Low-Level Gonosomal Mosaicism in Women Undergoing ICSI Cycles

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Purpose: Females with low-level gonosomal mosaicism (LLGM) scheduled for ICSI were retrospectively analyzed and compared to control groups. The prevalence of this mosaicism as well as its impact for an ICSI treatment were evaluated.

Methods: Routine cytogenetic analysis was done in 891 females scheduled for ICSI treatment. For comparison 294 females with recurrent abortions and 104 women with clinical or cytogenetical affected children, who also had routine chromosome analysis, were acquired.

Results: In the ICSI group the incidence of LLGM was significantly lower compared to the group of females with recurrent abortions (3.9% vs. 8.5%, $p < 0.01$). The incidence was similar between the groups of ICSI females and females with cytogenetical or clinically affected children (5.8%, $p = 0.43$). Neither anamnestic factors nor outcome parameters were different between females with and without LLGM undergoing ICSI.

Conclusion: There is no higher incidence of LLGM in females undergoing ICSI. In those who show LLGM, fertility capacity is not reduced.

KEY WORDS: ICSI; infertility; low-level mosaicism; treatment outcome.

INTRODUCTION

In industrial countries 10–15% of couples in the reproductive age suffer from fertility problems. Therefore it is important to examine the causes of infertility (1).

One possible reason for infertility is chromosomal abnormalities like numerical aberrations or translocations. Cytogenetic analyses of couples presenting with male factor infertility show a higher frequency of chromosomal abnormalities in infertile males compared to unselected male newborns (2).

In addition, some studies demonstrated an elevated frequency of chromosomal abnormalities in female partners of couples undergoing intracytoplasmic sperm injection (ICSI) (3–7). ICSI is the most effective therapeutic approach to severe male factor infertility (8). But still the genetic risk of this technique is discussed, especially in cases with parental chromosomal abnormalities.

Up to now, only a few studies have evaluated the risk of females with low-level gonosomal mosaicism for clinical abnormalities in the newborns or for failure of ICSI cycles (1,9). No significant differences in clinical outcome parameters between women with low-level gonosomal mosaicism and women with normal karyotypes were described (9).

To evaluate the question whether low-level gonosomal mosaicism has an impact on anamnestic fertility parameters, influences the outcome of assisted reproduction or leads to clinically affected children we investigated three groups of females for low-level gonosomal mosaicism in this study. The first group

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consisted of individuals with reduced fertility due to male factor infertility, the second group of females with recurrent abortions and the third group of females with clinical or cytogenetical affected children.

PATIENTS AND METHODS

Patients

We investigated 891 consecutive female patients, who were scheduled for an ICSI treatment. All of them had routine cytogenetic analysis in the Institute of Human Genetics before ICSI. For all women out of this group with low-level gonosomal mosaicism (study group) a control group of females (control group) out of the ICSI group with a normal chromosomal status was matched by the following parameters: chromosomal analysis during the same time period and a similar age (± 5 months). Two controls were matched by the age to one female with low-level gonosomal mosaicism. The person, who looked for the controls (F.H.), was not aware of any clinical data of that patient or couple. The control group consists of 65 females, since not for each individual two appropriate controls could be identified from the data base.

Data of anamnestic parameters and the clinical outcome after ICSI were collected and were put in a computerized data base in an anonymous way. To estimate the prevalence of low-level gonosomal mosaicism in females data of two further groups of females were collected who underwent routine cytogenetic analysis in the Institute of Human Genetics. One group consisted of 294 females with recurrent spontaneous abortions. In the other group 104 females with clinical or cytogenetical affected children were included.

Both control cohorts were analyzed within the same time period as the ICSI patients.

All patients had given their informed consent for anonymous analysis of their data within retrospective scientific projects.

Treatment Protocol

All patients out of the ICSI group included in the final analysis were treated according to a long luteal phase GnRH agonist protocol as usual.

Cytogenetic Analysis

Following cell culture and conventional metaphase spreading, peripheral blood lymphocytes obtained from all individuals were analyzed by Giemsa-staining

with a resolution of approximately 450–500 bands (ISCN). At least 20 cells were analyzed. In cases of suspected mosaicism the number of analyzed metaphases was increased using cytogenetic or fluorescence in situ hybridization (FISH) techniques. Gonosomal mosaicisms were defined as low-level when at least two or more of the analyzed cells but less than 15% displayed the karyotype 45,X or 47,XXX. For FISH analysis we used X specific centromere probes (DXZ1) from Qbiogene (Heidelberg, Germany). Hybridization, posthybridization washes, and detection were done according to the recommendation of the manufacturer. Cytogenetic analysis from fibroblasts was done following standard protocols (10) Cytogenetic lab-orientated quality testing is regularly done by BV Medgen (Eschweiler, Germany).

Statistics

Different results between the study and control group in the ICSI sample were tested for significance using the Mann–Whitney *U*-test and the chi-square test as appropriate. Relative frequencies of gonosomal mosaicisms between the ICSI group and the group of females with abortions or the group of females with suspicious children respectively were compared using Fisher's exact test. A *p*-value of *p* < 0.05 was considered to show a significant difference.

RESULTS

Chromosome analysis was performed in 891 couples with male factor infertility due to oligozoospermia (<20 million sperms/mL) during the study period. Forty-six chromosome anomalies (5.16%) were identified in the female group, namely translocations [6], structural anomalies [4] (Table I), and numerical abnormalities [35].

Table I. Chromosomal Anomalies Found in a Cohort of 891 Female Patients Planned for an ICSI Treatment Cycle (Excluding Low-level Gonosomal Mosaicism)

Karyotype	Case number
46,XX,t(6;15)(q16;q21)	1/97
45,XX,t(13;14)(q10;q10)	2/97
46,XX,inv(10)	1/98
46,XX,t(8;17)	2/98
46,XX,del(X)(q26>qter)	3/98
46,XX,t(2;8)	1/99
46,XX,inv(3)(p12;q13.2)	1/00
46,XX,t(3;X)(q21;q22)	1/01
46,XX,fra(16)(q22)	2/01
46,XX,t(1;10)(p22;q22)	3/01

Table II. Low-Level Mosaicism in Female Patients from Couples Undergoing an ICSI Cycle

Case number	Karyotype	Frequency
2/00	mos47,XXX[3]/46,XX[25] Lymphocytes control: 46,XX[50] FISH control:metaphases: 46,XX[50],interphases: XX[100]	3/25 0/50 0/50, 0/100
2/99	mos45,X[3]/47,XXX[1]/46,XX[46]	3/1/46
3/00	Lymphocytes control: 46,XX[30]	0/30
3/99	mos45,X[2]/46,XX[50]	2/50
4/99 I	mos45,Xps15[3]/46,XXps15[47]	3/47
5/99 II	Lymphocytes control: mos45,Xps15[1]/47,XXXps15[1]/46,XXps15[48]	1/1/48
6/99	mos45,X[2]/46,XX[51] Lymphocytes control: mos45X[1]/46,XX[49]	2/51 1/49
3/97	mos45,X[1]/47,XXX[1]/46,XX[53] Fibroblasts control: mos45,XX[2]/46,XX[48]	1/1/53 2/48
4/00	mos45,X[1]/46,XX[12] FISH control: mos47,XXX[1]/46,XX[49]	1/12 1/49
5/00	mos47,XXX[1]/45,X[3]/46,XX[29]	1/3/29
6/00	mos45X[2]/46,XX[28]	2/28
7/00	mos45X[1]/47,XXX[1]/46,XX[28]	1/1/28
8/00	mos47,XXX[2]/46,XX[29]	2/29
4/01	mos47,XXX[1]/46,X,delXq[1]/46,XX[49]	1/1/49
9/00	mos45X[2]/46,XX[38]	2/38
10/00	mos45,X[2]/46,XX[11] FISH control:normal	2/11
11/00	mos45,XX,-21[1]/45,X[2]/47,XXX[2]/46,XX[46] Lymphocytes control: 46,XX[20]	1/2/2/46 0/20
7/99	mos45,X[3]/46,XX[6]	3/6
12/00	Lymphocytes control: mos47,XXX[1]/46,XX[49]	1/49
8/99	mos47,XXX[2]/46,XX[48] Lymphocytes control: mos45,X[1]/46,XX[50]	2/48 1/50
1/96	mos45,X[2]/46,XX[31] Fibroblasts control: mos45,X[2]/46,XX[48]	2/31 2/48
4/98	mos47,XXX[2]/46,XX[53] Lymphocytes control 1: mos45,X[1]/46,XX[51] Lymphocytes control 2: mos45,X[2]/46,XX[50] Fibroblasts control: 46,XX[50]	2/53 1/51 2/50
2/96	mos47XXX[1]/47,XX+Chr1[1]/47XX+Marker[1]/45,X[2]/46,XX[57] Fibroblasts control: mos45,X[1]/46,XX[49]	1/1/1/2/57 1/49
10/99	mos45,X[3]/46,XX[47] Lymphocytes control: mos45,X[2]/46,XX[48]	3/47 2/48
4/97	45,X[1]/47,XXX[1](46,XX[50] Fibroblasts control: 46,XX[50]	1/1/50 0/50
5/97	45,X[1]/47,XXX[1]/46,XX[35]	1/1/35
6/97	47,XXX[2]/46,XX[31]	2/31
5/98	45,X[1]/47,XXX[1]/46,XX[50] Lymphocytes control: 47,XXX[2]/46,XX[48] Fibroblasts control: 49,XXXXXX[1]/46,XX[74]	1/1/50 2/48 1/74
6/98	45,X[2]/47,XXX[1]/46,XX[55] Lymphocytes control: 45,X[1]/46,XX[49] Fibroblasts control: 46,XX[50]	2/1/55 1/49 0/50
7/98	45,X[1]/47,XXX[1]/46,XX[50] Lymphocytes control: 46,XX[51]	1/1/50 0/51
11/99	47,XXX[2]/46,XX[50] Lymphocytes control: 46,XX[30]	2/50 0/30
12/99	45,X[1]/48,XXXX[1]/46,XX[48] Lymphocytes control: 46,XX[52]	1/1/48 0/52
13/99	45,X[1]/47,XXX[1]/46,XX[27] FISH control of STS region: 46,XX[20]	1/1/27 0/20
13/00	45,X[3]/46,XX[25] Lymphocytes control: 46,XX[13]; FISH control: 46,XX[50]	3/25 0/13; 0/50
14/00	45,X[1]/47,XXX[1]/46,XX[28]	1/1/28
15/00	45,X[2]/46,XX[27] FISH control: 46,XX[50]	2/27 0/50
5/01	45,X[1]/46,XX[11] FISH control: 45,X[4]/46,XX[46] Fibroblasts control: 45,X[1]/46,XX[94]	1/11 4/46 1/94
6/01	45,X[3]/46,XX[60] Lymphocytes control: 45,X[1]/46,XX[12] FISH control: 45,X[1]/46,XX[48]	3/60 1/12 1/48

In the male cohort out of 1172 males in 46 chromosomal abnormalities were found (3.92%). Twenty males (1.71%) showed structural abnormalities, 14 (1.19%) gonosomal aberrations, and 12 (1.02%) low-level gonosomal mosaicisms.

In 35 females a low-level gonosomal mosaicism was found (3.93%, 35/891) (Table II). From these 35 individuals two were not treated by ICSI due to total absence of sperms even in testicular biopsies. Two couples started the procedure, but cancelled the cycles due to low ovarian response, another three had no embryo transfer in their treatment cycles. Others did not start treatment for personal reasons. In these cases just the anamnestic data were included in this analysis. The remaining 22 females with low-level gonosomal mosaicisms underwent 36 ICSI cycles. The mean age was 34.89 ± 3.91 years.

Twenty-six out of 65 (40%) controls had no treatment. In two couples treatment was cancelled due to a low ovarian response. One couple experienced a spontaneous pregnancy. In two cases the male partner has been successfully refertilized, in one case no sperms were available by testicular biopsy, and in three cases a treatment by intrauterine insemination has been performed. For the couples who did not start treatment just the anamnestic data were taken into account. The other 39 individuals were treated in 85 ICSI cycles. The anamnestic data as well as treatment outcome were not different between the two groups (Tables III and IV). Especially the total amount of follicle stimulating hormone (FSH), the duration of treatment, the mean number of oocytes retrieved, or the pregnancy rate were not significantly different between the two cohorts. Therefore the low-level gonosomal mosaicism did not influence the fertilization

rate in the females investigated in our study. The mean age of the control group was 34.14 ± 4.16 years.

Out of 294 women who underwent chromosome analysis due to recurrent abortions 25 showed a low-level mosaicism (8.5%) (Table V). The frequency was significantly higher compared to the ICSI group ($p < 0.01$). The mean age of this group was 34.84 ± 4.18 years.

In 6 out of 104 women with clinically or cytogenetically affected children a low-level mosaicism was identified (5.8%) (Table VI). This result was not significantly higher compared to the ICSI group ($p = 0.43$). The mean age at analysis was 33.33 ± 4.61 years.

The age of the two control groups was not significantly different from the group of ICSI patients.

DISCUSSION

In the present study the prevalence of low-level mosaicism and its impact on the fertility potential was investigated. The main interest was focused on women undergoing an infertility treatment. Altogether three groups of females were analyzed. The first group consisted of individuals undergoing ICSI due to male factor infertility, the second group of females with recurrent abortions and the third group of females with clinically or cytogenetically affected children.

A total of 3.9% of the females from the ICSI group showed a low-level gonosomal mosaicism. In the group of females with recurrent abortions low-level gonosomal mosaicisms were found in 8.5% and the group of females with cytogenetical or clinical affected children showed low-level mosaicisms in 5.8%. The risk for low-level gonosomal mosaicism therefore

Table III. Anamnestic Data in the Study and Control Cohort Within the ICSI Patients

	Study group	Controls group	
<i>N</i>	35	65	
Age (years) ^a	34.89 ± 3.91	34.14 ± 4.16	ns
Menarche (years)	13.67 ± 1.64	13.22 ± 1.34	ns
Duration of menstrual cycle (minimal) (d)	27.27 ± 2.74	28.44 ± 9.74	ns
Duration of menstrual cycle (maximal) (d)	30.77 ± 4.82	30.70 ± 11.10	ns
Mean duration of menstrual cycle (d)	29.02 ± 3.12	29.86 ± 10.51	ns
Duration of bleeding (minimal) (d)	4.24 ± 1.26	4.27 ± 1.20	ns
Duration of bleeding (maximal) (d)	5.18 ± 1.33	5.00 ± 1.33	ns
Patients with previous pregnancies	22.2%	34.1%	ns
Patients with previous abortions	15.6%	13.6%	ns
Patients with previous births	6.7%	9.1%	ns

Note. Data are mean $\pm$ standard deviation if not otherwise defined; ns: not significant.

^a Age was calculated at the time of oocyte pick-up, or, in case that no treatment was done, at the time of chromosome analysis.

Table IV. Outcome of Infertility Treatment Cycles in the Study and Infertility Control Cohort

	Study group	Control group	
Patients (<i>n</i>)	35	65	
Patients with no treatment cycles (% of all patients)	13 (37.1%)	26 (40.0%)	ns
Started cycles (<i>n</i>)	36	85	
Duration of gonadotrophin stimulation (d)	13.4 ± 2.7	13.1 ± 2.6	ns
Total doses of gonadotrophin (IU)	4100 ± 2540	3747 ± 1987	ns
Peak estradiol on the day of hCG administration (pg/mL)	2153 ± 1250	1918 ± 1156	ns
Number of cycles with successful oocyte pick-up (% per started cycle)	34/36 (94.4)	84/85 (98.8)	ns
Number of oocytes retrieved	9.04 ± 4.94	9.74 ± 5.36	ns
Percentage of ICSI cycles (% of all cycles)	95%	82%	ns
Number of 2 PN oocytes	3.56 ± 2.91	3.55 ± 3.20	ns
Fertilization rate (%)	40%	43%	ns
Total fertilization failure (% of all oocyte retrievals with oocytes)	3/34 (8.8%)	8/84 (9.5%)	ns
Number of embryo transfers (% per started cycles)	31/36 (91.2%)	76/85 (89.4%)	ns
Number of embryos per transfer	2.38 ± 0.86	2.27 ± 0.92	ns
Cumulative embryo score (modified) ^a	24.10 ± 16.08	24.34 ± 11.60	ns
Clinical pregnancy rate per embryo transfer (% per embryo transfer)	8/31 (25.8%)	19/76 (25.0%)	ns
Abortion rate (% per clinical pregnancy)	3/8 (37.5%)	6/19 (31.6%)	ns
Ongoing pregnancies > 12th week of gestation (% per embryo transfer)	5/31 (16.1%)	13/76 (17.1%)	ns

Note. Data are mean ± standard deviation if not otherwise defined; ns: not significant.

^a The modified cumulative embryo score was used as previously described (16a,b).

was significantly higher in the abortion group ($p < 0.01$) compared to the ICSI group.

Several studies have shown the high incidence for chromosomal abnormalities in couples undergoing ICSI because of male factor infertility. Contrary to initial suggestions, the female partner of an infertile couple also may be at higher risk to suffer from chromosomal abnormalities (1,3,9,11). This could be confirmed by our own data. The incidence of chromosomal abnormalities in female partners was similar to that in the male partners (5.16% vs. 3.9%) and higher as expected in the general population.

Especially low-level gonosomal mosaicisms are found in the female partner undergoing ICSI (3). The effect on the fertilization rate is still discussed (1,3). For the interpretation of the impact of the low-level mosaicisms on fertility the different definitions of low-level mosaicisms are decisive. Meschede *et al.* (3) defined a low-level sex-chromosomal mosaicism as less than 10% of abnormal cells from the analyzed cells; Peschka *et al.* (6) described it as less than 6%. In some studies already one single cell with a chromosomal abnormality was included (11) even though a single abnormal cell can be discussed as a culture artefact.

Some authors used the definition that there had to be at least two cells with identical chromosomal aber-

rations to identify a low-level mosaicism (3,9). The number of metaphases analyzed was also different in all these studies.

Though the definition of low-level mosaicism and the amount of cells analyzed were different the percentage of females with low-level mosaicisms was similar in the mentioned studies: Meschede *et al.* (3) found a low-level mosaicism in 4.6% (20/436), Sonntag *et al.* (9) in 3.2% (26/811).

In our study the incidence of women undergoing ICSI with a sex-chromosomal mosaicism was 3.8%. Only cases with at least two of the analyzed cells, but only up to 15%, showing hypo- or hyperploidy for the X-chromosome were included. Because of the heterogeneity of the definition of low-level mosaicism and the different methodology described in the literature it is difficult to compare the results between different studies.

The effect of sex-chromosomal low-level mosaicism on the fertilization rate can only be discussed. In our study the group of women with low-level mosaicisms who underwent ICSI was matched with a control group of 39 women who underwent ICSI without chromosomal aberrations. Anamnestic data of study and control patients did not show any significant difference. Compared to newborn screenings the rate

Table V. All Females with Recurrent Abortions and Low-Level Mosaicism

Case number	Karyotype	Frequency
16/00	45,X[1]/47,XXX[1]/46,XX[30]	1/1/30
17/00	45,X[2]/46,XX[58]	2/58
18/00	45,X[1]/47,XXX[1]/46,XX[34]	1/1/34
19/00	45,X[1]/47,XXX[1]/46,XX[16]	1/1/16
20/00	45,X[2]/46,XX[48]	2/48
21/00	45,X[3]/46,XX[47]	3/47
7/01	45,X[3]/46,XX[34]	3/34
8/01	45,X[1]/47,XXX[1]/46,XX[47]	1/1/47
3/96	45,X[2]/46,XX[41]	2/41
4/96	45,X[1]/46,XXq-[1]/46,XX[23]	1/1/23
5/96	45,X[1]/47,XXX[1]/46,XX[28]	1/1/28
6/96	45,X[2]/46,XX[28]	2/28
7/97	45,X[2]/46,XX[47]	2/47
8/97	45,X[4]/46,XX[46]	4/46
9/97	45,X[1]/47,XXX[1]/46,XX[28]	1/1/28
8/98	45,X[2]/46,XX[48]	2/48
9/98	45,X[3]/46,XX[52]	3/52
10/98	45,X[2]/47,XXX[1]/46,XX[47]	2/1/47
11/98	45,X[1]/47,XXX[1]/46,XX[47]	1/1/47
14/99	45,X[2]/46,XX[98]	2/98
15/99	45,X[1]/47,XXX[1]/46,XX[48]	1/1/48
	Lymphocyte control: 46,XX[14]	14
16/99	45,X[4]/46,XX[96]	4/96
17/99	45,X[3]/46,XX[49]	3/49
	Fibroblasts control: 46,XX[50]	50
18/99	45,X[2]/46,XX[47]	2/47
	Lymphocytes control: 47,XXX[3]/46,XX[47]	3/47
19/99	45,X[2]/46,XX[51]	2/51

^a In these women control by lymphocyte or fibroblast culture was done in a few cases. The total number of women in this subgroup was 294.

of chromosomal abnormalities in couples with fertilization problems is increased (12). The elevated age of women undergoing ICSI can be one of the explanations for this phenomenon. The mean age of the women analyzed in our study undergoing ICSI is 34.23 years. It is known that sex-chromosomal loss shows a strong correlation with age (13). The loss of the second X-chromosome in females is more frequent than loss of the Y-chromosome in males which

explains the higher incidence of sex-chromosomal abnormalities in females than in males (13).

Because of the loss of X-chromosomes in women with elevated ages it would be really important to find a control group of fertile women of the same age with chromosomal analysis.

We are aware of the point, that of course the chosen control groups represent a highly preselected cohort of patients. However, no other control groups can be identified at the moment.

The frequency of sex-chromosome mosaicism in the study group of women with spontaneous abortions is significantly higher than in the group of women undergoing ICSI (8.5% vs. 3.8%, $p < 0.01$). This confirms data from Holzgreve *et al.* (14), who already described that women with recurrent abortions show sex-chromosomal abnormalities with a higher frequency. The hypothetical explanation of this situation is the possible impairment of chromosomal aneuploidies through nondisjunction by parental chromosomal mosaicism. For prenatal diagnosis this is of importance in parents with low-level mosaicism to detect numerical chromosomal aberrations of the fetus early during the pregnancy (14). Data of the frequency of these aberrations in germ cells do not exist to date. It is still considered possible that low-level mosaicism occurs in cells of the germ line and might have an effect on the development of the cells and therefore also on the fertilization ability.

The estimated risk for children with de novo chromosomal abnormalities as described by Bonduelle *et al.* (15) in a cohort of 1586 fetuses, is slightly higher after ICSI than in the fertile population. The risk for clinical or cytogenetical abnormal children because of maternal chromosomal low-level mosaicisms was not increased. The parents of the affected fetuses were cytogenetically analyzed and showed no chromosomal abnormality, not even a low-level mosaicism (15).

As a third group we chose women who had chromosomal analysis because they had children with abnormal karyotypes or children that showed clinical abnormalities. Comparing this group with the group of women undergoing ICSI, the amount of low-level mosaicisms was not significantly different (5.76% vs. 3.8%, $p = 0.43$). We chose this group, since it contains women without fertility problems because they already have children even though their children are clinical or cytogenetical abnormal. According to this group one can presume that low-level gonosomal mosaicism is not a serious limitation for the pregnancy rate.

Table VI. All Females With Clinical or Cytogenetical Affected Children and Low-Level Mosaicism^a

Case number	Karyotype	Frequency
10/97	45,X[1]/47,XXX[2]/46,XX[27]	1/2/27
20/99	45,X[2]/46,XX[50]	2/50
21/99	45,X[6]/47,XXX[3]/46,XX[42]	6/3/42
22/99	45,X[1]/47,XXX[1]/46,XX[48]	1/1/48
23/99	45,X[1]/47,XXX[1]/56,XX[99]	1/1/99
24/99	45,X[2]/46,XX[97]/47,XX+21[1]	1/97/1

^a In these women no control was done using FISH or fibroblast culture. The total number of women in this subgroup was 104.

A serious limitation of our study is the missing control group consisting of females from the general population, without any clinical abnormalities. Data on this topic, especially on control groups as included in the present study, have not been published yet.

To conclude, the results of the present study indicate that low-level gonosomal mosaicism in females has no impact on the fertility rate in couples undergoing ICSI. The risk for abortions may possibly be increased as seen in our second analyzed group of females with recurrent abortions. In the third analyzed group of females with children the incidence of low-level gonosomal mosaicism is the same as in the ICSI group. Therefore one may presume that the risk of low-level mosaicism in the ICSI group is not higher as it can be expected in fertile patients.

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