DEBATE

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

Inheritance of an abnormal paternal genome following ICSI

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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.

Keywords: 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., 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., 1998b; 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
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 Ménezo, 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.
ally inherited epigenetic programs dramatically in inheritance (epigenesis) of parental chromosomes. Such parentination studies led to the discovery of non-Mendelian functional (Beechey and Cattanach, 2000). Secondly, nuclear transplanta-fetal growth retardation, neurological abnormalities, etc lethality, overgrowth/reduced placenta, pre-, post-natal and of developmental disorders including early or late embryonic regions, with paternal or maternal de

Beechey, 1998). Uniparental duplication of these chromosomal 18 and 19) were functionally heterozygous (Cattanach and

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 paren-tally 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, p57kip2 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 paternally 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 inheritance of paternal chromosomal abnormalities known to be at mid-development in primordial germ cells in the genital (RNA binding motif, deubiquinating enzymes) are likely to be relevant to 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 cytosplasmic 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


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