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Fertility potential in a man with ankylosing spondylitis as revealed by semen analysis by light, electron and fluorescence microscopy

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Abstract

Ankylosing spondylitis affects 0.1%–0.5% of the adult population. The aim was to investigate the possible effects of both the disease and its treatment on semen quality by performing a highly detailed analysis in a man with ankylosing spondylitis, presenting for infertility. Sperm characteristics were evaluated by light microscopy, morphology by electron microscopy (transmission electron microscopy), DNA fragmentation by terminal deoxynucleotidyl transferase dUTP nick end labeling using fluorescence microscopy and chromosomal abnormalities by fluorescence in situ hybridisation using probes for chromosomes 13,15,16,18,21,22,X and Y. There was no evidence for an effect of either ankylosing spondylitis or its treatment with celecoxib and sulphasalazine on sperm quality as all parameters including concentration, motility, DNA fragmentation and aneuploidy incidence were within normal limits. Transmission electron microscopy, however, revealed a high incidence of head, neck and tail abnormalities, as well as the presence of immature sperm and phagocytes. Hysteroscopic removal of an endometrial polyp enabled the achievement of a spontaneous pregnancy and the delivery of a healthy boy.

Keywords

Ankylosing spondylitis, sperm, electron microscopy, DNA fragmentation, terminal deoxynucleotidyl transferase dUTP nick end labeling assay, aneuploidy screening

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Introduction

Ankylosing spondylitis (AS) has a prevalence of 0.1%–0.5% and most often affects men in their 20s.The vertebrae fuse and the spine becomes less flexible leading to a hunched-forward posture. Although AS has a genetic predisposition, its pathogenesis is also dependent on environmental factors. Carriers of the human leukocytic antigen B27 (HLA-B27) gene are at high risk, but variations in other genes, including endoplasmic reticulum aminopeptidase 1(ERAP1), Interleukin-1A (IL1A), Interleukin- 23R (IL23R), have also been associated with AS.[1] HLA-B27 heavy chain can form a homodimer, to activate natural killer and T-helper 17 cells, and induces endoplasmic reticulum stress to trigger the Interleukin-23 (IL-23) and Interleukin-17 (IL-17) axis for pro-inflammatory reactions.[1–4]

The frequency and severity of infertility vary among rheumatic diseases.[2,5,6] An increased frequency of varicocele was found in patients with AS associated with abnormal semen parameters, with no evidence of anti-sperm antibodies or hormonal alterations.[4] Patients with AS often have reduced sperm motility, increased incidence of sperm aneuploidies,

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higher plasma luteinising hormone (LH) and follicular stimulating hormone (FSH), and lower testosterone (T) concentrations compared to controls. There is an inverse correlation between disease activity and semen quality, which, after treatment, is restored.[7]

Inflammation in AS appears to be related to impaired testicular function and anti-tumor necrosis factor (TNF)α agents seem to constitute a safe option,[3,5–9] in terms of restoring Sertoli cell function[3] and fertility.[10] Other drugs commonly used for treatment of AS include celecoxib and sulphasalazine with variable effects on spermatogenesis.[11–15]

Although various publications have investigated fertility potential in patients with AS by performing standard semen analysis, no reports have examined the effect of AS management on sperm morphology by transmission electron microscopy (TEM). The chromosomal constitution of sperm in AS patients has only been previously investigated in one study by aneuploidy screening for five chromosomes.[7] The aim of the present study was to perform a highly detailed analysis on sperm count, motility, morphology (by both standard and TEM microscopy), DNA fragmentation and chromosomal abnormalities (by analysing eight chromosomes) in a male with AS.

Case

A 36-year-old couple with primary infertility (failure to conceive after unprotected intercourse for 2.5 years) presented for fertility treatment at the Unit for Human Reproduction at Papageorgiou General Hospital, Greece. The couple had previously undergone two unsuccessful intra-uterine inseminations and one unsuccessful in vitro fertilisation cycle at a different centre. A detailed semen analysis and sperm cryopreservation was performed because the man was suffering from AS (which he inherited from his father) and was due to start therapy with drugs that may have had an effect on sperm quality. Over the past 8 years and at the time of sperm collection, the man was under therapy with celecoxib and sulphasalazine.

Materials and methods

4.8 mL of semen were collected and processed for standard semen analysis, DNA fragmentation and molecular cytogenetic analysis following patient's informed consent. Standard semen analysis was performed according to World Health Organization (WHO) criteria: lower reference limits 1.5 mL for volume, 15 millions/mL for concentration, 32% for progressive motility and 4% for normal morphology.[16] Chromosomal abnormalities were assessed by fluorescence in situ hybridisation (FISH) using probes for chromosomes 13,15,16,18,21,22,X and Y according to Chatzimeletiou et al.,[17] DNA fragmentation was evaluated by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) using fluorescence microscopy according to Chatzimeletiou et al.,[17] and morphology was analysed by TEM, according to Chatzimeletiou et al.[17]

Standard semen analysis, FISH and DNA fragmentation assessment were performed as part of routine clinical practice. The sperm used was from the fresh semen sample and counting attained all cells. The electron microscopy analysis was approved by the Bioethics Committee of Aristotle University Medical School.

Hysteroscopy

The hysteroscopic intervention was performed in the eighth day of the cycle under paracervical block, with the Bettochi compact hysteroscope (Karl Storz GmbH) and normal saline as distending medium. During the diagnostic phase, a polyp located in the fundus was found. With semi-rigid operating instruments, the polyp was removed and sent for pathology. The patient was discharged after 2 h uneventfully.

Results

Standard semen analysis is shown in Table 1. TEM revealed a high incidence of abnormalities on the sperm head, neck and tail (Figure 1(a)–(d)). Several immature spermatids were also observed and several sloughed cells were evident. Phagocytes were also present (Figure 1(f)). Sperm DNA fragmentation as assessed by TUNEL-labelled nuclei was within normal limits (7%) (Figure 1(h)). Aneuploidy levels were low for all chromosomes tested (Table 2) (Figure 1(g)).

Discussion

The semen sample analysed had all parameters within normal limits according to WHO strict criteria, confirming that the primary infertility suffered by the couple was not due to male factor. The anti-inflammatory drugs celecoxib and sulphasalazine had no major effect on sperm quality as concentration, motility, morphology, chromosomal constitution and DNA fragmentation were within normal limits. However, the presence of several immature sperm as revealed by TEM may be in agreement with previous reports suggesting that sulphasalazine may have deleterious effects on spermiogenesis/spermatogenesis.[5,6,14,15]

The patient at the time of investigation was under treatment with celecoxib and sulphasalazine. Celecoxib, an inhibitor of cyclooxygenase-2 (COX-2; prostaglandin-endoperoxide synthase 2), is widely used in the treatment of chronic inflammation and pain. COX-2 is constitutively expressed in the testis, where it is responsible for prostaglandin production, so inhibition of this enzyme should have effects on testicular function. The effects of administering celecoxib in rats caused an important reduction in testicular interstitial fluid (IF) prostaglandin E(2) (PGE(2)) concentrations, accompanied by a compensatory increase in COX-2 mRNA expression, but had no effect on testis weight, testis morphology or serum testosterone levels. These results indicate significant roles for products of the COX-2 pathway in testicular vascular control and steroidogenesis, which may have implications for men with marginal fertility taking celecoxib for extended periods, but also highlight the

Table 1. Standard semen analysis.

		Lower reference limits
Volume	4.8 mL	1.5 mL
pH	8.5	7.2
Number	54×10^6 mL	15×10^6 mL
Total number	259×10^6 ejaculation	39×10^6 mL
Motility		
Linear progression	52%	32%
No progression – tail moving	25%	
Immotile	23%	
Morphology		
Normal	10%	4%
Abnormal	90%	
Big head	7	
Small head	5	
Long head	3	
Pear shaped head	25	
Round head	1	
Amorphous head	22	
Vacuoles	48	
Small acrosome	2	
Short tail	1	
Double tail	2	
Fourchette	4	
Broken tail	2	
Spiral tail	7	
Asymmetric tail extrusion	1	
Broken neck	16	
Cytoplasmic droplet	27	
Thick mid piece	29	
Round spermatids	15%	
White blood cells	4%	≤1%
DNA fragmentation		
TUNEL-labelled nuclei	7% (70/1000)	Normal range: 0%–29%

TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labeling.

potential of this drug to ameliorate testicular damage caused by systemic or local inflammation.[11] It may affect also some ion channels (T-type (CaV3) implicated in sperm physiology and can induce the acrosome reaction (AR), an intracellular Ca(2+) ([Ca(2+)]i) increase and a sperm depolarisation leading in a compromise fertilisation.[12]

Sulphasalazine is a common medication used for treatment of patients with AS, which reduces significantly the levels of (B27-HC) on peripheral blood mononuclear cells (PBMCs) including cytokines mRNA levels TNFα, Interleukin 17A (IL-17A), Interleukin 17F (IL-17F) and Interferon gamma (IFNγ), when it is used for a long period (up to 4 months).[13] Sulphasalazine treatment for inflammatory bowel disease in men causes oligospermia, reduced motility, increased incidence of morphologically abnormal spermatozoa[14] and in general has a deleterious effect on spermatogenesis.[15] It has been reported that in 27%–42% of men taking maintenance sulphasalazine, an increased number of abnormal sperm forms are observed.[18,19] However, these effects are found to be reversible 3 months after the removal of the drug.[14]

The low levels of diploid XX or YY bearing sperm may have been due to failure of second meiosis or due to endoreduplication. Diploid XY bearing sperm may have originated from non-separation of chromosomes during meiosis I or alternatively may be immature round spermatids. Endoreduplication of chromosomes occurring before meiosis has also been suggested as a mechanism leading to tetraploid meiocytes, which subsequently can enter meiosis and produce diploid sperm.[17] Fertilisation of an egg by diploid sperm inevitably results in the formation of a hydatidiform mole (molar pregnancy).[17]

Patients undergoing in vitro fertilisation have the option to undergo embryo biopsy and preimplantation genetic screening (PGS) in order to select genetically normal embryos for transfer. PGS is used for the detection of autosomal recessive and dominant diseases, X-linked conditions, translocations, aneuploidies (PGS) and HLA-matching. However, in the case of AS, PGS is not an option, as although there are genes predisposing to the disease, not all carriers of the genes will develop the disease. PGS is not

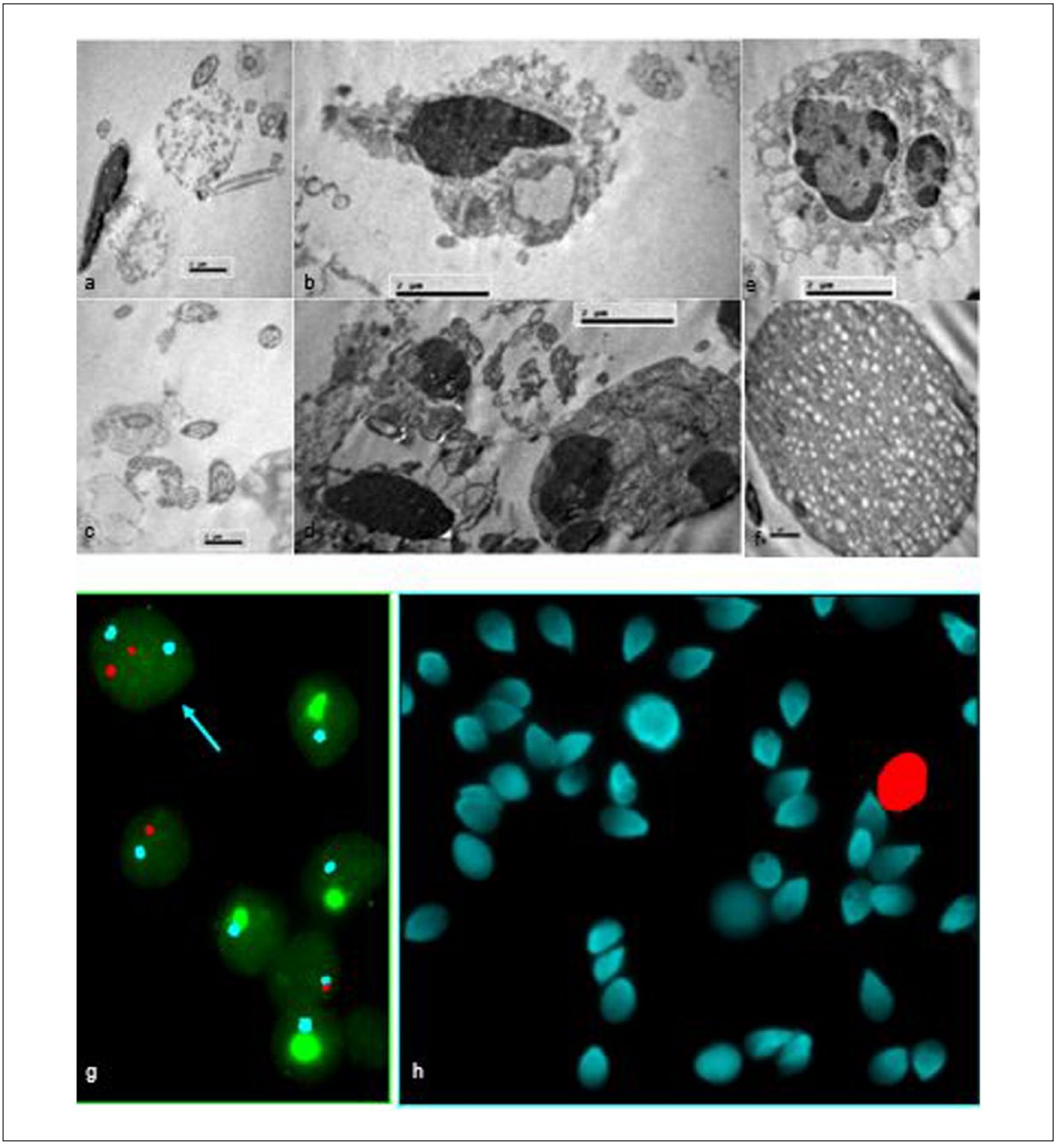

Figure 1. (a)–(f) TEM photomicrographs showing (a) sperm head without a tail, (b) immature sperm, (c) sections of tails with abnormal microtubule arrangement, (d) sloughed spermatids, (e) neutrophil, (f) phagocyte, (g) FISH photomicrograph showing sperm hybridised with a probe for chromosomes X-green, Y-red, 15-aqua. Note that all spermatozoa are normal haploid but the one on the top left is diploid (YY1515) arrow. (h) Photomicrograph showing TUNEL-labelled sperm. Note the normal spermatozoa in blue and one fragmented sperm in red.

therefore recommended for the identification and rejection from transfer to the uterus of embryos with the predisposing genes for AS, as in most cases, would result in the rejection of 'healthy' embryos.

In the current case, a female factor of infertility was eventually detected. Hysteroscopic removal of an endometrial polyp identified during examination, enabled the subsequent establishment of a pregnancy, following natural conception

Table 2. Fluorescence in situ hybridisation (FISH) analysis of spermatozoa (total number of sperm analysed in each probe set used = 1000).

Probe: MultiVysion PB								
Chromosomes					Haploid no (%)	Aneuploid no (%)	Diploid no (%)	Tetraploid no (%)
13	16	18	21	22	965 (96.5)	15 (1.5)	20 (2)	–
Probe: CEPX/Y/18								
Chromosomes					Haploid no (%)	Aneuploid no (%)	Diploid no (%)	Tetraploid no (%)
X		Y		18	970 (97.0)	10 (1.0)	8 (XY) 12 (XX,YY) (2.0)	–
Probe: CEPX/Y/15								
Chromosomes					Haploid no (%)	Aneuploid no (%)	Diploid no (%)	Tetraploid no (%)
X		Y		15	970 (97)	12 (1.2)	5 (XY) 13 (XX, YY) (1.8)	–

and the birth of a healthy baby boy. Thorough investigation of the couple is therefore of utmost importance for optimal outcome.

Finally, the association between leukocytes in the semen and semen quality remains a matter of debate in the literature. Leukocytospermia, defined by WHO as $>1 \times 106$ leukocyte/mL, has an incidence of 15% in the general population and is especially common in men with infertility.

Conclusion

We conclude that there is no evidence for an effect of either AS or its treatment with the anti-inflammatory drugs celecoxib and sulphasalazine on sperm quality, as all parameters including concentration, motility, DNA fragmentation and aneuploidy incidence were within normal limits. Nevertheless, the effect of AS on fertility needs further evaluation through prospective studies.

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Ethical approval

Ethics Committee of Aristotle University Medical School.

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Informed consent

Written informed consent was obtained from the patient(s) for their anonymised information to be published in this article.

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