a successful outcome, it became widely used by IVF centres with no experience of genetic testing. Not surprisingly, the results for the patients were variable and doubts began to be expressed as to the benefits of PGS, mostly applied to older women with fewer embryos for testing. In 2007 a paper was published that reported on the outcome of a randomised clinical trial of PGS that showed a negative effect of screening via PGS [14]; this paper has been widely quoted but also heavily criticised on technical grounds by scientists with extensive experience of the application of FISH to human blastomeres. There are two contributory problems: one that in order to test as many chromosomes as possible, several rounds of hybridisation with FISH probes may be carried out; thus reducing the efficiency and secondly the widespread mosaicism that affects the early human embryo will clearly lead to apparent ‘misdiagnoses’ when testing only a single cell for aneuploidy. In the meantime, research was progressing on methods for comprehensive chromosome testing, based upon analysis of DNA extracted from a single cell. The aim was to be able to apply the technique of comparative genomic hybridisation (CGH) as used in tumour cytogenetics, where karyotyping was not possible. For this to happen, the DNA from each cell had first to be amplified in a manner compatible with analysis via CGH. Two groups from opposite sides of the world (London UK and Melbourne Australia) were successful in achieving single cell CGH analysis of blastomeres and simultaneously both groups published their work in the year 2000 [15, 16]. The results obtained confirmed the early FISH data with respect to the incidence of full and mosaic aneuploidy in apparently normally developing human embryos. These results were achieved by classical metaphase analysis after the combined hybridisation of both test and control DNAs; even with the help of computer software, that analysis required the ability to karyotype and took 72 h for the hybridisation step alone. Although both innovator groups did apply the technique clinically, these factors clearly limited full clinical application. The final step needed was the refinement of array CGH so that it could be applied to the analysis of single cells; early results from this development were described in 2009 [17]. By 2013 it was evident that