Abaxial tail implantation in the emu, *Dromaius novaehollandiae*: morphological characteristics and origin of a rare avian sperm defect

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Abstract

Abaxial tail implantation is a defect occurring in the neck region of spermatozoa and is characterised by misalignment of the centriolar complex relative to the head base. This defect has been described in a number of mammalian species, but is rarely reported in birds. In this study, a detailed description of the defect in emu sperm is presented as well as morphological evidence of its origin in the testis. Despite their low incidence defective sperm could readily be identified using light (LM) and transmission electron microscopy (TEM). Affected sperm displayed obvious misalignment of the head and flagellum with many cells additionally showing unilateral swelling and caudal extension of the nuclear base. This material overlapped the anterior aspect of the centriolar complex. More subtle forms of the defect which were not resolved by LM were revealed by TEM. Abaxial sperm development could be identified in the testis during the early elongated spermatid stage of spermiogenesis. At this stage the centriolar complex was clearly misaligned in respect of the longitudinal axis of

the condensing and elongating nucleus. The rare occurrence and low incidence of this defect in the emu would suggest that it has little effect on fertility.

Keywords: emu, sperm morphology, abaxial tails

1. Introduction

Abaxial tail implantation is an anomaly occurring in the neck region of sperm involving the eccentric positioning of the centriolar complex relative to the head base [1]. It has been described in a number of mammalian species including the bull [2,3], buffalo [4,5], boar [6,7], dog [8,9], stallion [10] and man [11]. In general, it is not an uncommon defect in mammalian sperm, although the incidence of the anomaly is low [2]. However, a few cases with a high incidence have been reported in the bull [2,12,13] and boar [6]. The defect originates in the testis [6,7,14], but its influence on fertility has not been unequivocally established [5,9,10,12,15,16].

With the exception of an incidental reference to this defect in the turkey [17], a light micrograph of 'paraxial' sperm in the goose [18], and the presentation of a transmission electron micrograph illustrating abnormal articulation between the sperm head and tail in the duck [19], abaxial sperm tails have not, to our knowledge, been described in any other avian species. This would suggest that this defect is rarely observed in non-passerine birds. Considering the apparent paucity of this defect in birds and the total lack of descriptive data, this study reports on the incidence and morphological features of abaxial tails observed in emu (*Dromaius novaehollandiae)* spermatozoa. Information on the development of this defect during spermiogenesis is also presented.

2. Materials and methods

Semen samples were collected from the distal deferent duct of 15 sexually mature emus slaughtered at a commercial abattoir. The age of the birds ranged between 22 months and 4 years. The samples were immediately fixed in 2.5% glutaraldehyde in 0.13M Millonig's phosphate buffer, pH 7.4. Smears for light microscopy (LM) were prepared from the fixed semen samples and stained with Rapiddiff® (Clinical Sciences Diagnostics, Johannesburg, South Africa), a commercial Wright's stain. Smears from each bird were examined on an Olympus BX63 light microscope (Olympus Corporation, Tokyo) using a 100x oil immersion objective (bright field and phase contrast microscopy) to evaluate sperm morphology and determine the incidence of sperm defects by counting the number of abnormal sperm present in a total of 300 randomly selected cells.

Semen samples were prepared for transmission electron microscopy (TEM) as previously described [20]. Testis samples were also collected from each of the birds in 4% glutaraldehyde in 0.13M Millonig's phosphate buffer, pH 7.4 and prepared for TEM using standard techniques. Briefly, after fixation in the buffered glutaraldehyde, samples were post-fixed in similarly buffered 1% osmium tetroxide, dehydrated through a graded ethanol series and embedded in TAAB 812 resin. Thin sections were stained with lead citrate and uranyl acetate and viewed in a Philips CM10 transmission electron microscope (Philips Electron Optical Division, Eindhoven, The Netherlands) operated at 80kV.

Relevant features of the anomaly (LM and TEM) and stages of its development in the testis (TEM) were described and digitally recorded.

3. Results

3.1. Incidence

The incidence of the defect was low and was scored in only one of the 15 birds examined during the random count of 300 cells. This bird was one of the younger emus, i.e. one of those that were 22 months old. In this instance the defect formed 0.6% of the total cells counted. However, when examining the entire semen smear, occasional abaxial tails were noted in a further five birds.

3.2. Light microscopy (LM)

Abaxial sperm were observed on LM using both bright field and phase contrast microscopy, despite the relative scarcity of the defect. The defect was more easily resolved with phase contrast microscopy. In contrast to the normal axial alignment of the head and neck/midpiece (Fig. 1A-C), abnormal sperm demonstrated an abaxial relationship between the head and tail resulting in a non-linear or staggered arrangement of the two components (Fig.1). The base of the head appeared gently swollen and in some cells was observed to unilaterally overlap the neck region. The degree of overlapping varied from a subtle expansion of the nuclear base (Fig. 1C,D) to prominent bulging of the karyoplasm that extended along an appreciable part of the midpiece (Fig. 1A,B,E).

3.3. Transmission electron microscopy (TEM)

Despite the low incidence of the anomaly observed by LM, defective sperm were readily identified by TEM in affected birds. Using this technique, normal sperm revealed the typical ultrastructural features previously described for the head-base/neck interface in the emu [20] (Fig. 2A). Defective sperm displayed two basic forms of the defect, those with a flat nuclear (head) base and those demonstrating a caudally directed nuclear projection. In defective

sperm with a flat nuclear base the centriolar complex (proximal and distal centrioles) and attendant connecting piece were grossly misaligned in respect of the nuclear base. In these cells the nuclear base appeared to accommodate the abaxial centriolar complex by widening or by laterally extending a thin, horizontal sliver of karypolasm covered by the nucleolemma (Fig. 2B). In the other form of the defect the centriolar complex was more broadly yet abaxially attached to the nuclear base. In such instances the part of the nuclear base overlapping the misaligned centriolar complex extended caudally as a variably sized nuclear projection (Fig. 2C,D). In addition to the nuclear projection, that part of the nuclear base above the misaligned centriolar complex sometimes formed a thin lateral extension similar to that observed in grossly misaligned cells (Fig. 2D). In both forms of the defect the various components of the neck region (basal plate, capitellum, arms of the connecting piece) appeared normal. However, the shallow implantation fossa typical of emu sperm [20] was not always discernable in defective cells. An association between the connecting piece/centriolar complex and the base of the nucleus, no matter how tenuous, was always apparent in sperm with abaxial tails.

In some defective cells the nuclear projection was associated with a rudimentary, moderately electron-dense structure resembling components of the connecting piece (Fig. 3). These structures were associated with either the base or medial wall of the nuclear projection and were generally related to a narrow implantation fossa structurally similar to that of the adjacent fossa housing the abaxial tail. The structures were contained within the cytoplasm of the neck region and were not associated with an attendant centriolar complex or mitochondria.

3.4. Origin of the defect

Stages in the formation of abaxial sperm were observed in the testicular material from the six affected birds. The defect could be identified in early elongated spermatids which were characterized by lengthened nuclei containing moderately electron-dense, finely granular chromatin, and by the presence of the circular manchette (Fig. 4). The centriolar complex was clearly misaligned in respect of the longitudinal axis of the cell at this stage of spermiogenesis and both forms of the defect could be detected (Fig. 4B–D). The implantation fossa and structural features of the centriolar complex (Fig. 4B–D) were similar to those of normal spermatids (Fig. 4A) at the same stage of development. No lateral extension of the nuclear base was observed at this stage of development.

Late elongated spermatids typically displayed a condensed mass of chromatin and the late circular, transitional or longitudinal manchette (Fig. 5B–D). At this stage of development the two forms of the defect adopted the more definitive characteristics typical of mature sperm, including, where present, the formation of lateral extensions from the base of the nucleus (Fig. 5B,D). The structure of the components in the neck region of defective spermatids were again similar to those seen in normal cells at the same stage of development (Fig. 5A)

5. Discussion

Abaxial tail implantation in birds is rare and has only previously been reported in the turkey [17], goose [18] and duck [19]. No category for this defect is reflected in the numerous classification systems for sperm defects in poultry [17,21-26] and was not included in a recently proposed classification for ratite sperm abnormalities [20]. In the present study the defect was scored in only one of the 15 birds sampled, although careful examination of entire

semen smears (all cells scanned) identified isolated examples of the defect in a further five birds. This observation is in agreement with studies on mammalian sperm where the incidence of the defect is reported to be low [2,27]. It has been pointed out that abaxial tails in birds [18] and in the bull [3] may easily be overlooked on routine semen smears, particularly if the smears are poorly prepared or the resolution of the microscope inadequate. In the emu, this situation is further complicated by the cylindrical conformation of non-passerine bird sperm as incorrect orientation of the cell on the smear may mask the existence of the defect. It was clear from the present study that LM only revealed gross forms of the anomaly and that more subtle forms of the defect could only be identified by TEM. This may partially explain why abaxial tails were readily seen on TEM despite the low incidence of the defect revealed by LM. It has been noted that abaxial tails appear to increase in number in older bulls [28] and that certain sperm defects (larger heads, smaller heads, 180° bent heads) in the rooster also increase with age [26]. As the birds used in the present study were young (not older than 4 years), this may also explain the low incidence of the defect observed.

Some reports have suggested that abaxial tail implantation can have an adverse affect on fertility. Thilander et al [6] and Malmgren [29], in studies of boar and stallion sperm respectively, concluded that this anomaly negatively affects fertility due to its influence on sperm motility. Onstad [12] linked sterility in an 18 month-old bull to a 60% incidence of abaxial tail attachment in the ejaculate.

In contrast, this anomaly has been classified as a minor defect in the dog [8] and in man [11]. Semen evaluation studies in the dog [9], buffalo [4,5] and bull [2] have also indicated that abaxial tails have no influence on fertility, even when the incidence is greater than 50% [2]. Some authors even consider the anomaly to be a normal variation of sperm morphology

[5,9,10,16]. However, Sarlós et al [15] reported improved fertility in the boar with an increase in the incidence of abaxial tail implantation. The low incidence of the abnormality in the emu, coupled with the evidence from studies on mammalian sperm defects, would suggest that abaxial tail implantation has no affect on fertility in this bird.

Little is known about the morphological features of abaxial tail implantation in avian sperm. No descriptions of this defect have been provided and some doubt exists that the illustration by Wakely and Kosin [17] represents a true abaxially implanted tail, as the head/midpiece connection demonstrates normal alignment and it is only the principal piece of the flagellum that appears to be abaxially positioned. The electron micrograph of a defective sperm in the drake appears to represent an abaxially implanted tail although it is simply described as an "abnormality in the articulation between tail and head" [19].

This study revealed that abaxial tail implantation occurs in emu sperm. However, certain morphological peculiarities are obvious in defective emu sperm when compared to, for example, that of the bull [3] and boar [6]. In mammals the broad head base allows for abaxial attachment of the flagellum without the connecting piece overlapping the nuclear base [8,11]. In the emu, and presumably in other non-passerine birds, the similarity in width between the connecting piece and head base [20,30-35] results in a staggered alignment of the head and tail in defective sperm, with the connecting piece being positioned at a variable distance from the head base axis. In addition, the nuclear base of defective mammalian sperm remains morphologically unaltered in comparison to the situation in the emu where a thin lateral extension of the karyoplasm often covers the misaligned portion of the connecting piece, and a variably sized nuclear projection extends caudally in some cells from the nuclear base overlapping the centriolar complex. An additional implantation fossa has been reported in

abaxial sperm in the bull, and which is sometimes associated with an accessory tail [2,3]. However, the free nature of the accessory tail in bull sperm and its association with a centriolar complex, connecting piece and attendant mitochondria [2,3], differs markedly from the rudimentary structures observed in emu sperm. The association between abaxial tails and multiple tails suggested for the bull appears unlikely in the emu although some defective cells displayed a widened nuclear base typical of the multiflagellate sperm reported for this species [20]. Despite the structural differences outlined above, it is clear that abaxial tail attachment in the emu parallels that described for mammalian sperm thus further emphasizing the point that basic sperm anomalies can occur across a wide spectrum of vertebrate species [36].

In the emu the defect is clearly visible at the early elongated spermatid stage of spermiogenesis although there are indications that earlier predisposing factors concerning the movement of the centriolar complex precipitate the subsequent misalignment of the nucleus and flagellum. In human sperm abnormal head-tail alignment (abaxial sperm) occurs when the sperm centrioles "are unable to migrate and attach normally to the caudal pole of the spermatid nucleus" [37]. A similar mechanism may be involved during spermiogenesis in the emu.

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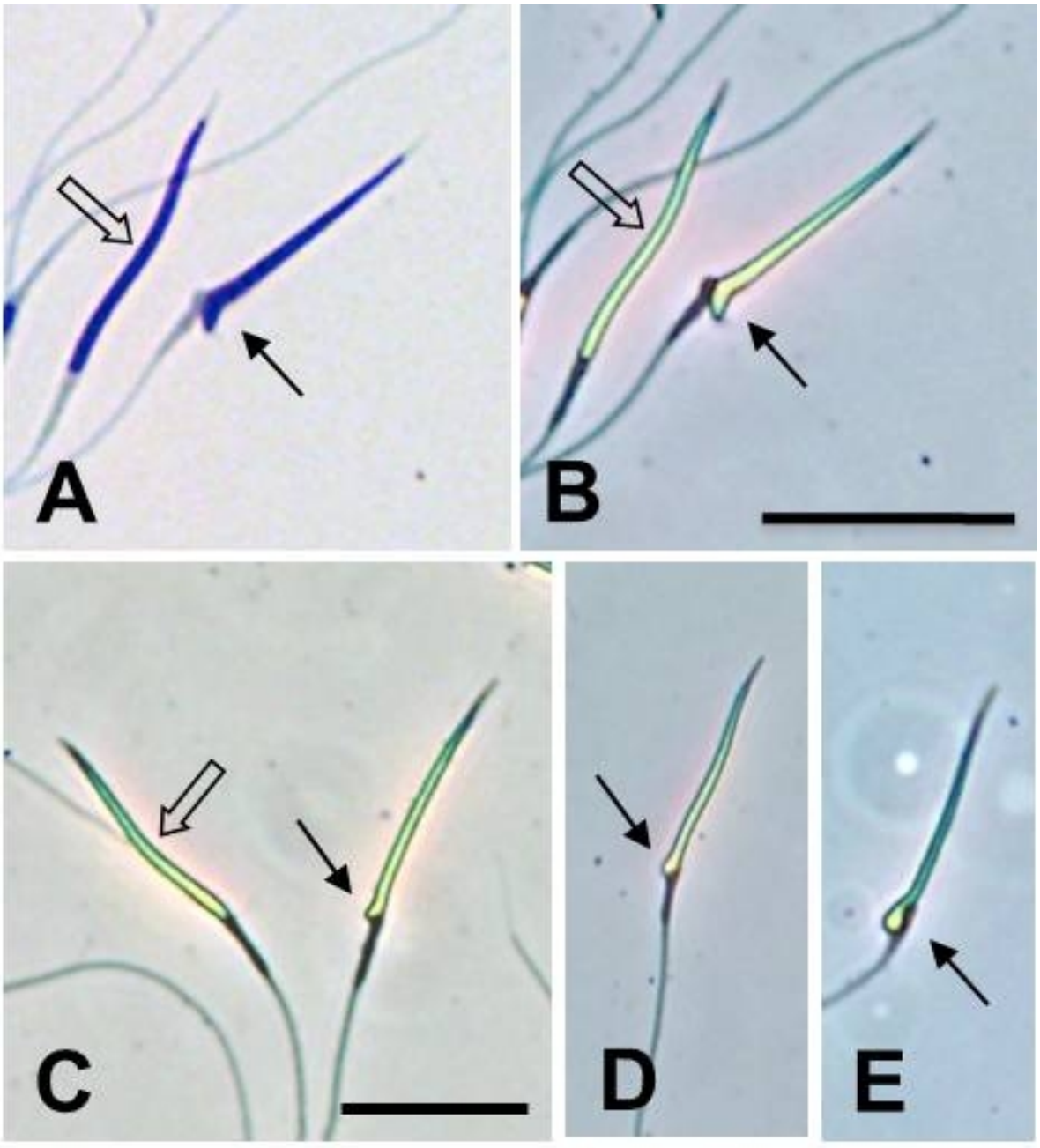

Fig. 1. Light micrographs of normal (block arrow) and defective sperm (arrow) as revealed by bright field microscopy (Wright's stain) (A) and phase contrast microscopy (B). Note the improved detail revealed by the latter technique. Figs. 1. C - E illustrate varying forms of the defect as observed by phase contrast microscopy. Compare the axial implantation of the tail in normal sperm (block arrows) with the abaxial connection in defective sperm. Note the prominent unilateral projection of the nucleus in Fig. 1E covering the proximal midpiece. Bar = 10 μm (A and B; C, D and E).

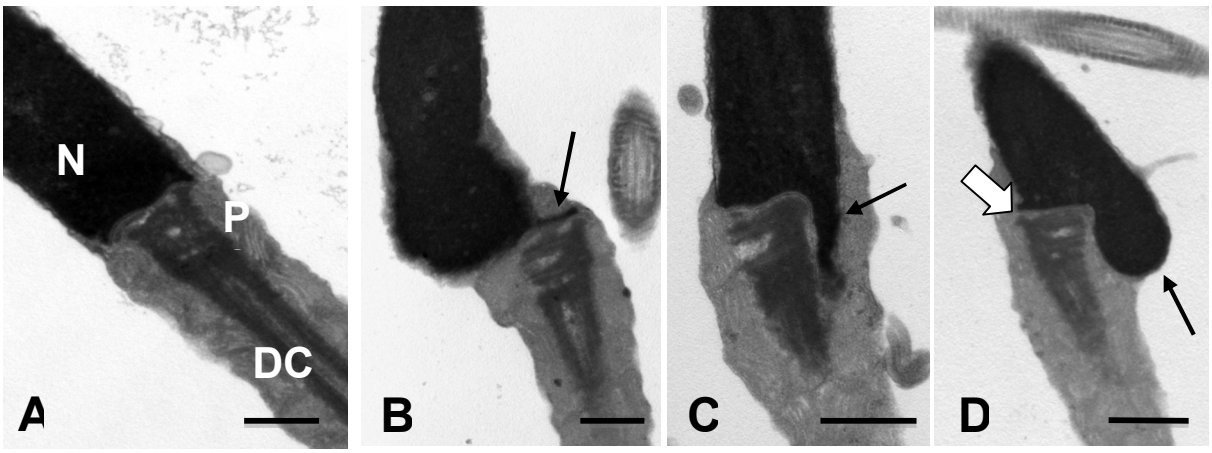

Fig. 2. Mid-saggital sections of normal (A) and defective (B – D) sperm demonstrating the ultrastructural features of the head-neck/midpiece region. In normal cells (A) the nuclear base and neck/midpiece are similar in width and the connecting piece of the centriolar complex attaches axially to the base of the nucleus via a shallow implantation fossa. In defective cells the centriolar complex is abaxially positioned in respect of the head (nuclear) base. In Fig. 2 (B) the nuclear base displays a thin nuclear extension (arrow) to accommodate the grossly misaligned centriolar complex whereas in Figs. 2(C), (D) the broadly connected yet abaxially positioned centriolar complex is unilaterally flanked by a bulbous projection (arrow) of nuclear material (compare to Figs. 1D, E). Note the short, lateral nuclear extension (block arrow) in Fig. 2D. (N) nucleus; (PC) proximal centriole; (DC) distal centriole. Bar = 0.5µm.

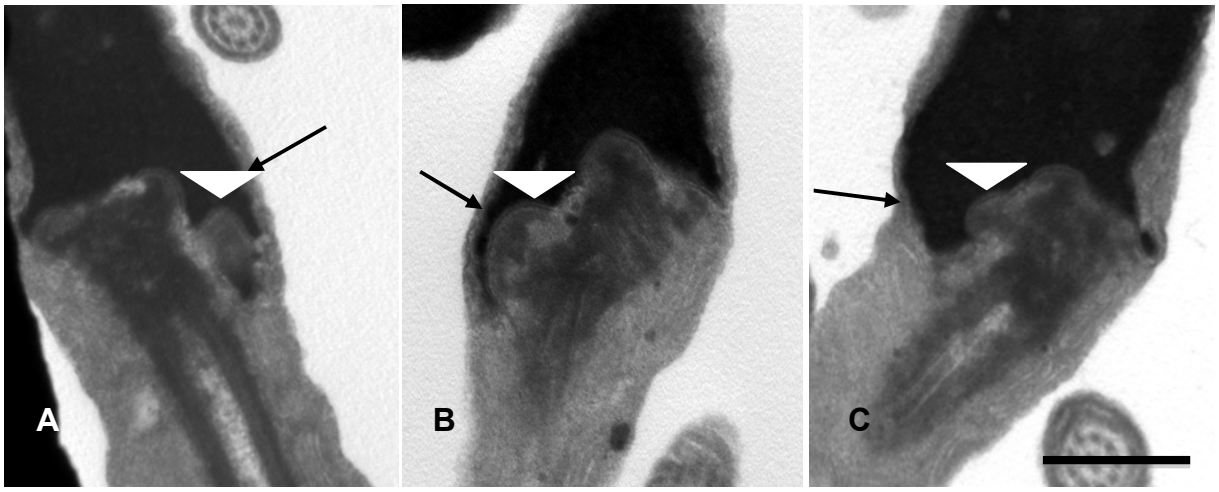

Fig. 3. Transmission electron micrographs of defective sperm illustrating attachment of presumptive elements of the connecting piece to the base (Fig. A) or medial surface (Fig. B,C) of the caudal nuclear projection (arrow). In each instance the nuclear membrane in contact with the centriole-associated structures forms a rudimentary implantation fossa (arrowheads). Note the thin lateral extension of the nuclear base in (A) and (C) which partially accommodates the abaxially positioned centriolar complex. Bar = 0.5µm.

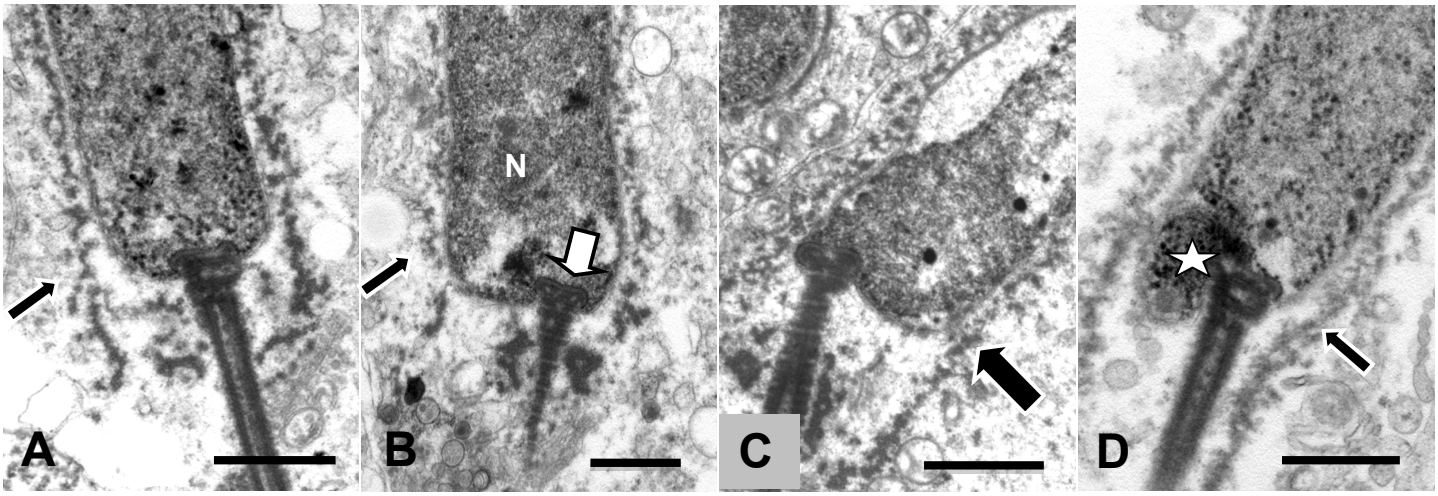

Fig. 4. Transmission electron micrographs of early stage elongated spermatids showing normal (A) and abaxially implanted flagella (B-D). In (B) the centriolar complex is abaxially attached to the base of the nucleus (N) via a clearly defined implantation fossa (white arrow). Fig. 4 (C) shows a similar stage in the development of the defect to that shown in B except that the centriolar complex is obliquely attached to the base of the nucleus. In Fig. 4(D) the abaxially positioned centriolar complex is unilaterally flanked by a projection of the nuclear base (star)(compare to Figs. 1E, 2D and 5C.). Note the circular manchette (black arrow) associated with both the normal and defective cells. Bar = 1μm.

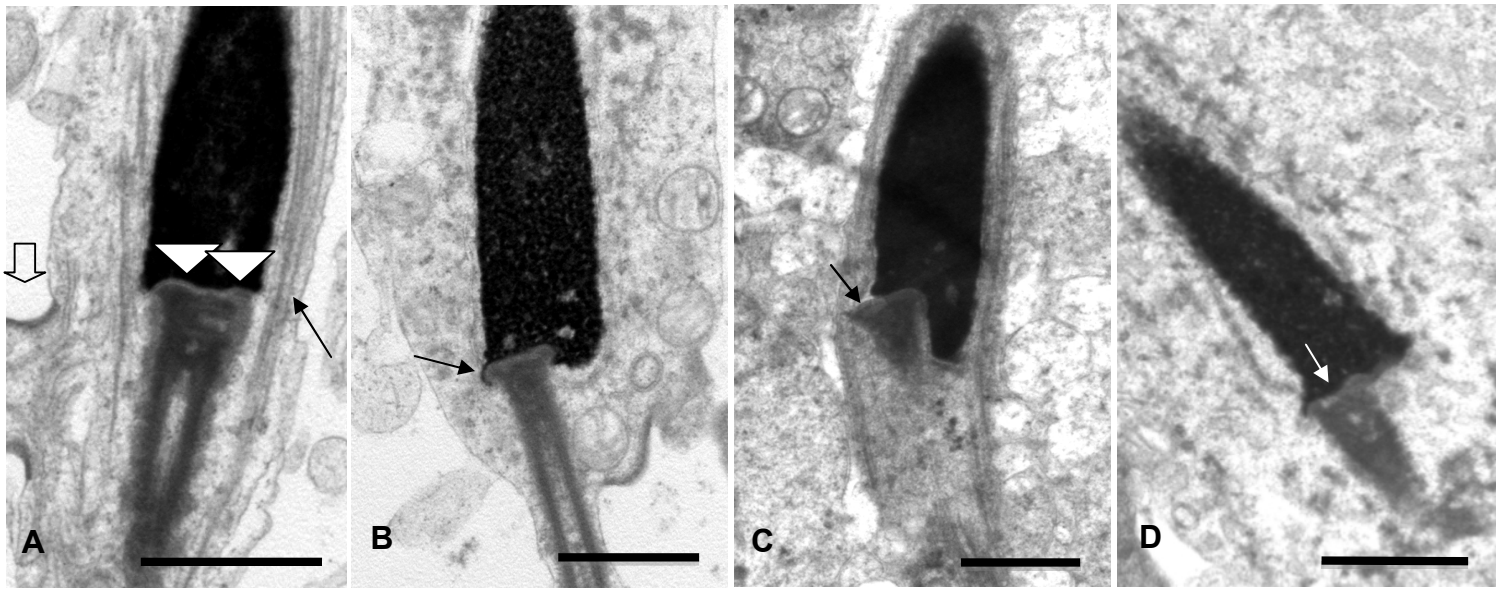

Fig. 5. Transmission electron micrographs of late stage elongated spermatids. Fig. 5(A) demonstrates a normally aligned head and tail. Note the shallow yet distinct implantation fossa (arrowheads), the prominent longitudinal manchette (arrow) and the cytoplasmic bridge (block arrow) connecting neighboring cells. In Fig. 5(B) the centriolar complex attaches abaxially to one half of the nuclear base. A thin strip of karyoplasm (arrow) completes the implantation fossa. In Fig. 5(C) the connecting piece (arrow) markedly overlaps the nuclear base which unilaterally extends a nuclear projection (compare with Figs. 1 D, E; Figs. 2 C, D and Fig. 4D). In Fig. 5(D) a widened nuclear base accommodates the abaxially positioned centriolar complex. Only a single implantation fossa (arrow) is obvious. Bar = 1μm.