

A Novel Meiotic Anomaly: Infertility Associated with Polyploid Germ Cells and Multi-tailed Sperm

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KEY WORDS Infertility; aberrant meiosis; polyploidy; multi-tailed sperm; premeiotic S-phase.

ABSTRACT Here we describe an apparently new meiotic anomaly in a healthy 35-year-old man with an oligoastheno-terato-zoospermy (OAT syndrome), normal male karyotype, and unremarkable endocrinological status. Molecular analysis of the Y chromosome did not reveal any disparity to normal controls. The analysis of cells from the ejaculate showed cells in all meiotic stages with pairing anomalies and a high percentage of polyploid germ cells including endoreduplicated metaphase I chromosomes. Most of the sperm were morphologically deviant with irregular large and/or binucleated heads and a variable number of up to 8 tails. To the best of our knowledge such a combination of meiotic disturbances has not been reported so far and points to a defect confined to the meiotic prophase, especially the S-phase.

INTRODUCTION

Meiosis is an evolutionarily highly conserved process in the eukaryotic life cycle. Generally, diploid cells entering meiosis undergo a single round of replication followed by a complex array of events with chromosome pairing, recombination, and segregation leading to four haploid progenies. It is also a highly specialized process. Thus, a number of genes involved herein are active only during this stage. As a consequence, mutations in these genes affect fertility without any other phenotypic abnormalities. Since these individuals are regularly childless and often the only affected in the family, it is difficult to prove the genetic basis of the disturbance, and almost impossible to identify the

underlying gene by positional cloning. It is therefore important to publish even single cases in order to collect similar observations. This might give insight into the pathway affected, point to similar meiotic disturbances in model organisms, and by this, pave the way to the gene.

Here we describe the complex meiotic abnormalities in an otherwise completely normal male individual characterized by a high percentage of polyploid germ cells and multi-tailed, morphologically aberrant sperms.

REPORT OF THE PROPOSITUS

The propositus is a 35-year-old healthy man who is married but childless. Physical examination revealed no abnormalities. Testis volume and hormone levels are all within normal levels, his medical history is unremarkable, also with respect to exposure to ionizing radiation or drug intake.

Chromosome preparations were performed from standard wholeblood cultures and analyzed after G- and C-banding. Semen analysis was carried out on six different samples over 16 months. An aliquot was taken for routine semen analysis (volume, sperm count motility, morphologic features, fructose content) according to standard procedures. Semen smears were stained with silver nitrate and examined for sperm morphology by light microscopy. In addition, the meiotic chromosomes were prepared from the ejaculate according to Sperling and Kaden (1971).

RESULTS AND DISCUSSION

The cytogenetic analysis revealed a normal male karyotype (not shown). Molecular studies,

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based on 22 probes recognizing different sites along the Y euchromatin, did not reveal any sub-microscopic deletions, including the AZF regions (Table 1). Repeated spermatograms proved an oligo-astheno-terato-zoospermy (“OAT syndrome”) with a sperm count below $20 \times 10^6/\text{ml}$ (Table 2). In three of six samples, leukocytes were observed, pointing to an infection and in two of these probes bacteria were observed which disappeared after antibiotic treatment. About 3/4 of the sperm were morphologically deviant. They showed abnormal heads, in particular with enlarged or two nuclei (Fig. 1 e,f) and a high proportion of multitailed forms with up to 8 tails (Table 3, Fig. 1a-d). In the cytogenetic preparations cells in almost all meiotic stages were found and, apart from a few apparently normal cells, pairing anomalies were visible at pachytene and as univalents in the first meiotic division (Fig. 2 a,b). The most striking finding concerned the frequency of polyploid

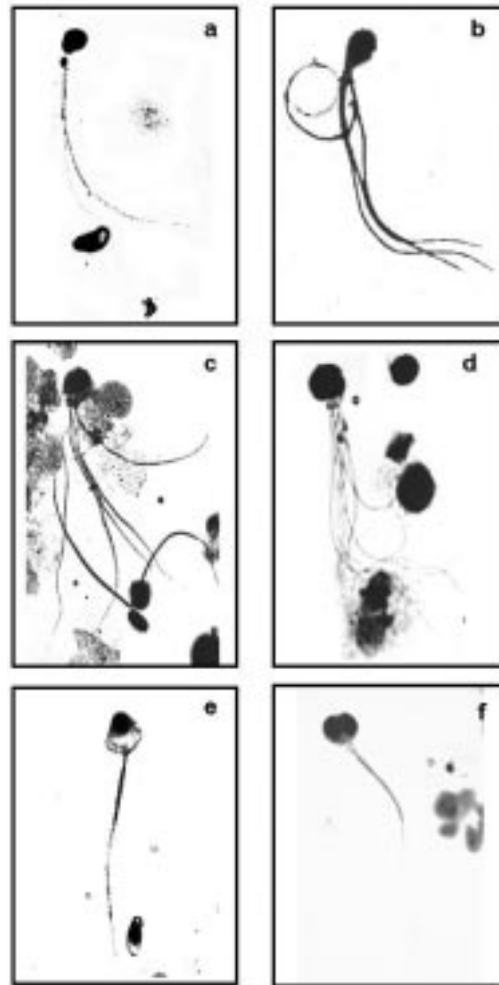


Fig. 1. Sperm cells of the propositus with anomalies of the head and/or of the tails after silver staining
a. 2-tailed sperm b. 4 tailed sperm
c. 6 tailed sperm d. 8 tailed sperm
e. Sperm with an enlarged head
f. Di-nucleated sperm head

Table 1: Molecular analysis of the Y Chromosome and characterization of the probes

Interval	Probe	Enzyme	Signal
1A	TDF-1	Eco RI	+
1C	Fr80-II	Taq I	+
2	47z	Taq I	+
3	52d B	Taq I	+
3	52d C	Taq I	+
3	50f2 A	Eco RI	+
3	50f2 B	Eco RI	+
3	Fr 35-II	Taq I	+
4A	pDP34	Taq I	+
4B	50f2 D	Eco RI	+
5/6	Fr15-II	Taq I	+
5/6	Fr25-II	Taq I	+
6	52d A	Taq I	+
6	50f2 C	Eco RI	+
6	50f2 E	Eco RI	+
7	pJA 1143	Hae III	+
7	pHY2.1	Hae III	+

Table 2: Results from 6 different semen analyses performed within 16 months

Probe	Spermatogram						
	Sperm/ml $\times 10^6$	Motile sperm (%)	Aberrant Spermatozoa (%)			Precursor Cells (%)	
			Total	Head	Midpiece		Tail
1	9	59	63	60	17	17	1
2	18	52	82	80	7	20	3
3	7	61	80	78	17	30	9
4	14	51	75	70	15	21	2
5	14	52	75	74	17	29	2
6	8	42	76	76	19	31	3

Table 3: No. of tails per sperm in smear preparations from the ejaculate of the propositus

N	No. of tails/sperm				
	1	2	3	4/6	8
1000	968	29	3	-	1

Table 4: Analysis of immature germ cells in the ejaculate of the propositus

N	Pachytene	diakinesis and metaphase I	
		diploid	polyploid
53	17 (32.1%)	15 (28.3%)	21 (39.6%)

germ cells (4n and 8n, Table 4) and endore duplicated meiotic chromosomes with incompletely replicated chromatids (Fig. 2,d). Even despite such abnormalities germ cell maturation did not cease completely as indicated by the occurrence of normal sperm.

To the best of our knowledge, such a combination of meiotic disturbances in combination with sperm defects has not been described before. Teratospermia with a high proportion of multi-tailed spermatozoa is occasionally observed in ejaculates from infertile men (Nistal

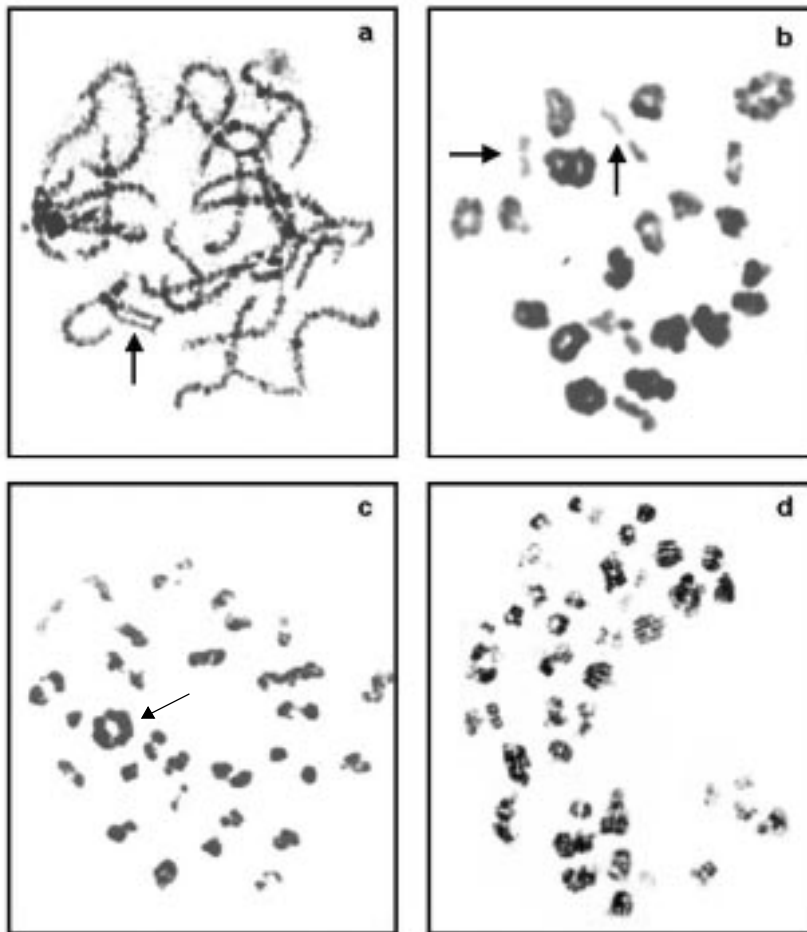


Fig. 2. Meiotic cells in the propositus ejaculate showing pairing anomalies or polyploidization
 a. Pachytene cell with pairing failure (arrow)
 b. Diakinesis with univalents and end to end association (arrows)
 c. Hyperhaploid metaphase I. The arrow points to a probable quadrivalent
 d. Hyperdiploid metaphase I with endoreduplicated chromosomes

et al. 1977; Escalier 1983; de Santi 1985; Zukerman et al. 1986), however data on the meiotic chromosome complement are completely missing in these cases. On the other hand, the analysis of meiotic chromosomes in infertile men with severe oligo- or azoospermia led to the detection of rare, phenotypically normal individuals, whose meiotic chromosomes showed greatly reduced chiasma counts in combination with pairing irregularities at pachytene, the occurrence of univalents at metaphase I and regularly spermatogenetic failure after the first meiotic division (Chandley 1981). These effects were attributed to the action of desynaptic or asynaptic mutations. Our case shows pairing abnormalities and sperm defects. It is unlikely that this unique combination is due to different causes or an exogenous factor. A more plausible explanation is that these disturbances are secondary events and, perhaps, the polyploid and endoreduplicated meiotic chromosomes point to the primary defect.

Principally, polyploid germ cells at metaphase I might arise from polyploid spermatogonia, from cell fusion, or as consequences of a defect in the premeiotic S-phase. Differentiation between these assumptions is not easy and even the possibility that they are all an artifact of preparation cannot usually be excluded a priori (Ford and Evans 1971). In our case the situation is obviously different: the occurrence of endoreduplicated metaphase I chromosomes is indicative of a defect in the premeiotic S-phase. The geometric series of diploid, tetra- and octaploid, but not hexaploid, cells is more easily explained by polyploidization than by cell fusion. In addition, we consider the large element in figure 2c as a quadrivalent, which can only be formed in polyploid spermatocytes, not after cell fusion. If one assumes that due to the polyploid complement the reduction divisions are affected but not the differentiation into sperm, this could explain the existence of sperm with large and double nuclei and up to 8 flagella. The latter phenomenon has been experimentally induced in the insect *Myrmecaelurus trigrammus* (Friedländer and Wahrman 1965) in

which the meiotic divisions were blocked by mercaptoethanol. The resulting $4n$ spermatids possessed 4 centrioles from which the 4 axis of the tails extended.

The premeiotic S-phase bears many similarities to its mitotic counterpart, nonetheless substantial differences also exist. This is most convincingly illustrated in yeast mutants uniquely affecting premeiotic S-phase (Stuart and Wittenberg 1998). It is tempting to speculate that in our case a gene might be affected that controls premeiotic DNA synthesis but is not required for the mitotic cell cycle.

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