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Homogeneous Microscopic Abnormalities in Sperm Morphology and Immotility as A Cause of Male Infertility

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Objective *To study the identification of the cause of specific sperm abnormalities.*
Methods *Two adult men with specific alterations in sperm morphology causing 100% immobility were included in this study. The study of sperm used: transmission electron microscopy (both patients); apoptotic markers, DNA fragmentation test and fluorescence in-situ hybridization (patient 1) and immunocytochemistry study of sperm flagellum using anti-β tubulin antibodies and ciliary activity test (patient 2).*
Results *Increased DNA fragmentation (52.6%) and apoptosis biomarkers were detected in patient 1, and loss of the central pair of microtubules in patient 2 ('9+0' axoneme); the nasal ciliary activity was normal.*
Conclusion *Results suggest an apoptotic origin of the abnormalities in the sperm from patient 1 and dysplasia of the fibrous sheath in patient 2.*

Key words: apoptotic changes; dysplasia of fibrous sheath; midpiece thickening; necrospermia; sperm immotility

Sperm abnormalities such as morphological and motility disorders are associated with male infertility. The two main causes of sperm immotility are necrozoospermia (the presence of non-viable spermatozoa) and ultrastructural abnormalities of sperm tails. The causes of necrospermia are varied, including testicular disorders, endocrinopathies, defects in androgen

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biosynthesis, toxins, drugs, chronic systemic diseases, infections and epididymal function or storage defect[1,2].

With regard to the morphological alterations, there are two main types of teratozoospermia: heterogeneous non-specific anomalies in different sperm components (the most frequent type) or a very homogeneous microscopic systematic sperm defect pattern which is present in most spermatozoa[3]. The latter can affect the sperm head or flagellum, with abnormalities in the head-neck region including varying degrees of misalignment, up to the most extreme defect resulting in acephalic spermatozoa[4].

In this paper, we present two cases of sperm immotility, one with thickening of the midpiece and anomalous head-neck attachment causing some headless sperm with severe necrospermia features we had not previously seen (patient 1), and another with short and thick tails , findings compatible with previously reported cases of dysplasia of the fibrous sheath (DFS) and/or primary ciliary dyskinesia (PCD) (patient 2). To reach the diagnosis of both cases, basic semen analysis and biochemical study of the seminal plama were insufficient. For this reason, ultrastructural studies and other techniques as appropriate were added:

In case 1, special stainings, fluorescence *in situ* hybridisation (FISH) and apoptosis markers on sperm were performed. FISH was carried out to estimate the proportion of aneuploidy and structural aberrations present in his ejaculate[5]. DNA fragmentation is the most characteristic feature of apoptosis, which is caused by the endogenous DNA degrading endonucleases activated by apoptotic responses, for example, caspase-associated cell signalling[6]. The DNA damage correlates well with the reproductive potential of sperm[7].

In case 2, to confirm the diagnosis of DFS and/or PCD, immunocytochemistry study of sperm flagellum and nasal ciliary beat and cellular rotation test were performed. Regarding flagellar structure, all eukaryotic cilia and flagella possess a central bundle of microtubules (formed by the polymerization of α- and β-tubulin), called the axoneme, which consists of nine doublet microtubules surrounding a central pair of singlet microtubules (the distinctive '9+2' microtubule arrangement). The fibrous sheath is a cytoskeletal structure encasing the axoneme and other dense fibres in the principal piece of sperm tail. It consists of two longitudinal columns linked to axonemal doublets 3 and 8[8].

PCD is a congenital disease in which the respiratory cilia are immotile, dysmotile, or both[9]; resulting in a range of chronic clinical manifestations such as bronchitis, rhino-sinusitis and otitis media, situs inversus[10]. These anomalies are observed in the flagella of spermatozoa of most patients, causing male infertility. Among the systematic sperm tail defects, DFS has been particularly well characterised[11,12]. It is a disorder related to PCD in which there is both widespread disarray of the sperm fibrous sheath and axonemal ciliary defects. In these cases the majority of spermatozoa are rigid, short, thick, and immotile and the fibrous sheath