

GENETICS OF ICSI

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LEARNING OBJECTIVES

1. The ART physician should become thoroughly knowledgeable about the rates of various chromosomal anomalies in the embryos and offspring derived from ICSI.
2. The ART physician should be familiar with chromosomal studies of sperm in normal versus infertile men.
3. The ART physician should become familiar with the results of conventional IVF versus ICSI in various clinical groups and the genetic implications.
4. The ART physician and laboratory personnel should understand the indications for PGD (pre-implantation genetic diagnosis) for male factor IVF cases.
5. The ART physician should be familiar with comparative results of ICSI-IVF with obstructive and non-obstructive azoospermia and severe and moderate oligospermia.

PRE-COURSE QUESTIONS

1. The results of ICSI with oligospermia or normospermia compared to IVF with normospermia:
 - a. Show more chromosomal errors with ICSI derived embryos.
 - b. Show better embryo quality with ICSI derived embryos.
 - c. Show a higher fertilization rate with ICSI derived embryos.
 - d. Show higher implantation rate with IVF embryos.
 - e. Show no significant differences.

2. Sperm aneuploidy:
 - a. Results in a higher miscarriage rate with ICSI.
 - b. Is dramatically increased in oligospermic men.
 - c. Is genetically transmitted from the infertile man's father.
 - d. Is minimally increased in oligospermic men.

Answers:

1. D
2. D

RELATIONSHIP OF SPERMATOGENIC DEFECT TO CHROMOSOMAL ABNORMALITIES IN SPERM AND IN ICSI DERIVED EMBRYOS

Since the introduction in 1992 of intracytoplasmic sperm injection (ICSI), men with the most severe impairments of spermatogenesis seem to be capable of fathering their own genetic children (1-3). It appeared initially that the most severe cases, e.g. with apparently 100% abnormal morphology, or even just rare motile sperm in the ejaculate, could have pregnancy and delivery rates not apparently different from conventional in vitro fertilization in men with normal sperm parameters. (4-6). It even became possible utilizing ICSI for men with no sperm whatsoever in their ejaculate to have children via sperm retrieval combined with ICSI. With obstructive azoospermia, sperm could be retrieved from the blocked epididymis, or from the seminiferous tubules of the testes in virtually every case (7-13). Even in men with non-obstructive azoospermia, up to 60% were found to have some sperm in the testis (not quantitatively sufficient to spill over into the ejaculate) that could be retrieved in tiny amounts from the testes and utilized for successful ICSI (3,14-24).

Subsequent to the growing popularity in the usage of ICSI in these severe cases of male infertility, there have been two conflicting trends of thought regarding the role of the defect in spermatogenesis in possibly influencing pregnancy rates, implantation rates, miscarriage rates, or the incidence of genetic abnormalities in the offspring. The initial concept was that poor sperm quality or quantity had little or no impact on the results. Assuming that the sperm were viable (as measured by finding some slight degree of motility, however slight) it appeared that the results of intracytoplasmic sperm injection were not related to any sperm parameters (4-6,25). There was no difference noted by Nagy et al. in clinical pregnancy rates related to sperm counts ranging from zero to greater than 5,000,000 per cc. There was no difference in pregnancy rates related to motility ranging from 0% to greater than 50%, (as long as a very rare motile sperm was seen). Even 0% normal morphology was associated with no difference in clinical pregnancy rates using ICSI (4). The presence or absence of Y chromosomal deletions also had no effect on ICSI results so long as a few sperm were present (26-27). When fertilization rates, embryo transfer rates, and delivered pregnancy rates, were compared in an extensive early series of men with obstructive azoospermia and normal spermatogenesis, versus men with non-obstructive azoospermia with the most severe impairment of spermatogenesis, there was still no difference in results when age-matched (22). The only difference in results was related to the age of the wife, and her ovarian reserve as measured by the number of eggs retrieved in the stimulation cycle (Tables 1-2).

The rate of chromosome abnormalities found in embryos produced by ICSI in cases of mild oligospermia versus conventional IVF has been shown to be comparable. Thus, it appeared that ICSI itself does not cause chromosomal defects (33-34). However, none of these reports detailed the karyotype or morphology of embryos generated by the most severe spermatogenic defects (as in non-obstructive azoospermia) versus more moderate spermatogenic defects (as in oligospermia). It is clear that ICSI offspring do have a slightly higher incidence of chromosomal abnormalities than a normal population of newborns. Most likely any chromosomal defects in ICSI offspring are thought to be related to sperm defects in the infertile male (28-32). There is certainly an increased incidence of chromosomal anomalies in ICSI offspring (30,35-38).

However, this is apparently not caused by ICSI, but rather is most likely a consequence of chromosomal aberrations in the sperm of infertile men (Tables 3-9).

Infertile males whose wives are undergoing ICSI are more likely to have chromosome abnormalities in their peripheral lymphocytes. An abnormal chromosome constitution has been found in almost 4% of males requiring ICSI, much higher than in a normal population of fertile men (39-44). Van Assche et al. summarized all of the chromosomal studies reported in various series of infertile men from 1974 to 1995 and came up with a very useful summary (Tables 3-8). Sex chromosomal abnormalities were present in the peripheral lymphocytes of 3.8% of the infertile male population as opposed to 0.14% in newborn infants, and autosomal abnormalities (mostly translocations) were found in 1.3% of infertile males as opposed to 0.25% of newborn infants. Even if the infertile male is chromosomally normal in his peripheral lymphocytes, nonetheless meiotic disruption may still generate high rates of sperm chromosome abnormalities (32).

A correlation has been suggested between poor semen parameters (concentration and progression) and sperm aneuploidy (45). However, the increase in sperm chromosomal abnormalities in infertile men is very small, and has not been correlated with an increase in spontaneous abortions or neonatal abnormalities (46). This slight increase in sperm chromosome aneuploidy seems to be produced by an increased frequency of pairing disruptions resulting in meiotic arrest (40,47). The sex chromosome bivalent is particularly susceptible to pairing abnormalities since there is generally only one crossover in the pseudoautosomal region. It has been speculated that infertile men have decreased recombination and pairing leading to both meiotic arrest (oligospermia) and non-disjunction of the sex chromosomes (31). This has been offered as the reason for the slightly higher risk of sex chromosome anomalies in ICSI offspring (0.8% compared to 0.17%) (35-36).

CLINICAL FOLLOW UP OF ICSI OFFSPRING

Pediatric follow-up and pre-natal karyotyping on almost 2,000 ICSI offspring (over 1,000 karyotypes) by the Brussels' group has shown only a 2.7% incidence of major congenital malformations, which was not significantly different from the incidence of major congenital malformations noted in a large variety of previous "newborn" studies (30,35-37,43,65). Other large follow-up studies of ICSI offspring continue to support this conclusion (66). Similarly reassuring results on pediatric follow-up of ICSI offspring have been reported more recently by Sutcliffe (67). Furthermore, early chromosome studies of embryos obtained after conventional in vitro fertilization versus intracytoplasmic sperm injection (in cases of moderate oligospermia), have shown no statistical difference in the chromosomal abnormalities of embryos derived from IVF in men with normal semen parameters versus embryos derived from ICSI in men with oligospermia (33-34).

However, there have been nonetheless gnawing concerns regarding possible chromosomal anomalies in ICSI offspring of men with the most severe spermatogenic defects. There has been consistently a 0.8% to 1% incidence of sex chromosomal anomalies in ICSI offspring, compared to a population norm of 0.14% to 0.2% (37,65). These newborns would naturally appear normal at birth, and the sex chromosomal anomaly (most frequently Klinefelter's) would not be identified without prenatal karyotypic screening. Although this 1% incidence of sex

chromosomal anomalies seemed to be reassuringly low, it was five times greater than what one should have expected in a normal population of newborns. Almost 2% of these newborns had autosomal chromosomal anomalies, including trisomy 21 and various translocations (35-37). But only 0.46% of these newborns were trisomy 21, and in large measure, these autosomal trisomies could be discounted as being related to maternal age. Similar results have more recently been reported by Aboulghar (38). A more recent follow-up from Brussels of almost 3,000 ICSI children still verifies no significant difference in congenital anomalies compared to IVF children (95). Nonetheless, in a much smaller recent study from France, it has been suggested that chromosomal abnormalities are more common in TESE-ICSI offspring than those derived from ICSI with epididymal sperm (96).

Perhaps a more alarming problem in ICSI-produced neonatal karyotypes was a 0.36% incidence of de novo balanced translocations, compared to the normal newborn population of 0.07% (36). There was, thus, a five times greater incidence of de novo structural aberrations, i.e., balanced translocations, in ICSI offspring. In addition, there was a 0.92% incidence of inherited translocations transmitted via ICSI from the father. Ten percent of those inherited translocations were unbalanced. Thus, there was a total incidence of chromosomal aberrations in the ICSI population of 2.5%, which might be attributed either to the ICSI procedure itself, or to the population of men who were now having offspring as a result of ICSI. Most evidence favors the view that it is due to the population of men who are now having offspring as a result of ICSI.

CONVENTIONAL IVF VERSUS ICSI AND CHROMOSOMAL ABNORMALITIES IN OFFSPRING

These sex chromosomal aneuploidies, autosomal de novo translocations, and transmitted balanced and unbalanced translocations occurred in a clinically tolerable, low frequency. They have been thought to be related either to problems in the sperm of the most severely infertile men in ICSI populations, or else to the very mechanism of the ICSI technique itself. Studies of embryos derived after ICSI versus IVF would make the latter speculation very unlikely (33). Embryos derived from ICSI versus those derived from regular IVF are quite similar in percentage of chromosomal errors. Thus, it is most likely that the sperm of infertile men might be the source of this low but definite increase in chromosomal abnormalities of ICSI offspring rather than ICSI itself.

Numerous studies continue to show no significant difference in ART results in couples undergoing regular IVF with normal semen parameters versus those undergoing ICSI. That is, ICSI does not seem to induce any negative results compared to regular IVF. Aboulghar in 1996 reported on 116 randomized prospective tubal factor infertile women with normal male fertility, 58 undergoing conventional IVF and 58 undergoing ICSI. The fertilization rate was 64.8% for IVF and 70% per injected oocyte (53.5% per retrieved egg) for ICSI. Clinical pregnancy rate for IVF was 31% and for ICSI was 32.8%. They concluded that the ICSI procedure itself had no beneficial, but also no negative, effect on clinical outcome (68). Staessen in 1999 actually compared conventional IVF to ICSI in sibling oocytes from couples with normal semen parameters and tubal infertility as well (69). Again, there was no apparent improvement in implantation or pregnancy with either IVF or ICSI in such a group. There was also no significant difference in 2PN fertilization and cleavage, except that ICSI embryos developed quicker.

Embryo morphology, and implantation and delivery rates were the same. They reported no detrimental effect of ICSI on overall reproductive outcome, but ICSI did reduce the incidence of unexpected fertilization failure. This study verified clinically the chromosomal study of Munne et al in 1998, which demonstrated no chromosomal differences between embryos generated by IVF versus those generated by ICSI.

The Norfolk group compared 211 ICSI cases to 211 conventional IVF cases. ICSI produced less “good quality” embryos than IVF, but there was no difference in pregnancy rate or miscarriage rate when adjusted for age and number of embryos transferred (70). Thus poor sperm quality (or ICSI) was associated with poorer embryo quality, but not with pregnancy or delivery rate. Others have found not only reduced blastocyst formation in association with poor quality of injected spermatozoa, but also a higher miscarriage rate (71-74). Some have speculated that this is caused not only by poor sperm quality, but also by the ICSI procedure itself; but in skilled hands, that is very unlikely because of all the clinical studies showing no difference in result using ICSI or IVF in normospermic couples (75-76).

CHROMOSOMAL STUDIES OF SPERM IN MEN WITH SEVERE SPERMATOGENIC DEFICIENCY

The awareness of these difficulties has thus resulted in an increased enthusiasm for chromosomal studies of peripheral lymphocytes and sperm of infertile males. Many studies have been reported since 1994 on chromosomal analysis of spermatozoa by FISH (28,32,45,77-85). There is a great deal of controversy generated by these studies about the percentages of aneuploid sperm in infertile men. McInnes et al. report a “highly significant increase in frequency of chromosome 13 disomy in 90,000 sperm from nine infertile men” as opposed to 182,000 sperm from 18 controls. However, that difference was only 0.28% in infertile men versus 0.13% in normal controls. Thus, although there was a mathematically statistically significant increase in sperm aneuploidy from infertile men, these differences were so slight as to not suggest a major biological impact (86). Bernardini found similar differences, as did Palermo, suggesting that sex chromosomal disomy was found in the sperm of 0.64% of infertile men and 0.46% of fertile men, nullisomy in 0.51% of infertile men, and 0.30% of fertile men, and autosomal disomy in 0.84% of infertile men as opposed to 0.63% of controls (66,78). All of these studies that seem to show an increased rate of sperm aneuploidy in infertile men, show such a tiny increased rate that they all support the position that sperm aneuploidy would not be likely to account for the high percentage of abnormal embryos (either mosaic or aneuploid) in the worst ICSI cases that have failed implantation. Overall, probably 5% to 10% of sperm have some aneuploid abnormality, whereas probably 90% of eggs are aneuploid.

There is a fair amount of inconsistency in the studies of sperm aneuploidy in infertile men. However, there seems to be some agreement that there is a slightly higher incidence of chromosome aneuploidy in the sperm of men with severe oligospermia, but so slight as to not suggest a major clinical impact for offspring. The frequency of nondisjunction of autosomes as well as the sex chromosomes in sperm was higher in men with severe oligospermia, but the increased incidence of aneuploidy in the sperm was so small as to readily explain that no difference was found in chromosome abnormalities between embryos obtained after ICSI versus IVF (34,87). The men who father offspring with abnormal karyotypes through an ICSI

procedure were not found by Vegetti et al. to have a higher aneuploidy rate for that particular chromosome in the spermatozoa compared to other male patients with oligospermia who did not bear children with abnormal karyotype (45). If anything, the analysis of sperm chromosome number by FISH in infertile and fertile men, though of some interest, has been more assuring than worrisome (86). Thus, the studies of sperm aneuploidy in men with severe oligospermia undergoing ICSI, may explain the higher incidence of sex chromosomal abnormalities in ICSI offspring (1%), but cannot explain the high frequency of chaotic embryos which we observe in patients undergoing TESE for non-obstructive azoospermia.

SPERM CHROMOSOME ANALYSIS IN TESE CASES

There have been two conflicting studies, one by Levron et al. and the other by Martin et al., of the sperm found in the testes of men with non-obstructive azoospermia (85). In Martin's study, only three patients were analyzed, and they all had normal FSH levels. Therefore, it might not be surprising that she found no increased incidence of aneuploidy in the testicular spermatozoa of the patients with non-obstructive azoospermia. In Levron's study, the opposite was found, i.e., there was a higher incidence of sperm aneuploidy in men with non-obstructive azoospermia. Thus, the only two studies on sperm from the testes of azoospermic men analyzed by FISH come to opposite conclusions (82,85). Both of these studies were hindered by the relative unavailability of sperm to study in men who have such severe quantitative defects in spermatogenesis. That is why we chose to study the embryos derived from TESE-ICSI with such patients. On the other hand, our PGD study was only with worst-case scenario patients with previous failure to implant or such a severe spermatogenic defect that only less than 30 sperm could be found.

STUDY OF MOSAIC CHAOTIC (COMPARED TO ANEUPLOID) EMBRYOS IN SEVERE MALE FACTOR

Most of the chromosome studies on the sperm of infertile men have focused on aneuploidy. However, chromosome abnormalities in human embryos are not limited to aneuploidy (48). In fact, in younger women, in whom aneuploidy is less common, the most common chromosome abnormality in cleavage-stage embryos is mosaicism and not aneuploidy (49-50). Mosaic embryos can reach blastocyst stage, but not result in viable offspring (48,51-52). Different mosaic types have been described in cleavage-stage embryos, and possible mechanisms producing mosaicism have been proposed (34,53-55). Laboratory conditions, hormonal stimulation and unsuitable follicular oxygen tension have been suggested as causes mosaicism (34,47,56). However, one detailed case report has been published of a couple in whom multiple IVF cycles, produced mostly chaotic mosaics, but when donor sperm was used, most embryos were found to be normal. This report suggested the possibility of a male factor origin for mosaicism (58). Therefore, it is appropriate to continue to study embryos derived from ICSI in cases of severe spermatogenic defect.

We studied a very select group of males with the severest case of non-obstructive azoospermia undergoing TESE with ICSI, versus males with moderate oligospermia whose wives were undergoing ICSI with ejaculated sperm (Tables 1-2). Since there is no significant difference in results in ICSI with TESE for non-obstructive azoospermia, obstructive azoospermia, or

oligospermia in a variety of clinical reports, we decided to look at the most difficult cases, with the most severe spermatogenic defects.

All patients underwent open testis biopsy whenever possible using microsurgical exposure (24). In all patients, the azoospermic semen was subjected on three separate occasions to centrifugation at 1800 g with careful, extended examination to determine the presence or absence of spermatozoa. If enough spermatozoa were so detected, these patients were excluded from the study and did not undergo TESE. The absence of ductal obstruction was verified in all patients at the time of the diagnostic biopsy or at the time of microsurgical TESE by direct observation. The diagnosis of testicular failure was based on the finding of azoospermia, the absence of obstruction, and histologic confirmation. The method of histologic analysis and the verification of non-obstructive azoospermia have been previously described (14,16-17,21-22,24,59). These were cases where extensive testicular exploration was necessary to find very few spermatozoa.

TESE always involved spermatozoa with elongated heads and the presence of a normal tail. What the pathologist refers to on histological sections as “mature spermatids” are, in fact, what appear to the embryologist at TESE to be spermatozoa. On histological sectioning, the tail of the spermatozoon is seldom seen, and usually only the thicker sperm head shows up in thin sections. We only injected spermatozoa with condensed, oval heads, and a normal tail. No “round cell” injection was performed. When spermatozoa were not found at TESE, there was no injection of “round cells” or any other “structure that had the appearance of sperm” (60). Of the azoospermic patients without obstruction undergoing TESE, only those who had a normal karyotype from peripheral blood were included. However, not all patients who underwent ICSI with ejaculated sperm had a karyotype performed. Of those oligospermic patients with a karyotype done, all were chromosomally normal.

Patients undergoing ICSI for male factor infertility caused by oligospermia (ICSI) were used as controls for the group of patients with the severest male factor requiring surgical recovery of sperm by TESE. ICSI patients (oligospermia) were compared to TESE (non-obstructive azoospermia) patients in order to control for chromosome abnormalities related to the ICSI procedure alone, versus those related to TESE-ICSI. Only female patients 39 and younger were included in the study to minimize the effect of maternal age on chromosome abnormalities. It should be noted that the equivalent pregnancy rates and delivery rates in TESE and ICSI series could be due to embryo selection (since chaotic embryos often have poor morphology) and to the replacing of more embryos in “desperate” cases.

FISH ANALYSIS

Non-transferred embryos were disaggregated and cells fixed individually following previously published protocols (34). All embryos were analyzed for chromosomes X, Y, 13, 16, 18, and 21. Some were also analyzed for chromosomes 15 and 22. All utilized previously published FISH protocols (61). During day three of development, one or two cells per embryo were biopsied by zona drilling using acidified Tyrode’s solution, and the embryos returned to culture as described elsewhere (62). All of the embryos were at the 4- to 12-cell stage of development at the time of biopsy. All blastomeres were fixed individually following our protocol (63). A scoring criterion for differentiating false-positives and false-negatives from

mosaicism was followed as previously described when analyzing all or most cells of each embryo (64). These same criteria were used to differentiate between close signals from two homologous chromosomes from two domains belonging to a split signal of a single chromosome. The criteria to classify embryos as normal, aneuploid, mosaic, polyploid or haploid based on FISH results of most or all the cells of an embryo was previously described by us and followed here without modification (33).

In addition, because not all embryos classified by PGD as chromosomally abnormal could be fully reanalyzed due to time constraints or the lack of patient consent, we followed the following classification of embryos based on single cells: 1) when the cell had two copies of each chromosome analyzed the embryo was classified as normal 2) when the cell had 3 or more copies of each chromosome the embryo was classified as polyploid 3) when the cell had 1 or less copies of each chromosome the embryo was classified as haploid 4) when the cell had one or two chromosomes with an abnormal number of copies the embryo was classified as aneuploid, and 5) when the cell had three or more chromosomes with an abnormal number of copies but the cell was not haploid or polyploid, the embryo was classified as mosaic. While criteria 1-3 may seem obvious, criteria 4-5 may seem arbitrary, but they are based on the observation that triple and higher multiple aneuploidies are extremely rare even in cleavage-stage embryos and that after full analysis of embryos with three or more abnormal chromosomes these are almost always mosaic (49-50).

Mosaic embryos were classified as follow: 1) embryos with a diploid cell line and one or more cell lines with different ploidies than diploid (N, 3N, 4N, 8N, etc) were considered diploid/polyploid mosaics, usually generated by endoreduplication, haploidization or karyokinesis arrest; 2) embryos with a diploid normal cell line and monosomic and/or trisomic cell line/s, these were considered aneuploid mosaics; 3) embryos with two cell lines that complement each other to form a diploid count of chromosomes, plus or not a normal cell line. These were classified as split mosaics and were generated by lack of DNA duplication previous to karyokinesis; 4) and finally chaotic embryos, were those that the mechanism of mosaicism formation could not be understood, and where usually every cell had a different chromosome count as if nuclear division had been at random.

The average maternal age was higher in the group undergoing ICSI with ejaculated sperm (35.1 ± 4.0 years) than in the azoospermic group in the worst TESE category (32.6 ± 4.2 years). The accuracy of FISH results was assessed by comparing PGD results with reanalysis results in non-replaced embryos. A total of 257 non-replaced embryos with PGD results were reanalyzed to compare with PGD results. Of those diagnosed by PGD as abnormal, 14.6% (30 / 206) were found normal after reanalysis, and of those diagnosed by PGD as normal, 7.8% (4/51) were found abnormal after reanalysis. The total error rate was 13.2% (34/257).

As shown in Table 10, of 775 embryos in the ICSI group (*i.e., men with oligospermia undergoing ICSI with ejaculated sperm*), 42.6% of embryos were normal, 26.3% were aneuploid, 26.1% were mosaic, 4.1% were polyploid, and 3.0% were haploid. In contrast, of the 86 embryos analyzed from the TESE group (*i.e., men with the severest non-obstructive azoospermia*), only 24.4% of embryos were normal, 16.3% were aneuploid, 55.8% were mosaics, 5.8% were polyploid, and 2.3% were haploid. The differences between both groups regarding normal and mosaic embryos were highly significant ($p < 0.05$ and $p < 0.001$, respectively) (Table

10 and 11). No statistically significant differences in aneuploidy were observed. Any slight difference was attributed to higher maternal age in the couples using ejaculated sperm.

The figure shows a cell from a chaotic embryo. Embryos are classified as mosaic based on single cell analysis if more than two chromosomes exhibited an abnormal number (but the embryo was neither haploid nor polyploid) (Fig. 1). Reanalysis (3 or more cells analyzed) of non-transferred embryos that were diagnosed by PGD as mosaic showed that there was also a higher rate of chaotic mosaics (see definition in material and methods) in the TESE group (95%) than in the ICSI group (62%) ($p < 0.001$) (Table 2). The remaining reanalyzed mosaics were diploid/polyploid, diploid/haploid, diploid/aneuploid, or other more rare combinations.

SPERMATOGENIC DEFECTS AND CHAOTIC MOSAICISM OF ICSI EMBRYOS

It is known that aneuploidy of embryos is not closely associated with, or correlated, with embryo morphology. As women age and the rates of aneuploidy increase, abnormalities in embryo morphology do not increase (34). However, chaotic mosaicism and polyploidy are associated with an increase in morphologic abnormalities in the embryos. The failure to observe differences between chromosomal abnormalities in embryos derived from standard IVF versus ICSI are most likely related to the fact that in the studies heretofore reported, ICSI was performed for more moderate degrees of oligospermia and non-obstructive azoospermia than for the most severe spermatogenic defects (33-34). Thus, aneuploidy appears to increase with maternal age and is related to defects in the egg, but chaotic mosaicism may be more related to defects in the sperm, and may result in a higher percentage of chaotic embryos derived from ICSI with non-obstructive azoospermia.

It is attractive to theorize that chaotic embryos that have been observed resulting from defective sperm may be more related to defects in the sperm centriole than to a higher incidence of numerical chromosome abnormalities. Our TESE-ICSI-derived embryos had no greater incidence of aneuploidy than ICSI with ejaculated sperm from men with higher sperm production rates. However, there was a dramatically increased rate of chaotic errors in these embryos resulting from abnormal mitosis, which could conceivably be related to defects in the sperm centriole (88). Similarly, an early report on MESA-ICSI for obstructive azoospermia in which distal (senescent) epididymal sperm were utilized, demonstrated an inexplicably high miscarriage rate despite the young age of the female partners (10,12). This phenomenon might also be explained by defects in embryo cleavage related to centriole dysfunction (89-90). The most severe degrees of spermatogenic defect, resulting in non-obstructive azoospermia, and requiring testicular sperm extraction, may result in a higher frequency of chromosomal abnormalities, but those abnormalities may be more related to errors in mitosis during early cleavage of the embryo, than to sperm aneuploidy.

Chaotic mosaics do not increase with maternal age and may be more commonly produced in association with male factor (34,58). The present data points toward a male origin of chaotic embryos. We hypothesize that sperm with immature or suboptimal mid-pieces, not apparent under microscopic observation, will result in abnormal centrosomes and chaotic mosaicism. Because the first mitotic divisions are controlled by the spermatozoon centrosome (91), this may result in abnormal chromosome distribution among sister cells. For instance, dispermic embryos have high rates of first mitotic mosaicism appearing as chaotic mosaics, and they are produced by

an abnormal number of male centrioles (haploids none, polyspermics two), or suboptimal centriole function (91). In both cases, the first mitotic spindle will not form properly, creating two different chromosomally abnormal cells.

Chaotic mosaics may also be produced by over-expression of factors regulating the centrioles. For instance, human cells that over-express a serine/threonine kinase named STK15 (92) may have an amplified number of centrosomes thus mis-segregating chromosomes and producing mosaic tissues (93). Fibroblast cultures from Huntington disease patients have shown a high frequency of cells with three or more centrosomes (18% versus 2% in controls), resulting in mosaicism, and morphological abnormalities such as large cells, multinucleated cells, anucleated cells, tetraploid cells, and other abnormalities commonly seen in cleavage-stage embryos (94).

Nonetheless, evidence is mounting that very severe spermatogenic defects, as in non-obstructive azoospermia, may result in a higher percentage of chaotic mosaic embryos, resulting in less efficient implantation and delivered pregnancy rates. This may only be observed in the most severe cases, since pregnancy rates and delivery rates are similar in ICSI compared to IVF, and even with most series of testicular sperm compared to ejaculated sperm. PGD may thus be useful in severe spermatogenic defects, especially with poor results in previous cycles.

POST-COURSE QUESTIONS

1. Chromosomally mosaic embryos:
 - a. Result most commonly from meiosis one in the oocyte at the time of egg maturation.
 - b. Can be diagnosed with reasonable accuracy from a single blastomere if more than two different chromosomes are aneuploid.
 - c. Are “chaotic” if different blastomeres have aneuploidy of different chromosomes.
 - d. Result most commonly from post-meiotic events.
 - e. B, C, and D.
2. The sperm centriole:
 - a. Contributes the substance from which the egg’s spindle is constructed.
 - b. Is the organizer of the spindle in the mouse embryo.
 - c. Is present in human round spermatids.
 - d. Is defective in Huntington’s patients.
 - e. None of the above.

Answers:

1. E
2. E

TABLE 1

	TESE/ICSI Non-Obstructive w/Sperm Present	ICSI with <2 Million Sperm	ICSI with ≥2 Million Sperm	MESA/ICSI Obstructive Azoospermia
# Patients	47	253	343	85
# Cycles	72	384	456	137
# Transfers	64	357	430	134
# Eggs (MII)	820	3,906	4,376	1,513
# 2PN	469	2,261	2,858	1,015
# Pregnant	28 (39%)	142 (37%)	189 (41%)	69 (50%)
# Deliveries	19 (26%)	100 (26%)	139 (30%)	50 (37%)
# Sacs	39 (16%)	192 (14%)	259 (15%)	89 (17%)
# Babies	25	128	179	56
# Boys	9	61	81	24
# Girls	16	67	98	32

TABLE 2

Age and # of Eggs Breakdown	TESE/ICSI Non-Obstructive w/Sperm Present	ICSI with <2 Million Sperm	ICSI with ≥2 Million Sperm	MESA/ICSI Obstructive Azoospermia
<30, <10	0% (0/1)	45% (5/11)	35% (6/17)	33% (1/3)
<30, ≥10	69% (9/13)	44% (24/55)	59% (27/46)	67% (16/24)
30-35, <10	20% (2/9)	33% (12/36)	44% (28/63)	42% (5/12)
30-35, ≥10	50% (9/18)	43% (57/134)	51% (70/138)	54% (26/48)
36-40, <10	33% (2/6)	41% (14/34)	30% (20/66)	32% (6/19)
36-40, ≥10	55% (6/11)	47% (23/49)	43% (26/60)	50% (9/18)
>40, <10	0% (0/2)	14% (4/29)	17% (5/29)	25% (1/4)
>40, ≥10	0% (0/4)	33% (3/9)	64% (7/11)	83% (5/6)

TABLE 3: Percentage Of Chromosome Abnormalities Observed In Seven Series Of Infertile Men (Azoospermic and Oligospermic) Compared To Normal Newborn Population

<u>All References</u>	<u>Number</u>	<u>Sex Chromosomes</u>	<u>Autosomes</u>	<u>Total</u>
Total	7,876	295 (3.8)	104 (1.3)	399 (5.1)
Newborn Infants	94,465	131 (0.14)	232 (0.25)	366 (0.38)

Van Assche *et al.*, 1996

TABLE 4: Percentage Of Chromosome Abnormalities Observed In Seven Series Of Infertile Men (Azoospermic and Oligospermic) Compared To Normal Newborn Population

All References	Number	Sex Chromosomes	Autosomes	Total
Koulischer and Schoysman, 1974	1,000	27 (2.7)	6 (0.6)	33 (3.3)
Chandley, 1979	2,372	33 (1.4)	18 (0.7)	51 (2.1)
Zuffardi and Tiepolo, 1982	2,542	175 (6.9)	40 (1.6)	215 (8.6)
Abramsson et al., 1982	342	6 (1.8)	4 (1.2)	10 (2.9)
de Gardelle et al., 1983	318	13 (4.1)	7 (2.2)	20 (6.3)
Matsuda et al., 1989	295	0 (0)	5 (1.7)	5 (1.7)
Yoshida et al., 1995	1,007	41 (4.1)	24 (2.4)	65 (6.5)
Total	7,876	295 (3.8)	104 (1.3)	399 (5.1)
Newborn Infants	94,465	131 (0.14)	232 (0.25)	366 (0.38)

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TABLE 5: Percentage Chromosome Abnormalities Reported In Five Series Of Oligozoospermic Males (No Azoospermic Cases)

<u>Reference</u>	<u>Number</u>	<u>Sperm Count (10⁶/ml)</u>	<u>Sex Chromosomes</u>	<u>Autosomes</u>	<u>Total</u>
Hendry <i>et al.</i> (1976)	108	< 20	1 (0.9)	1 (0.9)	2 (1.9)
Micic <i>et al.</i> (1984)	464	< 20	--	8 (1.7)	8 (1.7)
Retief <i>et al.</i> (1984)	390	< 10	14 (3.6)	10 (2.6)	24 (6.2)
Bourrouillou <i>et al.</i> (1985)	569	< 10	11 (1.9)	28 (4.9)	39 (6.9)
Matsuda <i>et al.</i> (1989)	170	< 20	2 (1.2)	4 (2.4)	6 (3.5)
Total	1,701		28 (1.6)	51 (3.0)	79 (4.6)

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TABLE 6: Percentage Of Chromosome Abnormalities Reported In Five Series Of Oligozoospermic Males (NoAzoospermic Cases)

<u>All</u> <u>References</u>	<u>Number</u>	<u>Sperm</u> <u>Count</u> <u>(10⁶/ml)</u>	<u>Sex</u> <u>Chromosomes</u>	<u>Autosomes</u>	<u>Total</u>
Total	1,701		28 (1.6)	51 (3.0)	79 (4.6)
Newborns	94,465		131 (0.14)	232 (0.25)	366 (0.38)

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TABLE 7: Percentage Of Chromosome Abnormalities Reported In Six Series Of Azoospermic Males

<u>Reference</u>	<u>Number</u>	<u>Sex Chromosomes</u>	<u>Autosomes</u>
Hendry et al. (1976)	54	3 (5.6)	2 (3.7)
Micic et al. (1984)	356	28 (7.9)	2 (0.6)
Retief et al. (1984)	106	19 (1.9)	--
Bourrouillou et al. (1985)	383	54 (14)	5 (1.3)
Rivas et al. (1987)	163	36 (22.1)	2 (1.2)
Matsuda et al. (1989)	89	5 (5.6)	2 (2.2)

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TABLE 8: Percentage Of Chromosome Abnormalities Observed In SEVEN Series Of Infertile (Azoospermic And Oligospermic) Men Compared To Normal Newborn Population

<u>All References</u>	<u>Number</u>	<u>Sex</u>		<u>Total</u>
		<u>Chromosomes</u>	<u>Autosomes</u>	
Total	7,876	295 (3.8)	104 (1.3)	399 (5.1)
Newborn Infants	94,465	131 (0.14)	232 (0.25)	366 (0.38)

Van Assche *et al.*, 1996

**Our experience (unpublished)
in 884 men with azoospermia
or severe oligospermia**

4%

2%

TABLE 9: Summary of Karyotype Anomalies In 1,082 Prenatal Diagnoses (Bonduelle et al., 1995, 1996, 1998, 1999)

<u>Abnormal Karyotypes</u>	<u>Maternal Age (years)</u>	<u>Number</u>	<u>Percentage</u>	<u>Percentage In Literature</u>
De Novo Sex-Chromosomal:	25, 26, 28 28, 32, 32, 37, 37, 44	9	0.83	0.19, 0.23
De Novo Trisomy 21 (5 children):	32, 33, 37, 41, 41	5	0.46	Age Dependent
De Novo Translocations	30, 30, 36, 39	4	0.36	0.07
Inherited Translocations		10	0.92	0.47
Balanced		9	0.83	0.45
Unbalanced		1	0.09	0.023

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TABLE 10: Chromosome abnormalities in ICSI and TESE embryos

	<u>ICSI-Oligospermia</u>	<u>ICSI-TESE</u>
Normal	330 (42.6%) ^a	21 (24.4%) ^a
Polyploid	32 (4.1%)	5 (5.8%)
Haploid	23 (3.0%)	2 (2.3%)
Aneuploid	188	14
Aneuploid and Mosaic	16	0
Mosaic	186	44
Total Aneuploid	204 (26.3%)	14 (16.3%)
Total Mosaic	202 (26.1%) ^b	44 (51.2%) ^b
Total	775	86

^a P<0.05, ^b P<0.001.

TABLE 11: Specific mosaicism types

	<u>ICSI</u>	<u>TESE</u>
Total number of mosaic embryos*:	202/775 (26.1%) ^a	44/86 (51.2%) ^a
Mosaic embryos fully analyzed:	92	20
Chaotic	57/92 (62.0%) ^b	19/20 (95.0%) ^b
Other **	35/92 (38.0%)	1/20 (5.0%)

^a P<0.001, ^b P<0.005

*Embryos were classified as mosaics after full embryo analysis or when based on a single cell that cell showed 3 or more abnormal chromosomes and the abnormality was not consistent with polyploidy or haploidy. If it had one or two abnormal chromosomes it was considered aneuploid.

**These being diploid/polyploid, diploid/aneuploid, or other rarer combinations.

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Figure 1 FISH on a chaotic embryo using probes for chromosomes 13 (red), 16(light blue), 18 (dark blue), 21 (green), 22 (yellow). Nucleus 1 shows one signal for chromosome 13, one 16, two 18, two 21 and one 22 or for short, 1[13],1[16],2[18],2[21],1[22]; Nucleus two has 2[13],1[16],2[18],2[21],2[22]; Nucleus 3 has 1[13],1[16],2[18],2[21],1[22]; micronuclei 4 has only one signal for chromosome 16; Nucleus 5 has 2[13],2[16],2[18],2[21],2[22] and a yellowish debris at 10 o'clock; Nucleus 6 has 2[13],1[16],2[18],1[21],1[22]; Nucleus 7 has 2[13],0[16],3[18],1[21],1[22]; Nucleus 8 has 3[13],1[16],2[18],1[21],3[22]; Nucleus 9 has 1[13],1[16],1[18],1[21],1[22]; and Nucleus 10 has 3[13],1[16],2[18],1[21],3[22] being the 21 signal split.

