

Cytogenetic Investigations of Infertile Men With Low Sperm Counts: A 25-Year Experience

Minireview

MARIE-CLAIRE VINCENT,* MYRIAM DAUDIN,†‡
PHILIPPE DE MAS,* GERARD MASSAT,‡
ROGER MIEUSSET,‡ FRANCIS PONTONNIER,†
PATRICK CALVAS,* LOUIS BUJAN,†‡ AND
GEORGES BOURROUILLOU*†

*From the *Service de Génétique Médicale, Hôpital Purpan; †C.E.C.O.S. Midi-Pyrénées and ‡Laboratoire sur la Fertilité Humaine Centre de Stérilité Masculine, Hôpital La Grave, Toulouse, France.*

Intracytoplasmic sperm injection (ICSI) is a providential therapeutic alternative for men with severe sperm count abnormalities. This method is being used increasingly and has given rise to numerous debates (Lamb, 1999; Schlegel, 1999). In fact, male infertility may be associated with several constitutional defects in genes and chromosomes (excluding Yq microdeletions). The existence of cytogenetic abnormalities in blood lymphocytes from infertile men has been reported in several surveys (Chandley, 1979; Croquette and Fourlinnie, 1980; Bourrouillou et al, 1985; Koulischer and Gillerot, 1985; De Braekeler and Dao, 1991; Yoshida et al, 1997). Most of these studies report wide frequencies of chromosomal anomalies, from 2.2% to 10.3%, due to different ascertainment procedures. Nevertheless, all of them point to an increasing percentage of chromosomal abnormalities concomitant with decreasing sperm count. In addition, the nature of chromosomal anomalies differs depending on whether a patient has oligozoospermia or azoospermia. In other respects, because cytogenetic investigative procedures are expensive and time-consuming, some authors (Pandiyani and Jequier, 1996) have aimed to determine the break-even point for the best cost:benefit ratio in the chromosomal survey of infertile men. In the present paper, based on cytogenetic findings in 2651 infertile men, we conclude that blood karyotyping should be performed on infertile probands when the sperm count is less than 5 million/mL.

Patients and Methods

Since 1972, 13 154 men have been clinically examined and biologically investigated at the Centre de Stérilité

Masculine Midi-Pyrénées in an effort to accurately determine the cause of their infertility. Among them, 2651 patients had sperm counts ≤ 20 million/mL in 3 distinct ejaculate specimens during a 1-year period, and agreed to a blood cytogenetic investigation. To comply with the laws of France, each patient gave an informed consent for genetic testing. Our population was classified as follows: one group of patients with azoospermia, either with non-obstructive and obstructive azoospermia; and another group of patients with oligozoospermia, categorized according to sperm count into those with severe (less than 5 million/mL), moderate (from 5 to 10 million/mL), or mild oligozoospermia (ranging from >10 to 20 million/mL). All groups contained a similar number of patients, with the exception of the obstructive azoospermia group. Blood lymphocyte karyotyping was performed at the Service de Génétique Médicale. At least 30 metaphases were analyzed by GTG-banding, RHG-banding, or both; and by QFQ-banding when Y chromosome abnormality was observed. More than 50 metaphases were screened when mosaicism was observed.

Since 1996, we have used fluorescence in situ hybridization (FISH) to examine structural abnormal karyotypes, through the use of FISH chromosome-painting with total chromosome probes (Oncor, Strasbourg, France), or with FISH-specific Chromoprobe T XpYp and XqYq probes (Cytocell, Oxford, United Kingdom) and CEP Y probe (Vysis, Downer's Grove, Ill). Molecular characterization of Yq deletions was not considered in this study. All patients bearing an abnormal karyotype were offered genetic counseling. For statistical analysis, percentages were compared with the use of the Chi-square test. Results were considered statistically significant when $P < .05$.

Results

Table 1 shows population characteristics, and the number and main types of chromosomal abnormalities found in our population. We observed 204 chromosomal anomalies. All were analyzed by banding techniques: 96 by GTG-banding; 43 by RHG-banding; 36 by both GTG- and RHG-banding; 23 by GTG- and QFQ-banding; 5 by RHG- and QFQ-banding; and 1 by GTG-, RHG-, and QFQ-banding. FISH was used to specify 2 autosomal structural complex rearrangements; 3 autosomal reciprocal translocations; and 8 cases of Y structural anomaly, Y mosaicism, or both. In one infertile man with 46,XX

Correspondence to: Dr Georges Bourrouillou, Service de Génétique Médicale, Hôpital Purpan, 31059 Toulouse Cedex, France (e-mail: bourrouillou.g@chu-toulouse.fr).

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Table 1. *Chromosomal anomalies and sperm count distribution in our series of 2651 infertile men**

	Azoospermia		Oligozoospermia			Total sample
	Obstructive	Non-obstructive	Severe <5 × 10 ⁶ /mL	Moderate 5-10 × 10 ⁶ /mL	Mild >10-20 × 10 ⁶ /mL	
No. of subjects	144	648	648	628	583	2651
Sex Chromosomes anomalies						
X		91	10			101
Y	1	10	11	7	1	30
Autosomal anomalies						
Robertsonian translocations		3	26	8	1	38
Reciprocal translocations	2	4	10	6	1	23
Others			6	6		12
Total No. anomalies	3	108	63	27	3	204
Percentage	2.1 ^d	16.7 ^{a,d}	9.7 ^{a,b}	4.3 ^{b,c}	0.5 ^c	7.7

* Meaningful statistically different groups are tagged with the same superscript letters (a, b, c, d; $P < .001$).

karyotype, we used the CEP Y probe in FISH analysis to find Y chromosome material after having detected the SRY sequence by molecular investigation.

Somatic cytogenetic abnormalities were observed in 7.7% ($\pm 1.0\%$) of the entire population of patients with infertility probands. This percentage was variable and decreased as the sperm count increased, from 16.7% ($\pm 2.9\%$) in the group with nonobstructive azoospermia; to 9.7% ($\pm 2.3\%$), 4.3% ($\pm 1.6\%$) and 0.5% ($\pm 0.6\%$) in the groups with probands that demonstrated severe, moderate, and mild oligozoospermia, respectively. All values differed significantly from each other ($P < .001$). The rate of chromosomal anomalies was 2.1% ($\pm 2.3\%$) in the group of patients with obstructive azoospermia. However, considering the large standard deviation and small sample size of this group, this rate cannot be accurately inter-

preted and statistically compared with that of the oligozoospermia groups.

According to sperm characteristics, the nature of chromosomal abnormalities was variable. Sex chromosome anomalies were observed more frequently in patients with nonobstructive azoospermia (77.1%) versus those with oligozoospermia and obstructive azoospermia ($P < .001$; Table 1). Among sex chromosome anomalies (Table 2), Y-chromosome structural aberrations were primarily associated with nonobstructive azoospermia and severe oligozoospermia, which is significantly different from the other groups ($P < .001$). Conversely, autosomal anomalies were observed more frequently in the oligozoospermia groups (87.7%) than in the nonobstructive azoospermia group ($P < .001$; Table 1). Autosomal structural anomalies (Table 3) were, interestingly, encountered pri-

Table 2. *Sex chromosome anomalies and sperm count distribution in 2651 infertile men*

	Azoospermia		Oligozoospermia			Total sample
	Obstructive	Non-obstructive	Severe <5 × 10 ⁶ /mL	Moderate 5-10 × 10 ⁶ /mL	Mild >10-20 × 10 ⁶ /mL	
No. of subjects	144	648	648	628	583	2651
X chromosome		91	10			101
47, XXY		83	5			88
47, XXY/46, XY		2	5			7
46, XX		5				5
Structural anomalies		1				1
Y chromosome	1	10	11	7	1	30
47, XXY			2	7		
45, XO		1				
45, XO/46, XX		3	1			
Mosaics*		5	2			
Structural anomalies		1	6			
inv(Y) (p11 ; q11)	1				1	
Total No. anomalies†	1	101 ^a	21 ^{a,b}	7 ^b	1	131

* Mosaicism of structural and/or numerical anomalies.

† Meaningful statistically different groups are tagged with the same superscript letters; a indicates $P < .001$; b, $P < .01$.

Table 3. Autosomal anomalies and sperm count distribution in 2651 infertile men

	Azoospermia		Oligozoospermia			Total sample
	Obstructive	Non-obstructive	Severe <5 × 10 ⁶ /mL	Moderate 5-10 × 10 ⁶ /mL	Mild >10-20 × 10 ⁶ /mL	
No. of subjects	144	648	648	628	583	2651
Robertsonian translocations		3	26	8	1	38
t(13;14)		2	22	6		30
Others		1	4	2	1	8
Reciprocal translocations	2	4	10	6	1	23
Inversions			6	2		8
Markers				4		4
Total no. anomalies*	2	7 ^{b,c}	42 ^{a,c}	20 ^{a,b}	2	73

* Meaningful statistically different groups are tagged with the same superscript letters; a and b indicate $P < .01$; c, $P < .001$.

marily in severe oligozoospermia, and the difference observed between severe and moderate oligozoospermia ($P < .01$) appeared significant, whereas the difference between severe oligozoospermia and nonobstructive azoospermia was striking ($P < .001$). Not surprisingly, all types of structural aberrations could be observed. But, as has been mentioned in most previous studies, the frequency of Robertsonian translocations between chromosomes 13 and 14 is higher than that of balanced reciprocal translocations, and is 20 times higher than that observed in newborns (Hook and Hamerton, 1977).

Discussion

Due to the successful development of ICSI, several teams working in the field of human reproduction claim that a chromosomal analysis should be performed in infertile men. In fact, male infertility is significantly associated with sperm chromosomal aberrations and thus could lead to men bearing offspring with chromosomal imbalances. To date there is no consensus for the delineation of an at risk population, and some authors have proposed that karyotyping should be performed for all infertile men (Meschede et al, 1995), and especially for men with nonobstructive azoospermia before they undergo testicular sperm extraction (Rucker et al, 1998). Other authors propose to extend this investigation to female partners of all infertile men (Mau et al, 1997; Peschka et al, 1999). It is noteworthy that although the number of medically assisted procreation procedures is consistently increasing, the number of cytogenetic laboratories remains constant in most countries, as does the number of karyotype examinations they are able to carry out. In addition, karyotyping is relatively expensive and, as such, is not always offered to patients who have access to medically assisted reproduction. So, we agree with Pandiyan and Jequier (1996), that it would be of great interest to define the criteria, with a better cost:benefit ratio, that would be useful for triggering a directed chromosomal examination of a population of at risk infertile men.

The data presented summarize a 25-year survey of chromosomal anomalies in infertile men. We retained all subjects for chromosomal examination if within 1 year they had 3 sperm counts of less than $20 \times 10^6/\text{mL}$. Over the years, an increasing number of patients were included in the series, and we never observed a change in the rate or the nature of the chromosomal aberrations among 952 subjects (Bourrouillou et al, 1985), 1612 subjects (Bourrouillou et al, 1992), and the present study. In fact, when using identical criteria, other teams (Retief et al, 1984) obtained the same results. In patients with oligozoospermia, we can estimate that the lower the sperm count, the higher the rate of chromosomal abnormality. In the subgroup of patients with severe oligozoospermia, the rate of chromosomal aberrations reached 9.7%. Thus we believe that chromosomal examination is necessary in these patients. In contrast, the observed rate of chromosomal abnormalities in patients with mild oligozoospermia was not significantly different from the rate reported in previous studies and remained close to the rate of 0.6% observed in the general population (Hook and Hamerton, 1977). Today, ICSI offers men with nonobstructive azoospermia the opportunity to become fathers (Schlegel et al, 1997). Because the rate of chromosomal anomalies reached 16.7% in this group, we strongly recommend that chromosomal examination be performed in these patients. Such a high rate of genetic anomalies is in agreement with other articles on the subject (Chandley, 1979; Croquette and Fourlinnie, 1980; Retief et al, 1984; Koulischer and Gillerot, 1985; De Braekeler and Dao, 1991; Yoshida et al, 1997).

It is noteworthy that 7.7% of infertile men with non-obstructive azoospermia or severe oligozoospermia with no problems other than infertility have Klinefelter syndrome. So, beside the psychological benefit for these patients to know the etiology of their infertility, detection of Klinefelter syndrome allows for androgenic substitutive therapy and follow-up for other health problems that can accompany this genetic problem. In our experience

the majority of patients with Klinefelter syndrome chose assisted reproduction with a donor without reluctance, but some patients with nonmosaic Klinefelter syndrome can rely on ICSI with testicular sperm extraction after genetic counseling (Palermo et al, 1998; Reubinoff et al, 1998).

When patients repeatedly had a sperm count of less than $5 \times 10^6/\text{mL}$, we considered them to have severe oligozoospermia. This group is clearly distinct from the groups with moderate ($5\text{--}10 \times 10^6/\text{mL}$) and mild ($>10\text{--}20 \times 10^6/\text{mL}$) oligozoospermia by the number of chromosomal anomalies observed (ie, 9.7%, 4.3%, and 0.5% in severe, moderate, and mild oligozoospermia, respectively). The nature of the structural autosomal anomalies encountered did not differ between these groups. The rate of anomalies detected represents the best criteria to trigger a chromosomal investigation. In addition, as these aberrations could segregate in a balanced or nonbalanced way in the offspring of carriers and their relatives, these results have important implications for genetic counseling. Because it is easy to detect these aberrations, an efficient screening of carriers can be offered to all at risk family members who possess a particular proband. Women relatives are likely to be asymptomatic, whereas men may bear the same anomaly without concomitant infertility (Pauer et al, 1997). In children born with unbalanced chromosomal abnormalities, the carrier of the abnormality is in fact, more likely to be the mother than the father (Boue et al, 1984; Daniel and Hook, 1989; Guichaoua et al, 1990; Gardner and Sutherland, 1996). In cases in which carriers are fertile or can benefit from ICSI, a prenatal diagnosis should be discussed and several ways to manage the chromosomal risk in a fetus can be proposed. A diagnosis can be offered during pregnancy by mean of performing a chromosomal examination of the fetus (ie, via amniocentesis or chorionic villus sampling). Alternatively, a noninvasive prenatal test (maternal serum screening for Down syndrome and fetal ultrasonography) would efficiently lower the risk of a chromosomal anomaly in such pregnancies (Meschede et al, 1998). More recently, a preimplantation genetic diagnosis is being offered under certain circumstances (Conn et al, 1998). The screening policies in most developed countries warrant a fetal chromosomal investigation when the risk for unbalanced chromosomal abnormalities reaches 0.5% or more. According to our survey, a similar risk should be expected in pregnancies that occur from men with a sperm count of less than $5 \times 10^6/\text{mL}$.

Indications for cytogenetic analysis of patients with moderate oligozoospermia is controversial. Patients with a sperm count ranging from $5 \times 10^6/\text{mL}$ to $10 \times 10^6/\text{mL}$ have two times fewer chromosomal anomalies than subjects with severe oligozoospermia. Nevertheless, the 4.7% prevalence of chromosomal aberrations is not negligible and karyotyping may be proposed before assisted repro-

duction is offered, especially if a patient demonstrates a family history of reproductive problems. Moreover, the cost of a routine blood karyotype (\$375–\$600 in the United States; 120 euros in France) pales in comparison with the societal costs of caring for a disabled child and the psychological burdens on families.

The number of chromosomal anomalies in the group with obstructive azoospermia is too low to draw any conclusion, but one should keep in mind that many patients may have vas deferens aplasia, which is often the result of a gene defect, and although it is invisible on a karyotype, requires a genetic diagnosis and counseling (Daudin et al, 2000).

In conclusion, our 25-year gathering of data on cytogenetic investigations in infertile men undoubtedly confirms previous reports and points to a risk of chromosomal abnormalities that is 20-fold higher in patients with severe oligozoospermia or nonobstructive azoospermia, than in the general population. A precautionary principle and our arguments confirm that a blood karyotype analysis prior to assisted reproduction, especially ICSI, should be carried out in infertile men with severe oligozoospermia and nonobstructive azoospermia.

References

- Boue A, Gallano P. A collaborative study of the segregation of inherited chromosome structural rearrangements in 1356 prenatal diagnoses. *Prenat Diagn.* 1984;4(special issue):45–67.
- Bourrouillou G, Dastugue N, Colombies P. Chromosome studies in 952 infertile males with a sperm count below 10 million/ml. *Hum Genet.* 1985;71:366–367.
- Bourrouillou G, Bujan L, Calvas P, Colombies P, Mansat A, Pontonnier F. Place et apports du caryotype en infertilité masculine. *Prog Urol.* 1992;2:189–195.
- Chandley AC. The chromosomal basis of human subfertility. *Br Med Bull.* 1979;35:181–185.
- Conn CM, Harper JC, Winston RML, Delhanty JDA. Infertile couples with Robertsonian translocations: preimplantation genetic analysis of embryos reveals chaotic cleavage divisions. *Hum Genet.* 1998;102:117–123.
- Croquette M-F, Fourlinnie J-C. Intérêt du caryotype dans les azoospermies et les oligo-asthénospermies sévères. *J Gyn Biol Reprod.* 1980;9:177–178.
- Daniel A, Hook EB. Risks of unbalanced progeny at amniocentesis to carriers of chromosome rearrangements: data from United States and Canadian laboratories. *Am J Med Genet.* 1989;31:14–53.
- Daudin M, Bieth E, Bujan L, Massat G, Pontonnier F, Miesusset R. Congenital bilateral absence of the vas deferens: clinical characteristics, biological parameters, cystic fibrosis transmembrane conductance regulator factor gene mutations, and implications for genetic counseling. *Fertil Steril.* 2000;74;6:1164–1174.
- De Braekeleer M, Dao TN. Cytogenetic studies in male infertility: a review. *Hum Reprod.* 1991;2:245–250.
- Gardner RJM, Sutherland GR. *Chromosomes Abnormalities and Genetic Counseling.* 2nd ed. Oxford, United Kingdom: Oxford University Press; 1996.
- Guichaoua MR, Quack B, Speed RM, Noel B, Chandley AC, Luciani J-M. Infertility in human males with autosomal translocations: meiotic

- studies of 14;22 Robertsonian translocation. *Hum Genet.* 1990;86:162–166.
- Hook EB, Hamerton JL. The frequency of chromosome abnormalities detected in consecutive newborn studies. Results by sex and severity of phenotype involvement. In: Hook EB, Porter IH, eds. *Population Cytogenetics: Studies in Humans*. London: Academic Press; 1977:63–79.
- Koulischer L, Gillerot Y. Chromosomes et infertilité, étude de 7672 cas. *Contracept Fertil Sex.* 1985;13:561–568.
- Lamb DJ. Debate: is ICSI a genetic time bomb? Yes. *J Androl.* 1999;20:23–33.
- Mau UA, Bäckert IT, Kaiser P, Kiesel L. Chromosomal findings in 150 couples referred for genetic counseling prior to intracytoplasmic sperm injection. *Hum Reprod.* 1997;12:930–937.
- Meschede D, De Geyter C, Nieschlag E. Genetic risk in micromanipulative assisted reproduction. *Hum Reprod.* 1995;10:2880–2886.
- Meschede D, Lemcke B, Stüssel J, Louwen F, Horst J. Strong preference for non-invasive prenatal diagnosis in women pregnant through intracytoplasmic sperm injection (ICSI). *Prenat Diagn.* 1998;18:700–705.
- Palermo GD, Schlegel PN, Sills E, Veeck LL, Zaninovic N, Menendez S, Rosenwaks Z. Births after intracytoplasmic injection of sperm obtained by testicular extraction from men with non-mosaic Klinefelter's syndrome. *New Engl J Med.* 1998;338:588.
- Pandiyan N, Jequier AM. Mitotic chromosomal anomalies among 1210 infertile men. *Hum Reprod.* 1996;11:2604–2608.
- Pauer HU, Hinney B, Michelmann HW, Krasemann EW, Engel W. Relevance of genetic counseling in couples prior to intracytoplasmic sperm injection. *Hum Reprod.* 1997;9:1909–1912.
- Peschka B, Leygraaf J, Van der Ven K, Montag M, Schartmann B, Schubert R, Van der Ven H, Schwanz G. Type and frequency of chromosome aberrations in 781 couples undergoing intracytoplasmic sperm injection. *Hum Reprod.* 1999;14:2257–2263.
- Retief AE, Van Zyl JA, Menkveld R. Chromosome studies in infertile males with a sperm count below 10 million/ml. *Hum Genet.* 1984;66:162–164.
- Reubinoff BE, Abeliovich D, Werner M, Schenker JG, Safran A, Lewin A. A birth in non-mosaic Klinefelter's syndrome after testicular fine needle aspiration, intracytoplasmic sperm injection and preimplantation genetic diagnosis. *Hum Reprod.* 1998;13:1887–1892.
- Rucker GB, Mielnik A, King P, Goldstein M, Schlegel PN. Preoperative screening for genetic abnormalities in men with nonobstructive azoospermia before testicular sperm extraction. *J Urol.* 1998;160:2068–2071.
- Schlegel PN, Palermo GD, Goldstein M, Menendez S, Zaninovic N, Veeck LL, Rosenwaks Z. Testicular sperm extraction with intracytoplasmic sperm injection for nonobstructive azoospermia. *Urology.* 1997;49:435.
- Schlegel PN. Debate: is ICSI a genetic time bomb? No: ICSI is safe and effective. *J Androl.* 1999;20:18–22.
- Yoshida A, Miura K, Shirai M. Cytogenetic survey of 1,007 infertile males. *Urol Int.* 1997;58:166–176.