Oocyte karyotyping by comparative genomic hybrydization provides a highly reliable method for selecting "competent" embryos, markedly improving in vitro fertilization outcome: a multiphase study

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Objective: To assess the karyotypic relationship between prefertilized/postfertilized oocytes and embryos using comparative genomic hybridization (CGH) on polar body-1 (PB-1), PB2, and blastomere biopsies and to evaluate IVF outcomes after transfer of blastocysts derived from euploid oocytes.
Design: Prospective cohort.
Setting: Medical center.
Patient(s): Phase1: Fourteen oocyte donors (23–29 years). Phase 2: Forty-one healthy embryo recipients aged 29–43 years free of endometrial implantation dysfunction. In 30 cases own eggs were used. Eleven women used donated oocytes.
Intervention(s): Phase 1: PB-1 biopsies followed intracytoplasmic sperm injection (ICSI), PB-2, and day 3 blastomere biopsies. Phase 2: PB-1 biopsy followed by ICSI using normal sperm and the subsequent embryo transfer of ≤2 blastocysts derived from euploid oocytes. Comparative genomic hybridization on all DNA derived from phase 1 and 2 biopsies.
Main Outcome Measure(s): Pregnancy and implantation rate.
Result(s): Phase 1: 39% of oocytes and 88% of zygotes were euploid; >95% progressed to blastocysts. Mosaicism as evidenced by euploid oocytes developing into aneuploid zygotes or embryos occurred in 13% of concepti. Phase 2: Six of 30 women using own eggs, who failed to produce euploid oocytes, were cancelled. Thirty-five women underwent embryo transfers with ≤2 (mean, 1.3 ± 0.7) blastocysts derived from euploid oocytes. The ongoing pregnancy/implantation rates per embryo transfer were 74% and 82%, respectively.
Conclusion(s): Transferring euploid embryos markedly improved IVF outcome. These findings, if corroborated, could initiate a paradigm shift in assisted reproductive technology (ART). (Fertil Steril® 2007;87:1033–40. © 2007 by American Society for Reproductive Medicine.)

Key Words: Comparative genomic hybridization, karyotype, embryo transfer, pregnancy, implantation

The earliest attempts at karyotyping human oocytes in the early 1970s yielded limited data due to the inadequacy of material and the limitations of available technology (1). In the ensuing decade the introduction and development of IVF techniques, for the first time, made human oocytes readily available for cytogenetic analysis, thereby precipitating a resurgence of interest in oocyte karyotyping (2, 3). The numerous studies that followed were severely hampered by limited knowledge and technical inability to optimize chromosome preparations or achieve accurate chromosome identification.

However, since the mid-90s, the application of sophisticated molecular cytogenetic techniques to human oocytes has developed significantly. The development of polar body (PB) biopsy techniques using sequential, multicolor fluorescent in situ hybridization (FISH) labeling was a giant step forward (4–6).

Unfortunately, FISH has serious limitations in that it does not permit access to full karyotyping of all 23 chromosome pairs. The recent introduction of more advanced techniques, such as comparative genomic hybridization (CGH) (7–9), spectral karyotyping (10), primed in situ labeling (PRINS) (11), and peptide nucleic acid (PNA) (12) techniques, have made karyotyping of all 23 chromosome pairs possible.

These new methodologies proved to be rather intensive with respect to time, technology, and cost. Due to this, their clinical application has been severely circumscribed, but nevertheless, a large body of data has accumulated as a result of academic studies performed using these techniques.

Although it has long been assumed the karyotype of an embryo is in large part a function of the karyotype of its

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oocyte of origin, and that an embryo's karyotype determines its potential to propagate a viable, normal pregnancy (i.e., its "competence"), the exact propagation of karyotype from oocyte to embryo has until now remained a subject of contention (12, 13). Most authorities believe that embryos derived from aneuploid oocytes (meiotic aneuploidy) will inevitably lead to embryo aneuploidy and "incompetence" (14, 15). However, the role of mosaicism (i.e., postfertilization, mitotic aneuploidy) in the propagation of embryo incompetence and the extent to which an advanced embryo can overcome the ill effects of mosaicism, remains a subject of hot debate (16–18).

Needless to say, both the incidence and origin of embryo aneuploidy (meiotic vs. mitotic) and the potential of a developing embryo to overcome the effects of mitotic aneuploidy (mosaicism) are highly relevant to the ultimate determination of embryo competence. If embryo aneuploidy is primarily a function of meiotic aneuploidy, and it can be demonstrated conclusively that an oocyte's karyotype is, in most cases, linearly perpetuated in its postfertilized zygote and embryo, then evidence gained through full karyotyping of the PB-1 would provide a high level of confidence that the embryo in question is most likely to be euploid and thus competent. Such knowledge could herald a time where selectively transferring only one embryo to a receptive uterine environment would consistently result in a single viable pregnancy.

Such ability could transform many accepted techniques in human reproduction. It would rapidly reverse currently stagnant IVF success rates, eliminate the risk of multiple pregnancies, and minimize the occurrence of first trimester miscarriages (mostly due to aneuploidy), as well as many chromosomal birth defects. Improvement in IVF success rates might also lead to a reduction in the cost per IVF baby, currently estimated to be between $70,000 to $100,000 in the United States. All of these factors may serve as a strong motivating influence on health insurance providers to improve coverage for IVF services. In total, this new technology could make assisted reproductive technology (ART) a viable option to a host of women who currently could/would not consider it.

A two-phase study was completed. In Phase 1 we evaluated whether a linear karyotypic correlation exists between the prefertilized and postfertilized oocyte and embryo and, in Phase 2, we assessed how and to what extent the selective transfer of no more than two blastocysts derived from euploid oocytes, would affect IVF implantation potential and viable ongoing pregnancy rates (beyond the first trimester).

METHODS AND MATERIALS

No IRB approval was obtained for this research, but all participants were fully informed and signed a consent form, and all met the requirements of the 1975 Helsinki guidelines.

Patients

Phase 1 Fourteen normally ovulating healthy oocyte donors aged 23–29 years (mean age 27.5 ± 5.7 years) were recruited from a licensed agency, screened, counseled, and fully informed. All women undergoing controlled ovarian hyperstimulation (COH) were pretreated as previously described (19). A total of 132 mature (MII) oocytes were harvested. All oocytes underwent first polar body (PB-1) biopsy. Thereupon each MII oocyte underwent intracytoplasmic sperm injection (ICSI) using sperm derived from seven normospermic donors recruited from a licensed sperm bank. Oocyte injection and embryo culture were performed as described elsewhere (20). The PB-2 biopsy was performed approximately 16 hours after ICSI. All cleaved embryos were subjected to single cell blastomere biopsy at 64–72 hours after ICSI, and then incubated for an additional period of 48–72 hours. Embryos that progressed to the grade 1–2 blastocysts stage were cryopreserved for future dispensation. The DNA specimens derived from PB-1, PB-2, and blastomeres were fully karyotyped using CGH.

Phase 2 Forty-one women ranging in age from 29–43 years (mean age 37.5 ± 5.5 years) were included in this study. Thirty patients used their own eggs. In eleven cases, the embryos resulted from fertilization of oocytes derived from donors. All recipients [1] had normal, regular uterine cavities based on hysterosonography or hysteroscopy performed in the preceding 12 months, [2] tested negative for alloimmune or autoimmune immunologic implantation dysfunction (i.e., antiphospholipid antibodies, natural killer cell activation, and/or shared HLA similarities with their partners), and [3] had endometrial linings that measured ≥9.0 mm in sagittal thickness before embryo transfer. In 30 cases the patients' own oocytes were used. COH was performed as described for patients in Phase 1. After egg retrieval (ER), all metaphase II (MII) oocytes underwent PB-1 biopsy and the DNA specimens so derived were fully karyotyped using CGH. Oocyte injection and embryo culture were performed as in Phase 1 (20). Only fresh blastocysts (≤2 per embryo transfer) derived from euploid oocytes of origin were transferred. The remaining (abnormal) blastocysts were discarded. In cases of oocyte donation, the recipients' endometrial linings were prepared with E_2 valerate administered at a dosage of 4–8 mg IM every 3 days. The exact dosage was adjusted to achieve a plasma E_2 concentration of 500–1,000 pg/mL and an endometrial lining measuring ≥9 mm. Intramuscular P (100 mg/d IM) was administered starting 6 days before embryo transfer and continued with twice weekly IM Delestrogen administrations until the 10th week of pregnancy or until pregnancy was discounted. Daily, oral dexamethasone (0.75 mg) was given throughout the cycle until a negative pregnancy test or the completion of the 10th week of pregnancy, whereupon it was tailed off over a period of 1 week and discontinued.

An informed consent form for embryo transfer based on American Society For Reproductive Medicine (ASRM) guidelines was signed. Up to two competent blastocysts were transferred per woman (mean 1.3 ± 0.7 per embryo trans-

fer). The cervical canal was cleansed and gently flushed before transfer using warmed culture media. All embryos were transferred atraumatically on day 5 of culture under transvaginal ultrasound guidance using a flexible catheter (Wallace, Smith Medical, Kent, UK) in <20 μL of media. Serum hCG levels were measured 7 and 9 days after blastocyst embryo transfer. Two values above 5.0 IU (doubling during 48 hours) were considered positive. Ongoing gestation was defined by fetal heart activity and appropriate growth at 12 weeks of gestation.

Comparative Genomic Hybridization

Extraction and amplification of genomic DNA Genomic DNA was obtained by laser dissection and needle aspiration from three sources: oocyte PB-1, oocyte PB-2, and embryonic blastomeres. The cellular material was aspirated into a 200-μL thin-walled polymerase chain reaction (PCR) tube (catalog #82006-602; VWR, West Chester, PA) for analysis and amplification using the Qiagen Repli-g kit (catalog 59045) as described in the manufacturers' manual.

Genomic DNA confirmation and quantification For confirmation of whole genomic amplification products, 5-μL reaction aliquots were mixed with 1 μL 6X Blue-Orange Loading Dye (Promega G1881, Mountain View, CA) and electrophoresed through a 1.0% agarose gel containing 0.1% ethidium bromide in TBE buffer (90 mM Tris-HCl, 90 mM boric acid, and 2 mM EDTA, pH 8.0). For quantification of the genomic DNA, 5-μL reaction aliquots were diluted with 95 μL of molecular biology-grade, nuclease-free water and analyzed with an Eppendorf BioPhotometer (Westbury, NY).

Nick translation of genomic DNA The whole genomic amplification products were fluorescently labeled using the Vysis Nick Translation Kit (catalog 32-801024; Des Plaines, IL).

Probe Preparation and Comparative Genomic Hybridization

The Spectrum Green-labeled probes were purified by ethanol precipitation. Briefly, 10 μL/200 ng nick-translated reference DNA, 1 μL/100 ng SprectrumRed reference DNA (Vysis 32-80423 or 32-804024), and 10 μL/10 μg Cot-1 DNA (Vysis 32-800028) used to suppress repetitive sequences and prevent nonspecific hybridization, were added to a 1.5-μL microcentrifuge tube, and hybridization was carried out as instructed in the manufacturers' manual. The CGH normal metaphase slides (Vysis 30-806010) were prepared following the manufacturer's protocol.

Image Capture and Analysis

The slides were mounted in DAPI II (Vysis) containing 4′,6-diamidino-2-phenylindole and antifade. Metaphase preparations were examined using an Olympus BX 61 fluorescence microscope (San Jose, CA). An average of 10 metaphases per hybridization were captured and analyzed using Cytovision v3.7 software (Applied Imaging, San Jose, CA). The average red:green fluorescence ratio for each chromosome was determined by the CGH software. Deviations of the ratio <0.8 (where the test DNA is under-represented) or >1.2 (where the test DNA is over-represented) were scored as loss or gain of material in the test or reference DNA, respectively.

The following optical filters visualized the fluorochromes used in the hybridization: a filter set specific for DAPI, Texas Red (catalog 30-150491) and FITC (fluorescein isothiocyanate) (catalog 30-150291) to view the counterstain; Spectrum-Red or Spectrum-Green DNA, respectively, a triple bandpass filter set designed to simultaneously excite and emit light specific for DAPI. Telomeric, centromeric, and heterochromatic regions were excluded from the analysis for being noninformative.

Statistical Analysis

Differences between groups were evaluated using Student's *t*-tests. Differences in rates and proportions were evaluated using the χ^2 test. Significance was set at $P<.05$.

RESULTS

Phase 1

Table 1 demonstrates that 65% (60/92) of the MII oocytes were aneuploid, whereas 35% (32/92) were euploid. Approximately 88% (28/32) of the postfertilized euploid oocytes were likewise euploid and 96% (27/28) of these developed into euploid embryos. Ninety-three percent (25/27) of the latter progressed to grade 1–2, day 5–6 blastocysts. Thus, approximately 89% (25/28) of postfertilized euploid MII oocytes developed into grade 1–2 blastocysts, all of which were also euploid. In comparison, the postfertilized aneuploid oocyte-to-blastocyst conversion rate was 20% (11/54), all of which were aneuploid ($P<.05$, confidence interval [CI] = 1–4). Ninety-three percent (25/27) of euploid embryos progressed to grade 1–2 blastocysts as compared to 21% (11/52) for conversion of aneuploid embryos ($P<.05$, CI = 1–4). The overall embryo-to-blastocyst conversion rate was 46% (36/79).

Approximately 13% (4/32) of euploid MII oocytes, after ICSI, developed into one or more aneuploid zygotes or embryos. This would suggest that the incidence of mitotic aneuploidy (mosaicism) was about 13% in this study.

Forty-three embryos failed to progress to blastocyst. In 95% of these cases (41/43) the embryos of origin were aneuploid. In contrast, only 3% (2/43) of cases where embryos failed to reach blastocysts, involved cases where the original embryos were euploid.

Table 2 illustrates that the common aneuploidies involved chromosomes 2, 4, 5, 9, 10, 12, 16, and 22, with abnormalities in chromosomes 4, 5, 16, and 22 the most prevalent. Our data further illustrate that in general trisomies were more frequently encountered than monosomies (57% vs. 43%) and

TABLE 1

Correlation between the karyotype of embryos and the prefertilized and postfertilized oocytes of origin: study involving serial comparative genomic hybridization on PB-1, PB-2, and blastomeres.

	%		
	PB-1	PB-2	Blastomere
% of MII oocytes successfully karyotyped	91 (92/102)		
% MII oocytes that were aneuploid	65 (60/92)		
% MII oocytes that were euploid	35 (32/92)		
% euploid MII oocytes propagated euploid zygotes	88 (28/32)		
% MII aneuploid oocytes propagated aneuploid zygotes	95 (57/60)		
% euploid oocytes propagated aneuploid zygotes	9 (3/32)		
% aneuploid oocytes propagated euploid zygotes	0 (0/60)		
% zygotes successfully analyzed		96 (82/85)	
% zygotes that were euploid		34 (28/82)	
% zygotes that were aneuploid		62 (54/82)	
% euploid zygotes propagated euploid embryos		96 (27/28)	
% euploid zygotes propagated aneuploid embryos		4 (1/28)	
% aneuploid zygotes propagated aneuploid embryos		100 (54/54)	
% embryo biopsies successfully karyotyped			96 (79/82)
% embryos that were euploid			34 (27/79)
% embryos that were aneuploid			66 (52/79)
% embryos that propagated G1-2 blastocysts			46 (36/79)
% euploid embryos that propagated grade1–2 blastocysts			93 (25/27)
% aneuploid embryos that propagated grade 1–2 blastocysts			21 (11/52)

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that of 72 oocytes examined, 48% had complex aneuploidies (>1 chromosome affected).

Phase 2

Table 3 presents the demographic characteristics of the 41 patients treated in Phase 2. Six women who used their own eggs did not have euploid embryos available for transfer. Thirty-five women each had ≤2 blastocysts transferred (mean 1.3 ± 0.7 per embryo transfer). The mean age of the egg providers was 37.5 ± 5.5 years. The indications for IVF in the 35 women who underwent embryo transfers are presented in Table 3. There were 30 cases where the patients' own eggs were fertilized and 11 cases of ovum donation. Twenty-eight (80%) ultrasound-confirmed pregnancies comprised 19 (68%) singletons and 9 (32%) twins. The ongoing pregnancy rate (PR) beyond the first trimester was 74% (26/35) and the implantation rate per blastocyst transferred was 82% (37/45). There were 2 miscarriages; one of which was associated with monosomy X and the other had a normal karyotype.

DISCUSSION

Numerical chromosome irregularities (aneuploidies) are common in early human embryos and contribute significantly to implantation failure as well as to early pregnancy loss (13, 21). Although the spermatozoon certainly contributes, it is primarily the oocyte that is the driving force in the establishment of embryo competence (6, 7, 9). The karyotype of the mature (MII) oocyte has emerged as being the most important single determinant of oocyte developmental competence with aneuploid oocytes rendered unfertilizable, arresting at various stages after fertilization, producing embryos that fail to implant, resulting in early miscarriages, or producing a variety of chromosomal birth defects (12, 13, 22). Assessment of embryo morphology with phase-contrast microscopy, although helpful, fails to provide reliable information by which to define the developmental competence of the embryo (23).

Traditional karyotyping of metaphase chromosomes using commercially available FISH is too inefficient and unreliable at the single cell level to be of practical use in fresh IVF (5, 6, 8, 24). Although widely used to identify embryo chromosome irregularities, FISH is of limited value because only a few chromosomes can be identified in each cell (usually X, Y, 13, 15, 16, 18, 21, and 22). An attempt to increase the number of chromosomes analyzed by simultaneously adding more FISH probes, reduces the accuracy of differentiating between various chromosomal elements and can lead to misdiagnosis (25). The matter is further complicated by the fact that the chromosome probes used in commercial FISH were designed to detect aneuploidies involved in spontane-

TABLE 2

Spectrum of oocyte karyotypic abnormalities detected by comparative genomic hybridization (CGH) on DNA derived from PB-1, PB-2, and blastomere biopsies of human oocytes.

	Autosomal chromosomes	Monosomy (%)			Trisomy (%)		
		PB-1	PB-2	Blastomere	PB-1	PB-2	Blastomere
A	1	7	4		7		9
	2		12		2	2	
	3	3			7		9
	4	7	15		4	11	13
	5	7		4	9	13	18
	6					9	2
	7	8			9	7	4
	8	3			4	4	2
	9			13			7
	10		12		7	4	
	11				7	2	
	12		12		4	9	4
	13	7	8		7	7	4
	14				9	7	
	15		8		4	2	2
	16	10		29		7	4
	17	7	8	4	9	2	2
	18	7		4	4		
	19	2	8	4	4	9	2
	20	8		4	2	2	9
	21	8	4	8		2	
	22	15	12	29	2	2	7

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ous miscarriages, not necessarily the often lethal chromosomal aneuploidies that lead to dysfunctional embryogenesis and failed implantation.

Newer technologies such as CGH, facilitate single cell karyotyping (7, 9). Comparative genomic hybridization, both enumerates and provides a comprehensive assessment of each chromosome, thereby identifying breakages and even partial aneuploidies. There have been reports of healthy IVF babies born after preimplantation genetic diagnosis (PGD) using full CGH karyotyping (26).

An often cited limitation of CGH is that it cannot differentiate between certain ploidy errors, such as haploidy, triploidy, and tetraploidy. However, this would not preclude its use in IVF, where such defects are readily identified by closely observing the number of pronuclei present in the zygote. The main problems associated with the use of CGH in determining the ploidy of blastomeres are: [1] it is very time consuming, taking at least 5–6 days to complete, such that the embryos so tested would have to be cryopreserved and stored until CGH results were available and thereupon be thawed for subsequent transfer to the uterus, [2] the process itself is expensive, and [3] it is very technology intensive (27, 28). These factors have all but precluded clinical use of CGH for blastomere PGD in clinical IVF.

In 2004, Gutiérrez-Mateo et al. (29) reported an oocyte aneuploidy incidence of 48% after CGH performed on DNA derived from 25 oocytes' first PBs. In a separate study using both CGH and FISH performed on 54 oocytes (30) derived from 36 women, the same researchers found the incidence of oocyte aneuploidy to be 57% and the chromosomes more frequently involved in aneuploidy to be chromosomes 1, 4, and 22, followed by chromosome 16. Twenty-five percent (25%) of the aneuploid doublets would have been misdiagnosed as normal using FISH with probes for nine chromosomes. Our findings indicate that the incidence of oocyte aneuploidy, even in young women (mean age 27.0 ± 2.5 years), is considerably higher (i.e., 65%). Our study further revealed aneuploidies of chromosomes 4, 5, 16, and 22, and predominantly in the form of trisomies.

Table 1 depicts that aneuploid oocytes fertilized by ICSI (using normal sperm), consistently propagated aneuploid zygotes (57/57) as well as embryos (57/57), and that in 87% (27/31) of cases, euploid oocytes developed into euploid embryos. These findings establish that embryo ploidy is

TABLE 3

Demographics and characteristics in 29 patients participating in Phase 2.

Number of women (n)	41
Number of women who did not have ET	6 (15%)
Number of women who underwent ET	35 (86%)
Age (y)	37.5 ± 5.5
Number of prior IVF failures	1.9 ± 1.4
Indications for IVF with own eggs in women who underwent Embryo transfer	
o Tubal factor	7
o Endometriosis	5
o Age (>39 y)	6
o Unexplained	7
o Ovum donation	10
Number of blastocysts transferred per embryo transfer	1.3 ± 0.7 (45/35)
Number of ongoing gestations per embryo transfer	26 (74%)
Number of singleton pregnancies	19 (68%)
Number of twin pregnancies	9 (32%)
Number of 1st trimester pregnancy losses	2/28 (7%)
Implantation rate/blastocyst transferred	37/45 (82%)

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linearly propagated after fertilization, suggesting that it could be possible, by selecting embryos derived from karyotypically normal oocytes to improve IVF outcome. It also illustrates that euploid embryos are far more likely to propagate blastocysts than were aneuploid embryos (93% vs. 21%).

In our Phase study, 95% of embryos that failed to progress to blastocysts were aneuploid. This latter observation challenges the widely held belief that embryos are better off being transferred earlier, in the cleaved state (i.e., 2–3 days after fertilization) rather than later, as blastocysts. This is an important observation because, if confirmed by other investigators, it could establish a rational basis for routinely waiting for embryos to attain the blastocyst stage of development before embryo transfer as many aneuploid embryos would be culled out in the process.

Aneuploidy, which arises during meiosis I, will inevitably affect all of the cells of the embryo. The rank and the number of chromosomes so affected would likely impact embryo survival, the incidence of miscarriage, as well as the risk of a birth defect (31). Conversely, embryo aneuploidy arising during mitosis (i.e., mosaicism), depending on the stage of embryonic development at which it occurs, is often compatible with the subsequent propagation of a viable offspring (32). Chromosome studies in blastocysts indicate that mosaicism is in fact quite a common finding in concepti and that the preponderance of aneuploid cells might decrease with advancing development (33).

The high incidence of oocyte aneuploidy (65%) in the relatively young population group comprising our Phase 1 study helps to explain the relatively low fecundicity of humans. Our findings also clearly indicate that in young women with fertile male partners, it is the karyotype of the oocyte, rather than that of the spermatozoon that likely determines the embryo's ploidy and competence. We have also confirmed that oocyte karyotype is in the vast majority of cases linearly conveyed to the zygote and embryo, and that although mitotic aneuploidy (mosaicism) occurred in 13% of cases, its disruptive impact on embryogenesis is likely to be much less significant than meiotic (oocyte) aneuploidy.

The relatively low occurrence of mitotic aneuploidy in our Phase 1 study might, at least in part, be attributable to the relatively young age of the oocyte providers and the fact that all oocytes were fertilized with sperm derived from normospermic males. It is equally plausible to conclude that mosaicism is a less common contributor to embryo aneuploidy than hitherto believed. Moreover, the later in embryogenesis that the mitotic aneuploidy insult occurs, the fewer the number of aneuploid cells, and the lower the likelihood that it might compromise the offspring. We postulate that the number and rank of chromosomes involved might also play a significant role in determining the nature and severity of the mitotic aneuploidy-related insult.

The single cell amplification technique employed in our Phase 1 and Phase 2 studies yielded inconclusive results in about 15% of analyses performed. We have found that it is possible to reduce this incidence to <5% when repeated hybridization can be performed as needed. The fact that complete CGH analysis takes 5–6 days to complete presents a formidable barrier to the routine adaptation of such egg/embryo competency testing in the conventional, fresh

IVF setting, where there is rarely more than 5 days available from performance of ER to fresh blastocyst transfer. Clearly, more latitude in time is needed to allow for transportation of specimens from the IVF laboratory to a centralized CGH laboratory, as well as for repeated CGH testing in the event that initial analyses fail to yield conclusive results. This problem could readily be overcome by temporarily cryobanking blastocysts derived from euploid oocytes and/or embryos in preparation for transfer during a subsequent natural or hormone replacement cycle. We predict that the process of separating the cycle of ER/ICSI/PGD-CGH/and blastocyst vitrification from the ET cycle (i.e, Staggered-in Vitro Fertilization [St-IVF]) will eventually find a permanent place in the ART therapeutic armamentarium.

The recent introduction of ultra-rapid freezing (vitrification) (34) has markedly improved post-warming survival of both oocytes and embryos, as well as their subsequent pregnancy-generating potential. As such it represents a significant improvement over hitherto conventional slow-freezing methods, which commonly resulted in intracellular ice formation and damage to the cytoskeleton of the cell. Thus, vitrification is ideally suited for use in St-IVF. Studies are currently underway in both the United States and the United Kingdom to evaluate ST-IVF.

The results of Phase 2 confirm that by selecting embryos derived from euploid eggs, it is possible to minimize the number of embryos transferred and significantly improving IVF outcome. Since most early pregnancy loss, as well as late chromosomal fetal defects are attributable to embryo aneuploidy, our approach to selecting competent embryos for transfer (or modifications thereof), if confirmed through larger studies, could establish PB-1 and/or blastomere biopsy with CGH as a valuable method by which to significantly improve IVF success rates, minimize the likelihood of multiple pregnancies, and bring us closer to the goal of one embryo, one baby. At the same time, it would likely reduce the incidence of early pregnancy loss and meiotic aneuploidy-related chromosomal defects, regardless of the age of the egg provider.

These findings might also improve the current disappointing results reported with oocyte cryopreservation, as it is possible, and perhaps even likely, that euploid oocytes would better tolerate cryopreservation than would aneuploid oocytes.

Finally, an ability to identify euploid oocytes, which upon fertilization would propagate euploid embryos and blastocysts, might also provide euploid inner cell mass cells, and present a much needed "shot in the arm" for human embryonic stem cell research (35).

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