

Lower sperm aneuploidy frequency is associated with high pregnancy rates in ICSI programmes

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BACKGROUND: A large proportion of patients undergoing ICSI have been shown to have an increased sperm aneuploidy rate. This study was undertaken to evaluate the impact of sperm aneuploidy on ICSI outcome. **METHODS:** To accomplish this, 48 consecutive unselected male patients (median age 34 years) had their sperm aneuploidy rate evaluated in the same swim-up preparation used for ICSI. Chromosomes 8, 12, 18, X and Y were evaluated by fluorescence in-situ hybridization. Patients were divided into two groups (A and B) based on the sperm aneuploidy frequency in their sperm. Group A had values below and group B above the upper limit of normal [1.55%, determined in 14 healthy men (median age 25 years) with normal semen parameters by WHO 1999 criteria (control group)]. **RESULTS:** Group A consisted of 12 patients (25%) whose sperm aneuploidy rates fell below the cut-off value of the control group (median 1.25%; range 0.85–1.52). Group B consisted of the remaining 36 patients (75%), who had an elevated sperm aneuploidy rate (median 3.25%; range 1.64–23.60). Fertilization (93 versus 85%) and cleavage (100 versus 98%) rates were similar for both groups. Group A had significantly higher clinical pregnancy (75 versus 34%; $P < 0.001$) and implantation (34 versus 13%; $P < 0.001$) rates compared with group B. In addition, group A had a lower overall miscarriage rate (11.1 versus 38.9%). Other factors that affect pregnancy and implantation, such as patient age and conventional semen parameters, were similar for both groups. **CONCLUSION:** This study showed that chromosomally abnormal sperm have a negative impact on ICSI outcome.

Key words: abortion rate/ICSI/male infertility/pregnancy rate/sperm aneuploidy

Introduction

Several studies have shown that infertile patients with oligoasthenoteratozoospermia (OAT) and normal blood karyotype have an increased sperm aneuploidy rate (for a review see Calogero *et al.*, 2003) as a consequence of synaptic chromosome anomalies restricted to the germ cell line (Egozcue *et al.*, 1983; Vendrell *et al.*, 1999; Egozcue *et al.*, 2000). An inverse correlation between sperm aneuploidy rate and conventional semen parameters (sperm density, motility and morphology) has been reported (Ushijima *et al.*, 2000; Vegetti *et al.*, 2000; Calogero *et al.*, 2001b). Therefore, patients with the most severe degree of OAT, who are more likely to undergo ICSI cycles for their infertility, also have the greatest proportion of aneuploid sperm. Accordingly, we have shown that a large proportion of non-selected ICSI patients have increased sperm aneuploidy rates (Calogero *et al.*, 2001a). The use of ICSI in these patients has consequently generated considerable debate regarding the genetic risk for the offspring (Meschede *et al.*,

1997). In addition, recent observations suggest that sperm chromosome abnormalities may also have a negative impact on the success rate of IVF programmes. Indeed, a higher incidence of sex chromosome aneuploidy has been reported in infertile patients who needed ICSI compared with men requiring conventional IVF (Storeng *et al.*, 1998). In another study, five patients with elevated sperm aneuploidy rates had fertilization failure, lack of pregnancy, preclinical abortion or first trimester spontaneous abortion (Pang *et al.*, 1999). A normal fertilization rate after ICSI was found in 10 patients with increased sperm aneuploidy rate, but a low pregnancy rate resulted (Pfeffer *et al.*, 1999). Van Dyk *et al.* (2000) reported an increased sperm diploidy rate in patients whose partners did not achieve pregnancy through ICSI, compared with those who did. We showed that ICSI patients whose partners did not achieve a pregnancy had a tendency towards a higher sperm aneuploidy rate compared with patients whose partners became pregnant (Calogero *et al.*, 2001a). These data therefore suggest

Table I. Sperm aneuploidy and diploidy rates for chromosomes X, Y, 12, 8 and 18 in 48 consecutive infertile patients undergoing ICSI sorted according to their sperm aneuploidy rate

	Sperm aneuploidy rate	
	Normal ($\leq 1.55\%$) ^a (n = 12)	Elevated ($> 1.55\%$) ^a (n = 36)
X (%)	49.78 (48.12–51.20)	49.24 (31.25–57.43)
Y (%)	49.64 (49–51.12)	49.09 (39.56–62.50)*
XY (%)	0.08 (0–0.50)	0.06 (0–1.64)
XX (%)	0.26 (0–0.44)	0.69 (0.20–6.25)**
YY (%)	0.23 (0–0.37)	0.56 (0–2.50)**
Total sex disomy (%)	0.57 (0.45–0.74)	1.48 (0.66–6.27)**
Total sex nullisomy (%)	0 (0–0.37)	0 (0–1.02)
Disomy 12 (%)	0 (0–0.05)	0 (0–0.48)
Nullisomy 12 (%)	0 (0–0)	0 (0–0.63)
Aneuploidy chromosome 12 (%)	0 (0–0.05)	0 (0–0.63)
Disomy 8 (%)	0.33 (0.11–0.49)	0.73 (0–8.70)**
Nullisomy 8 (%)	0 (0–0)	0 (0–4.79)
Aneuploidy chromosome 8 (%)	0.33 (0.11–0.49)	0.73 (0–8.70)**
Disomy 18 (%)	0.27 (0–0.49)	0.65 (0–8.70)**
Nullisomy 18 (%)	0 (0–0)	0 (0–2.39)
Aneuploidy chromosome 18 (%)	0.27 (0–0.49)	0.77 (0.28–3.61)**
Total aneuploidy rate (%)	1.25 (0.85–1.52)	3.26 (1.64–23.65)**
Diploidy rate (%)	0.07 (0–0.24)	0.03 (0–2.40)
Median number of sperm scored	3741 (403–4030)	1965 (39–4113)

^aUpper range obtained from 14 normozoospermic healthy men. Ranges are shown in parentheses.

* $P < 0.05$ versus normal sperm aneuploidy rate; ** $P < 0.005$ versus normal sperm aneuploidy rate.

Table II. ICSI outcome of 48 consecutive infertile patients with normal or elevated total sperm aneuploidy rate

	Sperm aneuploidy rate	
	Normal ($\leq 1.55\%$) ^a	Elevated ($> 1.55\%$) ^a
Number of subjects	12	36
Number of cycles	12	38
Male partner age [years (range)]	33 (23–40)	35 (23–52)
Female partner age [years (range)]	31 (20–39)	33 (20–41)
Total number of oocytes retrieved	76	253
Total number of oocytes injected	58	220
Total number of two pronuclei	54 (93.1%)	188 (85.4%)
Total number of oocytes cleaved	54 (100%)	185 (98.4%)
Total number of embryo transfers	12	38
Total number of embryos transferred	41	127
Percentage of grade A embryos transferred	80.5	78.8
Total number of grade B embryos transferred	17.1	16.5
Total number of grade C embryos transferred	2.4	4.7
Biochemical pregnancy	9/12 (75%)	17/38 (44.7%)*
Clinical pregnancy	9/12 (75%)	13/38 (34.2%)*
Implantation rate	14/41 (34.1%)	17/127 (13.4%)*
Preclinical abortion	0	4/17 (23.5%)*
Clinical abortion	1/9 (11.1%)	2/13 (15.4%)*
Ongoing pregnancies/deliveries	8/12 (66.7%)	11/38 (28.9%)*
Number of singleton	2	5
Number of twins	6	4
Number of triplets	0	0

^aUpper range obtained from 14 normozoospermic healthy men.

* $P < 0.001$ versus normal sperm aneuploidy rate.

that oocyte fertilization can be achieved in the presence of aneuploid sperm, but that it results in a low pregnancy rate. However, studies in a greater number of patients are necessary to establish the impact of sperm aneuploidy on ICSI clinical outcome. In this study, we evaluated ICSI outcomes of infertile men with normal peripheral karyotypes subgrouped according to their level of sperm aneuploidy. Fertilization,

implantation, clinical pregnancy and miscarriage rates were examined.

Materials and methods

Sperm aneuploidy rate was evaluated by fluorescence in-situ hybridization (FISH) in 48 consecutive male patients attending the

Table III. Sperm aneuploidy and diploidy rates for chromosomes X, Y, 12, 8 and 18 in 33 infertile patients sorted according to their sperm aneuploidy rate undergoing ICSI with ejaculated sperm

	Sperm aneuploidy rate	
	Normal ($\leq 1.55\%$) ^a ($n = 11$)	Elevated ($> 1.55\%$) ^a ($n = 22$)
X (%)	49.82 (48.12–51.20)	49.06 (46.74–50.53)*
Y (%)	49.58 (49–51.12)	49.45 (44.78–52.10)
XY (%)	0.10 (0–0.50)	0.19 (0–1.64)
XX (%)	0.24 (0–0.44)	0.58 (0.20–4.78)**
YY (%)	0.22 (0–0.30)	0.52 (0.12–1.49)**
Total sex disomy (%)	0.57 (0.45–0.71)	1.27 (0.66–6.27)**
Total sex nullisomy (%)	0 (0–0)	0 (0–0)
Disomy 12 (%)	0 (0–0.05)	0 (0–0.24)
Nullisomy 12 (%)	0 (0–0)	0 (0–0)
Aneuploidy chromosome 12 (%)	0 (0–0.05)	0 (0–0.24)
Disomy 8 (%)	0.31 (0.11–0.49)	0.71 (0.39–4.01)**
Nullisomy 8 (%)	0 (0–0)	0 (0–0.06)
Aneuploidy chromosome 8 (%)	0.31 (0.11–0.49)	0.71 (0.39–4.01)**
Disomy 18 (%)	0.29 (0–0.49)	0.59 (0.34–3.61)**
Nullisomy 18 (%)	0 (0–0)	0 (0–0.12)
Aneuploidy chromosome 18 (%)	0.29 (0–0.49)	0.68 (0.34–10.64)**
Total aneuploidy rate (%)	1.22 (0.85–1.48)	2.64 (1.64–10.75)**
Diploidy rate (%)	0.07 (0–0.24)	0.05 (0–1.11)
Median number of sperm scored	3758 (403–4030)	3692 (830–4113)

^aUpper range obtained from 14 normozoospermic healthy men. Ranges are shown in parentheses.

* $P < 0.05$ versus normal sperm aneuploidy rate; ** $P < 0.005$ versus normal sperm aneuploidy rate.

Andrology and Reproductive Endocrinology Unit, University of Catania. These patients underwent ICSI as treatment for their male factor infertility. Their median age was 34 years (range 23–52). Semen samples were collected by masturbation on the day of oocyte retrieval, usually after 4–5 days of abstinence. Semen parameters were evaluated according to the WHO guidelines (WHO, 1999). Morphology assessment was also performed in the same swim-up preparation used for the oocyte injection.

Fifteen patients were azoospermic. Of these, nine had congenital bilateral absence of the vas deferens and underwent percutaneous epididymal sperm aspiration. The other six had non-obstructive azoospermia and underwent testicular sperm aspiration ($n = 5$) or microsurgical testicular sperm extraction ($n = 1$).

To define the reference values of sperm aneuploidy rate in the ejaculate, we studied 14 healthy men with a median age of 25 years (range 19–35), normal semen parameters and normal somatic karyotypes. Their median sperm aneuploidy rate was 0.91% (range 0.30–1.55). The upper range in the normal men was established as the upper limit of normal sperm aneuploidy rate. The patients subsequently were divided into two groups. Group A had sperm aneuploidy rates $\leq 1.55\%$ and group B had rates $> 1.55\%$. The age of the female partners of the patients of groups A and B was not statistically different [31 years (range 20–39) and 33 years (range 20–41) respectively].

Ovulation induction was achieved with a GnRH analogue and FSH in all the female partners according to our previously described protocol for ICSI (Calogero *et al.*, 2001a). We confirmed biological pregnancy by measuring serum β -HCG on at least two occasions 14 days after embryo transfer.

FISH analysis, DNA hybridization and scoring

Sperm were prepared for FISH analysis as reported previously, using an aliquot of the swim-up preparation or of the sperm retrieved from the epididymis or testis (Calogero *et al.*, 2001a; b). Likewise, we have previously reported our methods for sperm head decondensation, DNA hybridization and visual scoring of the sperm. Sperm were fixed

and spread on slides washed in $2\times$ standard saline citrate solution and incubated in dithiothreitol. Sperm structure, including the tail, was preserved, allowing for morphological differentiation between the sperm and other cells present in the ejaculate.

Alpha-centromeric probes for chromosomes 8, 12, 18, X and Y were used for both patient and control samples. The probe mixture for triple FISH consisted of a repetitive DNA sequence of centromeric probes for chromosome X (pDMX1), labelled FITC, for chromosome Y (pLAY5.5), labelled Cy3 and for chromosome 12 (pBR12), labelled FITC and Cy3. The probe mixture for the double-colour FISH also consisted of a repetitive DNA sequence of centromeric probes for chromosome 8 (pZ8.4) and chromosome 18 (2Xba), labelled FITC or Cy3, respectively. The probes were provided by Professor M.Rocchi, (University of Bari, Bari, Italy). The slides were scored using an Axiophot fluorescence microscope (C.Zeiss, Oberkochen, Germany) with single-band DAPI, FITC and Cy3 filters.

Sperm were scored as reported elsewhere (Calogero *et al.*, 2001a; b). Only intact sperm with clear hybridization signals were scored. We excluded disrupted or overlapping sperm. Sperm were considered polysomic if they presented two or more distinct hybridization signals of equal intensity separated by at least one signal domain. Diploid sperm displayed two signals for each tested chromosome with normal head and tail morphology.

Statistical analysis

We analysed the data with SPSS 9.0 for Windows statistical software. The Mann–Whitney or χ^2 -test determined significance as appropriate. A P -value < 0.05 was considered statistically significant. Results are shown as median and range.

Results

Twelve out the 48 patients (25%) enrolled in this study were found to have a total sperm aneuploidy rate within the normal range (group A), whereas the remaining 36 patients (75%) had

Table IV. ICSI outcome performed with ejaculated sperm from 33 infertile patients with normal or elevated total sperm aneuploidy rate

	Sperm aneuploidy rate	
	Normal ($\leq 1.55\%$) ^a	Elevated ($> 1.55\%$) ^a
Number of subjects	11	22
Number of cycles	11	23
Male partner age [years (range)]	34 (23–40)	35.5 (23–52)
Female partner age [years (range)]	30 (20–39)	32 (20–41)
Total number of oocytes retrieved	69	156
Total number of oocytes injected	51	132
Total number of 2 pronuclei	47 (92.1%)	116 (87.9%)
Total number of oocytes cleaved	47 (100%)	115 (99.1%)
Total number of embryo transfers	11	23
Total number of embryos transferred	36	73
Percentage of grade A embryos transferred	86.1	74.0
Percentage of grade B embryos transferred	11.1	17.8
Percentage of grade C embryos transferred	2.8	8.2
Biochemical pregnancy	9/11 (81.8%)	9/23 (39.1%)*
Clinical pregnancy	9/11 (81.8%)	7/23 (30.4%)*
Implantation rate	14/36 (38.9%)	10/75 (13.3%)*
Preclinical abortion	0	2/9 (22.2%)*
Clinical abortion	1/9 (11.1%)	0*
Ongoing pregnancies/deliveries	8/11 (72.7%)	7/23 (30.4%)*
Number of singleton	2	4
Number of twins	6	2
Number of triplets	0	0

^aUpper range obtained from 14 normozoospermic healthy men.

* $P < 0.001$ versus normal sperm aneuploidy rate.

Table V. Conventional semen parameters of 33 infertile patients undergoing ICSI sorted according to their sperm aneuploidy rate

	Sperm aneuploidy rate	
	Normal ($\leq 1.55\%$) ^a ($n = 11$)	Elevated ($> 1.55\%$) ^a ($n = 22$)
Volume (ml)	2 (2–4)	3 (0.8–7)
Density ($\times 10^6$ /ml)	20 (0.01–210)	17 (0.01–190)
Total number of spermatozoa ($\times 10^6$)	48 (0.02–420)	40 (0.008–570)
Total motility (%)	56 (10–80)	40 (1–63)
Progressive motility (%)	13.5 (3–50)	22 (4–50)
Normal oval forms (%)	17.5 (10–30)	20 (10–35)

^aUpper range obtained from 14 normozoospermic men. Ranges are given in parentheses.

values above the cut-off (group B). Patients of group B had significantly higher ($P < 0.005$) disomy rates for sex chromosomes and chromosomes 8 and 18 compared with group A. The total aneuploidy (disomy + nullisomy) rate for group B patients was also significantly higher ($P < 0.005$). Diploidy rate, however, was similar for the two groups (Table I). Among the azoospermic patients, 14 out of 15 (93%) were included in group B because of the higher aneuploidy rate in their epididymal or testicular sperm. Compared with ejaculated specimens of other patients in group B, there was a higher total aneuploidy rate among the harvested sperm (3.82 versus 2.65%), but the difference was not statistically significant ($P = 0.07$).

The ICSI parameters of patients are detailed in Table II. Although the fertilization rate was similar between the two groups, the biochemical and clinical pregnancy rates, as well as the implantation rate, were significantly higher in group A ($P < 0.001$). The preclinical and total abortion rates were significantly higher in group B.

ICSI clinical outcome with the use of epididymal or testicular sperm was worse than with ejaculated sperm (Vicari *et al.*, 2001). To account for the azoospermic patients, we recalculated the data comparing only ICSI cycles performed with ejaculated sperm. The sperm aneuploidy rate (Table III) and the ICSI clinical outcomes (Table IV) found in the two groups of patients using ejaculated sperm were comparable to the results found when considering all patients. The biochemical and clinical pregnancy rates and the implantation rate were significantly higher in group A compared with group B. Other conventional sperm parameters, such as sperm density, motility and morphology, were similar in groups A and B (Table V), thus ruling out other possible factors that may negatively influence ICSI outcome.

Discussion

In this study, we evaluated the impact of high sperm aneuploidy rate on clinical ICSI outcome. To accomplish

this, we stratified patients undergoing ICSI for the treatment of their infertile condition into two groups according to their normal or elevated sperm aneuploidy rate. In comparing the ICSI outcomes of these two groups of patients, we found several significant differences. Patients with normal sperm aneuploidy rates had higher pregnancy and implantation rates. The rates for preclinical (23.5 versus 0%) and total (38.9 versus 11.1%) abortion were significantly higher in group B patients. Sperm aneuploidy may affect early steps of embryo development, which may explain its impact on embryo implantation and miscarriage. High sperm aneuploidy rates did not, however, completely preclude a couple from having their own offspring with a normal chromosome complement.

The two groups of patients did not differ regarding the main parameters known to affect ICSI outcome. Maternal and paternal age as well as semen analysis parameters were similar in the two groups. Because epididymal and testicular sperm have a greater sperm aneuploidy rate (Burrello *et al.*, 2002) and negatively affect the ICSI outcome (Vicari *et al.*, 2001), we re-evaluated the data excluding these patients, and there were no significant changes. We therefore conclude that the only difference that affected the pregnancy rate was the greater number of genetically abnormal sperm in the group B patients, which was >2-fold that found in group A patients. It is noteworthy that sperm examined in this study were taken from the swim-up fraction used for oocyte injection, thus confirming that swim-up did not eliminate aneuploidy sperm (Pfeffer *et al.*, 1999). The embryologist may therefore unknowingly select aneuploid sperm for oocyte injection. However, similar to what has been reported previously (Pfeffer *et al.*, 1999), the diploidy rate found in sperm recovered after swim-up was lower than that found in the whole semen of OAT patients (Calogero *et al.*, 2001b).

Other authors have suggested a negative trend of gamete chromosomal abnormalities on implantation rate (In't Veld *et al.*, 1997; Pang *et al.*, 1999; Rubio *et al.*, 2001), pregnancy rate (Pang *et al.*, 1999; Pfeffer *et al.*, 1999; Rubio *et al.*, 2001), and fetal survival (Rubio *et al.*, 2001). We have previously observed a higher percentage of men with elevated sperm aneuploidy rate among infertile couples with pregnancy failure after ICSI (Calogero *et al.*, 2001a). That study and the present one therefore support a negative impact of aneuploidy in achieving a pregnancy after ICSI. In addition, Gianaroli and colleagues have shown that pregnancy success after IVF is influenced significantly by embryo chromosome aneuploidy (Gianaroli *et al.*, 1997).

The pregnancy rate observed in patients of group B is commensurate with ICSI results reported by IVF centres worldwide. The pregnancy rate of a similar subset of infertile patients selected with normal sperm chromosomal constitution (group A) was more than optimal. This high value may be due to the relatively low number of patients found with an normal sperm aneuploidy rate, since the vast majority of the patients requiring ICSI have an abnormal sperm chromosomal complement (Calogero *et al.*, 2001a; Rubio *et al.*, 2001). Sperm aneuploidy may play a major role in explaining the suboptimal pregnancy rates in many infertile couples undergoing ICSI, but our findings need confirmation in larger series.

Normal morphology and good motility are two of the main criteria for selecting sperm to be injected during micromanipulation. Therefore, the overall likelihood that an embryologist may select a genetically abnormal spermatozoon for ICSI would be low. However, Pfeffer *et al.* (1999) and the results in this study demonstrate that the swim-up preparation does not completely eliminate genetically abnormal sperm. In addition, a recent study by Ryu and colleagues suggests that morphologically normal sperm in infertile patients with <4% normal forms (Kruger's strict criteria) have 2–3-fold higher aneuploidy rates compared with normally shaped sperm from normozoospermic men (Ryu *et al.*, 2001). Based on these results, even morphologically normal spermatozoa may have an abnormal chromosome asset.

In conclusion, this study showed that higher total sperm aneuploidy rates are associated with lower implantation and pregnancy rates, and higher rates of miscarriage in patients undergoing ICSI. Future development of methods to identify genetically abnormal sperm may allow for better selection and improved ICSI outcomes. Studies in a larger series of patients are needed to confirm these findings.

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