Analysis of chromosomal aneuploidies in sperm of infertile males by using FISH technique

O. Liamani¹, S. Moulessehou¹, I. Benzeguir².

¹ Département de Biologie, Facultés des Sciences de la nature et de la vie, Université Djillali Liabes, Sidi Bel Abbes, Algérie.
² Laboratoires d’analyses de biologie médicale.

ABSTRACT

Male factors leading to infertility account for at least half of all cases of infertility worldwide. In this study, we used multicolor fluorescent in situ hybridization (FISH) probes for chromosomes 13, 18, 21, X and Y to evaluate the aneuploidy incidence in sperm cells. The study group included 12 males with infertility and oligoasthenoteratozoospermia (OATs). FISH revealed a significantly higher incidence of sperm aneuploidies compared with controls. By comparing the incidence of the disomy in the OAT group, the highest incidence was the sex chromosome disomy, followed by the disomy of chromosomes 13, 21 and then 18. The nullisomy incidence in the OAT group was higher for sex chromosomes, followed by the nullisomy of autosomes 13, then 21 and 18. In conclusion FISH may be considered as an additional assay for the evaluation of spermatozoa beside standard analysis, thus playing an important role during proper diagnosis and treatment of infertility. As in these days, for patients with OAT, intracytoplasmic sperm injection (ICSI) is frequently used, it is important to inform the patients if they might have an increased risk of aneuploidies in embryos.

Key words: Oligoasthenoteratozoospermia, aneuploidies, FISH, infertility.

INTRODUCTION:

The infertility onus has been increasing over recent decades and it is estimated to affect 15% of couples worldwide. (WHO, 2010) There is conspicuous evidence that male partners account for the etiology of half the cases. The evaluation of male infertility is based on routine semen analysis, which measures both semen production and sperm quality. However, normal values of these parameters do not accurately mirror the fertilisation capability of the sperm. Moreover, there are numerous known causes of male infertility that this analysis provides no information about (Sa et Sousa, 2015).

Sperm aneuploidy is considered a major cause of pregnancy loss, aneuploid births, and developmental defects (Hassold et Hun, 2001). Recent reports demonstrate a significant increase of the sperm aneuploidy rate in infertile men when compared with fertile counterparts, although this did not exceed 2% with regard to chromosomes X, Y, 18, and 21 (Tempest et Martin, 2009; Harton et Tempest, 2012).

These findings suggest that differences between paternal and maternal contribution to aneuploidy is not due to differences in the chromosome segregation errors, but rather more effective control point in spermatogenesis than oogenesis. Recent studies have showed that synaptic and recombination errors not only cause abnormal chromosome segregation but also lead to blocking meiosis. If a partial blockage, the result will be oligozoospermia, whereas a complete blockade affects all germ cells and lead to azoospermia (Gonnsalves et al., 2004; Egozcue et al., 2005). As for many cases of spontaneous abortion and infertility the causes are chromosomal aberrations of the embryo was suggested that the better estimation of the aneuploidy rate at conception can be done by assessing the gametes chromosomes (Templado et al., 2013).

In this study, we used multicolor FISH probes for chromosomes 13, 18, 21, X and Y based on the evidence that these chromosomes are responsible for the most frequent found aneuploidies. We used strict scoring criteria and a minimum of 5000 sperm analyzed per chromosome for 12 patients with oligoasthenoteratozoospermia (OAT) and 08 individual with normal fertility.
Materials and Methods

Patients
The group of OAT patients included males referred to the Medicals Laboratories. The control group included males having at least two children and no assisted reproductive techniques applied for them. Our patients were included in the present study after having a clinical urological examination, a dosage of hormonal of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and testosterone. The study group included 12 males with infertility. In this group were included males presenting less than 10 million sperm/mL, sperm mobility less than 40% and normal sperm morphology less than 20%. The control group included 08 males with normal fertility and normal sperm concentration, morphology and mobility.

Semen analysis:
Semen sample of patients was collected by masturbation after 3 days of sexual abstinence and examined after liquefaction for 30 min at 37ºC. Volume, pH, concentration, motility and morphology where evaluated according to WHO guidelines (2010). Sperm morphology was evaluated by the May Grünwald-Giemsa (MGG) method. After removal of seminal liquid, the spermatozoa were washed twice with sterile water (300g for 10 mn), fixed with carnoy’s solution and then spread on a slide for FISH and TUNEL.

FISH analysis:
The sperm sample was washed in phosphate-buffered saline, and 20 mL of sperm was dropped and fixed on a slide with Carnoy’s solution (methanol–acetic acid; 3:1, vol/vol). The sperm nuclei were partially decondensed for 3 minutes by using a solution of NaOH (1 mol/L), then they were washed in 2XSSC for 10 minutes. The sperm sample was analyzed by using dual FISH (chromosomes 13 and 21) with a specific cocktail probe of 13q14 and 21q22 (Abbott, Rungis, France) and by using triple FISH (chromosomes X, Y, and 18) with specific alphoid probes of the X chromosome (probe DXZ1, spectrum green; Abbott), Y chromosome (probe DYZ3, spectrum orange; Abbott), and chromosome 18 (D18Z1, spectrum aqua; Abbott). Before hybridization, the sperm DNA slides were immersed in a jar of 2X SSC/0.4% NP40 solution for 30 minutes at 37ºC and then were passed through an ethanol series of increasing concentrations before being allowed to air dry. The denaturation was performed simultaneously on sperm nuclei and probes for 1 minute at 72ºC. The slides were incubated overnight at 37ºC. Posthybridization washes included 45 seconds in 0.4X SSC/0.3% NP40 at 72ºC, followed by 20 seconds in 2X SSC/0.1% NP40 at room temperature. The slides were counterstained with 4,6-diamino-2-phenylindole and observed by using a Zeiss Axioplan microscope (Zeiss, Le Pecq, France), with the appropriate set of filters. Subsequent image acquisition was performed by using a CCD camera with Isis (In Situ Imaging System; MetaSystems, Altlussheim, Germany).

Statistical analysis
Statistical analysis was performed using the Statistical Package for Social Sciences, version 22 (SPSS, Chicago, IL, USA). The comparisons between the controls and patients were calculated using Student’s t-test. Spearman’s correlation coefficient \( r \) was used to assess the correlations between variable. A significant statistical difference was accepted when \( P < 0.05 \).

RESULTS:
Semen analysis:
Sperm parameters and ages of the infertile patients are presented in Table 1 (values from the most recent semen analysis were used).

Table 1: semen parameters in study groups

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Mean age (years)</th>
<th>Sperm concentration (X 10^6 mL)</th>
<th>Progressive motility (%)</th>
<th>Normal morphology (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OAT 1</td>
<td>31</td>
<td>4.60</td>
<td>25</td>
<td>16</td>
</tr>
<tr>
<td>OAT 2</td>
<td>37</td>
<td>2.80</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>OAT 3</td>
<td>27</td>
<td>0.18</td>
<td>00</td>
<td>2</td>
</tr>
<tr>
<td>OAT 4</td>
<td>37</td>
<td>0.80</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>OAT 5</td>
<td>35</td>
<td>8.80</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>OAT 6</td>
<td>32</td>
<td>7.80</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>OAT 7</td>
<td>45</td>
<td>0.38</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>OAT 8</td>
<td>44</td>
<td>2.60</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>OAT 9</td>
<td>38</td>
<td>1.70</td>
<td>30</td>
<td>17</td>
</tr>
<tr>
<td>OAT 10</td>
<td>31</td>
<td>1.41</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>OAT 11</td>
<td>37</td>
<td>1.40</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>OAT 12</td>
<td>28</td>
<td>0.12</td>
<td>05</td>
<td>5</td>
</tr>
<tr>
<td>Control group</td>
<td>28-45</td>
<td>129±49.95</td>
<td>55.41±3.39</td>
<td>51.33±7.65</td>
</tr>
</tbody>
</table>

OAT: Oligoasthenoteratozoospermia.
Analysis of meiotic segregation:
The age for the patients from the OAT group was between 27–45 years, with a mean of 35.17 years, while for the control group varied between 28–45 years and the mean age was 38.87 years. No correlation between paternal age and the rate of sperm aneuploidy was found.

All the OAT patients, as well as the individuals included in the control group, exhibit chromosomal aneuploidies of the semen, but a large variability of the aneuploidy rates was found. For each individual included in the study were found sperm chromosomal numerical aberrations involving all the chromosomes evaluated. The average sperm parameters of the OAT patients as well as the control group are presented in the Table 1.

Incidence of disomy and nulisomy for chromosome 18 in OAT group (Table 2) was significantly higher than in the control group. The disomy of chromosome 18 varied between 0.19% and 0.43%; mean value was 0.30%. When compared with the disomy of chromosome 18 in the control group, 0.30% vs. 0.06% was found that there is a significant statistical difference, \( p=0.000 \).

The nulisomy of chromosome 18, in the OAT group varied between 0.32% and 0.69%, the mean value was 0.45% vs. 0.06% the nulisomy 18 in the control group and it was also documented a significant statistical difference, \( p=0.000 \).

The overall sexual chromosome disomy and nulisomy in OAT group (Table 2) is higher than the one identified in the control group. Gonosomes aneuploidy rate presented large variations for the study group. Gonosomes nulisomy varied between 2.99% and 6.57%, with a mean value of 4.51%. Rate of nulisomy for sex chromosomes was significantly higher in the OAT compared to the control (4.51% vs. 0.34%, \( p=0.000 \)).

For the disomy of chromosomes gonosomes larger variations were registered, between 0.84% and 2.63%, the mean value was 1.54% vs. 0.24% for the control group.

The differences between the sexual chromosome incidence of disomy for OAT patients and the controls were statistically significant (\( p=0.001 \)).

Incidence of disomy and nulisomy for chromosome 13 in OAT group was significantly higher than in the control group (Table 2). The disomy of chromosome 13 varied between 0.19% and 1.21%, with a mean value of 0.52%. The statistical difference between the OAT group and the control group was significant, 0.52% compared to 0.14% (\( p=0.047 \)).

Chromosome 13 nulisomy in the OAT group varied between 1.21 and 2.35%, with a mean value of 1.61%. The incidence of chromosome 13 nulisomy was higher in the OAT group as compared with the control group: 1.61% to 0.15% (\( p=0.000 \)).

For the OAT group was found a large variation of the Disomy 21, between 0.01 and 1.12%, the mean value was 0.62% (Table 2), while in the control group it was 0.15% (\( p=0.000 \)).

The variation of the chromosome 21 nulisomy ranged between 1.12 and 2.37% with a mean value of 1.71% while for the control group it was only 0.18% (\( p=0.000 \)).

The variation of the chromosome 21 disomy ranged between 0.01 and 1.12% with a mean value of 0.62% while for the control group it was only 0.15% (\( p=0.010 \)).

Diploidy frequency in the OAT group varied between 0.35 and 0.93%, with a mean value of 0.50%. The incidence of Diploidy was higher in the OAT group as compared with the control group: 0.50% to 0.34% (\( p=0.030 \)).

By comparing the incidence of the disomy in the OAT group, the highest incidence was the sex chromosome disomy, followed by the disomy of chromosomes 21,13 and then 18. In the control group, the highest incidence was the sex chromosome disomy, followed by the disomy of chromosomes 18, 21 and then 13. Large interindividual variations were found in the group of OAT patients. The highest incidence of chromosome 13 disomy (Figure 1) was found in patient OAT 7 (1.21%), while patient OAT 8 had the lowest incidence of disomy 13 (0.19%). For chromosome 18, the incidence of disomy varied between 0.43% (OAT 1) and 0.21% (OAT 6). The highest incidence of autosomal disomy was found for chromosome 21 (Figure 1), for this chromosome, the disomy incidence was 1.12% (OAT 12). The lowest incidence of chromosomes 21 disomy was 0.01% (OAT 7).

The incidence of sexual chromosome disomy (Figure 4) was higher than the rate of autosomal disomy and the interindividual variance for gonosome disomy (Figure 2) was very large. Patient OAT 7 presented the highest incidence of gonosome disomy (2.63%), while patient OAT 2 showed the lowest incidence for sex chromosome disomy (0.84%). The highest incidence of diploidy (Figure 3) was found in patient OAT 6 (0.93%), while patient OAT 1 OAT 10 had the lowest incidence of diploidy (0.35%).
Table 2: Incidence of disomy, nullisomy and diploidies for chromosomes 13, 18, 21 and sex chromosomes:

<table>
<thead>
<tr>
<th></th>
<th>Disomy frequency (%)</th>
<th>Total rate of sex chromosomes disomy (%)</th>
<th>Diploidy frequency (%)</th>
<th>Nullosomy frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1313 1818 2121 XX YY XY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OAT 1</td>
<td>0.29 0.43 0.34 0.10 0.59 0.18</td>
<td>0.87</td>
<td>0.35</td>
<td>0.50 4.53 1.35 1.06</td>
</tr>
<tr>
<td>OAT 2</td>
<td>0.25 0.35 0.44 0.25 0.30 0.29</td>
<td>0.84</td>
<td>0.45</td>
<td>0.39 3.47 1.21 1.84</td>
</tr>
<tr>
<td>OAT 3</td>
<td>0.18 0.28 0.58 0.54 0.49 0.38</td>
<td>1.41</td>
<td>0.79</td>
<td>0.45 2.99 1.06 1.63</td>
</tr>
<tr>
<td>OAT 4</td>
<td>0.42 0.19 0.69 0.48 0.23 0.59</td>
<td>1.30</td>
<td>0.45</td>
<td>0.41 4.21 1.18 1.48</td>
</tr>
<tr>
<td>OAT 5</td>
<td>0.56 0.34 0.79 0.33 0.41 0.36</td>
<td>1.10</td>
<td>0.50</td>
<td>0.32 5.11 1.29 1.93</td>
</tr>
<tr>
<td>OAT 6</td>
<td>0.33 0.21 0.36 0.45 0.29 0.45</td>
<td>1.19</td>
<td>0.39</td>
<td>0.36 4.54 1.39 1.47</td>
</tr>
<tr>
<td>OAT 7</td>
<td>1.21 0.39 0.01 0.72 0.82 1.09</td>
<td>2.63</td>
<td>0.93</td>
<td>0.69 6.57 2.03 1.98</td>
</tr>
<tr>
<td>OAT 8</td>
<td>0.19 0.29 0.98 0.41 0.53 0.98</td>
<td>1.92</td>
<td>0.45</td>
<td>0.56 3.84 1.92 1.12</td>
</tr>
<tr>
<td>OAT 9</td>
<td>0.53 0.32 0.83 0.27 0.50 0.29</td>
<td>1.06</td>
<td>0.37</td>
<td>0.35 4.62 1.84 1.60</td>
</tr>
<tr>
<td>OAT 10</td>
<td>0.31 0.22 0.21 0.30 0.46 0.61</td>
<td>1.37</td>
<td>0.35</td>
<td>0.43 3.91 1.65 1.92</td>
</tr>
<tr>
<td>OAT 11</td>
<td>0.92 0.23 1.09 0.66 0.72 1.06</td>
<td>2.44</td>
<td>0.46</td>
<td>0.52 5.38 2.35 2.19</td>
</tr>
<tr>
<td>OAT 12</td>
<td>1.08 0.33 1.12 0.70 0.63 1.08</td>
<td>2.41</td>
<td>0.52</td>
<td>0.47 4.92 2.11 2.37</td>
</tr>
<tr>
<td>Means</td>
<td>0.52 0.30 0.62 0.43 0.50 0.61</td>
<td>1.55</td>
<td>0.50</td>
<td>0.45 4.51 1.61 1.71</td>
</tr>
<tr>
<td>Control group</td>
<td>0.14 0.06 0.15 0.09 0.07 0.08</td>
<td>0.24</td>
<td>0.34</td>
<td>0.06 0.34 0.15 0.18</td>
</tr>
</tbody>
</table>

Figure 1 – Disomy of chromosome 13 – two green signals corresponding to chromosome 13 and one red signal corresponding to chromosome 21, Disomy of chromosome 21 – one green signal corresponding to chromosome 13 and two red signals corresponding to chromosome 21.

Figure 2 – Disomy of chromosome Y – two red signals corresponding to chromosome Y, one blue signal corresponding to chromosome 18 and one green signal corresponding to chromosome X.

Figure 3 – Diploidy – two green signals corresponding to chromosome 13 and two red signals corresponding to chromosome 21.

Figure 4 – Disomy of chromosome Y and X – one green signal corresponding to chromosome X, one red signal corresponding to chromosome Y and one blue signal corresponding to chromosome 18.
DISCUSSION:
The association of maternal advanced age with an increased risk for having an offspring with aneuploidy is well documented, while the effect of paternal advanced age is still unclear (Erikson et al., 1978). There are several reports (Griffin et al., 1995; Ländetie at al., 1996) showing that the incidence of sex chromosomes disomy is higher in cases of advanced paternal age. Further studies try to find correlation between the quality of semen and the incidence of chromosomal aneuploidies in sperm. This hypothesis was raised after observing a higher incidence of chromosomal abnormalities in cases where intra-cytoplasmic sperm injection (ICSI) was performed due to low concentration/motility/morphology of semen (Bonduelle et al., 2002).

For the control group, the rates of aneuploidy were similar with those reported by Templado et al., 2013. For the OAT group, the overall rate of chromosomal aneuploidy was 11.78%, which is comparable with the reports of Andreescu et al. in Romania (14.63%)( Andreescu et al., 2016), Pylyp et al. in Ukraine (Pylyp et al. 2013), Kumtepe et al., in Turkey (12%)(Kumtepe et al., 2009). Lower rates of chromosomal aneuploidy were reported by Wang et al., in China (8.5%) (Wang et al., 2010), Rao et al., in India (7.9%) (Rao et al., 2005), Gekas et al., in France (6.9%) (Gekas et al., 2001).

In this study, we have recorded also the incidence of nulisomy, which is not often reported. There is a debate regarding the correct assessment of nulisomy and its status as a failure of hybridization. Taking in consideration chromosomal non-disjunction during meiosis as the mechanism underlying the occurrence of disomy/ nulisomy, the incidence of nulisomy should be similar to the rate of disomy. We consider that our results regarding the incidence of nulisomy are not due to artifacts during the procedure so because in both groups, for the autosomal chromosomes studied, the rate of disomy/nulisomy is close to 1:1 (0.52% vs. 1.61%, 0.30% vs. 0.45%, 0.62% vs.1.72%, 0.14% vs. 0.15%, 0.06% vs. 0.06%, 0.15% vs. 0.18%). In the OAT group, the rate of nulisomy for sex chromosomes compared with the disomy is 2.90 (4.51% vs. 1.55%) explained by the high levels of sex chromosomes nulisomy. These findings can be explained by the anaphase lag that can occur in spermatogenesis (cimini et al., 2003; Cupiste et al. 2003).

We have studied the hypothesis of a possible correlation between sperm parameters and the incidence of aneuploidy. Between the semen parameters and aneuploidy of the studied chromosomes were found weak negative correlations. The correlation coefficients were: -0.30 for sperm concentration and aneuploidy rate, -0.24 for morphology and chromosomal aberrations, -0.40 for sperm motility and aneuploidy. The overall incidence of disomy in the OAT group showed a weak to moderate correlation with the semen parameters. Previous studies have reported negative correlation between the rate of chromosome aneuploidy and oligospermia (Durak Aras et al., 2012; Mougou-Zerelli et al., 2011).

In this study, we found a weak negative correlation between the disomy incidence and the sperm concentration (r=-0.51). By comparing the disomy incidence and the progressive motility and the normal morphology, we found a moderate to weak negative correlation, the correlation coefficients were r=-0.54 and r=-0.26 respectively. Different results in regards with the correlation between low motility and rate of aneuploidy were found. There are researchers that reported modest correlation between those two parameters (Aran et al., 1999; Vegetti et al., 2000), while in other cases, no correlation was found (Serrate et al., 2010). In regards with the correlation between the high incidence of teratozoospermia and the rate of aneuploidy, several reports indicated a positive correlation (Tang et al., 2010; Brahem et al., 2011), while in other cases no correlation was documented (Serrate et al., 2010).

CONCLUSION:
The results of our study support the importance of sperm FISH analysis for the patients with OAT, which usually undergoes assisted reproductive techniques. The molecular cytogenetic analysis allows the evaluation of sperm aneuploidy rates and should be recommended before the application of any assisted reproductive procedure. These investigations allow the identification of patients with an increased risk for reproduction failure and facilitate an appropriate counseling in order to inform the patients about their reproductive options, the genetic preimplantation testing and the prenatal genetic tests that are available.

REFERENCES:


