

DEBATE

Does blastocyst culture eliminate paternal chromosomal defects and select good embryos?

Inheritance of an abnormal paternal genome following ICSI

Subhasis Banerjee¹, Scott Lamond, Aidan McMahon, Stuart Campbell and Geeta Nargund

Centre for Reproductive Medicine, Department of Obstetrics & Gynaecology, St. George's Hospital Medical School, Cranmer Terrace, University of London, London SW17 ORE, UK

¹To whom correspondence should be addressed.

This debate was previously published on Webtrack, September 11, 2000

Following intracytoplasmic sperm injection (ICSI), ~60–70% of oocytes are fertilized and of these embryos, ~45% withstand in-vitro culture conditions to produce healthy blastocysts. The efficiency of implantation of 2–4-cell embryos selected at the pronuclear stage and that of blastocysts are comparable. However, prolonged selection of embryos *in vitro* (4–5 days), has been proposed to eliminate chromosomal abnormalities, more specifically those inherited by defective spermatozoa. This hypothesis is based upon the assumption that the paternal genetic contribution is indispensable for blastocyst development. Here we examine this hypothesis and suggest that phenotypic manifestation of paternal genomic abnormalities might not occur prior to implantation. In addition to the parent-of-origin effect during embryogenesis, blastocyst transfer may not prevent the inheritance of genetic defects involving 'male factor' loci.

Key words: abnormal paternal chromosomes/blastocyst culture/genomic imprinting/ICSI

Introduction

Intracytoplasmic sperm injection (ICSI) is an effective therapeutic intervention used worldwide to assist conception. The direct deposition of the spermatozoon into the oocyte circumvents the necessity of sperm–oocyte fusion. While ICSI continues to be a key therapeutic measure, the risks of transmitting damaged genes and abnormal chromosomes (Relief *et al.*, 1984; Sakkas *et al.*, 1996; In't Veld *et al.*, 1997; Twigg *et al.*, 1998a; Vegetti *et al.*, 2000) most commonly found in infertile spermatozoa, to the offspring are of major concern (Bonduelle *et al.*, 1998a,b; Bowen *et al.*, 1998). Numerous cytogenetic and fluorescent in-situ hybridization

(FISH) analyses including a recent report (Relief *et al.*, 1984; Martini *et al.*, 1997; Vegetti *et al.*, 2000) suggest that the chromosomal anomalies in higher age-group oocytes are far greater than those in spermatozoa of infertile patients. The assay systems employed in these studies are quite informative on numerical or structural aberrations of the chromosomes. However, minor but global chromosomal damage (DNA nicks, double-stranded breaks, cross-linking) or de-novo micro-deletions and point mutations, for example, those at 'male factor' loci would remain underestimated in these assays. The phenotypic consequences of these kinds of chromosomal damage are often undetected, probably due to the very efficient repair mechanisms operating in normal oocytes (Twigg *et al.*, 1998b). Whether this is true for high age-group oocytes is not known. In recent years, the possible adverse genetic consequences of ICSI have been fully recognized and in addition to genetic screening and counselling, other suggestions have been made in order to counter this problem (Behr, 1999; Edwards and Beard, 1999; Sakkas, 1999). The abiding principle of these suggestions is the selection of healthy embryos prior to uterine transfer. Selection could be at the pronuclear stage, where spatial distribution, polarity of the nuclei and organelles are critical parameters to be assessed (Scott and Smith, 1998), or at the blastocyst stage (Bavister and Boatman, 1997; Gardner and Lane, 1998; Tsirigotis, 1998; see Figure 1). While the final outcome (pregnancy to term) of selected 2–4-cell embryos and blastocysts transfers are comparable (Edwards and Beard, 1999), the latter is more desirable for two reasons: (i) the technology of cultivating human blastocysts without co-culture has improved considerably in the last 2–3 years (Gardner *et al.*, 1998; Behr *et al.*, 1999) and, more importantly; (ii) the development of blastocysts from the zygote, *in vitro*, underscores the successful inheritance of relatively undamaged paternal chromosomal complements following ICSI (Janny and Ménézo, 1994; Sakkas, 1999).

Human blastocyst culture is undoubtedly a great technological development, which will continue to facilitate our understanding of human early embryogenesis. Moreover, blastocysts have proved invaluable for preimplantation genetic diagnosis (Veiga *et al.*, 1997) and to a certain extent in avoiding multiple pregnancies following uterine transfer. However, as we discuss here, blastocyst transfer by no means guarantees preventing inheritance of abnormal paternal chromosomes after ICSI. This hypothesis is based on our current knowledge of molecular and genetic mechanisms involved in mammalian fertilization, and pre- and post-implantation development in mammals. We also discuss here how and when paternally inherited chromosomal abnormalities involving bi- or mono-allelically (imprinted) expressed genes and those in 'male

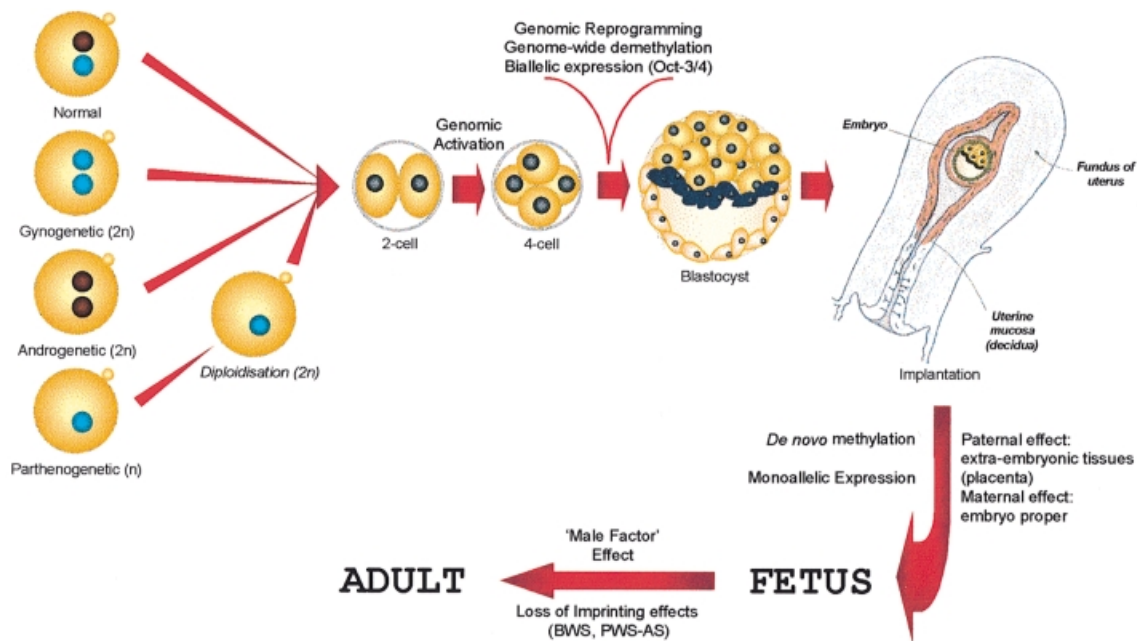


Figure 1. Parental genomic contributions in blastocyst formation and mammalian development.

factor' loci on the Y chromosome could possibly interfere with fertilization, early embryogenesis, fetal and post-natal development.

Transformation of the transcriptionally silent sperm head to a male pronucleus consists of a series of macromolecular events: sperm chromosome decondensation, release of protamines, DNA repair, chromosomal remodelling, assembly of organelles and a nuclear envelope around the reprogrammed haploid chromosomes (Collas, 1998; Collas and Poccia, 1998). All these events are accomplished by molecular chaperones, histones, non-histone structural proteins, DNA-repair enzymes and factors transiently accumulated in the ooplasm. Indeed, this maternal stockpile, in amphibians and flies, is so extensive that the zygote can replicate several thousand times independently of parental gene activation (Newport, 1987; Berrios and Avilion, 1990). In humans and higher mammals, successful male pronuclear assembly and fertilization are therefore largely determined by the quality of the oocyte cytoplasm (chromosome decondensation, DNA repair, demethylation and remodelling factors) rather than by the integrity of the paternal genome. Moreover, a direct correlation of sperm DNA damage with failed human fertilization remained debatable (Janny and Ménéz, 1994; Sakkas *et al.*, 1996; Hammadeh *et al.*, 1998; Twigg *et al.*, 1998b), possibly because the non-genetic factors contributed by the spermatozoa were not considered in these studies. Activation of endogenous metabolic pathways producing reactive oxygen species (ROS) is the major cause of human sperm DNA damage (Aitken *et al.*, 1998; Twigg *et al.*, 1998a). ROS, however, can simultaneously damage sperm membrane lipoproteins and the proximal centriole, which are essential for optimum chromosome decondensation and maternal chromosome separation prior to female pronuclear assembly respectively (unpublished data; Simerly *et al.*, 1995; Sathananthan *et al.*, 1996; Van Blerkom, 1996).

In order to evaluate the effect of sperm chromosomal abnormalities on pre-implantation development, it is imperative

to reconcile what specific contribution the parental genome could possibly have in blastocyst formation. This has been studied extensively in the mouse system where parthenogenetic, gynogenetic and androgenetic embryos can be readily created and cultivated (Barton *et al.*, 1984; McGrath and Solter, 1984; Surani *et al.*, 1986). These studies have revealed that parthenogenetically activated haploid oocytes (diploidized by preventing first cleavage division), diploid gynogenones (maternal chromosomes) and androgenones (paternal chromosomes), could all (except XX and YY androgenones because paternal X is inactivated in blastocysts by imprinting and lack of X-linked gene products, respectively) produce morphologically healthy blastocysts (see Figure 1). However, the efficiency of androgenetic blastocyst development was invariably poor compared with those embryos derived from maternal only (parthenogenones and gynogenones) and biparental (normal) genomic contributions. Nevertheless, analysis of the newly synthesized proteins as a measure of genomic activation (2–4-cell stage) revealed no qualitative differences between androgenones and parthenogenones or gynogenones. Therefore, development of the fertilized oocyte to the blastocyst stage is relatively independent of the parental genotype. Additionally, the maternal genome is more competent for embryonic activation and blastocyst development compared to its paternal counterparts. This is important given the high frequency (25–50%) of aneuploidy in human oocytes (Martini *et al.*, 1997). Failure of blastocyst development from 2–4-cell embryos following ICSI most possibly arises from inadequate genomic reprogramming (for instance genome-wide demethylation) necessary for subsequent gene activation, cleavage and cell determination (Walsh and Bestor, 1999). This is a function of the oocyte cytoplasm as amply evidenced in animal nuclear (for a review, see Kikyo and Wolffe, 2000) and human cytoplasmic (Cohen *et al.*, 1998) transfer studies.

The consequences of inheriting abnormal paternal chromosomes are most likely to be manifested during implantation

Table I. Developmentally regulated human imprinted loci/genes (for a detailed catalogue of other human imprinted genes see <http://cancer.otago.ac.nz>; for a complete list of imprinted mouse genes and chromosomal map see <http://www.mgu.har.mrc.ac.uk>)

C/S locus ^a	Imprinted genes	Imprinting ^b	Parent-of-origin effect on development and diseases
1p31	<i>NOEY2, ARIII</i>	Maternal	A ras-related growth regulator in ovarian and breast cancers
1p36.3	<i>P73</i>	Paternal	Fetal pancreas and thymus; LOH in neuroblastomas
6q22-23	<i>UPD</i>	Maternal	Agnesis of pancreatic β cells and neonatal diabetes mellitus
6q24	<i>ZAC/PLAGL1</i>	Maternal	Transient neonatal diabetes
6q25-27	<i>IDDM8</i>	Maternal	Type 1 diabetes
7q11.23	Unknown	Paternal	Severe growth retardation and microcephaly
7q32	<i>MEST, PEG1</i>	Maternal	Mesoderm-specific transcript, member of α/β hydroxylase fold family, expressed in fetal tissues
11p15	<i>ZNF214, ZNF215</i>	Paternal	Zinc-finger proteins within BWS/CR2 region
11p15.5	<i>IGF-II</i>	Maternal	Fetal growth factor co-regulated by H19 locus; prenatal overgrowth, embryonal malignancies and paediatric disorder, hemihypertrophy, macroglossia, Beckwith–Weidmann syndrome (BWS)
	<i>H19</i>	Paternal	Co-ordinate regulation of IGF-II, tumour suppression
	<i>ASCL2, HASH2</i>	Paternal	Helix–loop–helix transcription factor, expressed in extravillous trophoblasts
	<i>KvLQT1</i>	Paternal	A potassium channel protein involved in long QT Syndrome
	<i>IPL, TSSC3, BWR1C</i>	Paternal	Imprinted expression in placenta and fetal liver, involved in apoptosis
14	<i>UPD</i>	Maternal	Hypotonia, motor development delay, dysmorphic facial features, low birth weight, growth abnormalities and precocious puberty
		Paternal	Severe mental and musculo–skeletal disorders
15q11-13	<i>ZNF127</i>	Maternal	Zinc-finger motif
	<i>NDN, Necdin</i>	Maternal	Expressed in brain, differentiated neurons and fibroblasts
	<i>SNRPN, SNRUF</i>	Maternal	Two proteins encoded by a bicistronic transcript, pathogenesis of Prader–Willi syndrome (PWS)
	<i>PARI, PAR5, PAR-SN, IPW</i>	Maternal	Untranslated transcripts, involved in PWS
	<i>UBE3A, E6-AP</i>	Paternal	Ubiquitin protein ligase, expressed monoallelically in brain; involved in Angelman syndrome (AS)
	'Autism'	Maternal	Social adjustment problem
	<i>MAGEL2</i>	Maternal	Exclusive monoallelic expression in brain and fibroblasts
20q13.11	<i>GNAS1</i>	Maternal	Guanine nucleotide binding protein, endocrine disturbance, pseudohypoparathyroidism type 1a
	<i>NESP55</i>	Paternal	Neuroendocrine secretory granule protein in Golgi membrane
	<i>XL alphas</i>	Maternal	Large G protein in Golgi network of neuroendocrine cells, formation of secretory vesicles
20q13.3	<i>PHP-1b</i>	Maternal	Pseudohypoparathyroidism type 1b
X	Maternal UPD	Unknown	Clinical stigmata
	Paternal UPD	Unknown	Impaired gonadal function and short stature
Xp11.23	<i>Qter</i>	Maternal	Turner syndrome: a neurodevelopmental disorder, poor secondary sexual characteristics
Xq13.2	<i>XIST</i>	Maternal	Silencing of the paternal X in trophoblastic cells

UPD = uniparental disomy; LOH = loss of heterozygosity; IGF = insulin-like growth factor.

^aChromosomal locus.

^bTranscriptionally silent allele.

and post-implantation development. Two lines of experimental evidence support this notion. Firstly, the Harwell group (Mammalian Genetics Unit, MRC, Oxford, UK) made extensive use of mouse mutants, carrying Robertsonian and reciprocal translocations, by creating animals uniparentally disomic for specific regions of chromosomes (Pat.Dp or Mat.Dp). These studies showed that parental genes in at least 15 different regions (each region spanning several megabases of DNA) of 10 chromosomes (nos. 2, 6, 7, 9, 11, 12, 14, 17, 18 and 19) were functionally heterozygous (Cattanach and Beechey, 1998). Uniparental duplication of these chromosomal regions, with paternal or maternal deficiency, leads to a variety of developmental disorders including early or late embryonic lethality, overgrowth/reduced placenta, pre-, post-natal and fetal growth retardation, neurological abnormalities, etc (Beechey and Cattanach, 2000). Secondly, nuclear transplantation studies led to the discovery of non-Mendelian functional inheritance (epigenesis) of parental chromosomes. Such parentally inherited epigenetic programs dramatically influence embryogenesis after implantation. In general, paternal and maternal genomes contribute to the proliferation of extra-embryonic tissues and embryo proper, respectively (McGrath and Solter, 1984; Surani *et al.*, 1986).

The functional inequality of haploid genomes is due to

imprinting of specific chromosomal loci/genes during gametogenesis when the parental alleles are physically separated (Tucker *et al.*, 1996; Banerjee and Smallwood, 1998; Surani, 1998; Tilghman, 1999). The imprints are established in the gamete, passed on to the zygote and must withstand embryonic activation (2–4-cell stage) for the successful development of the embryo to term (Surani *et al.*, 1986). Unlike the mouse, data on the developmental consequences due to loss of imprinting (LOI) or loss of heterozygosity (LOH) in human are far less comprehensive (see Table I; Ledbetter and Engel, 1995). The most extensively studied chromosomes are 11 (11p15) and 15 (15q11-13) where duplication, translocation or deletion of these regions led to paediatric disorders, e.g. Beckwith–Wiedemann and Prader–Willi/Angelman syndromes. The functional imprints of genes located at human chromosome 11p15.5 (*IGF2*, *H19*, *p57^{kip2}* and *KvLQT* genes) or those controlled by imprinting centres at 15q11-13, are generally established following implantation of the embryo (Latham *et al.*, 1993; Szabo and Mann, 1995; Walsh and Bestor, 1999). Paternal chromosomal damage, or failure to maintain the imprinting markings during early activation, could lead to aberrant development of the embryo. For example, paternal or maternal duplication of the *Igf-II/H19* genes located at the distal end of mouse chromosome 7

(dist.7) and simultaneous maternal or paternal deficiencies, respectively, severely affects embryo development. Embryos paternally disomic for the distal 7 (Pat. Dp.Dist.7) die within 7–10 days of gestation, whereas, those maternally disomic for *Igf-II/H19* region (Mat.Dp.Dist. 7) have retarded growth and die at late gestation or immediately after birth (Sasaki *et al.*, 1992; Cattanach and Beechey, 1998; Banerjee *et al.*, 2000).

Blastocyst transfer would almost certainly fail to prevent inheritance of paternal chromosomal abnormalities known to affect the fertility of the male offspring following ICSI. The chromosomal abnormalities in human male infertility include aneuploidy (47,XXY and 47, XXY, Klinefelter's syndrome), X- or Y-autosome translocations and deletions of the Y chromosome. Analysis of micro-deletions in azoospermia/oligospermia using sequence-tagged sites (STSs) resulted in the identification of three loci (AZFa, AZFb and AZFc) containing a number of 'male factor' genes (Kostiner *et al.*, 1998). The earliest expression of candidate genes at these loci (RNA binding motif, deubiquinating enzymes) are likely to be at mid-development in primordial germ cells in the genital ridge, destined to proliferate in the gonads (Ruggiu *et al.*, 1997). It should be noted, however, that genetic defects in 'male factor' loci or Klinefelter's syndrome can be diagnosed by multiplex PCR analysis of sperm DNA (Vogt, 1998) or FISH of preimplantation embryos (Reubinoff *et al.*, 1998) respectively.

Finally, one might ask if there is any possibility of abnormal paternal chromosomes interfering with the development of healthy blastocysts. There are scenarios where this possibility could exist. As mentioned above, rapid cleavage and cell determination in preimplantation embryos are entirely reliant upon embryonic gene activation. The activation of housekeeping genes (polymerases, cell-cycle kinases and phosphatases, etc) and Oct-3/4 gene products, critical for maintaining pluripotency of the embryo and determination of cell fate in blastocysts (Niwa *et al.*, 2000), could occur from either of the two alleles. Maternal loss of function of any of these genes, resulting from high frequency of aneuploidy in human oocytes (Martini *et al.*, 1997), could be lethal in the absence of functional complementation by an activated paternal allele. Under such circumstances, inheritance of abnormal paternal chromosomal complements would lead to degeneration of 2–4-cell embryos. Genetic rescue of this type would however fail to prevent inheritance of defective parental alleles.

To summarize, blastocyst development reflects the macro-molecular and enzymatic competence of the oocyte cytoplasm and is relatively independent of paternal genomic effect. The simplest way to overcome the possible adverse genetic consequences of abnormal paternal chromosomes in assisted reproduction, would be either minimizing sperm DNA damage or repairing damage prior to ICSI. This, however, is a daunting task because: (i) we do not know to what extent the structural genome (centromeric and non-centromeric heterochromatin) and protein coding genes are mutated in damaged spermatozoa; and (ii) the technology of repairing a damaged haploid genome is almost non-existent. The recent development of cytoplasmic transfer and oocyte fusion methods (Cohen *et al.*, 1998; Tesarik *et al.*, 2000), raised the possibility of developing in-vitro sperm

DNA repair systems and delivering the remodelled sperm nucleus to the oocytes.

References

- Aitken, R.J., Gordon, E., Harkiss, D. *et al.* (1998) Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. *Biol. Reprod.*, **59**, 1037–1046.
- Banerjee, S. and Smallwood, A. (1998) Chromatin modification of the imprinted *H19* gene in mammalian spermatozoa. *Mol. Reprod. Dev.*, **50**, 474–484.
- Banerjee, S., Singh, P.B., Rasberry, C. *et al.* (2000) Embryonic inheritance of the chromatin organisation of the imprinted *H19* domain in mouse spermatozoa. *Mech. Dev.*, **90**, 217–226.
- Barton, S.C., Surani, M.A.H. and Norris, M.L. (1984) Role of paternal and maternal genomes in mouse development. *Nature*, **311**, 374–376.
- Bavister, B.D. and Boatman, D.E. (1997) The neglected human blastocyst revisited. *Hum. Reprod.*, **12**, 1607–1610.
- Beechey, C.V. and Cattanach, B.M. (2000) Genetic and physical imprinting map of the mouse. Mammalian Genetics Unit, Harwell, UK (<http://www.mgu.har.mrc.ac.uk>).
- Behr, B. (1999) Blastocyst culture and transfer. *Hum. Reprod.*, **14**, 5–6.
- Behr, B., Pool, T.B., Milki, A.A. *et al.* (1999) Clinical experience with human blastocyst development *in vitro* without co-culture. *Hum. Reprod.*, **14**, 454–457.
- Berrios, M. and Avilion, A.A. (1990) Nuclear formation in a *Drosophila* cell-free system. *Exp. Cell Res.*, **191**, 64–70.
- Bonduelle, M., Aytoz, A., Van Assche, E. *et al.* (1998a) Incidence of chromosomal aberrations in children born after assisted reproduction through intracytoplasmic sperm injection. [Editorial.] *Hum. Reprod.*, **13**, 781–782.
- Bondelle, M., Joris, H., Hofmans, K. *et al.* (1998b) Mental development of 201 ICSI children at 2 years of age. *Lancet*, **351**, 1553.
- Bowen, J.R., Gibson, F.L., Leslie, G.I. *et al.* (1998). Mental and developmental outcome at 1 year for children conceived by intracytoplasmic sperm injection. *Lancet*, **351**, 1529–1534.
- Cattanach, B.M., Beechey, C.V. (1998) Genomic imprinting in the mouse: possible final analysis. In Reik, W. and Surani, A. (eds), *Genomic Imprinting: Frontiers in Molecular Biology*. Vol. 18. IRL Press, Oxford, UK, pp. 118–145.
- Cohen, J., Scott, R., Alikani, M. *et al.* (1998) Ooplasmic transfer in mature human oocytes. *Mol. Hum. Reprod.*, **4**, 269–280.
- Collas, P. (1998) Cytoplasmic control of nuclear assembly. *Reprod. Fertil. Dev.*, **10**, 581–592.
- Collas, P. and Poccia, D. (1998) Remodelling the sperm nucleus into a male pronucleus at fertilisation. *Theriogenology*, **49**, 67–81.
- Edwards, R.G. and Beard, H.K. (1999) Is the success of human IVF more a matter of genetics and evolution than growing blastocysts? *Hum. Reprod.*, **14**, 1–6.
- Gardner, D.K. and Lane, M. (1998) Culture of viable human blastocysts in defined sequential serum-free media. *Hum. Reprod.*, **13** (Suppl. 3), 148–159.
- Gardner, D.K., Vella, P., Lane, M. *et al.* (1998) Culture and transfer of human blastocysts increases implantation rates and reduces the need for multiple embryo transfers. *Fertil. Steril.*, **69**, 84–88.
- Hammadeh, M.E., Al-Hassani, S., Doerr, S. *et al.* (1998) Comparison between chromatin condensation and morphology from testis biopsy extracted and ejaculated spermatozoa and their relationship to ICSI outcome. *Hum. Reprod.*, **14**, 263–267.
- In't Veld, P.A., Broekmans, J.M., de France, H.F. *et al.* (1997) Intracytoplasmic sperm injection (ICSI) and chromosomally abnormal spermatozoa. *Hum. Reprod.*, **12**, 752–754.
- Janny, L. and Ménéz, Y.J.R. (1994) Evidence for a strong paternal effect on human preimplantation embryo development and blastocyst formation. *Mol. Reprod. Dev.*, **38**, 36–42.
- Kikyo, N. and Wolffe, A.P. (2000) Reprogramming nuclei: insights from cloning, nuclear transfer and heterokaryons. *J. Cell Sci.*, **113**, 1–20.
- Kostiner, D.R., Turek, P.J. and Reijo, R.A. (1998) Male infertility: analysis of the markers and genes on the human Y chromosome. *Hum. Reprod.*, **11**, 3032–3038.
- Latham, K.E., Doherty, A.S., Scott, C.D. *et al.* (1993) *Igf2r* and *Igf2* gene expression in androgenetic, gynogenetic and parthenogenetic preimplantation mouse embryos: absence of regulation by genomic imprinting. *Genes Dev.*, **8**, 290–299.

- Ledbetter, D.H. and Engel, E. (1995) Uniparental disomy in humans: development of an imprinting map and its implications for prenatal diagnosis. *Hum. Mol. Genet.*, **4**, 1757–1764.
- Martini, E., Flaherty, S.P., Swann, J. *et al.* (1997) Analysis of unfertilised oocytes subjected to intracytoplasmic sperm injection using two rounds of fluorescence in-situ hybridization and probes to five chromosomes. *Hum. Reprod.*, **12**, 2011–2018.
- McGrath, J. and Solter, D. (1984) Completion of mouse embryogenesis requires both maternal and paternal genomes. *Cell*, **37**, 179–183.
- Newport, J. (1987) Nuclear reconstitution *in vitro*: Stages of assembly around protein-free DNA. *Cell*, **48**, 205–217.
- Niwa, H., Miyazaki, J.-I. and Smith, A.G. (2000) Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. *Nature Genet.*, **24**, 372–376.
- Relief, A.E., van Zijl, J.A., Merkveld, R. *et al.* (1984) Chromosome studies in 496 infertile males with a sperm count below 10 million/ml. *Hum. Genet.*, **66**, 162–164.
- Reubinoff, B.E., Abeliovich, D., Werner, M. *et al.* (1998) A birth in non-mosaic Klinefelter's syndrome after testicular fine needle aspiration, intracytoplasmic sperm injection and preimplantation genetic diagnosis. *Hum. Reprod.*, **13**, 1887–1892.
- Ruggiu, M., Speed, R., Taggart, M. *et al.* (1997) The mouse *Dazl* gene encodes a cytoplasmic protein essential for gametogenesis. *Nature*, **389**, 73–77.
- Sakkas, D. (1999) The use of blastocyst culture to avoid inheritance of an abnormal paternal genome after ICSI. *Hum. Reprod.*, **14**, 4–5.
- Sakkas, D., Urner, F., Bianchi, P.G. *et al.* (1996) Sperm chromatin anomalies can influence decondensation after intracytoplasmic sperm injection. *Hum. Reprod.*, **11**, 837–843.
- Sasaki, H., Jones, P.A., Chaillet, R. *et al.* (1992) Parental imprinting: Potentially active chromatin of the repressed maternal allele of the mouse insulin-like growth factor II (*Igf2*) gene. *Genes Dev.*, **6**, 1843–1856.
- Sathananthan, A.H., Ratnam, S.C., Ng, S.C. *et al.* (1996) The sperm centriole: its inheritance, replication and perpetuation in early human embryos. *Hum. Reprod.*, **11**, 345–356.
- Scott, L.A. and Smith, S. (1998) The successful use of pronuclear embryo transfers the day following oocyte retrieval. *Hum. Reprod.*, **13**, 1003–1013.
- Simerly, C., Wu, G.J., Zoran, S. *et al.* (1995) The paternal inheritance of the centrosome, the cell's microtubule-organizing center, in humans and the implications for infertility. *Nature Med.*, **1**, 47–52.
- Surani, M.A.H. (1998) Imprinting and the initiation of gene silencing in the germ line. *Cell*, **93**, 309–312.
- Surani, M.A.H., Barton, S.C. and Norris, M.L. (1984) Development of mouse eggs suggests imprinting of the genome during gametogenesis. *Nature*, **308**, 548–550.
- Surani, M.A.H., Barton, S.C. and Norris, M.L. (1986) Nuclear transplantation in the mouse: heritable differences between parental genomes after activation of the embryonic genome. *Cell*, **45**, 127–136.
- Szabo, P.E. and Mann, J.R. (1995) Biallelic expression of imprinted genes in the mouse germ line: implications in erasure, establishment, and mechanisms of genomic imprinting. *Genes Dev.*, **9**, 1857–1868.
- Tesarik, J., Nagy, J.P., Mendoza, C. *et al.* (2000) Chemically and mechanically induced membrane fusion: non-activating methods for nuclear transfer in mature human oocytes. *Hum. Reprod.*, **15**, 1149–1154.
- Tilghman, S.M. (1999) The sins of the fathers and the mothers: Genomic imprinting in mammalian development. *Cell*, **96**, 185–193.
- Tsirigotis, M. (1998) Blastocyst stage transfer: pitfalls and benefits. *Hum. Reprod.*, **13**, 3285–3289.
- Tucker, K.L., Beard, C., Dausman, J. *et al.* (1996) Germ-line passage is required for establishment of methylation and expression patterns of imprinted but not of nonimprinted genes. *Genes Dev.*, **10**, 1008–1020.
- Twigg, J., Fulton, N., Gomez, E. *et al.* (1998a) Analysis of the impact of intracellular reactive oxygen species generation on the structural and functional integrity of human spermatozoa: lipid peroxidation, DNA fragmentation and effectiveness of antioxidants. *Hum. Reprod.*, **13**, 429–436.
- Twigg, J.P., Irvine, D.S. and Aitkin, R.J. (1998b) Oxidative damage to DNA in human spermatozoa does not preclude pronucleus formation at intracytoplasmic sperm injection. *Hum. Reprod.*, **13**, 1864–1871.
- Van Blerkom, J. (1996) Sperm chromosome dysfunction: a possible new class of male factor infertility in the human. *Mol. Hum. Reprod.*, **2**, 349–354.
- Vegetti, W., van Assche, R., Frias, A. *et al.* (2000) Correlation between semen parameters and sperm aneuploidy rates investigated by fluorescence in-situ hybridization in infertile men. *Hum. Reprod.*, **15**, 351–365.
- Veiga, A., Sandalinas, M., Benkhalifa, M. *et al.* (1997) Laser blastocyst biopsy for preimplantation diagnosis in the human. *Zygote*, **5**, 351–354.
- Vogt, P.H. (1998) Human chromosome deletion in Yq11, AZF candidate genes and male infertility: history and update. *Mol. Hum. Reprod.*, **4**, 739–744.
- Walsh, C.P. and Bestor, T.H. (1999) Cytosine methylation and mammalian development. *Genes Dev.*, **13**, 26–34.