

CHROMOSOMALLY ABNORMAL HUMAN EMBRYOS

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Extensive studies over the last 15 years have confirmed that chromosomal errors and aneuploidies are extremely common in early human embryos. Embryonic aneuploidies may be inherited from an aneuploid oocyte or sperm or arise *de novo* after fertilisation. The advent of in vitro fertilization (IVF) as a treatment for infertility has meant that some surplus early human embryos have been used to study the extent and frequency of chromosomal abnormalities that exist at, or soon after, conception. This has led to the application of aneuploidy testing using preimplantation genetic diagnosis (PGD) techniques in IVF programs to identify embryos that are predicted to be free of errors of certain chromosomes. In this presentation I will review the current state of knowledge of the incidence of chromosomal errors, excluding inherited rearrangements and translocations, in early human embryos.

Much of the early information about the frequency of aneuploidy in embryos was gleaned from studies using fluorescent in situ hybridization (FISH). FISH enables the simultaneous enumeration of 8 or 9 chromosomes and is limited primarily by the number of fluorochromes available to label the probes. The chromosomes commonly analysed using FISH include X, Y, 13, 16, 18, 21 and 22. Such studies showed that more than 50% of human blastomeres contained a chromosomal error. Some of the most interesting information was revealed when multiple cells from individual embryos were analysed. This demonstrated that embryos contained significant chromosomal mosaicism in that different cells within embryos had a different chromosomal constitution. Sometimes all cells had different aneuploidies and in other embryos there was a mixture of euploid and aneuploid cells.

It is difficult to get a complete picture of the extent of aneuploidy in human embryos when only a small number of chromosomes can be analysed. To rectify this we have developed the molecular karyotyping technique of comparative genomic hybridization (CGH) to enable the assessment of all chromosomes in single human blastomeres. CGH demonstrated that errors of every chromosome exist in human blastomeres and many of these occur at much higher frequencies than might have been expected from analysis of later stage conceptuses. This has exposed the current limitations of PGD for aneuploidy using FISH as many embryos are being diagnosed as euploid when in fact they harbour aneuploidies of chromosomes that weren't tested.

CGH analysis of human blastomeres revealed that about 15% of these cells had partial aneuploidy and were missing or had an excess of large segments of chromosomes. Presumably this occurred when there had been a chromosomal break in a previous cell division.

Chromosomal aneuploidies that are consistent in every cell from an individual embryo have probably arisen from a meiotic error in an oocyte, or less frequently a spermatozoon. Aneuploidies that are present in some, but not all, cells must arise after fertilisation during the early cleavage divisions of the embryo. There is some evidence that women with certain types of infertility, such as recurrent implantation failure are more likely to produce embryos with post-zygotic mosaicism and older women are more likely to have meiotic errors in their embryos (Voullaire et al., 2007). The exact causes of these chromosomal errors in embryos are unknown but may involve defective cell cycle checkpoints which usually prevent abnormal cells from continuing cell division.

Almost all of the human embryos that have been analysed for chromosomal constitution have come from couples with fertility problems and it is possible that the information obtained is not representative of the general population. Some patients who are fertile access IVF in order to have PGD for monogenic disease. A small number of embryos affected with the disease have been analysed for chromosomal errors and found to have meiotic aneuploidies and chromosomal mosaicism at frequencies similar to those from infertility patients. However to obtain these embryos the women have been exposed to hormones used to produce multiple ovarian follicles. It is possible that this hormonal stimulation may induce some aneuploidies and a recent study (Baart et al., 2007) has suggested that using minimal stimulation can result in lower frequencies of embryonic aneuploidy. The only way to unequivocally determine the impact of the IVF process and infertility on chromosome errors in embryos would be to study *in vivo* derived embryos from fertile women. Clearly these studies cannot be done.

Although traditional CGH studies have been extremely valuable in identifying chromosomal abnormalities in human embryos, the technique is laborious and time-consuming. For this reason it has not been extensively used for clinical PGD (Wilton et al., 2003). A number of workers are now applying array-CGH to single cells from human embryos. This should enable a much larger number of embryos from patients with different types of infertility to be examined and should greatly increase our understanding of the frequency and impact of chromosomal aberrations on early human development.

References: Baart EB, Martini E, Eijkemans MJ, Van Opstal D, Beckers NG, Verhoeff A, Macklon NS, Fauser BC. *Hum Reprod.* 2007 Apr;22(4):980-8. Epub 2007 Jan 4. Voullaire L, Collins V, Callaghan T, McBain J, Williamson R, Wilton L. *Fertil Steril.* 2007 May;87(5):1053-8. Epub 2007 Apr 6. Wilton L, Voullaire L, Sargeant P, Williamson R, McBain J. *Fertil Steril.* 2003 Oct;80(4):860-8.