Diploid-aneuploid mosaicism in human embryos cultured to the blastocyst stage

Magdalena Bielanska, Ph.D.,[a,b] *Shaoguang Jin, Ph.D.,*[b] *Martin Bernier, M.D.,*[b] *Seang Lin Tan, M.D.,*[a,b] *and Asangla Ao, Ph.D.*[b,c]

[a] McGill Reproductive Centre, [b] Departments of Obstetrics and Gynecology, and [c] Human Genetics, Royal Victoria Hospital, McGill University, Montreal, Quebec, Canada

Objective: To examine diploid-aneuploid mosaicism in human in vitro cultured blastocysts.
Design: A laboratory study on spare blastocysts from an IVF program.
Setting: University hospital laboratory.
Patients(s): Forty-three couples undergoing IVF or intracytoplasmic sperm injection.
Intervention(s): Ninety-one blastocysts were spread for fluorescence in situ hybridization using the HCl–Tween 20 method. A total of 6,664 nuclei were analyzed for aneuploidy using fluorescent DNA probes specific to chromosomes 2, 7, and 18.
Main Outcome Measure(s): The proportion of aneuploid cells within each blastocyst.
Results(s): The incidence of diploid-aneuploid mosaicism among 91 blastocysts examined was 17.6%. All of the mosaic blastocysts were abnormal for only one of the three chromosomes tested, with the incidence of involvement of chromosomes 2, 7, and 18 being 3.3%, 8.8%, and 5.5%, respectively. The majority of the mosaic blastocysts had low proportions of aneuploid cells. Ten of the 16 (62.5%) affected blastocysts were of morphology compatible with implantation.
Conclusion(s): A considerable proportion of human IVF blastocysts show a form of mosaicism that has been observed in fetal and placental tissues. This mosaicism often arises at the final stage of preimplantation development in vitro and is present in blastocysts of morphology compatible with implantation. (Fertil Steril® 2005;84:336–42. ©2005 by American Society for Reproductive Medicine.)

Key Words: Aneuploidy, blastocysts, chromosomes, confined placental mosaicism, fluorescence in situ hybridization, mosaicism

Chromosomal mosaicism is the coexistence of karyotypically distinct cell lineages derived from a single zygote. This condition may result either from the development of a chromosomal abnormality within a diploid embryo or from the correction of a proportion of abnormal cells to diploidy in an originally chromosomally abnormal embryo. The most common form of chromosomal mosaicism observed in human recognized pregnancies and live borns involves diploid cells and cells showing aneuploidy for a gonosome or one of the 22 autosomes.

Diploid-aneuploid mosaicism has been detected in approximately 5% of trisomic or monosomic spontaneous abortions (1). Mosaicism, most often involving a diploid and an aneuploid cell line has been found in approximately 0.3% of viable pregnancies ascertained by amniocentesis (2, 3). It is also diagnosed in up to 2% of pregnancies screened by chorionic villi sampling (CVS) (4, 5). The majority of these pregnancies show both cell lineages in the placenta but only the diploid cells in the fetus, a form of mosaicism referred to as confined placental mosaicism (CPM) (6).

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Reprint requests: Asangla Ao, Ph.D., Departments of Obstetrics and Gynecology, Royal Victoria Hospital, McGill University, 687 Pine Avenue West, F3.16, Montreal, Quebec H3A 1A1, Canada (FAX: 514-843-1662; E-mail: asangla.ao@muhc.mcgill.ca).

The clinical outcome of an aneuploid mosaic pregnancy depends on the chromosome involved and on the proportion, distribution, and survival of the abnormal cells within the embryonic and the extraembryonic tissues and organs (7–11). For most chromosomes, generalized mosaicism affecting the fetus and the placenta usually results in severe malformations and fetal demise (9, 11). Mosaicism confined to placental tissues is more viable. However, CPM may also lead to serious consequences, including perinatal mortality, premature labor, and very often intrauterine growth retardation (IUGR) (12, 13). An abnormal outcome is more likely when high levels of aneuploidy persist to term in the placenta. Confined placental mosaicism originating from a reduction of trisomy to disomy during embryonic development carries a risk of uniparental disomy (UPD) in the fetus, which may lead to an abnormal phenotype through homozygosity for recessive genes or parent specific imprinting (14).

In vitro fertilization has become a well established treatment for male and female infertility. The availability of the nontransferred, or "spare," embryos for research has enabled the study of chromosomes at the earliest stages of embryonic development. Multiple fluorescence in situ hybridization (FISH) studies have concurred that the incidence of the mosaic karyotype in the IVF day 2–3 embryos is very high, affecting 15% to over 50% of embryos analyzed (15–19).

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The mosaic forms detected in cleavage stage embryos are much more diverse than those detected parentally. The most frequent forms of mosaicism observed in the embryos comprised of diploid cells and cells with gains and/or losses for either one or for multiple chromosomes tested. An analysis of mosaicism at all stages of preimplantation development showed that imbalances for multiple chromosomes (diploid-chaotic mosaicism) are associated with embryonic arrest before the blastocyst stage and are therefore not compatible with pregnancy. However mosaicism involving a trisomy and/or monosomy for only one of the chromosomes analyzed (diploid-aneuploid mosaicism) was not limited to early development and was also found among blastocysts. This form of mosaicism has also been reported in blastocysts examined in several other studies (20–22).

To determine whether diploid-aneuploid mosaicism in IVF blastocysts is clinically relevant and related to the form of mosaicism reported parentally, this study investigated aneuploid mosaicism in 91 blastocysts using FISH probes for chromosomes 2, 7, and 18. Chromosomes 2 and 7 were chosen for the study because, although they are the two autosomes most frequently involved in mosaicism detected by CVS (4, 7), their involvement in mosaicism at the blastocyst stage has not yet been examined. Chromosome 18 was used for two reasons. First it was selected as a third probe of high hybridization efficiency which would help to distinguish aneuploid mosaicism from chaotic abnormalities involving multiple chromosomes. The second reason for choosing chromosome 18 was that its contribution to mosaicism among viable pregnancies screened by CVS is lower than that of chromosomes 2 and 7; therefore, it was of interest to determine whether there were differences in the relative rates of mitotic errors for the above three chromosomes at the last stage of preimplantation development in vitro.

MATERIALS AND METHODS

Embryos

Embryos supernumerary to those transferred to the uterus were donated with written consent by patients undergoing IVF or intracytoplasmic sperm injection (ICSI) at the McGill Reproductive Centre. The study was approved by the research ethics board of McGill University Hospital Centre, Royal Victoria Hospital. The mean maternal age of patients was 36.6 years (range, 32 to 42 years). All embryos included in the study had been normally fertilized (2PN). Embryos were cultured in G1 and G2 sequential media under oil (IVF Science Scandinavia, Gothenburg, Sweden) to day 5 or day 6 postinsemination in humidified incubator with 5% CO_2, atmospheric oxygen, at 37°C. Fertilized oocytes were cultured individually in 20 μL drops of G1 medium. On day 3, cleavage stage embryos were transferred to fresh 40 μL drops of G2 medium.

Embryos showing a blastocoel cavity were classified as blastocysts and were categorized into two groups and scored according to Gardener and Schoolcraft, 1999 (23): "Good" quality blastocysts with expanded cavity, distinct inner cell mass (ICM), and trophectoderm (TE) layer were scored as >3 BB, and "poor" quality blastocysts with poorly developed cavity, less defined or no apparent ICM, presence of dark cells, and fragmentation were scored as <3 BB.

Embryo Spreading and Fixation

Blastocysts were washed in phosphate-buffered saline (PBS) and transferred onto poly-L-lysine coated glass slides. They were dissolved in 0.01 N HCl, 0.1% Tween 20 solution and the nuclei spread and fixed on the slides (24). The preparations were air dried, washed in PBS for 5 min, and dehydrated in 50%, 70%, and 100% ethanol. Fixed nuclei were viewed using a phase contrast microscope and their location marked with a diamond pen. Embryonic nuclei were pretreated with pepsin (0.1 mg/mL in 0.01 N HCl) for 10 min at 37°C and again dehydrated.

FISH

Probes used for the study were commercially available from Vysis (Downers Grove, IL). The probes had been tested on normal male lymphocytes and had been successfully used for hybridization in embryonic cells (18, 19, 25). A 3-color hybridization mixture specific to chromosomes 2, 7, and 18 was prepared by combining individual probes for chromosome 2 (CEP 2, alpha satellite in spectrum orange), chromosome 7 (CEP 7, alpha satellite in spectrum green), and chromosome 18 (CEP 18 alpha satellite in spectrum aqua or in spectrum blue) in hybridization buffer (50% formamide; Oncor, Gaithersburg, MD) in a ratio of 2:1:1. The nuclei and probe mixture were combined on the glass slide and co-denatured by heating to 76°C for 6 min. Hybridization was carried out in a moist chamber overnight at 37°C. The unbound probe was washed off using 0.4× SSC–0.3% Tween 20, solution at 73°C, for 2 min, followed by rinsing in 2× SSC–0.1% Tween 20 at room temperature. Preparations were mounted in antifade solution (p-phenylenediamide dihydrochloride in PBS; Vector, Burlingame, CA) containing DAPI (Sigma, Oakville, Canada) nuclear stain (0.25 ng/mL).

Signal analysis. Nuclei were viewed using a fluorescence Olympus DX 60 microscope equipped with a filter wheel containing filters for red and green emission (Applied Imaging, Santa Clara, CA), and a single filter for aqua emission (Vysis). Images were captured using a CCD camera and Cytovision software (Applied Imaging). Only intact undamaged nuclei were scored. Two signals of the same color, separated by a distance of less than one signal domain, were scored as a split signal from a single chromosome. Nuclei with a dim signal for more than one of the chromosome pairs tested were considered as a hybridization failure and were not included in results. Fluorescence spots of lower intensity or diameter noticeably smaller than other signals in same nucleus were considered nonspecific probe binding and were not scored as chromosome specific signals. Fluorescence