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Chromosome Studies in Patients with Defective Reproductive Success

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PROBLEM: The objective of this study was to evaluate the contribution of chromosomal anomalies to decreased fertility in humans.

METHOD OF STUDY: In order to investigate the aetiology of infertility in our population and to assess the karyotype in a group of infertile couples and individuals with fertility problems, 782 persons (259 couples, 158 male and 106 female) with different clinical diagnoses of sterility and infertility were analysed cytogenetically.

RESULTS: The overall frequency of major chromosomal aberration was 13.1% (103/783), which suggests that fertility or sterility problems in this population are due to chromosomal aberrations. Couples experiencing repeated spontaneous abortions, having malformed children or having sterility problems had chromosomal abnormalities in 18.0% (47/259 couples) of the population studied, and constituted chromosomal disorders occured in couples seeking IVF and ICSI with prevalence of 22.2% (8/38 couples), especially minor mosaicism of sex chromosomes in the female partners. The prevalence of chromosome abnormalities in infertile men was 17.7% (28/158), and in subfertile females, it was 26.4% (28/106).

CONCLUSIONS: These results could indicate an increased tendency to miotic sex chromosome non-disjuction in humans.

Key words:
Chromosomal aberration, fertility, spontaneous abortions, sterility

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INTRODUCTION

Mutant genes and chromosomal disorders can disturb gamete formation and impair normal embryonic development. Besides aneuploid segregation due to paternal chromosomal aberration, a post-zygotic factor can lead to uncontrolled chromosome distribution in early cleavage stages producing mosa-

icism. The reported frequency of chromosome aberration in the general population was less than 1%,[1] while in a group of patients with altered reproductive fitness the percentage was always higher. Ten years ago, the prevalence of chromosomal abnormalities in couples with repeated spontaneous abortions was 2.4–6.8%.[2–5]. Patients having two or more spontaneous abortions or those undergoing in vitro fertilisation-intracytoplasmic sperm injection (IVF-ICSI), have approximately 10% aberrant karyotypes.[6–9] It seems that the prevalence of chromosomal aberrations is increasing slightly with time.

Sex chromosomes are the most commonly involved in human chromosomal aberrations[1] and these aberrations disturb reproductive fitness. The chromosome changes in autosomes, especially reciprocal translocations involving acrocentrics such as Robertsonian translocations, can disturb sperm density by some central effect during spermatogenesis. The presence of a small extra marker chromosome in the karyotype is also a condition associated with the disruption of the human spermatogenic sequence.[10,11] In all of these abnormal chromosomal situations, the sterility of male carriers appears to stem from a defect in spermatogenesis, which leads to the production of few or no spermatozoa. Gametogenesis in female carriers of the same abnormalities appears, however, to be unaffected. Therefore, the risk of having spontaneous abortions or malformed children for female carriers is present, whereas in male carriers, chromosomal aberrations lead to subfertility or sterility. Constitutional chromosomal abnormalities were found in 14.1% azoospermic[12] and 5.1–11.4% oligospermic[11,12] males. Female patients with one of the Turner syndrome symptoms – short stature – had an overall prevalence of chromosomal aberration of 28.3%,[13] and those with impaired reproductive fitness, such as amenorrhea, had up to 63.3%.[14]

PATIENTS AND METHODS

We cytogenetically analysed 782 persons (259 couples, 158 male and 106 female) with different clinical diagnoses of infertility and sterility. Of these, 38 couples were patients in the Family Planning Department (Clinic for Obstetrics and Gynecology) and were in the program for IVF or ICSI. The karyotype analyses were performed on preparations made from short-term lymphocyte cultures and GTG-banded metaphase chromosomes. Standard methods employing hypotonic treatment before fixation with methanol–acetic acid and slide preparation by the air-drying technique were used.[15] Silver-nucleolar organizer regions[16] (Ag-NOR) staining was performed for cases where acrocentric chromosomes were involved in aberration or for the identification of an extra marker chromosome.

Both spouses were analyzed in the cases of spontaneous abortions and/or couples with malformed child. One spouse was analyzed if oligozoospermia (sperm count below 10×10^6/mL), azoospermia, hypogonadism or unexplained sterility was diagnosed in the male or oligomenorrhea, amenorrhea or unexplained sterility was diagnosed in the female. Some male patients were referred with a clinical diagnosis of Klinefelter syndrome, gynecomastia or chryptorchidism. We categorized women referred with the clinical diagnoses of Turner syndrome, hypogonadism diagnosed by ultra sound or short stature, as separate test groups.

For the each patient, 20 mitosis were analyzed routinely. If one aneuploid cell was found, at least an additional 100 mitoses were analyzed.

RESULTS

The overall prevalence of major chromosomal aberration was 13.1% (103/783), and this finding suggests that defective reproductive success is a major symptom of the chromosomal aberration.

The 259 couples were divided into groups having: (1) a normal and/or malformed child (NC/MC), (2) two or less spontaneous abortions ($\leq$ 2SAB), (3) two or less spontaneous abortions and a malformed child ($\leq$ 2SAB + MC), (4) three or more spontaneous abortions ($\geq$ 3SAB) and (5) sterility (Table I). The overall prevalence of aberration was 18.0%, and in the 38 couples seeking IVF and ICSI it was 22.2% (8/38); minor mosaicism of sex chromosomes in female partners was the most common finding in the latter group.

TABLE I. Incidence of Major Chromosomal Aberrations in 259 Couples with Defective Reproductive Success

Clinical diagnosis	Normal karyotype No. (%)	Chromosome aberrations No. (%)
NC/MC	34 (89.5)	4 (10.5)
$\leq$2SAB	111 (82.3)	23 (17.7)*
$\leq$2SAB + MC	9 (75.0)	3 (25.0)
$\geq$3SAB	29 (78.4)	8 (21.6)
Sterility	30 (79.0)	8 (21.0)
Total	213 (82.0)	46 (18.0)

MC, malformed child.
NC, normal child.
SAB, spontaneous abortion.
* *One couple with chromosomal aberration found in both spouses.*

TABLE II. Incidence of Chromosomal Aberrations in 158 Men with Defective Reproductive Success

Clinical diagnosis	Normal karyotype No. (%)	Chromosome aberrations No. (%)
Sterility	14 (77.8)	4 (22.2)
Azoospermia	8 (57.2)	6 (42.8)
Oligozoospermia	10 (83.4)	2 (16.6)
Oligoathenozoo-spermia	7 (63.7)	4 (36.3)
Hypogonadism	67 (89.4)	8 (10.6)
Klinefelter syndrome	5 (62.5)	3 (37.5)
Gynecomastia	11 (91.7)	1 (8.3)
Cryptorchidism	8 (100)	0
Total	130 (82.3)	28 (17.7)

In 158 men with defective reproductive fitness, 17.7% had some chromosomal aberration ($P < 0.0001$, compared to normally fertile men; Table II). No aberration was found in men with a clinical diagnosis of pure chryptorchidism.

In 106 women with defective reproductive success, 26.4% had chromosomal abberations ($P < 0.001$, compared to normally fertile females; Table III). The abberant karyotypes of 259 couples, 158 males and 106 females having defective reproductive fitness are shown in Table IV.

DISCUSSION

The prevalence of chromosomal aberrations in 782 persons with different clinical diagnoses of sterility and fertility (13.1%, 103/783) is considerably greater than that in the general population (less than 1%[1]) and the prevalence of major chromosomal aberrations in couples with decreased reproductive success (18%) is also greater than that previously reported (2.4–6.8%).[2–5] Aberrant karyotypes occured in 22.2% of the couples undergoing an assisted reproductive procedure which is comparable to other reports with couples attending IVF clinics (18%).[17] Forty percent of all chromosomal aberration in patients with impaired reproductive fitness were mosaics (Table IV). Improved techniques alone can not explain these differences and post-zygotic factors which lead to mosaicism in early embryonic development may be implicated.

TABLE III. Incidence of Chromosomal Aberration in 106 Women with Defective Reproductive Success

Clinical diagnosis	Normal karyotype No. (%)	Chromosome aberrations No. (%)
Sterility	4 (80.0)	1 (20.0)
Amenorrhea	29 (78.4)	8 (21.6)
Oligomenorrhea	5 (100)	0
Turner syndrome	13 (65.0)	7 (35.0)
Hypogonadism	5 (50.0)	5 (50.0)
Short stature	22 (75.9)	7 (24.1)
Total	78 (73.6)	28 (26.4)

The prevalence of chromosomal abnormalities in infertile men was 10–14 times higher than expected in the normal population (Table II).[1] The consistency of results[10,11] seems to justify a diagnostic chromosomal analysis in subfertile men (sperm count below 10×10^6/mL). Three structural chromosomal aberrations of autosomes were found in males with normal sperm counts among the couples with repeated spontaneous abortions. These results suggest that some chromosomal changes, mostly in men, may cause infertility by gametic selection. The only phenotypic manifestation in some chromosomal aberrations may be disturbed spermatogenesis (which does not occur in oogenesis). Besides major chromosomal aberrations, large blocks of duplicated heterochromatin can be a factor disturbing reproduction.[18]

The most commonly involved chromosomal aberrations in subfertile and sterile males and in females are sex chromosomes (Table IV). Two inversions of chromosome 9 and extrabisatelit chromosome in a man with a sperm count of 13×10^6/mL were also found. This rare aberration has been reported in oligoasthenozoospermic males, as a cause of disrupted spermatogenesis[19] and in normal individuals with apparently no phenotype effect but with increased risk of aneuploid gametes.[20]

The overall frequency of chromosomal abnormalities in subfertile females group was 26.4% (28/106) which is significantly different from the control frequency of 0.4% for women in general.[1] Amenorrhea, hypogonadism and Turner syndrome are clear manifestations of female chromosomal aberrations involving the X chromosome (Table I).

We conclude that chromosomal disorders are the underlying bases of infertility and sterility in a higher proportion of cases than had previously been expected. An important contributing factor is the number of chromosomal abnormalities due to hidden mosaics, which may be detectable in tissues other than peripheral blood lymphocytes. Detailed cytogenetic analyses of both males and females with decreased reproductive fitness is essential for predicting the success of assisted reproductive procedures.

TABLE IV. Chromosomal aberrations in 782 persons with defective reproductive success

	No. of patients	Structural aberration	No. of patients	Numerical aberration	No. of patients	Mosaicism (mos)	No. of patients
Couples	518	46,XX,inv(9)	5	47, XXY	1	45,X/46,XY	2
		46,XY,inv(9) #	4	47, XYY	1	45,X/46,XX #	8
		46,XX,t(1;15)	1			47,XXY/46,XY	1
		46,XX,t(1;11)	1			47,XXX/46,XX	5
		46,XX,t(8,18)	1			47,XX,+21/46,XX	1
		46,XX,t(3;15)	1			47,XX,+22/46,XX	1
		46,XX,t(1;21)	1			45,X/47,XXX/46,XX	2
		46,XY,t(6;8)	1			45,X/48,XXXX/46,XX	2
		46,XY,t(14;?)	1			45,X/49,XXXXX/46,XX	1
		45,XY,der(13;14)	1			47,XY+?17/47,XY,+mar/46,XY	1
		46,XY,t(11,14)	1			45,X,inv(9)/47,XXX,inv(9)/46,XX,inv(9)	1
						45,X/47,XXX/49,XXXXX/46,XX	2
		Subtotal	18		2		27
Male	158	45,XY,der(14;21)	1	47,XXY	16	46,XY/46,XX	1
		45,XY,der(13,15)	1	47,XY,+mar	1	45,X/46,XY	1
		46,X,del(Y)(q11 → qter)	1	47,XXY,inv(9)	1	47,XXY/48,XXXY/50,XXXXXY/ 49,XXXXY/46,XY	1
		46,XY,inv(9)	1				
		46,X,+mar	1				
		46,XX *	2				
		Subtotal	7		18		3
Female	106	46,XX,inv(9)	3	45,X	4	45,X/46,XY	1
		46,XX,del(14)(q12 → q13)	1	47,XXX	2	45,X/46,XX	2
		46,X,del(X)(q13)	1			47,XXX/46,XX	1
		46,X,i(Xq)	1			46,X,i(Xq)/45,X	2
		46,XX,t(12;19)	1			46,X,del(X)(pter → q13)/46,XX	1
		46,XY *	4			45,X/46,X,der(X)(:p11 → q23:)	1
						45,X/47,XXX/46,XX	1
						47,XXX/45,X/46,XX	1
						45,X/47,XXX/48,XXXX/46,XX	1
		Subtotal	11		6		11
		Total	36		26		41

* *phenotype different from chromosomal sex.*
\# *chromosomal aberrations found in both spouses.*

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