**Sperm macrocephaly syndrome in a patient without AURKC mutations and with a history of recurrent miscarriage.**

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(Article begins on next page)
Sperm macrocephaly syndrome in a patient without AURKC mutations and with a history of recurrent miscarriage

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Abstract

This paper reports a case of recurrent miscarriage in a patient affected by a variant phenotype of sperm macrocephaly syndrome (SMS). SMS is usually related to specific sperm characteristics (large head, multiple tail) and homozygous mutations in the aurora kinase C gene (AURKC). However, the present case observed large-headed spermatozoa with no flagellar abnormalities and no mutations detectable by AURKC sequencing. Furthermore, the patient had repeatedly conceived by intracytoplasmic sperm injection, but pregnancy always aborted. This study performed morphological analysis (Papanicolau staining), annexin V/propidium iodide staining, sperm chromatin structure assay (SCSA), fluorescence in-situ hybridization (FISH) and transmission electron micros-copy. This study observed large-headed, monotailed, mono-centriolar spermatozoa characterized by abnormal chromatin and swollen mitochondria. SCSA revealed a high ratio of late apoptotic cells with fairly intact amount of DNA. The FISH analysis showed 100% disomy rate. As far as is known, this is the first study to include gene sequencing, TEM, cytogenetic analysis and sperm DNA fragmentation in a case of SMS and also to report recurrent miscarriage related to this specific condition. SMS may be associated with important abnormalities of the sperm subcellular structure and with disomy even in the absence of mutations in the AURKC coding sequence.

Sperm macrocephaly syndrome (SMS) is a rare condition that affects spermatozoa and is related to infertility. It is characterized by a specific phenotype of large-headed, multi-tailed spermatozoa with an abnormal chromosomal status. A very few pregnancies have been obtained so far in SMS patients by means of IVF procedures. We present a case of SMS that differs from the classical syndrome as we observed large-headed spermatozoa without tail abnormalities. The affected patient had achieved three pregnancies following IVF, but all aborted. We carried out a detailed examination of the patient’s spermatozoa – morphological, cytogenetic, DNA fragmentation and ultrastructural analysis – and we observed that his spermatozoa are characterized by a large head whose texture appears apoptotic, a single tail and a midpiece whose mitochondria appear swollen. The DNA content within the spermatozoa was altered, as well as the chromosomal status, suggesting that some error must have occurred during spermagenesis. Interestingly, the genetic sequencing of the specific gene usually related to SMS syndrome (AURKC) revealed no mutations in our patient, suggesting that other genes may be involved in determining this syndrome. As far as is known, this is the first study in which spermatozoa of a SMS patient have been observed using morphological analysis, ultrastructural analysis, cytogenetic analysis and sperm DNA fragmentation analysis together. Moreover, it is believed that this is first report of recurrent miscarriage due to this specific syndrome.

KEYWORDS: AURKC gene, FISH, macrocephalic spermatozoa, recurrent miscarriage, sperm DNA fragmentation, TEM
Sperm macrocephaly syndrome (SMS) is a rare infertility-related condition (prevalence in the subfertile population <1%) that was first described in 1977 (Nistal et al., 1977). It is commonly associated with severe oligozoospermia (<2 million/ml) and is characterized by large-headed and multi-tailed spermatozoa that show abnormal ploidy and increased chromatin decondensation (Benzacken et al., 2001; Escalier, 1983; Guthauser et al., 2011; Viville et al., 2000).

The vast majority of patients showing this peculiar pheno-type display a homozygous truncating mutation in the aurora kinase gene (AURKC), which is known to play a crucial role in meiotic chromosomal segregation and cytokinesis (Dieterich et al., 2007). The mutated transcript leads to a blockage of both meiotic divisions, finally causing the presence of tetra-ploid, multi-flagellated spermatozoa; a North-African population was detected as a possible ‘genetic imprint’ carrying this alteration (Ben Khelfa et al., 2011, 2012; Dieterich et al., 2009; El Kerch et al., 2011).

SMS is related to the impairment of spontaneous conceptions and to a poor outcome of IVF treatment. Macrocephalic spermatozoa are unable to enter into the oocyte, and intra-cytoplasmic sperm injection (ICSI), although more effective in obtaining fertilization, is risky because of the relevant chance to generate a genetically altered offspring. Some new techniques, such as intracytoplasmic morphologically selected sperm injection or polarized light microscopy analysis of spermatozoa (Magli et al., 2012; Wilding et al., 2011) could possibly help in eventually selecting rare normal spermatozoa in these patients, but their real effectiveness in identifying euploid spermatozoa is this specific situation is uncertain (Celik-Ozenci et al., 2004; Chelli et al., 2010).

It has recently been reported that SMS may sometimes appear in a milder, incomplete form in which fertility is not fully compromised and the birth of a healthy baby may occasionally happen after IVF (Guichaoua et al., 2009; Shimizu et al., 2012). Herein is reported the case of a man with a variant of SMS, who had repeatedly conceived after ICSI, but whose pregnancies always ended with a spontaneous abortion in the first trimester. This case, possibly a mild variant of the classical SMS, is peculiar for some specific features: (i) the absence of other sperm morphological abnormalities (e.g. multi-tail) that are characteristic of the classical form of the syndrome; (ii) the absence of detectable mutations in AURKC; and (iii) the ease of obtaining a pregnancy by ICSI and the repeated abortions that followed. A thorough study of the sperm characteristics of the patient was carried out.

Materials and methods

Patient

A 46-year-old man married to a 39-year-old woman was referred to the study clinic because of a long-lasting (10 years) history of primary infertility and two spontaneous abortions in the first trimester of pregnancy. He had no history of orchitis, testicular torsion, post-traumatic testicular damage, cryptorchidism or drug abuse. His karyotype, as well as that of his wife, was normal. The patient informed us that his only brother had a 12-year history of infertility, but any other information regarding his family was missing and the patient did not allow any member of his family to be interviewed.

Two previous ICSI attempts performed in other IVF units had resulted in a singleton pregnancy followed by miscarriage before 12 weeks of gestational age. A third, recently performed ICSI attempt was also followed by the conception of a singleton pregnancy and by a spontaneous miscarriage at 10 weeks of gestation: this time a karyotype of the aborted conceptus was performed and revealed a 69 XXX aberration.

After obtaining the patient’s consent and the approval of OIRM S. Anna Hospital ethical committee (reference number CEA2316, 24 February 2012), a routine sperm analysis and a series of specific tests was performed on the patient’s semen: sperm chromatin structure assay (SCSA), annexin V/propidium iodide (AnnV/PI) assay, fluorescence in-situ hybridization (FISH), transmission electron microscopy and AURKC sequencing.

Routine sperm analysis

A sperm sample was obtained after 3–5 days of ejaculatory abstinence. After complete liquefaction at 37°C, semen parameters were assessed in accordance to WHO guidelines (WHO, 2010). Evaluation of concentration and motility was performed under bright light microscopy at ·20 magnification. After Papanicolaou staining, morphological estimation was accomplished on 200 replicates at ·100 magnification.
Sperm chromatin structure assay (SCSA)

DNA damage was measured by SCSA following the criteria established by Evenson et al. (2002). An aliquot of 2 · 10⁶ sperm cells was diluted with 200 lI TNE buffer (0.15 mol/l NaCl, 0.01 mol/l Tris HCl, 1 mmol/l disodium EDTA, pH 7.4, 4LC) and immediately mixed with 400 lI acid detergent solution (0.08 mol/l HCl, 0.15 mol/l NaCl, 0.1% Triton X 100, pH 1.2, 4LC) and 1.2 ml acridine orange staining solution (0.037 mol/l citric acid, 0.126 mol/l Na₂HPO₄, 0.0011 mol/l disodium EDTA, 0.15 mol/l NaCl, pH 6.0, 4LC) containing 6 lg/ml acridine orange (Sigma–Aldrich, St Louis, MO, USA). The sample was analysed using a FACScan Flow cytometer (BD Immunocytometry System, San Jose, CA, USA). The green fluorescence (FL1) was collected through a 515–545 nm band-pass filter and the red fluorescence (FL3) through a 650 nm long-pass filter. Data acquisition of 10,000 cells was initiated 3 min after the addition of the acid detergent solution and collected using BD CellQuest Pro version 4.0 (BD Biosciences, San Jose, CA, USA).

Annexin V/propidium iodide assay

The annexin V/propidium iodide (AnnV/PI) assay was performed using the Alexa Fluor 488 Annexin V/Dead Cell Apoptosis Kit (Invitrogen, Eugene, OR, USA). An aliquot of 4 · 10⁵ sperm cells was washed twice with 1 phosphate-buffered saline (PBS), then resuspended in 200 lI 1· annexin binding buffer and divided into two fractions of 100 ll each. Cells were incubated at room temperature for 15 min in the dark with 5 ll Alexa Fluor 488 annexin V and 1 ll 100 lg/ml PI working solution. After the incubation period, 400 ll 1· annexin binding buffer was added to the cell suspension and kept on ice, and immediately analysed by flow cytometry, measuring the fluorescence emission at 530 nm and 575 nm using 488 nm excitation.

FISH analysis

Semen (1 ml) was washed twice in 1· PBS and centrifuged at 160 g for 10 min (Martin, 1998). The pellet was suspended carefully in 1 ml of fresh cold fixative solution (methanol/acetic acid, 3:1), stored at -20°C and fixed with Carnoy's solution. Spermatozoa were spread on a slide, air dried and stored at -20°C overnight. The slides were washed in 1 mol/l NaOH solution, in standard saline citrate solution for additional 4 min, washed in PBS and air dried. After dehydration, FISH was performed with DNA probes mapping on chromosomes 13, 16, 21 and 22 (Vysis MultiVario, Abbott Laboratories, Chicago, IL, USA). Slides were washed and counterstained with 6 diaminophenylindole solution (DAPI) and spermatozoa were analysed with an epifluorescence microscope equipped with single band-pass filters and counted with Metafer software (Carl Zeiss). Observation and interpretation criteria were based on the number of spots for chromosomes 13, 16, 21 and 22 in the sperm nuclei. A spermatozoon with the nucleus containing more than one spot for each probe was considered as diploid.

Propidium iodide flow cytometry

Spermatozoa were frozen and stored at -80°C according to the laboratory routine procedures. After thawing, cells were flushed twice in PBS and centrifuged for 10 min at 800 g at room temperature. To obtain nuclear decondensation and allow PI staining, cells were suspended in 4% para-formaldehyde (PFA) for 30 min at 4°C. Cells were then centrifuged (800 g, 5 min) and resuspended in 100 ll Perm buffer (Ca- and Mg-depleted PBS, 1% of 10· BSA, 0.1% sodium azide, 0.1% saponine) with 1 ll of 1 g/l PI (Sigma–Aldrich). After 1 h incubation at 4°C, sperm cells were washed using the staining buffer (45 ml Ca- and Mg-depleted PBS, 5 ml of 10· BSA, 250 ll sodium azide; Sigma–Aldrich) and resuspended in 500 ll of the staining buffer. The sample was then immediately analysed using a FACScan Flow cytometer. The red fluorescence (FL2) was collected through a 585 nm long pass filter. Data acquisition of 10,000 cells was collected using CellQuest.

Transmission electron microscopy

Spermatozoa were centrifuged (10 min, 250 g) and the pellet was fixed in 1% paraformaldehyde (Merck, Darmstadt, Germany), 1.25% glutaraldehyde (Fluka, St Louis, MO, USA) and 0.5% sucrose in So¨rensen phosphate buffer (0.4 mol/l, pH 7.4; prepared by adding 56 g K₂HPO₄·pH₃O (Sigma–Aldrich) and 10.6 g NaH₂PO₄·pH₃O (Merck, Darmst-tad, Germany) to 1 l double-distilled water for 2 h). The pellet was then washed in 1.5% sucrose in So¨rensen phos-phate buffer (0.4 mol/l, pH 7.4) for 6–12 h, post-fixed in 2% osmium tetroxide, dehydrated and embedded in Glau-ert's embedding mixture, which consisted of equal parts of Araldite M and Araldite Ha¨rter, HY 964 (Merck) and finally supplemented with 2% of the accelerator DY 064 (Merck). The plasticizer dibutyl-phthalate was added at 0.5%. Thin sections (70 nm) were cut using a Leica Ultracut UCT, stained with uranyl-acetate and lead citrate and examined in a JEM-1011 transmission electron microscope (JEOL, Tokyo, Japan) equipped with a Mega-View-III digital camera and a Soft-Imaging-System (SIS, Mu¨nster,
Germany) for computerized image acquisition.

**AURKC amplification and sequencing**

Genomic DNA was extracted from 5–10 ml of frozen EDTA venous blood using the EZ1 Advanced XL instrument (Qiagen, Mannheim, Germany). This study sequenced the seven coding exons, intron–exon boundaries and 5'– and 3'-untranscribed regions (UTR) of AURKC (NM_001015878.1). Fragments were amplified by PCR using seven different primers (Table 1) in a total volume of 25 ll, using 50 ng DNA, 144 lmol/l dNTP, 2 mmol/l MgCl₂ and 1 U KAPA2G Fast Polymerase (KAPA Biosystems, Woburn, MA, USA). Thermal cycling conditions were 1 min at 95LC, followed by 30 cycles of 10 s at 95LC, 10 s at 61LC and 1 s at 72LC, and final extension of 30 s at 72LC. Fragments were directly sequenced using the Big-Dye Terminator Cycle Sequencing Kit version 1.1 on an ABI Prism 3130 automatic sequencer (Applied Biosystems, Foster City, CA, USA).

Table 1: Primers for AURKC mutation screening.

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<th>Exon</th>
<th>Primers (5'–3')</th>
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| 1    | Forward: TCAGGAATTTTCAGCCAATAGGA
       | Reverse: AGAGGGAGGGGAGATGTCAAC |
| 2    | Forward: CCCTATTCCCTTCTCTACTTCTCC
       | Reverse: CCACACACCAGTCTTCTTTC |
| 3    | Forward: TGAAGAGGAAGGGGAGCATT
       | Reverse: TGCAGGTTTACTTGAGGGT |
| 4    | Forward: GTGTGACCAGGCACTGACG
       | Reverse: CACCAGCCCACTAACCTACAG |
| 5    | Forward: TCCACCTCAGACGGAAATTGT
       | Reverse: AAACTGGGTCATTTCTACACTG |
| 6    | Forward: AAGGAGAATTTCCCTCATCCTG
       | Reverse: CAGCCATCACTGACCAGCTC |
| 7    | Forward: AGGCTCAAGAAGAGGGAGGAC
       | Reverse: GCAGCTCTCATGGGAAAGAGT |

**Results**

**Sperm analysis**

Sperm analysis at bright light microscopy revealed a normal sperm count (80 million/ml) and progressive motility (35%) according to WHO guidelines (WHO, 2010). The 200 spermatozoa analysed for morphology after Papanicolaou staining showed 100% abnormal head morphology: 95% of cells had macrocephaly, whilst the remaining spermatozoa were acephalic (Figure 1). The prevalence of other structural anomalies, such as midpiece or flagellar alterations, was below 25%. The diagnosis of SMS was made, but interestingly enough, no multiple tails (a feature typical of SMS) were observed.
Figure 1. Analysis of sperm morphology. In the patient’s semen (A), macrocephalic spermatozoa represent 95% of the sperm population; control spermatozoa (B) are shown at the same magnification. Bars = 10 μm.

**SCSA and AnnV/PI assays**

The SCSA analysis revealed a high percentage of debris and aggregates; in addition, the increased size of spermatozoa required a different setting for data acquisition. This analysis observed 65.5% of spermatozoa with native DNA, with an overall low DNA fragmentation rate (Figure 2).

**Figure 2.** Sperm chromatin structure assay (SCSA). Cytographs of acridine orange-denatured spermatozoa of the patient (A) and of a normospermic control (B). Left panels, gated sperm population; right panels, SCSA cytograms. Spots in region 2 (R2) correspond to the population of normal spermatozoa with acceptably low amount of single-stranded DNA; spots in region 3 (R3) correspond to the population of spermatozoa with an unacceptable amount of single-stranded DNA; spots in region 4 (R4) correspond to the population of spermatozoa exhibiting a high amount of staining associated with double-stranded DNA. The whole population of spermatozoa (the sum of R2 + R3 + R4) is named R1; the R3:R1 ratio is termed DNA fragmentation index, whereas the R4:R1 ratio is termed high DNA stainability. About 65% of spermatozoa in the patient’s semen belonged to the R2 category. FL1-H = green fluorescence (515–545 nm band-pass filter); FL3-H = red fluorescence (650 nm longpass filter); SSC-H: side scatter height and FSC-H: forward scatter height.
Table 2 Percentages of the sperm subpopulations detected by annexin V/propidium iodide assay after flow cytometry analysis.

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<th>Subpopulation</th>
<th>Case</th>
<th>Control</th>
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<tr>
<td>Live (Ann-PI⁻)</td>
<td>3.96</td>
<td>75.90</td>
</tr>
<tr>
<td>Late apoptotic (Ann⁺PI⁺)</td>
<td>94.95</td>
<td>19.05</td>
</tr>
<tr>
<td>Early apoptotic (Ann⁺PI⁻)</td>
<td>0.36</td>
<td>1.11</td>
</tr>
<tr>
<td>Late necrotic (Ann-PI⁺)</td>
<td>1.01</td>
<td>3.95</td>
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The AnnV/PI assay identified four types of spermatozoa, whose relative proportions are presented in Table 2: (i) live cells with no membrane alteration (Ann-PI⁻); (ii) early apoptotic but still viable spermatozoa (Ann⁺PI⁻); (iii) late apoptotic spermatozoa (Ann⁺PI⁺); and (iv) dead necrotic spermatozoa with no binding to annexin V (Ann-PI⁺) (Fig-ure 3). Overall, this study observed a very high proportion of late apoptotic spermatozoa (Ann⁺PI⁺) compared with control sperm sample and a significant (P < 0.0001) reduction of viable cells (Ann-PI⁻).

FISH analysis

A total of 500 spermatozoa were analysed. Chromosome 16 was labelled with aqua signal, chromosome 22 with gold signal (Figure 4), chromosome 13 with red signal and chromosome 21 with green signal. Among the observed spermatozoa, no euploid forms were detected; the chromosome 13 signal was always detectable and showed disomy in 83% of spermatozoa, whereas the chromosome 16 signal (aqua) showed disomy in 92% of the analysed spermatozoa (Table 3).

Propidium iodide flow cytometry

The DNA index was calculated using the ratio between the mean fluorescence intensity of propidium iodide fixed by sperm cells of the investigated patient (M_p) and that of spermatozoa belonging to a normospermic donor (M_I). The fluorescence peak around 750 indicated that most of the sperm cells of the patient contained an aberrant quantity of DNA: this observation was further confirmed by the DNA index value of 3.3 that showed a 3-fold higher DNA content in the patient’s cells compared with the normospermic control (Figure 5).

Transmission electron microscopy

In the large head of macrocephalic spermatozoa, TEM revealed the presence of a nucleus whose chromatin was mostly uncondensed and of a normally shaped acrosome (Figure 6). The midpiece appeared normally constituted by an axoneme associated with a set of nine outer dense fibres; however, a large cytoplasmic residual was detected in 78% of cells. Abnormal mitochondrial appearance was also observed. The mitochondria appeared swollen and compartmentalization of the inner membrane was almost absent, with a limited number of cristae.

AURKC analysis

Direct sequencing of AURKC was unable to identify point mutations, including the c.144delG and the c.436-2A>G, that were previously reported in patients with classical SMS (Ben Khelifa et al., 2011; Dieterich et al., 2007). Five polymorphisms in homozygosis in the dbSNP135 region were identified in the patient: rs11084490, rs58264281, rs58682946 and rs74179426 in the 5'-UTR; and rs758098 in intervening sequence 2. The same polymorphisms, however, are found in homozygosis in most healthy subjects, as they are particularly common in the Italian population.

Discussion

Sperm macrocephaly syndrome is a rare condition related to different mutations in AURKC, all causing a relevant impair-ment of sperm function and infertility (Ben Khelifa et al., 2011). Classical SMS is usually associated with a negative reproductive prognosis and also with a poor outcome of IVF treatment; however, a
few successful deliveries with birth of healthy babies after IVF were reported (Guichaoua et al., 2009; Shimizu et al., 2012), leading to acceptance of the possibility that milder phenotypes of SMS exist and are not fully incompatible with fertility.

Figure 3 Annexin V/propidium iodide assay. Dot plots of spermatozoa of the patient (A) and of a normospermic control (B) stained by AnnV/PI. Left panels, gated sperm population; right panels, AnnV/PI cytograms. Spots in the lower left quadrant represent live cells (Ann-PI-); spots in the upper right quadrant represent late apoptotic spermatozoa binding both annexin V and PI (Ann+PI+); spots in the lower right quadrant represent early apoptotic, but still viable spermatozoa, labelled with annexin V but not with PI (Ann+PI-); spots in the upper left quadrant represent necrotic, dead cells excluding annexin V (Ann-PI+). A high proportion of late apoptotic spermatozoa (Ann+PI+) and a significant reduction of viable cells (Ann-PI-) was observed in the patient’s semen with respect to control (P < 0.0001). FL1-H = green fluorescence (515–545 nm band-pass filter); FL3-H = red fluorescence (650 nm longpass filter); SSC-H: side scatter height and FSC-H: forward scatter height.

Figure 4 FISH analysis of the patient’s spermatozoa. Chromosome 16 was labelled with aqua signal, chromosome 22 with gold signal, chromosome 13 with red signal and chromosome 21 with green signal. Disomic spermatozoa for chromosome 13 or 21 (A), as well as for chromosome 16 or 22 (B). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Table 3 Disomy rate detected by FISH in the patient’s spermatozoa.

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<th>Disomy</th>
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<tr>
<td>13</td>
<td>83 (415)</td>
</tr>
<tr>
<td>16</td>
<td>92 (460)</td>
</tr>
<tr>
<td>21</td>
<td>71 (355)</td>
</tr>
<tr>
<td>22</td>
<td>60 (300)</td>
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Values are % (n).

Figure 5 Representative flow cytometry histograms obtained by propidium iodide staining of semen samples. (A) Sperm sample from a normospermic donor; (B) spermatozoa from the SMS patient. In the right panels, the X-axes represent the intensity of fluorescence on a linear scale, directly proportional to the DNA amount obtained after fixation for each cell analysed, and the Y-axes indicate the number of cells analysed on a linear scale. In each, the small peak between 100 and 200 corresponds to cell debris. The peak around 750 in B indicates that most of the cells contain an aberrant quantity of DNA. The DNA index calculated for the patient (Mp) compared with the mean florescence of control semen (Mi) is 3.3, suggesting a 3-fold increased DNA amount in the patient’s sperm nuclei. FL2-H= red fluorescence (585 nm longpass filter); SSC-H: side scatter height and FSC-H: forward scatter height.
The case of SMS reported herein is peculiar because it was characterized by a very high prevalence (95%) of spermatozoa with giant heads, but without multiple tails, a feature that is characteristic of the classical SMS. Moreover, the patient had no detectable mutations in both the translated and untranslated regions of AURKC, which is altered in classical SMS. A similar case of apparently isolated morphological defect of sperm head was previously reported (Guthauser et al., 2011), but it was not as thoroughly studied as the present one. Indeed in the present case the ultrastructural TEM analysis showed that the large head was not an isolated defect: a disorganized disposition of mitochondria in the midpiece was also noticed, and all mitochondria had altered morphology. Also in the current case, the structure of the tail was normal, and this likely accounts for the normal motility that was observed in the patient’s semen sample.

Despite the extremely high rate of nuclear abnormality, that was confirmed by TEM, the total amount of fragmented DNA detected by SCSA assay within the sperm nuclei was surprisingly, and differently from previous reports (Brahem et al., 2011, 2012), only slightly higher than that of normospermic controls. However, AnnV/PI analysis showed a very high rate of late cellular apoptosis, that suggests that tests detecting apoptosis-related DNA strand breaks (e.g. TdT-mediated dUDP nick-end labelling assay) could have been more sensitive in detecting DNA alterations.

In contrast to other authors, who reported that 40% of spermatozoa from a SMS patient were euploid (Yurov et al., 1996), this study observed a 100% proportion of diso-mic spermatozoa and frequently the presence of multiple disomy in the same cell. Moreover, the fetal karyotype of one of the abortions following ICSI was 69,XXX, suggesting fertilization with a diploid spermatozoon. Overall, these data suggest that a severe, systematic error should have occurred during meiosis in the current patient. In fact, the propidium iodide cytofluorimetry analysis showed that the total amount of DNA detected within the patient’s sperm heads was 3-fold higher than normal. It is therefore fairly realistic to assess that a blockage of the second meiotic division could have occurred, resulting in disomic (and probably diploid) spermatozoa. This error was apparently not due to AURKC mutations, that are associated with the classical SMS but not detected in the case presented herein. It is speculated that mutations of other genes involved in meiotic segregation could have determined the very high aneuploidy rate reported herein, or that the patient’s phenotype could depend on post-transcriptional errors or other dysfunctions of the AURK protein.
Another interesting peculiarity of the case showed herein is the ease to conceive with ICSI; indeed, in the three ICSI attempts that this couple underwent, almost all inseminated oocytes were fertilized and the embryos obtained were judged of good quality according to the morphological score that was used (Holte, 2007). This is likely related to the absence of multi-tailed spermatozoa: although macrocephalic, mono-centriolar spermatozoa allow the formation of a single meiotic aster, and consequently even if a spermatozoon is dysomic or diploid, two pronuclei are seen in the zygotes (Rawe et al., 2008). The spontaneous miscarriage that always followed conception was the unavoidable consequence of a severe sperm aneuploidy.

In conclusion, this paper presents herein a new type of male-related infertility that consists in a rare variant of the classical SMS, not linked to the AURKC mutation, in which all spermatozoa were macrocephalic, mono-tailed, dysomic and able to cause an apparently normal fertilization and zygote development, followed by a pregnancy bound to miscarriage for severe fetal genetic aberrations. Further studies are needed to understand the molecular mechanisms involved in this altered meiotic segregation and to detect other genes that, once mutated, may lead to an abnormal chromosomal pattern. Based on these observations: (i) spermatozoa with AURKC mutations should not be used for ICSI due to the high genetic risk for the off-spring; (ii) a careful genetic counselling should be mandatory for patients with SMS; and (iii) FISH analysis should be proposed to all macrocephalic sperm patients in order to assess the ploidy of their spermatozoa before allowing an ICSI attempt.

Acknowledgements

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References


