FERTILITY AND STERILITY®
VOL. 79, NO. 1, JANUARY 2003

Published by Elsevier Science Inc.
Printed on acid-free paper in U.S.A.

Chromosomal abnormalities in embryos derived from testicular sperm extraction

Sherman Silber, M.D.,[a] *Tomas Escudero, B.Sc.,*[b] *Kathleen Lenahan, R.N.,*[a] *Iman Abdelhadi, B.Sc.,*[c] *Zaid Kilani, M.D.,*[c] *and Santiago Munné, Ph.D.*[b]

Infertility Center of St. Louis at St. Luke's Hospital, St. Louis, Missouri; Institute for Reproductive Medicine and Science of Saint Barnabas, Livingston, New Jersey; and the Farah Hospital, Amman, Jordan

Objective: To compare the rate of chromosome abnormalities in embryos obtained from karyotypically normal patients with nonobstructive azoospermia undergoing testicular sperm extraction (TESE) to those from patients undergoing intracytoplasmic sperm injection (ICSI) with ejaculated sperm.

Design: Retrospective analysis.

Setting: IVF centers.

Patient(s): Male partners had either nonobstructive zoospermia or oligospermia.

Intervention(s): Preimplantation genetic diagnosis. Chromosome enumeration was performed by fluorescence in situ hybridization (FISH). Embryos classified as abnormal were reanalyzed to study mosaicism.

Main Outcome Measure(s): Chromosome abnormalities in embryos.

Result(s): Embryos from ICSI cycles with ejaculated sperm (group 1) were 41.8% normal, 26.2% aneuploid, and 26.5% mosaic. In contrast, the embryos from ICSI cycles with TESE for nonobstructive azoospermia (group 2) were 22% normal, 17% aneuploid, and 53% mosaic. The difference in mosaicism rate between the two groups of embryos was highly significant.

Conclusion(s): The present study results indicate a high incidence of mosaicism in embryos derived from TESE in men with a severe deficit in spermatogenesis. Sperm derived from TESE for nonobstructive azoospermia may have a higher rate of compromised or immature centrosome structures leading to mosaicism in the embryo. (Fertil Steril® 2003;79:30–8. ©2003 by American Society for Reproductive Medicine.)

Key Words: Mosaicism, chaotic, aneuploidy, PGD, preimplantation genetic diagnosis

Received February 2, 2002; revised and accepted July 25, 2002.

Presented at the annual meeting of the European Society of Human Reproduction and Embryology, Vienna, Austria, June 29–July 4, 2002.

Reprint requests: Santiago Munné, Ph.D., The Institute for Reproductive Medicine and Science of Saint Barnabas, 101 Old Short Hills Road, Suite 501, West Orange, New Jersey 07052 (FAX: 973-322-6235; E-mail: santi.munne@embryos.net).

[a] Infertility Center of St. Louis at St. Luke's Hospital.

[b] The Institute for Reproductive Medicine and Science of Saint Barnabas.

[c] The Farah Hospital.

0015-0282/03/$30.00
PII S0015-0282(02)04407-2

Since the introduction of intracytoplasmic sperm injection (ICSI) in 1992, 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, for instance, those 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 IVF in men with normal sperm parameters (4–6). It even became possible using 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 nonobstructive azoospermia, ≤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 used for successful ICSI (3, 14–24). The original reports on ICSI in these most severe cases of spermatogenetic impairment seemed to indicate that pregnancy rates and delivery rates were related more to the age of the wife and her ovarian reserve than to the actual sperm count, or sperm quality (5, 6, 22, 25). In fact, it appeared that the results of intracytoplasmic sperm injection were not related to any sperm parameters (4–6, 25)

Nonetheless, subsequent follow-up studies have indicated that this easy supposition that sperm parameters were not correlated with ICSI outcome might, in fact, be misleading (26–30). The rate of chromosome abnormalities found in embryos produced by ICSI in cases of mild oligospermia vs. conventional

IVF has been shown to be comparable. Thus ICSI itself does not appear to be a teratogenic agent (31, 32).

However, none of these early reports on the results of ICSI detailed the karyotype or morphology of embryos generated by the most severe spermatogenic defects (as in nonobstructive azoospermia) vs. more moderate spermatogenic defects (as in oligospermia). There does appear to be a slightly lower fertilization rate and clinical pregnancy rate for testicular sperm derived from men with nonobstructive vs. obstructive azoospermia (33). Furthermore, there is clearly an increased incidence of chromosomal anomalies in ICSI offspring compared with the case of a normal newborn population (28, 33–37). Additionally, there is an increased incidence of abnormalities found in peripheral lymphocytes of males requiring ICSI (38–43). Sex chromosomal abnormalities are present in the peripheral lymphocytes of 3.8% of the infertile male population as opposed to in 0.14% in newborn infants, and autosomal abnormalities (mostly translocations) are 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, there is a concern that meiotic disruption may nonetheless generate high rates of sperm chromosome abnormalities (30).

For this reason, there has been great interest in studying the chromosomal constitution of the sperm of infertile men. Most fluorescence in situ (FISH) studies on sperm of infertile males have found higher rates of aneuploidy than in fertile males; for instance, a correlation has been suggested between poor semen parameters (concentration and progression) and sperm aneuploidy (44). However, with the exception of the most severe defects in spermatogenesis requiring testicular sperm extraction (TESE), the increase in sperm chromosomal abnormalities was very small and was not correlated with an increase in spontaneous abortions or neonatal abnormalities (45). However, it does probably explain the slight but definite increase in sex chromosomal anomalies found in ICSI offspring (35). This slight increase in sperm chromosome aneuploidy seems to be produced by an increased frequency of pairing disruptions that results in meiotic arrest (29, 39, 46).

Most chromosome studies of the sperm of infertile men have focused on aneuploidy. However, chromosome abnormalities in human embryos are not limited to aneuploidy (47). In fact, in younger women, the most common chromosome abnormality in cleavage-stage embryos is mosaicism and not aneuploidy (48, 49). These mosaic embryos can reach blastocyst stage but still not result in viable offspring (47, 50, 51). Different mosaic types have been described in cleavage-stage embryos, and possible mechanisms producing mosaicism have been proposed (32, 52–54). Occasionally, an infertile male in multiple IVF cycles may produce mostly chaotic mosaics, but when donor sperm is used, may produce normal embryos (55). The purpose of the present study is to see whether a sperm defect might be associated with mosaicism in extreme cases of infertile males, by comparing patients with moderate male infertility requiring ICSI, with severe cases of nonobstructive azoospermia requiring testicular biopsy (TESE) with ICSI.

MATERIALS AND METHODS

Patient Population

The patient population consisted of men with severe nonobstructive azoospermia undergoing TESE with ICSI (group 2) and of men with oligospermia whose wives were undergoing ICSI with ejaculated sperm (group 1). All patients underwent open testicular biopsy whenever possible using microsurgical exposure (Figs. 1 and 2) (24). In all patients, the azoospermic semen was subjected on three separate occasions to centrifugation at $1800 \times g$ 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 nonobstructive azoospermia have been previously described (14, 16, 17, 21, 22, 24, 56).

Testicular sperm extraction always involved spermatozoa with elongated heads and the presence of a normal tail. No round-cell injection was performed (57). All of the patients with nonobstructive azoospermia undergoing TESE in this study had a normal karyotype from peripheral blood. However, not all oligospermic patients who underwent ICSI with ejaculated sperm had a karyotype performed. Of those oligospermic patients with a karyotype done, all were chromosomally normal.

Embryo Source and Control Group

The embryos used for this study all belonged to patients undergoing preimplantation genetic diagnosis (PGD) for chromosome abnormalities and were analyzed in accordance with guidelines approved by our respective internal review boards, including informed written consent in each case. Some of these embryos already were described in previous studies (32, 49). Embryos classified by PGD as chromosomally abnormal usually had all or most of their cells analyzed after PGD, whereas those classified as normal were usually replaced in the patient. PGD patients therefore normally had all their embryos analyzed, although not all embryos were analyzed fully.

Intracytoplasmic sperm injection, embryo culture, and embryo biopsy were performed in three different centers, but the PGD analysis and embryo reanalysis were performed by the same individuals for all three centers. Patients undergo-

ing ICSI for male-factor infertility caused by oligospermia (ICSI) were used as controls for the group of patients with severe male factor requiring surgical recovery of sperm by TESE. ICSI patients (with oligospermia) were compared with TESE patients (with nonobstructive azoospermia) to control for chromosome abnormalities related to the ICSI procedure alone, vs. those related to TESE-ICSI. Only female patients aged ≤39 years were included in the study to minimize the effect of maternal age on chromosome abnormalities.

Fluorescence In Situ Hybridization Analysis

Nontransferred embryos were disaggregated, and cells were fixed individually according to protocols published elsewhere (32). All embryos were analyzed for chromosomes X, Y, 13, 16, 18, and 21. Some were also analyzed for chromosomes 15 and 22. All used previously published FISH protocols (58). During day 3 of embryo development, one or two cells per embryo were biopsied by zona drilling using acidified Tyrode's solution, and the embryos were returned to culture as described elsewhere (59). All of the embryos were at the 4- to 12-cell stage of development at the time of biopsy. All blastomeres were fixed individually according to our protocol (60).

A scoring criterion for differentiating false positives and false negatives from mosaicism was followed as described elsewhere (61) when analyzing all or most cells of each embryo. These same criteria were used to differentiate between close signals from two homologue 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 described by us elsewhere (31) and was followed without modification here.

In addition, because not all embryos classified by PGD as chromosomally abnormal could be fully reanalyzed because 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 chromosomes analyzed, the embryo was classified as normal; [2] when the cell had three or more copies of each chromosome, the embryo was classified as polyploid; [3] when the cell had one 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. Whereas criteria 1–3 may seem obvious, criteria 4 and 5 may seem arbitrary, but those 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 (48, 49).

Mosaic embryos were classified as follows: [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 lines 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, were classified as split mosaics and were generated by lack of DNA duplication that occurred before karyokinesis; [4] and finally, chaotic embryos, or those for which the mechanism of mosaicism formation could not be understood, usually were cases in which every cell had a different chromosome count, as if nuclear division had been at random.

Statistical Analysis

The proportion of abnormal embryos was computed for each patient for each type of abnormality being studied. These proportions were then subjected to a two-factor analysis, the factors being the type of procedure (ICSI, TESE) and the fertility centers (1–3). The results are summarized by mean incidences and the appropriate standard errors, together with an indication of the importance of the relevant effect. The mean incidences represented back-transformations from the logistic scale, by which the analysis was carried out.

RESULTS

A total of 111 cycles of ICSI with ejaculated sperm and 19 cycles of TESE–ICSI were included in this study. The average maternal age was higher in the ICSI (35.4 ± 4.2 years) than in the TESE (32.4 ± 4.0 years) group.

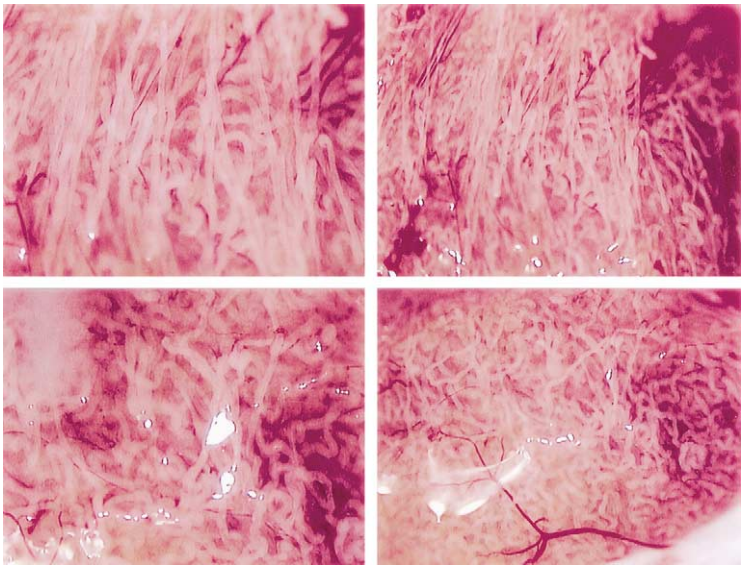
The accuracy of FISH results was assessed by comparing PGD results with the reanalysis results in nonreplaced embryos. A total of 306 nonreplaced embryos was reanalyzed to compare with PGD results. Of those diagnosed by PGD as abnormal, 11.8% (30/255) were found to be normal after reanalysis, and of those diagnosed by PGD as normal, 7.8% (4/51) were found to be abnormal after reanalysis. The total error rate was 11.1% (34/306).

As shown in Table 1, of 830 embryos in the ICSI group (i.e., men with oligospermia undergoing ICSI with ejaculated sperm), 41.8% of embryos were normal, 26.2% were aneuploid, 26.5% were mosaic, 4.7% were polyploid, and 3.0% were haploid. In contrast, of the 100 embryos analyzed from the TESE group (i.e., men with nonobstructive azoospermia), only 22% of embryos were normal, 17% were aneuploid, 53% were mosaics, 5% were polyploid, and 4% were haploid. The differences between both groups regarding normal and mosaic embryos were highly significant ($P<.001$; Table 1).

No statistically significant differences in aneuploidy rates were observed, and the slightly higher rate of aneuploidy in

FIGURE 1

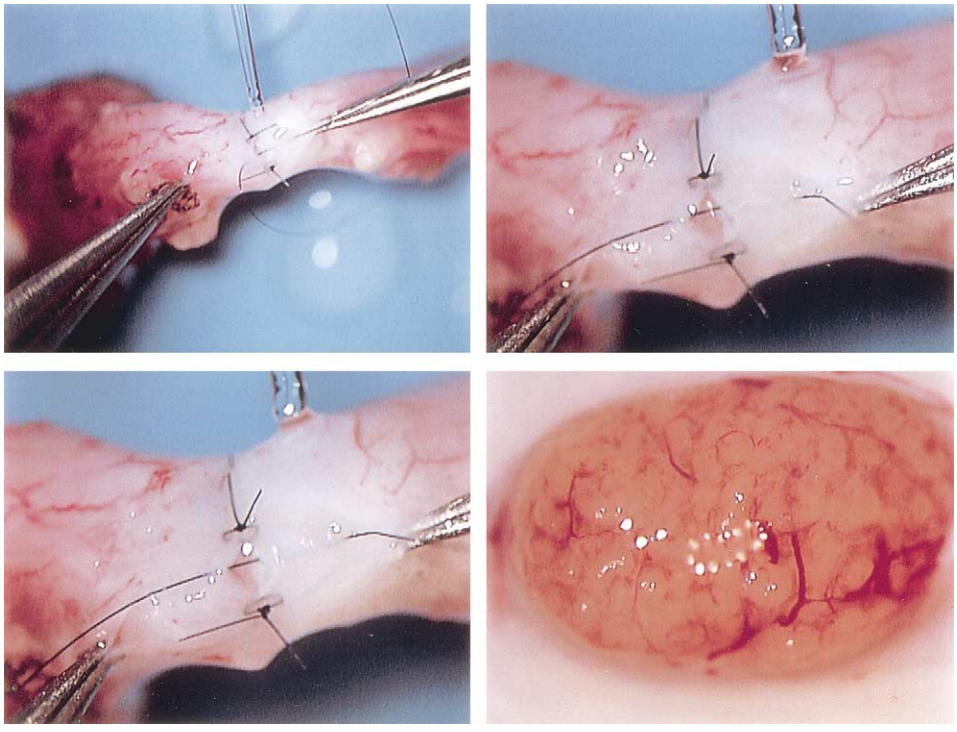
View of microsurgical TESE of patient with Sertoli cell only and thin, empty seminiferous tubules.



Silber. Chromosome abnormalities in TESE embryos. Fertil Steril 2003.

FIGURE 2

View of microsurgical TESE of patient with maturation arrest and full-appearing seminiferous tubules, which are similar to the full tubules seen with obstructive azoospermia.



Silber. Chromosome abnormalities in TESE embryos. Fertil Steril 2003.

TABLE 1

Chromosome abnormalities in ICSI and TESE embryos.

Group	ICSI–oligospermia, n (%)	ICSI–TESE, n (%)
Normal	347 (41.8)[a]	22 (22)[a]
Polyploid	39 (4.7)	5 (5)
Haploid	25 (3.0)	4 (4)
Aneuploid	199 (23.9)	16 (16)
Aneuploid and mosaic	19 (0.2)	1 (1)
Mosaic	201 (24.2)	52 (52)
Total aneuploid	218 (26.2)	17 (17)
Total mosaic	220 (26.5)[b]	53 (53)[b]
Total	830	100

[a,b] $P<001$ between the ICSI and TESE embryo groups.

Silber. Chromosome abnormalities in TESE embryos. Fertil Steril 2003.

the controls can be attributed to the higher maternal age of the control group. Furthermore, the rates of aneuploidy per chromosome were similar in both groups, including 2.9% and 1% rates of sex chromosome aneuploidy in the ICSI and TESE groups, respectively (Table 2).

Figure 3 shows a cell from a chaotic embryo. Embryos were 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). Reanalysis (≥3 cells analyzed) of untransferred embryos that were diagnosed by PGD as mosaic showed that there was also a higher rate of chaotic mosaics (see definition in Materials and Methods) in the TESE group (82%) than in the ICSI group (64.1%; Table 3). However, this difference was not statistically significant. The remaining reanalyzed mosaics were diploid–polyploid, diploid–haploid, diploid–aneuploid, or other, more rare combinations.

TABLE 2

Specific aneuploidy types.

Group	ICSI–oligospermia, n (%)	ICSI–TESE, n (%)
Total analyzed	830	100
Embryos with one aneuploidy	179	15
Embryos with two aneuploidies	39	2
Total aneuploid embryos	218 (26.2)	17 (17)
Aneuploidies per chromosome		
Chromosomes XY	24 (2.9)	1 (1)
Chromosome 13	27	1
Chromosome 15	41	2
Chromosome 16	60	4
Chromosome 18	26	5
Chromosome 21	44	3
Chromosome 22	35	3
Total number of aneuploidies	297	19

Silber. Chromosome abnormalities in TESE embryos. Fertil Steril 2003.

TABLE 3

Specific mosaicism types.

Group	ICSI–oligospermia	ICSI–TESE
Total number of mosaic embryos[a]	200/830 (26.5)[b]	53/100 (53)[b]
Mosaic embryos fully analyzed	106	28
Chaotic	68/106 (64.1)[c]	23/28 (82.1)[c]
Other[d]	38/106 (35.9)	5/28 (17.9)

[a] Embryos were classified as mosaics after full embryo analysis or when based on a single cell that showed three 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.

[b] $P<.001$.

[c] $P<.005$.

[d] Diploid–polyploid, diploid–aneuploid, or other rarer combinations.

Silber. Chromosome abnormalities in TESE embryos. Fertil Steril 2003.

Table 4 shows results by center. There were significant differences in mosaicism rates in the oligospermia group between centers 1 and 2 vs. center 3; these differences cannot be explained by maternal age differences because mosaicism generally does not increase with maternal age. There were no other significant differences among the three centers. The higher mosaicism rates and lower normal rates in TESE-ICSI cases compared with oligospermia ICSI cases were maintained for all three centers.

DISCUSSION

Early chromosome studies of embryos obtained after conventional IVF vs. intracytoplasmic sperm injection (in cases of moderate oligospermia) have shown no difference in the incidence of chromosomal abnormalities (31, 32). However, there have been growing concerns regarding possible chromosomal anomalies in ICSI offspring of men with the most

TABLE 4

Aneuploidy, mosaicism, and normal embryos by center.

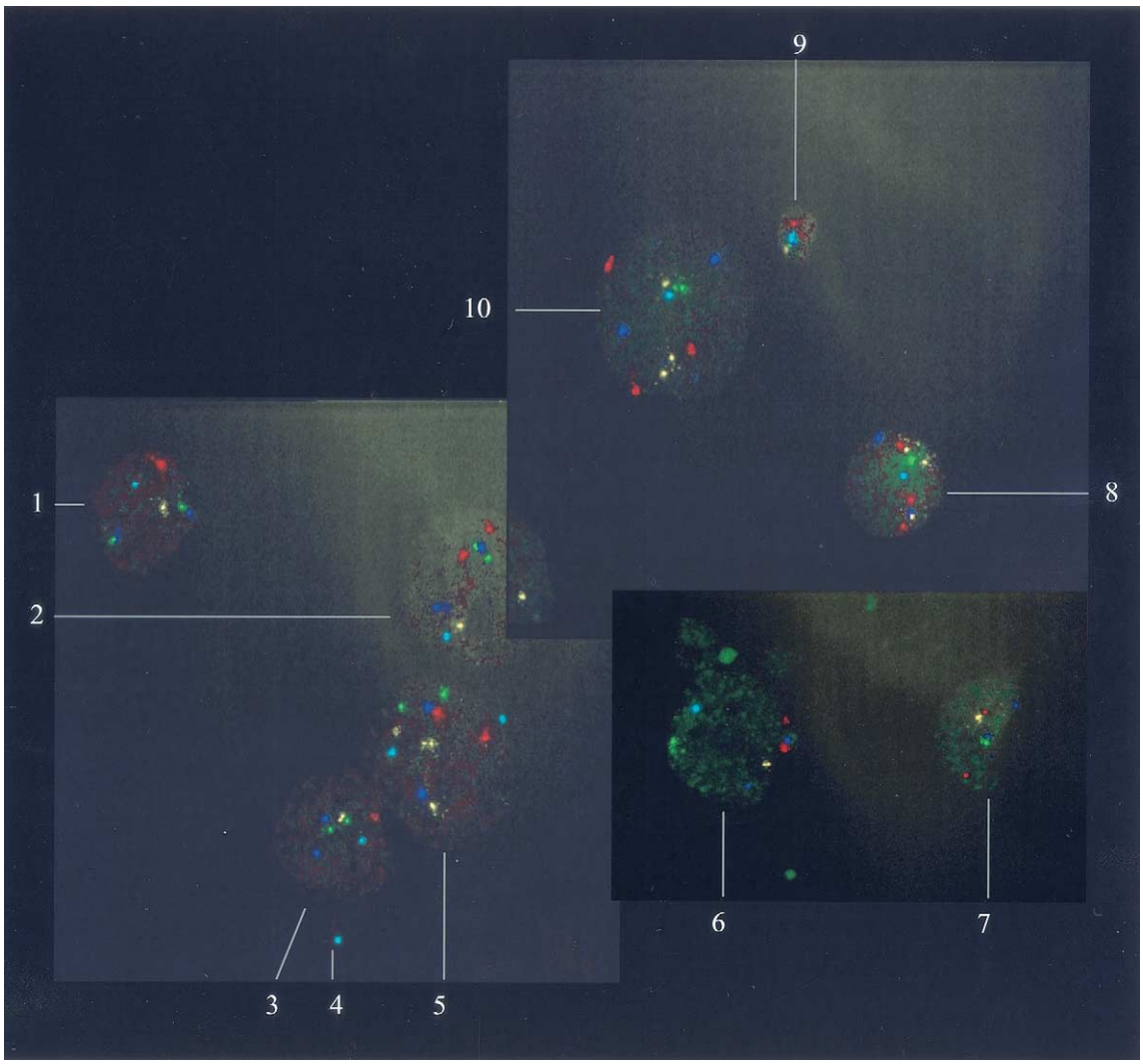
Group	ICSI–oligospermia, n (%)	ICSI–TESE, n (%)
Normal		
1	268/608 (44)	7/28 (25)
2	42/117 (36)	6/41 (15)
3	37/97 (38)	9/31 (29)
Total aneuploid		
1	157/608 (26)	7/28 (25)
2	39/117 (33)	9/41 (22)
3	16/97 (16)	1/31 (3)
Total mosaic		
1	149/608 (25)[a]	11/28 (39)
2	31/117 (26)[a]	22/41 (53)
3	36/97 (37)[a]	19/31 (61)

[a] $P<.05$, center 1 + center 2 result vs. center 3 result, this column.

Silber. Chromosome abnormalities in TESE embryos. Fertil Steril 2003.

FIGURE 3

Fluorescence in situ hybridization on a chaotic embryo using probes for chromosomes 13 (*red*), 16 (*light blue*), 18 (*dark blue*), 21 (*green*), and 22 (*yellow*). Nucleus 1 shows one signal for chromosome 13, one for chromosome 16, two for chromosome 18, two for chromosome 21, and one for chromosome 22; or for short, 1[13],1[16],2[18],2[21],1[22]; nucleus 2 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 have 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.



Silber. Chromosome abnormalities in TESE embryos. Fertil Steril 2003.

severe spermatogenic defects. There consistently has been a 0.8% to 1% incidence of sex chromosomal anomalies in ICSI offspring, compared with a population norm of 0.14% to 0.2% (34–37). These newborns would appear normal at birth, and the sex chromosomal anomaly (most frequently Klinefelter's) would not be identified without prenatal karyotypic screening.

Autosomal aneuploidies in this population were no different than what would be expected based on maternal age in a non-ICSI population (35). Perhaps a more alarming problem in ICSI-produced neonatal karyotypes was a 0.36% incidence of de novo balanced translocations, compared with the normal newborn population of 0.07% (35). 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%.

These low-frequency chromosomal abnormalities have been thought to be related either to the ICSI procedure itself or to problems in the sperm of the most severely infertile men in ICSI populations. It is thought by many that the sperm of infertile men might be the source of this low but definite increase in chromosomal abnormalities of ICSI offspring rather than the ICSI procedure itself (31). The awareness of these difficulties has thus resulted in an increased enthusiasm for chromosomal studies of the sperm of infertile males.

Many studies have been reported since 1994 on the chromosomal analysis of spermatozoa by FISH (26, 30, 44, 62–70). There is a great deal of controversy generated by these studies about the percentages of aneuploid sperm in infertile men. Although there seems to be a statistically significant increase in sperm aneuploidy from infertile men, these differences were so slight as to not suggest a major biological impact (71). The frequency of nondisjunction of autosomes and sex chromosomes was higher in the sperm of men with severe oligospermia than in normal men, but this increased incidence of aneuploidy in the sperm was so small as to readily explain why no difference was found in chromosome abnormalities between embryos obtained after ICSI vs. standard IVF (31, 32, 72). Furthermore, sperm aneuploidy would not be likely to account for the high percentage of abnormal embryos (either mosaic or aneuploid) in our TESE cases.

Testicular sperm extraction combined with ICSI has now enabled the treatment of couples with nonobstructive azoospermia (7, 8, 11, 18, 24, 56). Although there is a higher risk of chromosomal abnormalities in the peripheral lymphocytes of this population of men, the majority of these men are karyotypically normal. There have been several conflicting studies, by Levron et al. (70), by Martin et al. (67), and by Palermo et al. (68), of the sperm found in the testes of men with nonobstructive azoospermia. Martin et al. (67) showed no increased incidence of aneuploidy in the testicular spermatozoa of patients. But only three patients were analyzed, and they all had normal FSH levels. Levron et al. (70) showed the opposite; that is, there was a higher incidence of sperm aneuploidy in men with nonobstructive azoospermia. The results of Palermo et al. (68) agree with the findings of Levron et al. (67), but again only five patients, and very few sperm, were studied. These studies were all 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.

It is known that aneuploidy of embryos is not closely associated, or correlated, with embryo morphology. As women age and the rates of aneuploidy increase, abnormalities in embryo morphology do not increase (32). However, mosaicism, chaotic mosaicism, and polyploidy are associated with an increase in morphologic abnormalities in the embryos and do not increase with age. Aneuploidy appears to increase with maternal age and is related to defects in the egg, but mosaicism and chaotic mosaicism may be more related to defects in the sperm and may result in a high percentage of chaotic mosaic embryos derived from ICSI with nonobstructive azoospermia.

The high rate of mosaic embryos that have been observed resulting from TESE-ICSI may be related more 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 did ICSI with ejaculated sperm from men with higher sperm production rates. However, there was a dramatically increased rate of mosaic errors in these embryos resulting from abnormal mitosis; this rate could conceivably be related to defects in the sperm centriole (71). Similarly, an early report on microsurgical epididymal sperm aspiration–ICSI for obstructive azoospermia in which distal (senescent) epididymal sperm were used demonstrated an inexplicably high miscarriage rate despite the young age of the female partners (12). This phenomenon might also be explained by defects in embryo cleavage related to centriole dysfunction (72, 73). Thus, the most severe degrees of spermatogenic defect, resulting in nonobstructive azoospermia and requiring testicular sperm extraction, or even senescent, nonmotile, sperm from distal epididymis, may result in a higher frequency of chromosomal abnormalities. But those abnormalities may be related more to errors in mitosis during early cleavage of the embryo than to sperm aneuploidy.

The present data point toward a male origin of chaotic embryos. Because the first mitotic divisions are controlled by the spermatozoon centrosome (74), 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 by suboptimal centriole function (75–77). Sperm integrity is clearly necessary for normal mitotic division and early embryonic development (78).

Thus, severe spermatogenic defects, as in nonobstructive azoospermia, may result in a higher percentage of mosaic and chaotic mosaic embryos, resulting in less efficient implantation and delivered pregnancy rates.

References

1. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of a single spermatozoa into an oocyte. Lancet 1992;340:17–8.
2. Van Steirteghem AC, Nagy Z, Joris H, Liu J, Staessen C, Smitz J, et al. High fertilization and implantation rates after intracytoplasmic sperm injection. Hum Reprod 1993;8:1061–6.
3. Silber SJ, Van Steirteghem AC, Devroey P. Sertoli cell only revisited. Hum Reprod 1995;10:1031–2.
4. Nagy Z, Liu J, Janssenwillen C, Silber S, DeVroey P, Van Steirteghem AC. Comparison of fertilization, embryo development, and pregnancy rates after intracytoplasmic sperm injection using ejaculated, fresh, and frozen thawed epididymal and testicular sperm. Fertil Steril 1995;63: 808–15.

5. Liu J, Nagy Z, Joris H, Tournaye H, Devroey P, Van Steirteghem AC. Intracytoplasmic sperm injection does not require a special treatment of the spermatozoa. Hum Reprod 1994;9:1127–30.
6. Liu J, Nagy Z, Joris H, Tournaye H, Smitz J, Camus M, et al. Analysis of 76 total fertilization failure cycles out of 2,732 intracytoplasmic injection cycles. Hum Reprod 1995;10:2630–6.
7. Schoysman R, Vanderzwalmen P, Nijs M, Segal L, Segal-Bertin G, Geerts L, et al. Pregnancy after fertilization with human testicular spermatozoa. Lancet 1993;342:1237.
8. Schoysman R, Van Der Zwalmen P, Nijs M. Successful fertilization by testicular spermatozoa in an in vitro fertilization program. Hum Reprod 1993;8:1339–40.
9. Devroey P, Liu J, Nagy A, Tournaye H, Silber SJ, Van Steirteghem AC. Normal fertilization of human oocytes after testicular sperm extraction and intracytoplasmic sperm injection (TESE and ICSI). Fertil Steril 1994;62:639–41.
10. Silber SJ, Nagy ZP, Liu J, Godoy H, Devroey P, Van Steirteghem AC. Conventional IVF versus ICSI for patients requiring microsurgical sperm aspiration. Hum Reprod 1994;9:1705–9.
11. Silber SJ, Van Steirteghem AC, Liu J, Nagy Z, Tournaye H, Devroey P. High fertilization and pregnancy rates after ICSI with spermatozoa obtained from testicle biopsy. Hum Reprod 1995;10:148–52.
12. Tournaye H, Devroey P, Liu J, Nagy Z, Lissens W, Van Steirteghem A. Microsurgical epididymal sperm aspiration and intracytoplasmic sperm injection: a new effective approach to infertility as a result of congenital bilateral absence of the vas deferens. Fertil Steril 1994;61:1045–51.
13. Devroey P, Silber S, Nagy Z, Liu J, Tournaye H, Joris H, et al. Ongoing pregnancies and birth after intracytoplasmic sperm injection (ICSI) with frozen-thawed epididymal spermatozoa. Hum Reprod 1995;10: 903–6.
14. Steinberger E, Tjioe DY. A method for quantitative analysis of human seminiferous epithelium. Fertil Steril 1968;19:960–70.
15. Clermont Y. Kinetics of spermatogenesis in mammals: seminiferous epithelium cycles in spermatogonial renewal. Physiol Rev 1972;52: 198–236.
16. Zukerman Z, Rodriguez-Rigau L, Weiss DB, Chowdhury LJ, Smith KD, Steinberger E. Quantitative analysis of the seminiferous epithelium in human testicle biopsies and the relation of spermatogenesis to sperm density. Fertil Steril 1978;30:448–55.
17. Silber SJ, Rodriguez-Rigau LJ. Quantitative analysis of testicle biopsy: determination of partial obstruction and prediction of sperm count after surgery for obstruction. Fertil Steril 1981;36:480–5.
18. Devroey P, Liu J, Nagy Z, Goossens A, Tournaye H, Camus M, et al. Pregnancies after testicular extraction (TESE) and intracytoplasmic sperm injection (ICSI) in non-obstructive azoospermia. Hum Reprod 1995;10:1457–60.
19. Silber SJ, Nagy Z, Liu J, Tournaye H, Lissens W, Ferec C, et al. The use of epididymal and testicular spermatozoa for intracytoplasmic sperm injection: the genetic implications for male infertility. Hum Reprod 1995;10:2031–43.
20. Silber SJ, Alagapan R, Brown LG, Page DC. Y chromosome deletions in azoospermic and severely oligospermic men undergoing intracytoplasmic sperm injection after testicular sperm extraction. Hum Reprod 1998;13:3332–7.
21. Silber SJ, Nagy Z, Devroey P, Tournaye H, Van Steirteghem AC. Distribution of spermatogenesis in the testicles of azoospermic men: the presence or absence of spermatids in the testis of men with germinal failure. Hum Reprod 1997;12:2422–8.
22. Silber SJ, Devroey P, Camus M, Van Steirteghem AC. The effect of female age and ovarian reserve on pregnancy rate in male infertility: treatment of azoospermia with sperm retrieval and intracytoplasmic sperm injection. Hum Reprod 1997;12:2693–700.
23. Silber SJ. The varicocele dilemma. Hum Reprod Update 2001;7:70–7.
24. Silber SJ. Microsurgical TESE and the distribution of spermatogenesis in non-obstructive azoospermia. Hum Reprod 2000;15:2278–84.
25. Nagy Z, Liu J, Joris H, Verheyen G, Tournaye H. The result of intracytoplasmic sperm injection is not related to any of the three basic sperm parameters. Hum Reprod 1995;10:1123–9.
26. Moosani N, Pattinson HA, Carter MD, Cox DM, Rademaker AW, Martin RH. Chromosomal analysis of sperm from men with idiopathic infertility using sperm karyotyping and fluorescence in situ hybridization. Fertil Steril 1995;64:81–817.
27. Silber SJ. What forms of male infertility are there left to cure? Hum Reprod 1995;10:503–4.
28. Bonduelle M, Wilikens J, Buysse A, Van Assche E, Wisanto A, Devroey P, et al. Prospective study of 877 children born after intracytoplasmic sperm injection with ejaculated, epididymal, and testicular spermatozoa, and after replacement of cryopreserved embryos obtained after ICSI. Hum Reprod 1996;11:131–59.
29. Martin R. The risk of chromosomal abnormalities following ICSI. Hum Reprod 1996;11:924–5.
30. Huang WJ, Lamb DJ, Kim ED, de Lara J, Lin WW, Lipshultz LI, et al. Germ-cell nondisjunction in testes biopsies of men with idiopathic infertility. Am J Hum Genet 1999;64:1638–45.
31. Munné S, Marquez C, Reing A, Garrisi J, Alikani M. Chromosome abnormalities in embryos obtained after conventional in vitro fertilization and intracytoplasmic sperm injection. Fertil Steril 1998;69:904–8.
32. Munné S, Cohen J. Chromosome abnormalities in human embryos. Hum Reprod Update 1998;4:842–55.
33. Palermo GD, Schleel PN, Hariprashad JJ, Ergun B, Mielnik A, Zaniovic N, et al. Fertilization and pregnancy outcome with inracytoplasmic sperm injection for azoospermic men. Hum Reprod 1999;14: 741–8.
34. Bonduelle M, Aytoz A, Wilikens A, Buysse A, Van Assche E, Devroey P, et al. Prospective follow-up study of 1,987 children born after intracytoplasmic sperm injection (ICSI). In: Filicori M, Flamigni C, eds. Treatment of infertility: the new frontiers. Princeton, NJ: Communications Media for Education, 1998:445–61.
35. Bonduelle M, Aytoz A, Van Assche E, Devroey P, Liebaers I, Van Steirteghem A. Incidence of chromosomal aberrations in children born after assisted reproduction through intracytoplasmic sperm injection. Hum Reprod 1998;13:781–2.
36. Bonduelle M, Camus M, De Vos A, Staessen C, Tournaye H, Van Assche E, et al. Seven years of intracytoplasmic sperm injection and follow-up of 1,987 subsequent children. Hum Reprod 1999;14:243–64.
37. Aboulghar H, Aboulghar M, Mansour R, Serour G, Amin Y, Al-Inany H. A prospective controlled study of karyotyping for 430 consecutive babies conceived through intracytoplasmic sperm injection. Fertil Steril 2001;76:249–53.
38. Abyholm T, Stray-Pedersen S. Hypospermiogenesis and chromosomal aberrations. A clinical study of azoospermic and oligospermic with normal and abnormal karyotype. Int J Androl 1981;4:546–58.
39. Egozcue J, Templado C, Vidal F, Navarro J, Morer-Fargas F, Marina S. Meiotic studies in a series of 1100 infertile and sterile males. Hum Genet 1983;65:185–8.
40. Pandiyan N, Jequier AM. Mitotic chromosomal anomalies among 1210 infertile men. Hum Reprod 1996;11:2604–8.
41. Testart J, Gautier E, Brami C, Rolet F, Sedmon E, Thebault A. Intracytoplasmic sperm injection in infertile patients with structural chromosome abnormalities. Hum Reprod 1996;11:2609–12.
42. Van Assche EV, Bonduelle M, Tournaye H, Joris H, Verheyen A, Devroey P, et al. Cytogenetics of infertile men. Hum Reprod 1996;11: 1–26.
43. Yoshida A, Miura K, Shirai M. Chromosome abnormalities and male infertility. Assist Reprod Rev 1996;6:93–9.
44. Vegetti W, Van Assche E, Frias A, Verheyen G, Bianchi MN, Bonduelle M, et al. Correlation between semen parameters and sperm aneuploidy rates investigated by fluorescence in situ hybridization in infertile men. Hum Reprod 2000;15:351–65.
45. Colombero LT, Hariprashad JJ, Tsai MC, Rosenwaks Z, Palermo GD. Incidence of sperm aneuploidy in relation to semen characteristics and assisted reproductive outcome. Fertil Steril 1999;72:90–6.
46. Rosenmann A, Wahrham J, Richler C, Voss R, Persitz A, Goldman B. Meiotic association between the XY chromosomes and unpaired autosomal elements as a cause of human male sterility. Cytogenet Cell Genet 1985;39:19–29.
47. Verlinsky Y, Cieslak J, Ivakhnenko V, Lifchez A, Strom C, Kuliev A. Birth of healthy children after preimplantation diagnosis of common aneuploidies by polar body fluorescent in situ hybridization analysis. Fertil Steril 1996;66:126–9.
48. Munné S, Alikani M, Tomkin G, Grifo J, Cohen J. Embryo morphology developmental rates and maternal age are correlated with chromosome abnormalities. Fertil Steril 1995;64:382–91.
49. Marquez C, Sandalinas M, Bahce M, Alikani M, Munné S. Chromosome abnormalities in 1255 cleavage-stage embryos. Reprod Biomed Online 2000;1:17–27.
50. Evsikov S, Verlinsky Y. Mosaicism in the inner cell mass of human blastocysts. Hum Reprod 1998;11:3151–5.
51. Sandalinas M, Sadowy S, Alikani M, Calderon G, Cohen J, Munné S. Developmental ability of chromosomally abnormal human embryos to develop to the blastocyst stage. Hum Reprod 2001;16:1954–8.
52. Munné S, Weier HUG, Grifo J, Cohen J. Chromosome mosaicism in human embryos. Biol Reprod 1994;51:373–9.
53. Harper JC, Coonen E, Handyside AH, Winston RML, Hopman AHN, Delhanty JDA. Mosaicism of autosomes and sex chromosomes in morphologically normal, monospermic preimplantation human embryos. Prenat Diagn 1995;15:41–9.
54. Delhanty JDA, Harper JC, Ao A, Handyside AH, Winston RML. Multicolour FISH detects frequent chromosomal mosaicism and chaotic division in normal preimplantation embryos from fertile patients. Hum Genet 1997;99:755–60.

55. Obasaju M, Kadam A, Sultan K, Fateh M, Munné S. Sperm quality may adversely affect the chromosome constitution of embryos that result from intracytoplasmic sperm injection. Fertil Steril 1999;72:1113–5.
56. Silber SJ, Van Steirteghem A, Nagy Z, Liu J, Tournaye H, Devroey P. Normal pregnancies resulting from testicular sperm extraction and intracytoplasmic sperm injection for azoospermia due to maturation arrest. Fertil Steril 1996;66:110–7.
57. Silber SJ, Johnson L. Are spermatid injections of any clinical value? ROSNI and ROSI revisited. Hum Reprod 1998;13:509–23.
58. Bahce M, Escudero T, Sandalinas M, Morrison L, Legator M, Munne S. Improvements of preimplantation diagnosis of aneuploidy by using microwave-hybridization, cell recycling and monocolor labeling of probes. Mol Hum Reprod 2000;9:849–54.
59. Grifo JA. Preconception and preimplantation genetic diagnosis: polar body, blastomere, and trophectoderm biopsy. In: Cohen J, Malter HE, Talansky BE, Grifo J, eds. Micromanipulation of gametes and embryos. New York: Raven Press, 1992:223–49.
60. Munné S, Dailey T, Finkelstein M, Weier HUG. Reduction in signal overlap results in increased FISH efficiency: implications for preimplantation genetic diagnosis. J Assist Reprod Genet 1996;13:149–56.
61. Munné S, Weier HUG. Simultaneous enumeration of chromosomes 13, 18, 21, X and Y in interphase cells for preimplantation genetic diagnosis. Cytogenet Cell Genet 1996;75:263–70.
62. Miharu N, Best RG, Young SR. Numerical chromosome abnormalities in spermatozoa in fertile and infertile men detected by fluorescence in situ hybridization. Hum Genet 1994;93:502–6.
63. Bernardini I, Martini E, Geraedts JP, Hopman AH, Lanteri S, Conte N, et al. Comparison of gonosomal aneuploidy in spermatozoa of normal fertile men and those with severe male factor detected by in situ hybridization. Mol Hum Reprod 1997;3:431–8.
64. Rives N, Mazurier S, Sibert L. Incidence of aneuploidy in sperm nuclei of infertile men [abstract]. Hum Reprod 1998;13(Suppl):126–7.
65. Rives N, Mazurier S, Bellet D, Joly G, Macé B. Assessment of autosome and gonosome disomy in human sperm nuclei by chromosome painting. Hum Genet 1998;102:616–23.
66. Pang MG, Hoegerman SF, Cuticchin AJ, Moon SY, Doncel GF, Acosta AA, et al. Detection of aneuploidy for chromosomes 4, 6, 7, 8, 9, 10, 11, 12, 13, 17, 18, 21, X and Y by fluorescence in situ hybridization in spermatozoa from nine patients with oligoasthenoteratospermia undergoing intracytoplasmic sperm injection. Hum Reprod 1999;14:1266–73.
67. Martin RH, Greene C, Rademaker A, Barcley L, Ko E, Chernos J. Chromosome analysis of spermatozoa extracted from men with non-obstructive azoospermia. Hum Reprod 2000;15:1121–4.
68. Palermo GD, Colombero LT, Hariprashad JJ, Schlegel PN, Rosenwaks Z. Chromosome analysis of epididymal and testicular sperm in azoospermic patients undergoing ICSI. Hum Reprod 2002;17:570–5.
69. Yogev L, Paz G, Yavetz H. Chromosome analysis of spermatozoa extracted from testes of men with non-obstructive azoospermia. Hum Reprod 2000;15:2685–8.
70. Levron J, Aviram-Goldring A, Madjar I, Raviv G, Barkai G, Dor J. Sperm chromosomal abnormalities in men with severe male factor infertility who are undergoing in vitro fertilization with intracytoplasmic sperm injection. Fertil Steril 2001;63:479–84.
71. Sathananthan AH, Ratnam SS, Ng SC, Tarin JJ, Gianaroli L, Trounson A. The sperm centriole: its inheritance, replication and perpetuation in early human embryos. Hum Reprod 1996;11:345–56.
72. Schatten G. The centrosome and its mode of inheritance: the reduction of the centrosome during gametogenesis and its restoration during fertilization. Dev Biol 1994;155:299–335.
73. Schatten H, Schatten G, Mazia D, Balczon R, Simerly C. Behavior of centrosomes during fertilization and cell division in mouse oocytes and in sea urchin eggs. Proc Natl Acad Sci USA 1986;83:105–9.
74. Palermo G, Munné S, Cohen J. The human zygote inherits its mitotic potential from the male gamete. Hum Reprod 1994;9:1220–5.
75. Zhou H, Kuang J, Zhong L, Kuo WL, Gray JW, Shanin A, et al. Tumor amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. Nat Genet 1998;20:189–93.
76. Doxsey S. The centrosome—a tiny organelle with big potential. Nat Genet 1998;20:104–6.
77. Sathasivam K, Woodman B, Mahal A, Bertaux F, Wanker EE, Shima DT, et al. Centrosome disorganization in fibroblast cultures derived from R6/2 Huntington's disease (HD) transgenic mice and HD patients. Hum Mol Genet 2001;21:2425–35.
78. Moomjy M, Colombero LT, Veeck L, Rosenwaks Z, Palermo GD. Sperm integrity is critical for normal mitotic division and early embryonic development. Mol Hum Reprod 1999;5:836–44.