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Missing Single or Double Peripheral Microtubules in Amniote Axonemes

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Despite the universality of the axonemal structure, exceptions of the 9+2 pattern are normal for some eukaryotes and are often found in pathology. We studied the ultrastructure of thymic epithelial cilia of the snake *Coluber jugularis* and compared their axonemes to the diversity found in human teratozoospermic ejaculates. In the *Coluber* epithelial cilia, one or more peripheral doublets were sometimes replaced by single microtubules in the absence of other structural peculiarities. In the human pathological flagella, missing single peripheral microtubules were rarely seen and were always associated with other axonemal defects. We conclude that the single peripheral microtubules observed in some snake cilia could be a normal variation of axonemal structure in lower amniotes.

Key words: axoneme, ultrastructure, single peripheral microtubules.

Introduction

The axoneme consisting of 9 peripheral doublets and 2 central microtubules (9+2) is almost universal for eukaryotic cilia and flagella. However, exceptions are found to be normal for some organisms [1], and a diversity of axoneme peculiarities can be found in pathological cases [2]. We performed an electron microscopic study of snake thymic epithelial cilia and the variations found in their ultrastructure were compared to the diversity of abnormal human sperm axonemes.

Materials and Methods

The snake species studied by us was *Coluber jugularis* Laur, found in the region of Zlatni Pyassatsi. Thymus specimens were obtained by dissection. For comparison, teratozoospermic human ejaculates were used as a source of very diverse axonemal variants. Thymus samples and washed sperm cells were processed for routine transmission electron microscopy as described in [3]. Briefly, they were fixed with glutaraldehyde, postfixed with OsO_4, dehydrated, embedded in resin and sectioned.

Results

Most axonemes of thymic epithelial cells of *C. jugularis* were standard 9+2. However, one or more peripheral doublets were sometimes replaced by single microtubules (Fig. 1). No other peculiarities in the axonemal structure were observed.

In the pathological human spermatozoa, one or more missing whole peripheral doublets were often found. However, missing single peripheral microtubules were rarely seen and were always associated with other axonemal defects, such as displaced, partially disintegrated or entirely missing peripheral doublets (Fig. 2).

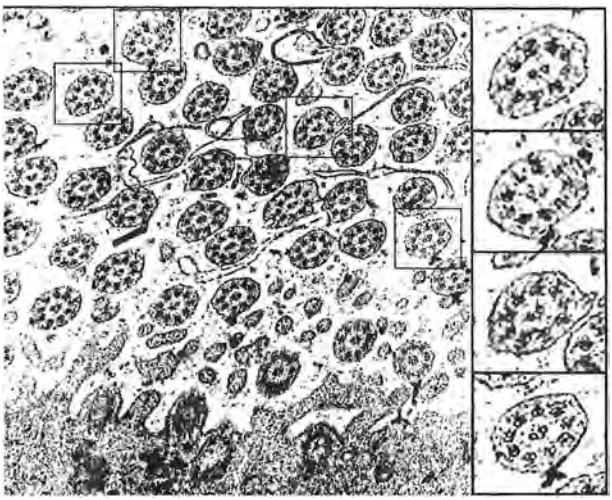

Fig. 1. *Coluber jugularis* thymic epithelial cilia in cross-section (× 15 000). Examples of axonemes with single peripheral doublets are indicated and shown as magnified insets (× 30 000)

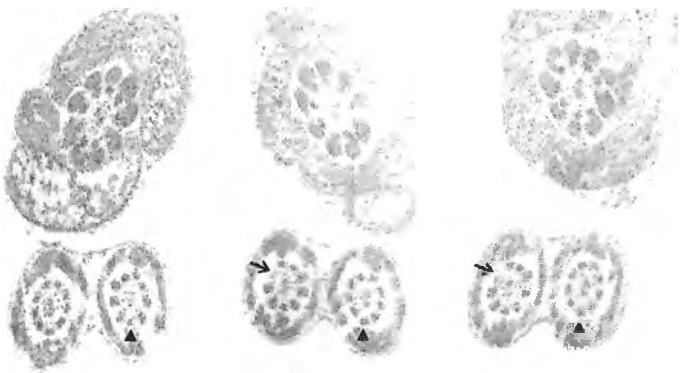

Fig. 2. Serial cross-sections of an abnormal bent or double sperm tail from a patient with teratozoospermia (× 26 000). One of the peripheral doublets is replaced by a single microtubule (arrowheads). Another peripheral doublet seems to disintegrate along the tail (arrows)

Discussion

Although the 9+2 pattern is strikingly universal among eukaryotes, variations are sometimes found in specific ciliary or flagellar structures. Single peripheral microtubules are normal for the cilia of some flatworm larvae, where the axoneme begins with the typical 9+2 structure but only single microtubules extend to the medial and distal regions [5]. We observed single peripheral microtubules in some thymic epithelial cilia of the snake *Coluber*, for which we found no literature reports concerning axonemal structure. Single peripheral microtubules, among other more common defects, were reported in cilia of human endometrial neoplastic cells [2]. However, we did not observe them in abnormal cilia of mouse thymic epithelial cells with oncornaviruses, although supernumerary central microtubules and peripheral triplets instead of doublets were common in the ciliary axonemes [4]. Even in the very diverse human teratozoospermic sperm axonemes, single peripheral microtubules were found rarely and always in the presence of other anomalies. In conclusion, missing single peripheral microtubules in the absence of other structural peculiarities are not typical for human axonemal pathology. This makes it probable that the single peripheral microtubules observed in some snake cilia are a normal variation of axonemal structure in lower amniotes.

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