

# **Head-Base Bending and Disjointed Spermatozoa in the Emu (*Dromaius novaehollandiae*): A Morphological Comparison of Two Closely Related Defects**

**Short title:** Head-Base Bending and Disjointed Sperm in the Emu

**Authors:** Lizette du Plessis<sup>1</sup> and John T. Soley<sup>2</sup>

**Address:** <sup>1</sup>Electron Microscope Unit, Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa

<sup>2</sup>Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa

**Corresponding author:** Lizette du Plessis

**e-mail:** lizette.duplessis@up.ac.za

## **Abstract**

The accurate assessment of avian sperm abnormalities is hampered by a lack of descriptive data and by the confusing terminology currently in use. Critical appraisal of semen samples from the distal *ductus deferens* of the emu revealed that two closely related yet separate (distinct) defects previously collectively referred to as “bent sperm” or “crooked-necked sperm” could be identified by light and electron microscopy. Head-base bending typically involved a 180° bend at the base of the nucleus which placed the head and midpiece into close apposition and parallel to each other. No part of the neck or midpiece was involved and bending was restricted exclusively to the base of the nucleus. Incomplete chromatin condensation was always associated with the bend. Disjointed sperm, which superficially resembled “bent” sperm, showed complete separation of the neck from

the head-base at the level of the connecting piece. All structural elements of the neck region appeared normal. In both defects the region of contact between the head (nucleus) and the neck/midpiece was enclosed as a unit by the plasmalemma. Both defects were observed to originate in the testis; however, their subsequent expression in the *ductus deferens* cannot be ruled out. These results confirm that head-base bending of emu sperm represents a head defect, whereas disjointed sperm should be classified as a tail (neck/midpiece) defect.

**Keywords:** emu, *Dromaius novaehollandiae*, abnormal spermatozoa, head-base bending, disjointed sperm, avian sperm defects

## 1. Introduction

Avian sperm defects, in particular those of poultry, have been extensively studied. Although some ultrastructural descriptions of abnormal avian sperm have been provided by scanning [1-4] and transmission electron microscopy [2,5-7], most information on this topic stems from light microscopic observations. The paucity of corroborative ultrastructural data and the resultant lack of critical appraisal of avian sperm defects have led to the use of disparate and often confusing terminology for their classification.

Of the various defects identified in non-passerine birds, bending of the filiform avian spermatozoon appears to be a common anomaly and various terms have been used to describe and categorize this defect. Some authors have adopted a generalized approach and simply refer to head, midpiece and tail (flagellum) bends [3,7,8], while others qualify the degree of bending, recognizing 90° and 180° head and tail bends as well as midpiece bending [9-11]. Other descriptions of bending are more specific and define the precise area where the bend occurs. Bakst and Sexton [2], for example, use the term “bent spermatozoa” to describe “those in which the head was reflexed at the neck, midpiece

or distal aspect of the nucleus”. This description was later extended to include the proximal part of the principal piece and further defined as an acute flexion of the tail bringing it adjacent and parallel to the head [12]. This anomaly was particularly evident in sperm subjected to adverse osmotic conditions, temperature variations and different storage times [2,4,12-18]. Earlier literature referred specifically to bent necks where the head was typically observed to lie back along the tail. This anomaly was originally described by Saeki [13] as crooked-necked sperm, the term being defined by Maeda et al [16] as an acute bend in the neck region or anywhere along the length of the midpiece. Various terms such as “broken-necked”, “bent-neck” and “neck-bending” have also been used and this defect has been described, but not always defined, in a number of avian species including the fowl, duck, goose, turkey, partridge, Japanese quail and parrots [15,17,19-23].

Although little attention has been given in the literature to the description of sperm anomalies in ratites, bending also appears to be a common defect in this commercially important group of birds. Reporting on the incidence of abnormal sperm in the emu, Malecki et al [24] provide a category for “bent sperm” without defining the term whereas Góes et al [25] simply report that bent heads, folded tails and strongly folded tails are observed in the rhea. Midpiece reflexes have been described in the ostrich [26,27] and Soley et al [28] reported an association between cytoplasmic droplets and bent tails in the same species. In a recent study of abnormal sperm in the emu it was noted that the majority of defects occurred at the base of the head and in the neck region. The head-base defects exclusively involved varying degrees of bending as well as looping and kinking of the nucleus, whereas in the neck region a defect referred to as “disjointed sperm” and which superficially resembled a form of “bending” was displayed [29].

In order to fully understand the distinction between head-base bending and “bending” in the neck region (disjointed sperm) in the emu, this paper presents a comprehensive, comparative morphological study of the two defects utilizing both light and electron microscopy. The incidence of the two defects is presented but as this study focused on establishing a morphological basis for the accurate assessment of the defects using abattoir material, functional data detailing the possible effects of the anomalies on fertility, although speculated on, are not provided. The results are compared with relevant information in the literature on avian sperm defects and definitive terminology is suggested for the two abnormalities in order to eliminate the confusing terminology currently in use. This study also provides previously unreported ultrastructural data relevant for future descriptions and comparisons of sperm defects in non-passerine birds.

## **2. Materials and Methods**

Semen samples were collected from 14 sexually mature and active emus within 30 min of slaughter at a commercial abattoir. The birds ranged in age from 2 to 4 years. Droplets of semen were gently squeezed from the distal *ductus deferens* directly into 2.5% glutaraldehyde in 0.13M Millonig’s phosphate buffer, pH 7.4 at room temperature. Small blocks of testicular tissue were also collected and immediately fixed in the same fixative at the time of semen collection. Thin smears for light microscopy were made from the fixed semen samples and stained with Wright’s stain (Rapidiff, Clinical Sciences Diagnostics, Johannesburg, South Africa) before viewing with a 100x oil immersion lens using both bright field and phase contrast microscopy. Smears were also stained with the Feulgen stain and acidic aniline blue according to the methods described by Vieytes et al [30] to evaluate the degree of chromatin condensation. The incidence of the two defects was determined by examining the smears using a 100x oil immersion objective and counting the number

of abnormal spermatozoa present in a total of 300 cells for each bird. The incidence is expressed as a percentage of the total cell count.

Semen samples were prepared for electron microscopy as previously described [29]. Tissue samples were routinely prepared for transmission electron microscopy (TEM) using standard techniques. Thin sections were stained with uranyl acetate and lead citrate and viewed in a Philips CM10 transmission electron microscope operated at 80kV. Samples for scanning electron microscopy were coated with gold and viewed at 10kV using a JEOL 840 scanning electron microscope. Relevant structural features revealed by the various microscopic techniques were described and digitally recorded.

### **3. Results**

#### *3.1 Light Microscopy (LM)*

Head-base bends and disjointed sperm were observed in all samples and could be readily distinguished using both bright field (Figs. 1A,C) and phase contrast (Figs. 1B,D) microscopy. However, phase contrast microscopy resolved the differences between the two defects more clearly. A total of 13.6% abnormal sperm were detected in the animals studied of which head-base bending and disjointed sperm represented 3.8% and 1.9% of the total cell count, respectively. The incidence of the defects in individual birds is shown in Figure 2.

Head-base bending typically involved a 180° bend at the base of the nucleus which placed the head and midpiece (and in some instances part of the principal-piece) into close apposition and parallel to each other. A few intermediate forms were observed. No part of the neck or midpiece was involved and bending was restricted exclusively to the base of the nucleus (Figs. 1A,B). The free aspect of the

bend (essentially the anterior aspect of the defective sperm) presented a typically rounded profile with nuclear material clearly visible at both ends of the bend (Figs. 1A,B). The principal-piece was often angled across the sperm head (Fig. 1A).

Disjointed sperm displayed some of the characteristics of head-base bending, notably, an apparently 180° reflex of the tail, parallel alignment of the head and midpiece, and angling of the principal-piece across the sperm head (Figs. 1C,D). The most striking difference, however, was the obvious disconnection of the head and tail at the head/tail interface resulting in the neck and base of the nucleus lying next to and level with each other. This arrangement produced a straight profile (Fig. 1C) in contrast to the rounded profile typical of head-base bending. The neck was occasionally positioned posterior to the nuclear base creating a staggered appearance or the impression that both components were completely separated (Fig. 1E). However, SEM and TEM confirmed that in both defects the parallel-aligned head and neck/midpiece remained firmly attached (see below). No loose heads or tails were observed in the smears.

Occasionally the orientation of the sperm head relative to the neck/midpiece made it difficult to accurately distinguish between the two defects (Fig. 1F). In such instances scoring of the defect depended on the experience of the observer although this involved a degree of subjectivity.

The use of specialized stains (Feulgen and acid aniline blue) to indicate the possible role of defective chromatin condensation in the formation of the defects produced inconclusive results. Feulgen staining of the emu sperm was weak and the nuclei exhibited a uniform light pink staining reaction with no indication of localized loss of the dye. Acid aniline blue staining produced more graphic, yet inconsistent results. Most cells (normal morphology) appeared pale indicating no uptake

of the dye (i.e. normal chromatin condensation). However, some spermatozoa with head-base bending showed localized accumulation of the dye indicating a lack of chromatin condensation, while others remained unstained.

### *3.2 Scanning Electron Microscopy (SEM)*

SEM confirmed the basic structural features of the two defects seen on LM. It was also clear using this technique that only the base of the nucleus was involved in the formation of head-base bending, and that the neck and midpiece remained unaffected (Fig. 3A). Disjointed sperm again revealed complete separation of the neck and head-base, with both structures lying parallel and level with each other (Fig. 3B,D) or staggered (Fig. 3C). It was sometimes possible to observe a shallow concavity at the base of the head indicating the implantation fossa, thus suggesting that separation of the neck from the head occurred at the connecting piece (Fig. 3D). In both defects the midpiece and head appeared to be attached to each other by a membrane and the principal piece of the tail was often seen to lie at an angle across the sperm head (Fig. 3A-D).

### *3.3 Transmission Electron Microscopy (TEM)*

A short description of the neck region of normal emu sperm (Fig. 4) is provided as background for the ultrastructural description of the two defects. This region displayed a shallow implantation fossa at the base of the nucleus lined by a thin layer of moderately electron-dense material, the basal plate. As in the ostrich [31] the fossa appeared in the form of individual impressions each housing one of the poorly defined segmented columns that formed the connecting piece. The tips of the segmented columns merged to form the capitellum which was separated from the basal plate by an intervening layer of clear material. The proximal centriole was enclosed by the connecting piece and lay on top

of, and at right angles to, the long distal centriole. Mitochondria of the *pars spiralis* extended into the neck region. The chromatin at the base of the nucleus was densely compacted.

In addition to the obvious morphological characteristics of the defects defined by LM and SEM, TEM revealed a number of significant structural peculiarities which further differentiated head-base bending (Fig. 5) from disjointed sperm (Fig. 6). In heads bent at the base, TEM clearly demonstrated the presence of nuclear material on both sides of the bend. The nuclear chromatin in the vicinity of the bend was generally incompletely condensed, with variably-sized gaps filled with flocculent or granular material and the occasional thread-like strands occurring in the karyoplasm. These gaps were generally positioned at the convex surface of the bend (Fig. 5A-D) and in most instances extended into both legs of the bend. Despite the acute bending of the nuclear base, the nucleus always remained firmly attached to the basal plate lining the implantation fossa. In some defective cells a lack of karyoplasm at the base of the nucleus created the erroneous impression of nuclear detachment. Although such cells superficially resembled disjointed sperm, they technically represented a form of head-base bending as the nuclear membrane was still firmly attached to the connecting piece (Fig. 5E,F). The region of contact between the head (nucleus) and the neck/midpiece resulting from the bend was enclosed as a unit by the plasmalemma (Figs. 5A-E, 7). Except for the absence of the plasmalemma on the surface apposed to the head, the structural elements of the neck and midpiece appeared normal. The nucleus and centriolar complex were always separated by the *pars spiralis* mitochondria of the midpiece.

As indicated by SEM, disjointed sperm (Fig. 6) showed complete separation of the neck from the head-base at the level of the connecting piece. A thin layer of moderately electron-dense material representing the basal plate and possibly elements of the capitellum remained associated with the



implantation fossa (Fig. 6A,B). The segmented columns (poorly developed in non-passerine birds) of the connecting piece and the enclosed proximal centriole remained associated with the proximal aspect of the midpiece. The structure of the connecting piece and the proximal centriole appeared normal. In most instances the disjointed nucleus appeared fully condensed (Fig. 6B), but occasional defective cells displaying zones of incomplete chromatin condensation were observed (Fig. 6A). As with head-base bending, the region of contact between the disjointed nucleus and the midpiece was contained as a unit within the plasmalemma even in sperm displaying a staggered arrangement of the head and neck/midpiece (Fig. 6C). This phenomenon was particularly obvious in transverse sections of this region, although it was not possible to determine whether such sections represented head-base bending or disjointed sperm (Fig. 7). TEM also revealed intermediate stages in the development of disjointed sperm with some cells displaying a tenuous, mal-aligned connection between the rim of the implantation fossa and the connecting piece (Fig. 6D).

Both defects were observed by TEM in the testicular material. Isolated sperm demonstrating head-base bending were randomly scattered in the testis parenchyma (Fig. 8A). These cells showed the typical characteristics described in defective sperm from the *ductus deferens*. In all instances a lack of chromatin condensation was again observed in the region of the bend (Fig. 8B). Intermediate forms of the disjointed sperm were also seen in the testis (Fig. 9), clearly indicating the misalignment of the head and neck region (compare with Fig. 6D).

#### **4. Discussion**

The present study indicated that when assessing emu sperm morphology a clear distinction should be made between head-base bending, which represents a true form of bending, and disjointed sperm which, although resembling bending, represents a separate sperm anomaly. The available literature

on avian sperm abnormalities does not differentiate between the two defects despite the fact that micrographs and illustrations in a number of papers appear to portray disjointed sperm as well as sperm bent at the head-base. These defects are generally grouped using generic terminology such as “crooked-necked sperm” and “bent sperm”. For example, Bakst and Sexton [2] illustrate a typical example of head-base bending as well as what appears to be a disjointed sperm, describing both as “bent spermatozoa”. Maeda et al [4] demonstrate sperm acutely bent at the neck region, at any point along the midpiece, and at the head-base, referring to them collectively as crooked-necked sperm. The sketches by Saeki [13] of crooked-necked sperm can in fact be interpreted as head-base bending and disjointed sperm respectively. Failure to recognize specific sperm defects can be attributed to the continuing use of established, inappropriate descriptive terminology coupled with a lack of accurate descriptions of avian sperm anomalies. The situation is further complicated by a dearth of supporting ultrastructural information on avian sperm defects.

Avian sperm appear prone to bending, especially in the region encompassing the base of the head, neck, midpiece and the adjacent segment of the principal piece [12]. A number of studies have reported on the fragility of the neck region of poultry sperm, which makes this region susceptible to damage [2,4,9,12-15,32], particularly when the cells are exposed to adverse physiological influences. Various external factors such as osmolarity, storage time and temperature have been implicated in sperm bending [13, 14]. However, the present study utilized semen from the *ductus deferens* which, barring the possible effects of glutaraldehyde fixation, would not have been influenced by potentially damaging external factors.

Head bending in the emu was, with few exceptions, confined to the base of the head [29] and was associated with incomplete chromatin condensation in the vicinity of the bend. The areas of

incomplete condensation were generally positioned towards the outside of the bend and displayed coarse granules and intervening spaces, similar to regions of uncondensed chromatin described in mammalian sperm [33-35]. This phenomenon is considered to be a morphological manifestation of chemical or molecular abnormalities in the condensation process [33,34,36] resulting in single stranded DNA being formed as opposed to the more stable double stranded form. The nucleus is therefore more fragile and as our results suggest, would make it more prone to bending. Attempts to consistently demonstrate incomplete chromatin condensation at the LM level with acid aniline blue and Feulgen stains were unsuccessful. Santiago-Moreno et al [37] similarly reported inconclusive results with the aniline blue stain when used to predict fertility in roosters.

Disjointed sperm clearly represent a separate anomaly which is characterized by the distinct separation of the nuclear base and connecting piece within the confines of the plasmalemma. An anomaly referred to as “dislocated” sperm and displaying similar morphological features to those reported in the present study, has been reported in epididymal sperm in mice [38]. It was speculated that this defect resulted from structural deficiencies and/or deficiencies in the assembly of periaxonemal proteins, causing the dislocation of the segmented columns of the connecting piece from the nuclear attachment sites. A number of other mammalian sperm defects, such as acephalic sperm, decapitated sperm and the flexed head defect, are believed to originate from eccentric implantation of the head and neck, or structural deficiencies of the neck region [33]. Similarly Chemes and Rawe [35,39] reported on sperm with misaligned head-midpiece junctions, stating that different degrees of incorrect alignment results in either acephalic sperm (the most severe form of the defect) or sperm where the head is misaligned. They further ascribed the misalignment to the lack or abnormal implantation of the centrioles during the early stages of spermiogenesis. In the emu, all structural elements in the neck region appeared to be present and morphologically normal, although the

connecting piece was not correctly related to the implantation fossa. This would appear to indicate failure of the connecting piece to establish proper contact with the head-base during spermiogenesis. The extremely shallow implantation fossa in this species may further compound this problem. Based on the above observations [33,38,39], we propose that disjointed sperm are closely related to dislocated, flexed, decapitated and acephalic sperm as described in mammals.

Although the two defects can generally be distinguished by LM, a small proportion of abnormal cells are difficult to categorize. As revealed by TEM, some defective cells which technically would be defined as “head-base bending” appear to be disjointed sperm. Such cells would be impossible to differentiate by LM. The orientation of some cells would also preclude their accurate classification. A slight degree of overlap between the two defects could therefore be expected when assessing semen samples. It was clear from this study that the two sperm defects made up only a small proportion of the total sperm count. An interesting observation was that the incidence of head-base bending (3.8%) was double that of disjointed sperm (1.9%). Although the present study was not designed to test functional parameters, it is doubtful whether the low incidence of the two defects would have had a marked influence on fertility.

The presence in the testis parenchyma of developing spermatids displaying head-base bending and misalignment of the head and flagellum points to a testicular origin for both defects in the emu. This is the first morphological evidence of any form of head (head-base bending) or neck (disjointed sperm) “bending” taking place in the avian testis. Despite numerous publications on “bent” or “crooked-necked” sperm [2,4,9-19,23,26] in birds, no information on the origin of this defect has been presented. Both defects could conceivably also develop in the *ductus deferens* due to a combination of inherent and external factors. The most probable inherent factors appear to be

defective chromatin condensation (for head-base bending) coupled with early misalignment of the flagellum (both defects) during the round spermatid stage of development (personal observations). Therefore, inherent deficiencies in the process of spermiogenesis may be responsible for the eventual manifestation of the defect in the excurrent duct system. The most likely external factors are those proposed by Yamane [14] where flagellar movement coupled with intrinsic weaknesses in the head-base (defective chromatin condensation) and neck (misalignment and/or missing elements of the connecting piece) could adversely affect sperm structure during their progress along the deferent duct.

In summary, terms such as “bent sperm” and “crooked-necked sperm” should be avoided when assessing emu sperm morphology as they are non-specific and do not reflect the existence of two separate and distinct abnormalities, namely head-base bending and disjointed sperm. Head-base bending is a true form of bending and as it involves only the head is classified as a head defect. In contrast, while disjointed sperm superficially appear bent, they actually reflect dislocation of the head and tail in the neck region, thus representing a tail defect. The lack of supportive morphological data for the accurate identification of avian sperm defects and the perpetuation of loosely used terminology for their classification requires critical reappraisal.

### **Acknowledgements**

The authors thank Mr. P. Bezuidenhout, Chairman of the Eastern Cape Emu Breeders Association and Mrs. P. Rough for making material available and the Grahamstown Ostrich Abattoir and Emu Ranch, Rustenburg, Northwest Province, South Africa for use of their facilities. This work was financially supported by the National Research Foundation (Incentive Funding grant number 73279) and the University of Pretoria (*ad hoc* funding).

## References

- [1] Marquez BJ, Ogasawara FX. Scanning electron microscope studies of turkey semen. *Poultry Sci* 1975;54:1139-43.
- [2] Bakst MR, Sexton TJ. Fertilizing capacity and ultrastructure of fowl and turkey spermatozoa before and after freezing. *J Reprod Fertil* 1979;55:1-7.
- [3] Hess RA, Hughes BL, Thurston RJ. Frequency and structure of macrophages and abnormal sperm cells in guinea fowl semen. *Reprod Nutr Dev* 1986;26:39-51.
- [4] Maeda T, Terada T, Tsutsumi Y. Studies of the factors causing abnormal acrosomes and crooked-necks in fowl spermatozoa during freezing and thawing. *Br Poultry Sci* 1986;27:695-702.
- [5] Maretta M. Ultrastructure of double and multiple sperm tails in drake. *Vet Med (Praha)* 1979;24:679-89.
- [6] Froman DP, Bernier PE. Identification of heritable spermatozoal degeneration within the ductus deferens of the chicken (*Gallus domesticus*). *Biol Reprod* 1987;37:969-77.
- [7] Ferdinand A. Licht- und electronenmikroskopische untersuchungen zur morphologie von ganterspermatozoen. Dissertation zur erlangung des grades eines Doctor Medicinae Veterinariae. Hannover, 1992.

- [8] Penfold LM, Wildt DE, Herzog TL, Lynch W, Ware L, Derrickson SE, Monfort SL. Seasonal patterns of LH, testosterone and semen quality in the Northern pintail duck (*Anas acuta*). *Reprod Fertil Dev* 2000;12:229-35.
- [9] Alkan S, Baran A, Özdaş ÖB, Evecen M. Morphological defects in turkey semen. *Turk J Vet Anim Sci* 2002;26:1087-92.
- [10] Tabatabaei S, Batavani RA, Talebi AR. Comparison of semen quality in indigenous and Ross broiler breeder roosters. *J Anim Vet Adv* 2009;8:90-93.
- [11] Tabatabaei S, Chaji M, Mohammadabadi T. Correlation between age of rooster and semen quality in Iranian indigenous broiler chickens. *J Anim Vet Adv* 2010;9:195-98.
- [12] Bakst MR. Fertilizing capacity and morphology of fowl and turkey spermatozoa in hypotonic extender. *J Reprod Fertil* 1980;60:121-27.
- [13] Saeki Y. Crooked-necked spermatozoa in relation to low fertility in the artificial insemination of fowl. *Poultry Sci* 1960;39:1354-61.
- [14] Yamane J. Neck-bending of cock sperm and its causal genesis. In: *Riproduzione animale e fecondazione artificiale: scritti in onore di Telesforo Bonadonna. Societa Italiana per il Progresso della Zootechnica*. Edizioni Agricole, Bologna, 1972, pp. 327-31.

- [15] Van Wambeke F. The effect of tonicity of storage media for fowl semen on the occurrence of neck-bending spermatozoa, fertility and hatchability. *Br Poultry Sci* 1972;18:163-68.
- [16] Maeda T, Terada T, Tsutsumi Y. Comparative study of the effects of various cryoprotectants in preserving the morphology of frozen and thawed fowl spermatozoa. *Br Poultry Sci* 1984;25:547-53.
- [17] Bozkurt HH, Alkan S, Arda O, Dağlioğlu S, Ileri IK. The effect of BPSE (Beltsville poultry semen extender) and PBS (phosphate buffered saline) semen extender on ultrastructure of turkey spermatozoa kept in the cold. *Vet Fak Dergisi (Istanbul)* 1998;24:343-54.
- [18] Siudzińska A, Łukaszewicz E. Effect of semen extenders and storage time on sperm morphology of four chicken breeds. *J Appl Poultry Res* 2008;17:101-08.
- [19] Humphreys PN. Brief observations on the semen and spermatozoa of certain passerine and non-passerine birds. *J Reprod Fertil* 1972;29:327-36.
- [20] Steltzer G, Crosta L, Bürkle M, Krautwald-Junghanns M-E. Attempted semen collection using the massage technique and semen analysis in various psittacine species. *J Avian Med Surg* 2005;19:7-13.
- [21] Patryka A, Jerysz A, Pokorny P. Lipid peroxidation in fresh and stored semen of green-legged partridge. *EJPAU* 2007;10:8. <http://www.ejpau.media.pl/volume10/issue2/art-08.h>



- [22] Chelmońska B, Jerysz A, Łukaszewicz E, Kowalczyk A, Malecki I. Semen collection from Japanese Quail (*Coturnix japonica*) using a teaser female. *Turk J Vet Anim Sci* 2008;32:19-24.
- [23] Łukaszewicz E, Jerysz A, Partyka A, Siudzińska A. Efficacy of evaluation of rooster sperm morphology using different staining methods. *Res Vet Sci* 2008;85:583-88.
- [24] Malecki IA, Cummins JM, Martin GB, Lindsay DR. Effect of collection frequency on semen quality and the frequency of abnormal forms of spermatozoa in the emu. *P Aus S Ani* 1998;22:406.
- [25] Góes PAA, Cavalcante AK da S, Nichi M, Perez EG de A, Barnabe RC, Barnabe VH. Reproductive characteristics of captive greater rhea (*Rhea Americana*) males reared in the state of São Paulo, Brazil. *Braz J Poult Sci* 2010;12:57-62.
- [26] Bertschinger HJ, Burger WP, Soley JT, de Lange JH. Semen collection and evaluation of the male ostrich. *Proc Biennial Congr S Afr Vet Assoc, Grahamstown, 1992*, pp. 154-58.
- [27] Irons PC, Bertschinger HJ, Soley JT, Burger WP. Semen collection and evaluation in the ostrich. In: Deeming DC (Ed.), *Improving our Understanding of Ratites in a Farming Environment*. Ratite Conference, Manchester, UK, 1996, pp. 157-99.
- [28] Soley JT, Bertschinger HJ, Els HJ, Burger WP. The morphology and incidence of retained cytoplasmic droplets in ostrich spermatozoa. In: Deeming DC (Ed.), *Improving our*

Understanding of Ratites in a Farming Environment. Ratite Conference, Manchester, UK, 1996, pp. 16-18.

- [29] Du Plessis L, Soley JT. Incidence, structure and morphological classification of abnormal sperm in the emu (*Dromaius novaehollandiae*). *Theriogenology* 2011;75:589-601.
- [30] Vиейtes AL, Cisale HO, Ferrari MR. Relationship between the nuclear morphology of the sperm of 10 bulls and their fertility. *Vet Rec* 2008;163:625-29.
- [31] Soley JT. Ultrastructure of ostrich (*Struthio camelus*) spermatozoa: I. Transmission electron microscopy. *Onderstepoort J Vet Res* 1993;60:119-30.
- [32] Lake PE. The relationship between morphology and function of fowl spermatozoa. *Proc Xth World's Poultry Congr*, Edinburgh, 1954, pp. 79-85.
- [33] Zamboni Z. Sperm ultrastructure and its relevance to infertility. *Arch Pathol Lab Med* 1992;116:325-44.
- [34] Dadoune J-P. The nuclear status of human sperm cells. *Micron* 1995;26:323-45.
- [35] Chemes HE, Rawe VY. Sperm pathology: a step beyond descriptive morphology. Origin, characterization and fertility potential of abnormal sperm phenotypes in infertile men. *Hum Reprod Update* 2003;9:405-28.

- [36] D'Occhio MJ, Hengstberger KJ, Johnston SD. Biology of sperm chromatin structure and relationship to male fertility and embryonic survival. *Anim Reprod Sci* 2007;101:1-17.
- [37] Santiago-Moreno J, López-Sebastián A, Castaño C, Coloma MA, Gómez-Brunet A, Toledano-Díaz A, Prieto MT, Campo JL. Sperm variables as predictors of fertility in Black castellana roosters; use in the selection of sperm donors for genome resource banking purposes. *Span J Agric Res* 2009;7:555-62.
- [38] Mochida K, Tres LL, Kierszenbaum AL. Structural and biochemical features of fractionated spermatid manchettes and sperm axonemes of the *Azh/Azh* mutant mouse. *Mol Reprod Dev* 1999;52:434-44.
- [39] Chemes HE, Rawe VY. 2010 The making of abnormal spermatozoa: cellular and molecular mechanisms underlying pathological spermiogenesis. *Cell Tissue Res* 2010;341:349-57.

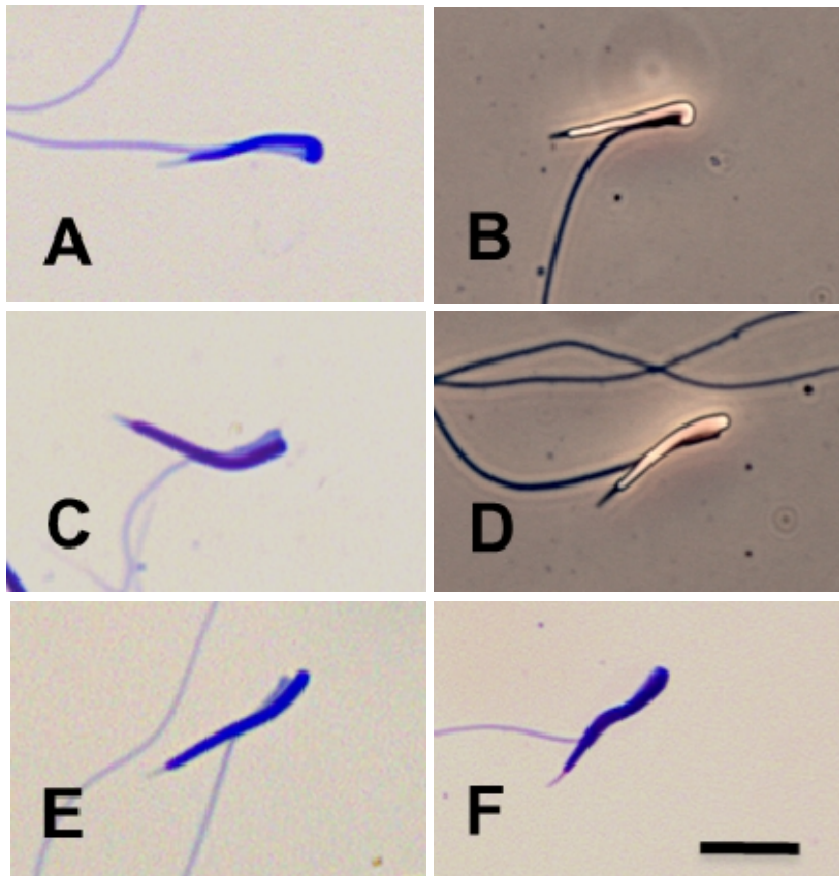


Fig. 1. Light microscopy of Wright's-stained smears. Head-base bending illustrated by bright field microscopy (A) and phase contrast microscopy (B). Note the rounded profile of the bend, the presence of nuclear material on both sides of the bend and the lack of involvement of the neck or midpiece. Disjunct heads revealed by bright field microscopy (C) and phase contrast microscopy (D). Note the straight profile of the parallel-aligned neck and nuclear base in (C) and the staggered appearance in (D). (E) A disjunct sperm showing a staggered orientation of the head and neck/midpiece, creating the erroneous impression of complete separation of the head and tail. (F) A presumptive disjunct sperm. As the nucleus lies on top of the midpiece obscuring their relationship, it is difficult to accurately classify the defect. (E) and (F) bright field microscopy. Bar = 10 $\mu$ m.

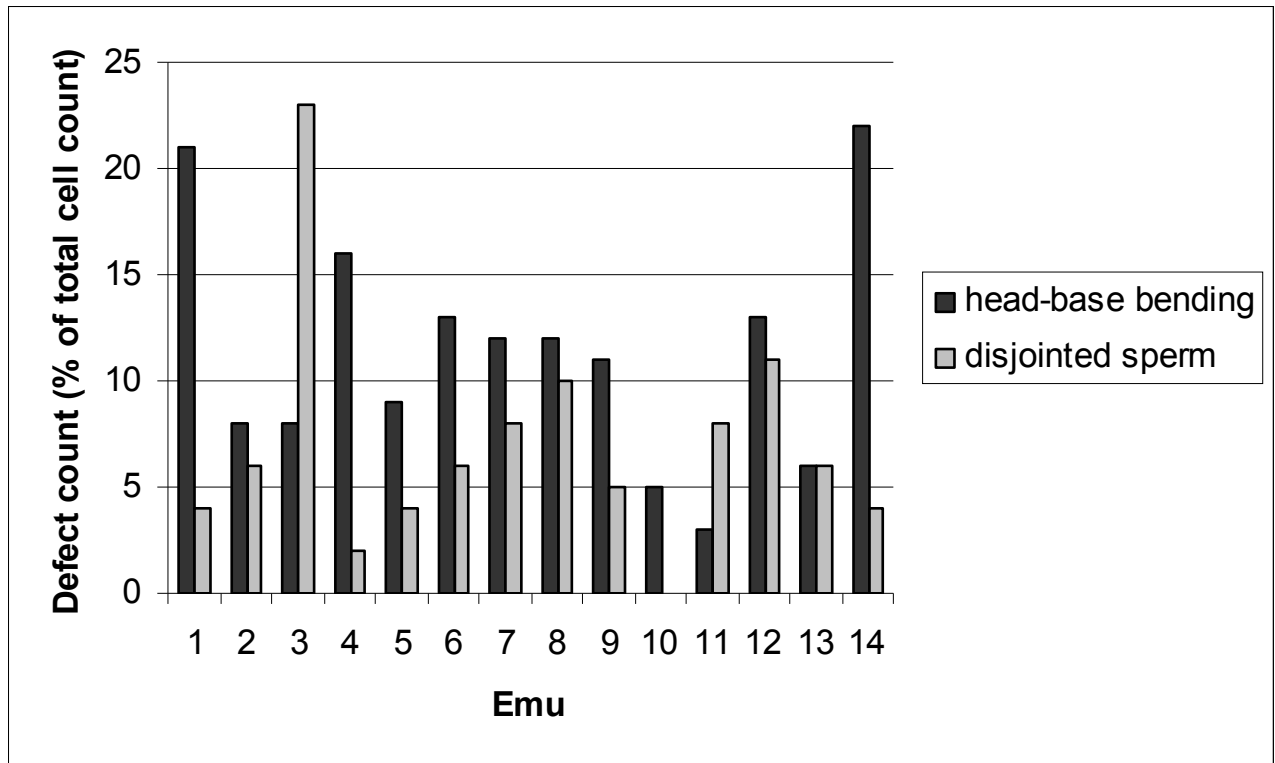


Fig. 2. Histogram showing the incidence of head-base bending and disjointed sperm in each of the 14 birds studied. Each bar represents the number of defective cells observed in a total of 300 cells counted. Cell counts were obtained from smears stained with Wright's stain.

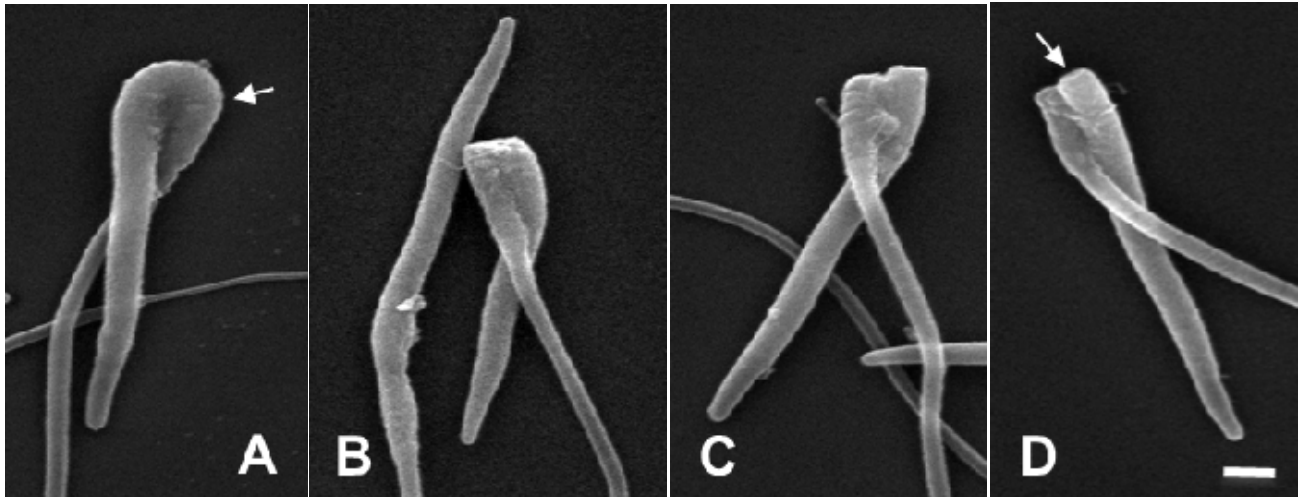


Fig. 3. Scanning electron micrographs of defective sperm showing head-base bending (A) and disjointed heads (B-D). In (A) the position of the nuclear/midpiece junction is indicated by an arrow. The midpiece and head appear to be attached by a membrane. Note the hollow (arrow) indicating the implantation fossa in (D). The disjointed sperm illustrated in Figs. 2B, D and in Fig. 2C are similar to those shown in Fig. 1C and Fig. 1E respectively. Bar = 1 $\mu$ m.

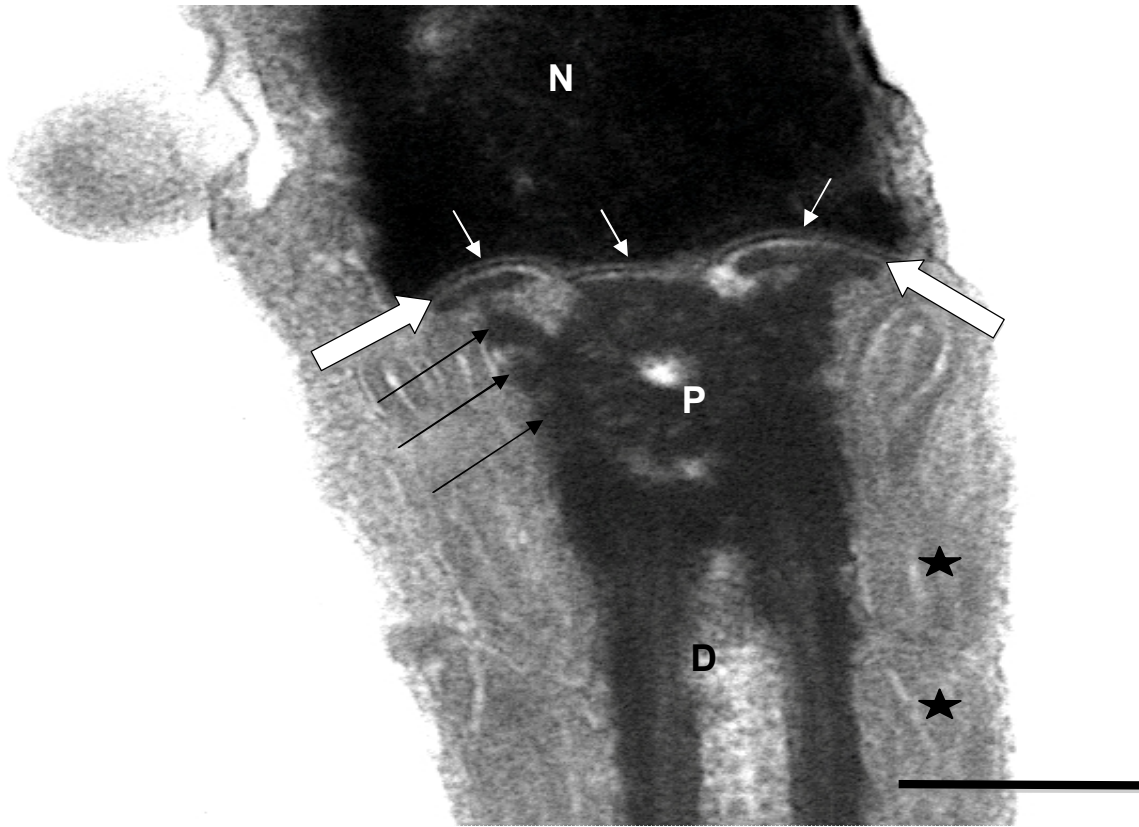


Fig. 4. Neck region of a normal emu sperm. Nucleus (N), basal plate (white arrows), capitellum (block arrows), segmented columns of connecting piece (black arrows), proximal centriole (P), distal centriole (D), mitochondria (stars). Bar = 0.25 $\mu$ m.

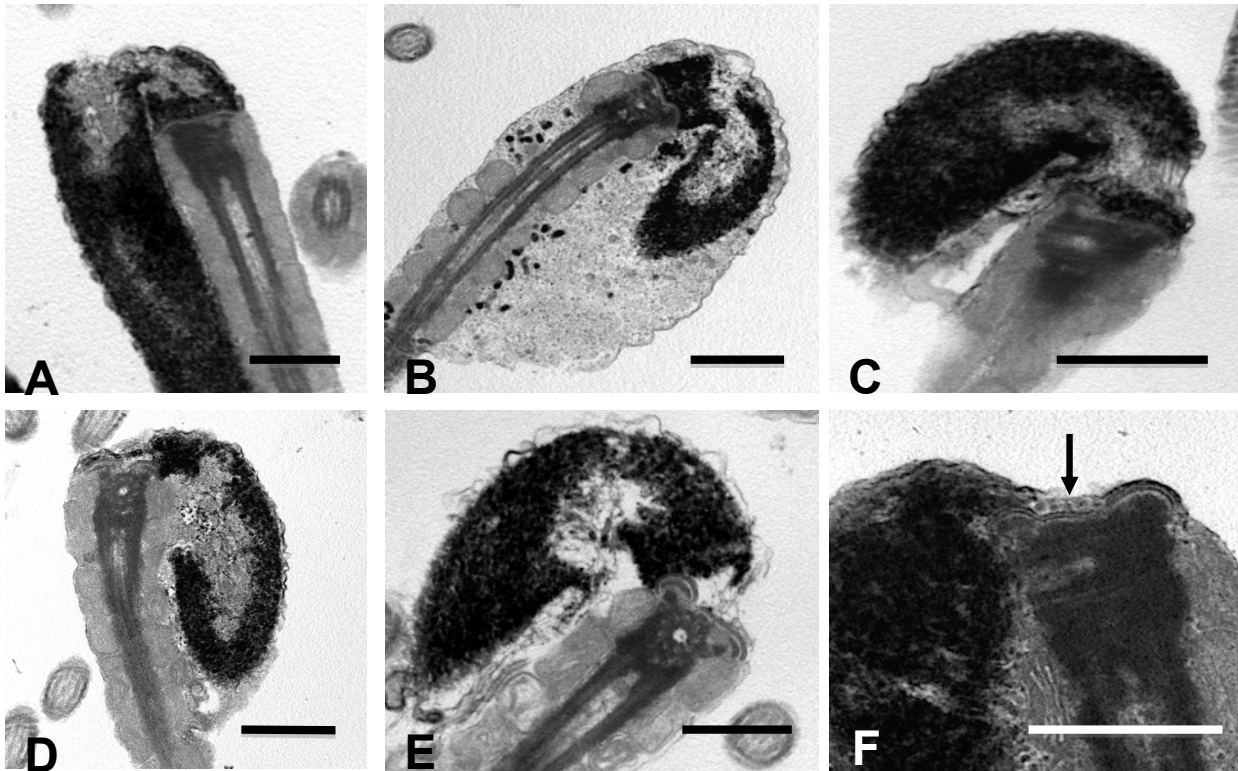


Fig. 5. Transmission electron micrographs showing various forms of nuclear base bending. (A-E) Regions of incompletely condensed chromatin are present in the vicinity of the bend. (F) One leg of the nuclear bend (arrow) is represented only by the nucleolemma enclosing a few chromatin granules. Bars = 0.5 $\mu$ m



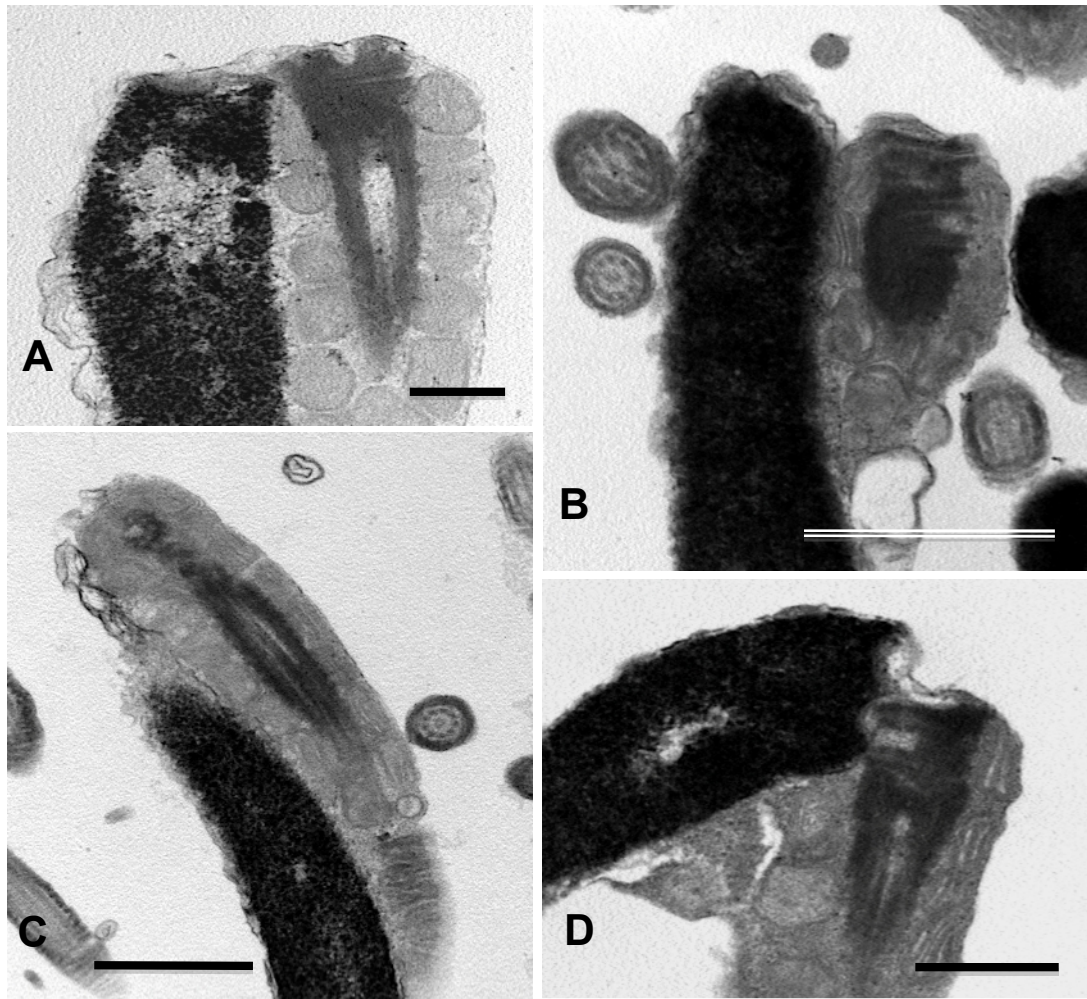


Fig. 6. Disjunct sperm. (A,B) Typical forms of the defect. Note complete separation of the connecting piece from the nuclear base. (C) Staggered form. (D) Intermediate form of the defect. Bar = 0.5 $\mu$ m.

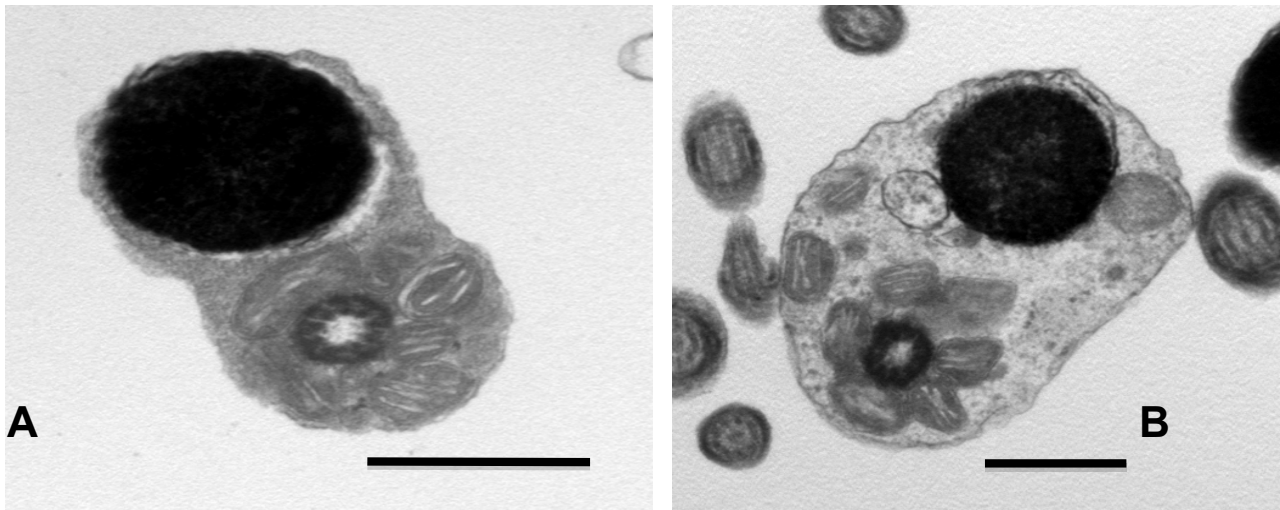


Fig. 7. (A, B) Transverse sections of head-base bending or disjointed sperm. Note the parallel arrangement of the nucleus and midpiece. The plasmalemma collectively surrounds both structures. Bar = 0.5 $\mu$ m.

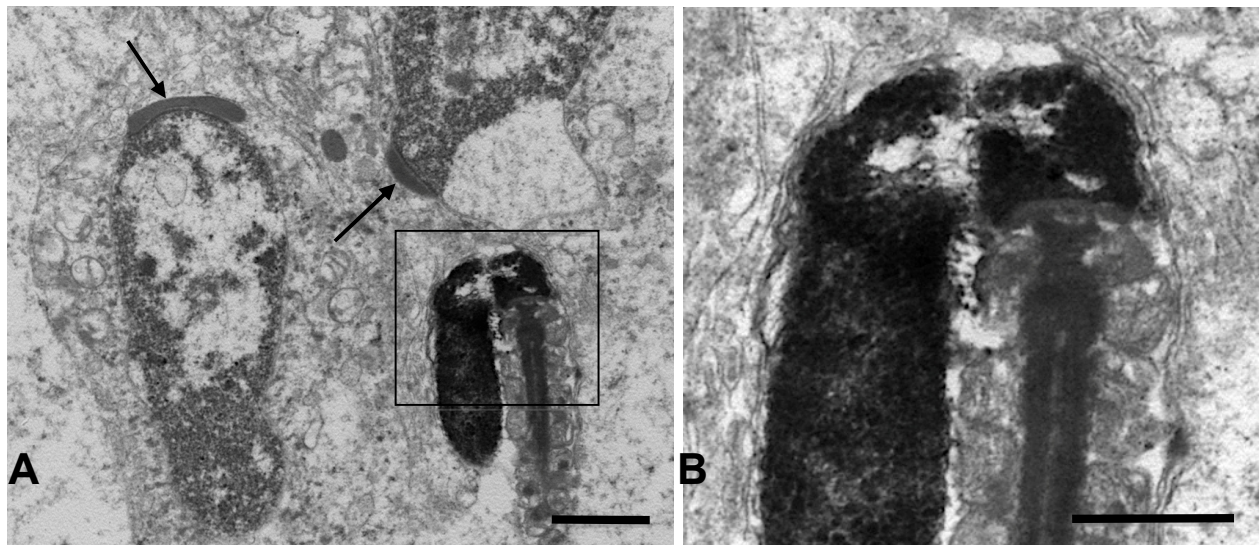


Fig. 8. (A) Section of the testis parenchyma revealing a cell with head-base bending positioned near two early elongating spermatids (arrows). Bar = 1 $\mu$ m. The area within the square is enlarged in (B). Note the similarity to the cells illustrated in Fig. 5. Bar = 0.5 $\mu$ m.

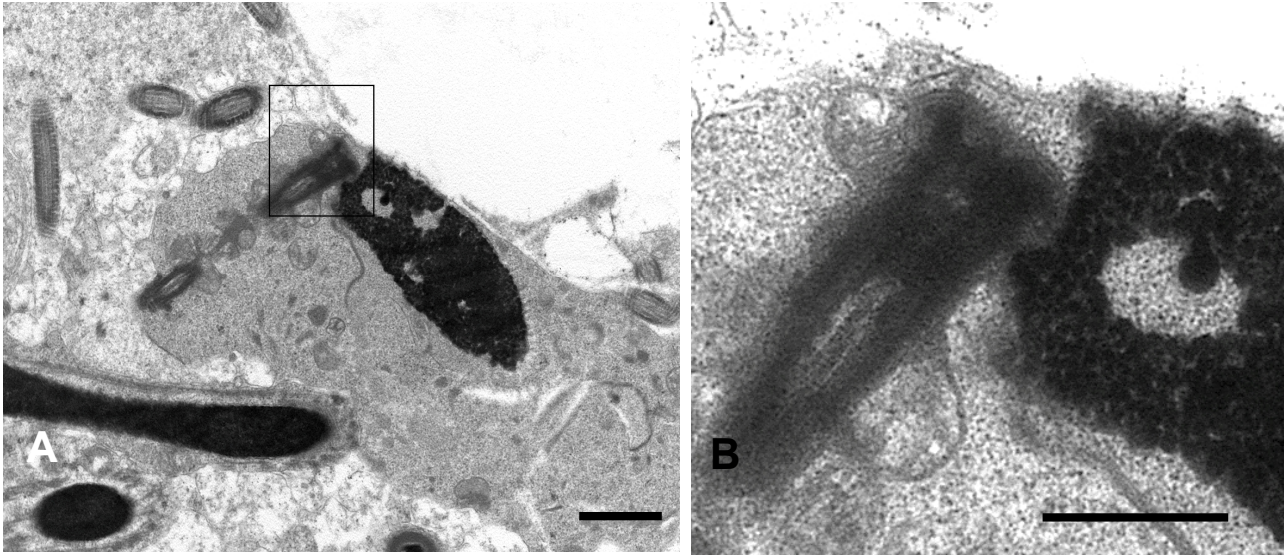


Fig. 9. (A) An intermediate form of a disjointed sperm in the testis. Bar =  $1\mu\text{m}$ . The area within the square is enlarged in (B) and shows misalignment of the head and flagellum. Bar =  $0.5\mu\text{m}$ .