

# Sex chromosome mosaicism in couples undergoing intracytoplasmic sperm injection

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**BACKGROUND:** Several studies have shown an increased frequency of constitutional chromosome aberrations in male and female partners of couples examined prior to ICSI. We conducted a cohort study to determine whether there was an increase in numerical sex chromosome mosaicism among couples undergoing ICSI compared with fertile couples. **METHODS:** Cytogenetic investigations were performed in 228 females and 208 males seen for ICSI between January 1997 and March 2001. They were matched to control females and males. **RESULTS:** Sex chromosome loss or gain was observed in at least one cell from 24.1% of ICSI women in comparison with 22% of controls (not significant). A significant difference between these two groups was found when X chromosome loss in at least two cells was considered, 9.6% for ICSI females versus 4.8% for controls ( $P = 0.01$ ). No significant difference was observed between male groups concerning loss or gain of the X or Y chromosome. **CONCLUSION:** Our results support previously published studies indicating that the loss of an X chromosome in a single cell in females undergoing ICSI is probably an artefact. However, they suggest that a woman could have true sex chromosome mosaicism when two 45,X0 cells are found.

*Key words:* chromosomal abnormality/cytogenetics/ICSI/sex chromosome mosaicism

## Introduction

Between 10 and 15% of all couples from industrially developed countries are faced with infertility during their reproductive lives. ICSI has become a highly effective therapeutic approach to male infertility and for couples who have experienced unsuccessful IVF (De Braekeleer and Dao, 1991).

Several cytogenetic studies have now shown a high incidence of chromosomal abnormalities, not only in the male partner of couples undergoing ICSI, but also in the female partner (Mau *et al.*, 1997; Meschede *et al.*, 1998; Scholtes *et al.*, 1998; Hocquet *et al.*, 1999; Peschka *et al.*, 1999; Schreurs *et al.*, 2000; Gekas *et al.*, 2001). Among them, the significance of numerical sex chromosome mosaicism remains to be elucidated (Meschede *et al.*, 1998; Scholtes *et al.*, 1998; Gekas *et al.*, 2001). Notably, when a sex chromosome abnormality is seen in a low percentage of cells, it is suspected by some workers to be an artefact.

We conducted a cohort study to determine whether there was an increase of numerical sex chromosome mosaicism among couples undergoing ICSI compared with fertile couples.

## Materials and methods

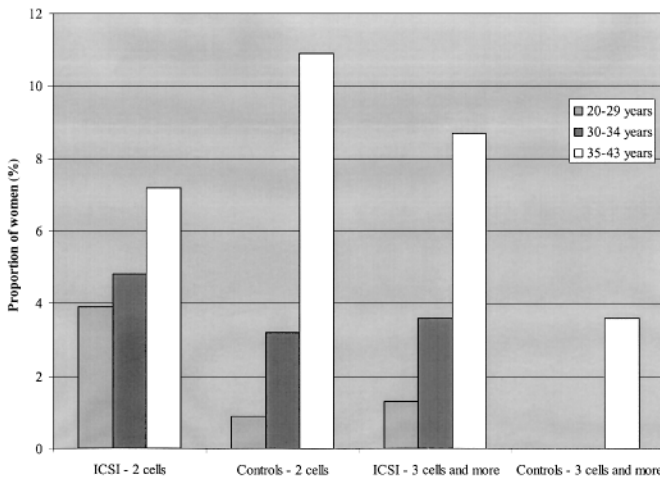
All of the 228 couples seen between January 1997 and March 2001 at the CHU Morvan, for whom ICSI was indicated, were included in the present study. Each couple underwent a routine diagnostic evaluation including an andrological examination and, for women, a standard endocrinological, gynaecological and ultrasound examination to rule out potential infertility factors.

A karyotype was systematically performed in both partners. However, because some individuals had their karyotype done outside our institution, all 228 females, but only 208 males, were included in the analysis.

The control group consisted of 227 adult females and 194 adult males who underwent a standard cytogenetic examination because of the presence of a chromosomal abnormality (excluding the gonosomes) or psychomotor retardation in their family.

Chromosome analysis was carried out on phytohaemagglutinin-stimulated peripheral lymphocytes cultured for 72 h using standard techniques (Morel *et al.*, 2002). The karyotype was confirmed using the R-banding technique. Sixteen cells were karyotyped, although when at least one of these 16 showed a loss or a gain of an X or Y chromosome, the number of analysed metaphases was increased to 25. If a second abnormal cell was observed, the analysis was considered to be complete, otherwise, the number of metaphases was increased to 50.

The statistical analysis was performed using the  $\chi^2$ -test or, in cases



**Figure 1.** Distribution of ICSI and control women having at least two cells with a 45,X constitution by age groups.

**Table I.** Distribution of ICSI and control women by age groups

Age groups (years)	ICSI		Controls	
	No. of women	Mean age (SD)	No. of women	Mean age (SD)
20–29	76	26.8 (1.9)	109	26.2 (2.3)
30–34	83	31.7 (1.3)	63	32.4 (1.4)
35–43	69	37.5 (2.2)	55	37.8 (2.4)
Total	228	31.9 (4.6)	227	30.8 (5.2)

of small numbers, Fisher's exact test. The mean ages of ICSI and control groups were compared using a two-tailed Student's *t*-test. The statistical significance was set at  $P \leq 0.05$ .

## Results

### Chromosomal findings in women

The mean age of the 228 ICSI females was 31.9 years (SD 4.6), with a range of 22–43 years. The mean age of the 227 control women was 30.8 years (SD 5.2), with a range of 20–43 years. The difference was not statistically significant. Women of both groups were distributed into three age groups (Table I). They spanned different time intervals in order to have somewhat comparable numbers of women in each group.

Sex chromosome loss or gain was observed in at least one metaphase from 24.1% (55/228) of ICSI women compared with 22% (50/227) of control females (not significant). X chromosome loss was observed in at least one cell from 20.6% (47/228) and 18.9% (43/227) of ICSI and control women respectively (not significant) (Table II).

However, a significant difference ( $P = 0.01$ ) was found between the ICSI and control groups when considering women with a 45,X karyotype in at least two metaphases (22/228 versus 11/227). The same conclusion applied when three or more 45,X cells were found ( $P = 0.01$ ). Four ICSI females had at least four cells with an X chromosome loss versus none in the control group (Table II).

Figure 1 shows an increase in the proportion of females

belonging to both ICSI and control groups and having at least two cells with a 45,X constitution as a function of age. However, in each age group, there were more ICSI women than controls with at least two 45,X cells ( $P = 0.001$ ), with the exception of 35–43 year old, 2-cell, controls.

No significant difference in X chromosome gain (47,XXX and 48,XXXX) was found between ICSI and control females (Table II). In most of the cases, X chromosome gain was associated with X loss in at least one cell (Table II).

### Chromosomal findings in men

The mean age of the 208 ICSI males was 34.3 years (SD 5.6), with a range of 23–59 years. The mean age of the 194 control men was 34 years (SD 6.4), with a range of 20–53 years. The difference was not statistically significant.

Y chromosome loss was observed in at least one metaphase from 6.2% (13/208) of ICSI men compared with 7.2% (14/194) of control males (not significant) (Table III). Only one man in the ICSI group had two cells with a 45,X constitution.

Other sex chromosome loss or gain was observed in a sole metaphase from a few ICSI and control males (Table III). No significant differences were found.

## Discussion

Several studies have now reported an overall increased frequency of chromosomal aberrations in male and female partners of couples referred for ICSI; they consist mostly of translocations, inversions and numerical sex chromosome aberrations (Mau *et al.*, 1997; Meschede *et al.*, 1998; Scholtes *et al.*, 1998; Hocquet *et al.*, 1999; Peschka *et al.*, 1999; Schreurs *et al.*, 2000; Gekas *et al.*, 2001).

However, the meaning of 'low level' mosaicism for a numerical gonosome anomaly observed in women prior to planned ICSI treatment is still debated (Meschede *et al.*, 1998; van der Ven *et al.*, 1998; Gekas *et al.*, 2001). One difficulty comes from the fact that there is no consensus on the definition of low level mosaicism, which has been considered by some authors as the presence of <10% of abnormal cells (Meschede *et al.*, 1998; Scholtes *et al.*, 1998; Gekas *et al.*, 2001; Sonntag *et al.*, 2001) and by others of <6% (Peschka *et al.*, 1999).

Mau *et al.* included patients with a single cell showing an abnormal gonosomal complement in their analysis of 150 couples undergoing ICSI (Mau *et al.*, 1997). Although recognizing that these abnormal single cells could be artefactual, those individuals were included in the frequency calculations. The number of metaphases studied varied from 14–60. Six women (3.9%) had a gonosomal mosaicism including three with a single cell abnormality. Numerical gonosomal abnormalities were found in seven men (4.6%), but these were present in more than one cell in a sole man.

Several authors considered as mosaics those individuals who had two cells or more with the same abnormality (Meschede *et al.*, 1998; Scholtes *et al.*, 1998; Sonntag *et al.*, 2001). However, the number of metaphases analysed was different between the studies. Meschede *et al.* and Sonntag *et al.* extended their analysis to include up to 150 metaphases

**Table II.** Sex chromosome numerical abnormalities in women

Abnormalities	Group		No. of women with		
			One cell	Two cells	Three or more cells
45,X	ICSI	Sole anomaly	20	8	6
		Association with X gain	5	4	4
	Control	Sole anomaly	23	6	2
		Association with X gain	9	3	0
47,XXX	ICSI	Sole anomaly	7	0	0
		Association with X loss	11	2	0
	Control	Sole anomaly	5	2	0
		Association with X loss	6	2	1
48,XXXX	ICSI	Sole anomaly	0	1	0
		Association with X loss	0	0	0
	Control	Sole anomaly	0	0	0
		Association with X loss	4	0	0

**Table III.** Sex chromosome numerical abnormalities in men

Abnormalities	Group	Number of men with	
		One cell	Two cells
45,X	ICSI	12	1
	Control	14	0
45,Y	ICSI	3	0
	Control	3	0
47,XXY	ICSI	3	0
	Control	1	0
47,YYY	ICSI	4	0
	Control	1	0

if at least two cells with the same abnormality were seen among the first 15 examined (Meschede *et al.*, 1998; Sonntag *et al.*, 2001). Low level mosaicism was detected in 4.1% (18/436) of women and in 0.5% (2/432) of men (Meschede *et al.*, 1998). Sonntag *et al.* increased the series to 811 women and reported a incidence of 3.2% of low level mosaicism (Sonntag *et al.*, 2001). The presence of one abnormal cell within the first 20 analysed prompted Scholtes *et al.* to increase their search to 100 metaphases (Scholtes *et al.*, 1998). They found mosaicism, including low level, in nine of the 1116 men studied (0.8%) and in 84 of the 1164 women investigated (7.2%).

Single cells with a sex numerical abnormality were disregarded by Peschka *et al.* if there was a sole abnormal cell line. However, if two cell lines with a numerical abnormality were found, the presence of a single abnormal cell of each cell line was sufficient to be classified as mosaicism (Peschka *et al.*, 1999). Should one abnormal cell be identified within the first 20 analysed, the analysis was extended to 50–100 metaphases (Peschka *et al.*, 1999). They identified low level mosaicism in just 0.7% of males (11/766), but in 3.3% of females (51/766).

A French collaborative study on 3208 patients included in ICSI programmes was published by Gekas *et al.* (Gekas *et al.*, 2001). At least 20 metaphases were analysed for each patient, and, in cases of suspected sex chromosome mosaicism, the number of metaphases analysed was increased to a minimum

of 30. Only individuals with sex numerical abnormalities present in more than two cells were considered as mosaics; however, a few individuals with only two cells bearing the same abnormality were included in the analysis (Gekas *et al.*, 2001). Twenty-eight of the 1012 women (2.77%) had a numerical sex chromosome mosaicism, including 22 with a low-level mosaicism; 17 of the 2196 men (0.77%) also had a gonosomal mosaicism.

In summary, the review of the literature showed a high heterogeneity, not only in the definition of low level mosaicism, but also in the methodology applied (number of metaphases analysed). This makes a comparison between the results obtained in the several studies very difficult. Furthermore, none of the studies, with one exception (Peschka *et al.*, 1999), used a control group to put the rate of sex chromosome mosaicism in a proper perspective. In this latter study, a comparable rate of low level sex chromosome mosaicism was found among women undergoing ICSI and fertile control females (Peschka *et al.*, 1999). It should be noted that the authors considered as having low level sex chromosome mosaicism 18 women with one 45,X cell and one 47,XXX cell; data on the control group was not provided in their paper.

Our approach was rather different from that of the previously published reports. Indeed, we used a standardized protocol in the number of metaphases examined in ICSI and control groups and compared the results obtained in both groups by means of statistical analyses.

In our study, most of the aneuploid cell lines represented <10% of the total number of metaphases analysed in women. The difference between ICSI and control females was statistically significant only when at least two cells with 45,X constitution were found. However, it should be noted that there was no significant difference between the groups of women when X chromosome loss was observed in only two cells (which could be due, at least partly, to the methodology used).

The presence of a sole abnormal cell is probably the result of culture and/or preparation artefacts. This explanation is unlikely when more than one metaphase is aneuploid for two reasons. Firstly, it is difficult to understand why artefacts



would preferentially affect samples from the ICSI women, but not those of controls. Secondly, it is even harder to explain why the female samples, but not the male samples, would exclusively be the target of artefacts.

Therefore, the presence of two or more 45,X cells in lymphocytes of ICSI women is the signature of true mosaicism, whether it is of low level or not. Indeed, the International System for Cytogenetic Nomenclature (ISCN, 1995) does not give the minimum number of metaphases to be analysed to exclude mosaicism, nor does it set the minimum and maximum percentages of aneuploid cells required to define low level mosaicism (ISCN, 1995).

It has been known for several decades that sex chromosome aneuploidy in lymphocyte cultures is age-related in both sexes (Jacobs *et al.*, 1961, 1963; Fitzgerald, 1975; Zijno *et al.*, 1996). The increase in aneuploidy is more marked in women than in men, the loss of an X chromosome being more frequent than its gain in females (Galloway and Buckton, 1978; Nowinski *et al.*, 1990; Guttenbach *et al.*, 1995). A faster rate of increase of hypodiploid cells was observed in women aged 45–60 years by some workers (Jacobs *et al.*, 1963; Guttenbach *et al.*, 1995) or aged >60 years by others (Galloway and Buckton, 1978), which is why, in our study, male and female partners of ICSI couples were age-matched with those of controls in order to eliminate the effect of age on the risk of aneuploidy.

In conclusion, the incidence of 45,X mosaicism is increased among women consulting for ICSI. However, several questions remain unanswered. We do not know whether the 45,X/46,XX mosaicism is confined to lymphocytes or expands to other tissues, possibly germ cells. The consequences of sex chromosome mosaicism in couples undergoing ICSI also require further studies. Indeed, conflicting results have been reported on its biological relevance for the course of ICSI treatment (Toncheva *et al.*, 1994; Scholtes *et al.*, 1998; Sonntag *et al.*, 2001). Finally, could these numerical sex chromosome mosaicisms be a possible explanation, at least partly, for the increase of gonosomal abnormalities observed among offspring conceived by ICSI (In't Veld *et al.*, 1995; Liebaers *et al.*, 1995; Van Opstal *et al.*, 1997; Bonduelle *et al.*, 1998, 1999)? Indeed, it seems important to know whether this increase is the result of medications used to induce ovulation and/or maintain the pregnancy in the early stages or if it is due to the underlying infertility (Hansen *et al.*, 2002).

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