CHAPTER 8

Avian Spermatozoa: Structure and Phylogeny

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8.1 INTRODUCTION

It was intended that this chapter would be confined to a review of ultrastructural works on bird spermatozoa and phylogenetic implications but it soon became apparent that the work, nearly a century old, of Gustaf Retzius (1909, 1911, 1912) and to a lesser extent the earlier publications of Emil Ballowitz (1886, 1888, 1913) still comprised a large proportion of our knowledge of avian sperm morphology. I have therefore included their light microscopical observations, and pertinent drawings of Retzius, with the later ultrastructural works in this chapter. Retzius' drawings are comparable with those of Ernst Haekel (1862) for the extraordinary visual acuity, the excellence of the optical systems, and the dedication of these authors, on which their production depended. The light microscopical investigations of McFarlane (1963) are also of great value. The illustrations by Ballowitz are drawn to a smaller scale than those of Retzius and, though providing significant information, are not reproduced in the present work. Avian species examined for sperm morphology by Ballowitz, Retzius and McFarlane are tablulated in Tables 8.1, 8.2 and 8.3 respectively.

This chapter is largely restricted to sperm morphology and ultrastructure and a phylogenetic analysis of these. For a consideration of spermatogenesis see Aire, Chapter **7** of this volume. For sperm biology see such works as Birkhead and Møller (1992), Briskie *et al*. (1997), Froman *et al*. (2002), and references therein, and, in this volume, Briskie and Montgomerie, Chapter **9**, who give an extensive bibliography, including the important works of Birkhead and colleagues; and Stepinska and Bakst, Chapter **10**.

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To place the structure of the avian spermatozoon in an evolutionary perspective it is useful to briefly consider the general characteristics of amniote spermatozoa and particularly of crocodile sperm as crocodiles are widely held to be the extant sister group of birds.

8.2 AMNIOTE SPERMATOZOA, BASIC FEATURES

From detailed comparative and cladistic considerations, the following characteristics of a hypothetical plesiomorphic amniote spermatozoon (Fig. 8.1) may be recognized. This model is virtually identical with that of the lowest extant amniotes, the Chelonia, Crocodylia and Sphenodontida. Amniote sperm are seen to have few basal synapomorphies relative to the tetrapod ground plan which is deduced from common features of the amniote and lissamphibian sperm (Jamieson 1995, 1999, and references therein) and reference to sperm of sarcopgterygian fish (Jamieson 1991).

8.2.1 Amniote Sperm Plesiomorphies

Plesiomorphic features of the generalized amniote spermatozoon, retained from their tetrapod ancestry (see also sarcopterygian fish in Jamieson 1991), and still seen in Chelonia, Sphenodontida, Crocodylia and to varying degrees in other amniotes, are as follows. The spermatozoon (Fig. 8.1) is elongate and filiform, with an anterior hollow conical acrosome vesicle overlying a simple subacrosomal cone. The base of the acrosome invests the tapered anterior tip (rostrum) of the nucleus and rests on pronounced nuclear 'shoulders'. The subacrosomal space within the acrosome contains two or three axial rods (putative perforatoria) or, less likely, only one rod. These penetrate the nucleus deeply, almost to its base, in endonuclear canals. The nucleus is plesiomorphically elongate and cylindrical in amniotes from Chelonia through *Sphenodon*, crocodiles, squamates, birds, monotremes and, in therian mammals, the pangolin alone (Leung and Cummins 1988), as in lissamphibians (Scheltinga and Jamieson 2003a, b; Scheltinga *et al.* 2003). At the base of the nucleus there is a compact fossa (implantation fossa) with which are associated two triplet centrioles of which the distal forms the basal body of the flagellar axoneme. Whether the presence of an annulus in amniotes is plesiomorphic or an apomorphic reversal is debatable. The terminal portion of the 9+2 axoneme forms a short endpiece distinguished from the principal piece by the absence of the fibrous sheath.

8.2.2 Amniote Spermatozoal Synapomorphies

The Chelonia and Sphenodontida are considered the most basal extant amniotes and have virtually identical spermatozoa (Healy and Jamieson 1992; Jamieson 1995; Jamieson 1999). The characteristics of these include features considered synapomorphies of the Amniota which are simultaneously symplesiomorphies for Chelonia and Sphenodontida and for the remaining amniotes, including birds. The amniote synapomorphies include:

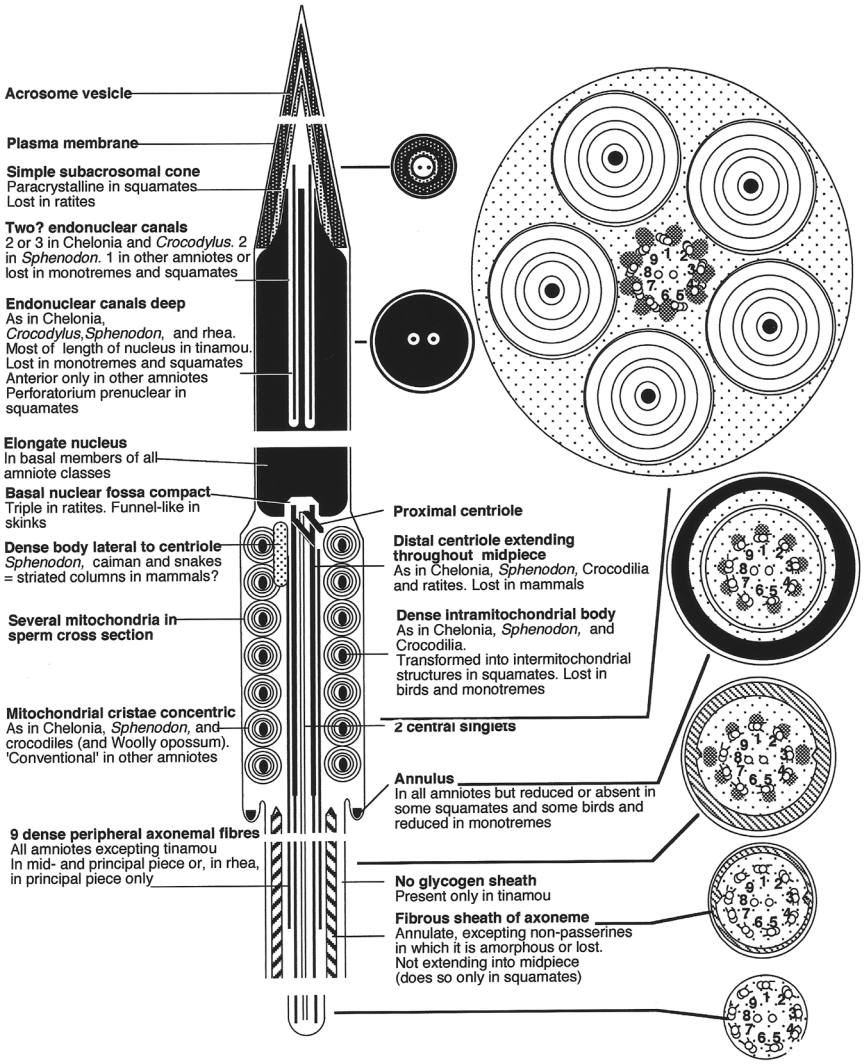

Fig. 8.1 Hypothetical plesiomorphic amniote sperm. From Jamieson, B. G. M. 1999. Pp. 303-331. In C. Gagnon (ed). *Spermatozoal Phylogeny of the Vertebrata. The Male Gamete. From Basic Science to Clinical Applications*, Cache River Press, Vienna, USA, Fig. 10.

Elongation of the distal centriole. The distal centriole is extremely elongate and extends the entire length of the long midpiece (the latter defined by its mitochondria) in turtles, the tuatara, crocodiles (Fig. 8.2), and paleognaths (Figs. 8.4-8.9), an apparent basal synapomorphy of amniotes. These elongate centrioles differ from most metazoan basal bodies in being penetrated by two central singlets from the axoneme. Thus in spermatids of the ratite *Rhea*, the distal centriole elongates and, late in spermiogenesis, becomes penetrated by

a central pair of tubules from the developing axoneme (Phillips and Asa 1989). The shorter, though still elongate distal centriole in the rooster and the somewhat shorter centriole in Guineafowl (0.6 μm) and *Geopelia striata* (0.5 μm) (Jamieson 1995), the short centriole in squamates, and the vestigial centriole in monotremes possibly represent secondary reduction in length of the distal centriole (Healy and Jamieson 1992), culminating in almost total reduction in therian mammals (Jamieson 1999).

Mitochondria, with concentric cristae. In turtles, tuatara (Healy and Jamieson 1992; 1994; Jamieson and Healy 1992; Jamieson 1995, 1999), *Caiman crocodylus* and *Crocodylus johnstoni* (Jamieson 1995; Jamieson *et al.* 1997; Jamieson 1999) (Fig. 8.2), the mitochondria have concentric cristae, known elsewhere in amniotes only in the sperm of some marsupials, notably the Woolly opossum, *Caluromys philander* (see Fawcett 1970; Phillips 1970) and the Virginia opossum, *Didelphis virginiana* (Temple-Smith and Bedford 1980) and also in the macropod *Lagorchestes hirsutus* (Jamieson 1999; Johnston *et al*. 2004).

The mitochondrial cristae in the three 'reptilian' taxa (Chelonia, Sphenodontida, Crocodylia) usually surround a large central dense body. In all other amniotes studied, the cristae have a "conventional" appearance, being linear or curved, as in Lissamphibia, but never concentric, and do not surround a dense body. In spermatids of *Sphenodon* (Healy and Jamieson 1992; Jamieson and Healy 1992), the cristae have the linear appearance usual for metazoan sperm and the concentric arrangement is a late development. Phylogenetic "reversion" of concentric cristae to the linear condition seen in other amniotes would need only suppression of this final transformation (Jamieson and Healy 1992). Concentric cristae also occur in the spermatozoa of Gymnophiona (Scheltinga *et al.* 2003) and Urodela (Scheltinga and Jamieson 2003a). Although noting that multiple, homoplastic origin of concentric cristae would not be dismissed with certainty, Scheltinga *et al*. (2003) proposed that concentric cristae are an autapomorphy of tetrapods and not of amniotes as had previously been suggested by Jamieson (1999). They would therefore be symplesiomorphic for amniotes. The concentric arrangement appears to have been lost in all birds.

The annulus. A dense ring, the annulus, at the posterior end of the midpiece is a feature of many metazoan sperm. It is clearly plesiomorphic for amniotes, occurring in all classes (Jamieson and Healy 1992), including paleognaths and several non-passerine orders, but absence in Dipnoi possibly indicates apomorphic re-acquisition in tetrapods. Irrespective of such reversal it is clearly a symplesiomorphy for Aves.

Fibrous sheath. A dense fibrous sheath (Fig. 8.1) must, clearly, have developed, as an annulated structure, in the earliest amniotes as it is present in all amniote classes. With the exception of squamates, in which it penetrates the midpiece, it commences immediately behind the midpiece, as in turtles, *Sphenodon* (Healy and Jamieson 1992; Jamieson and Healy 1992) and crocodiles (Jamieson 1995, 1999; Jamieson *et al.* 1997); in ratites (Figs. 8.4 - 8.8),

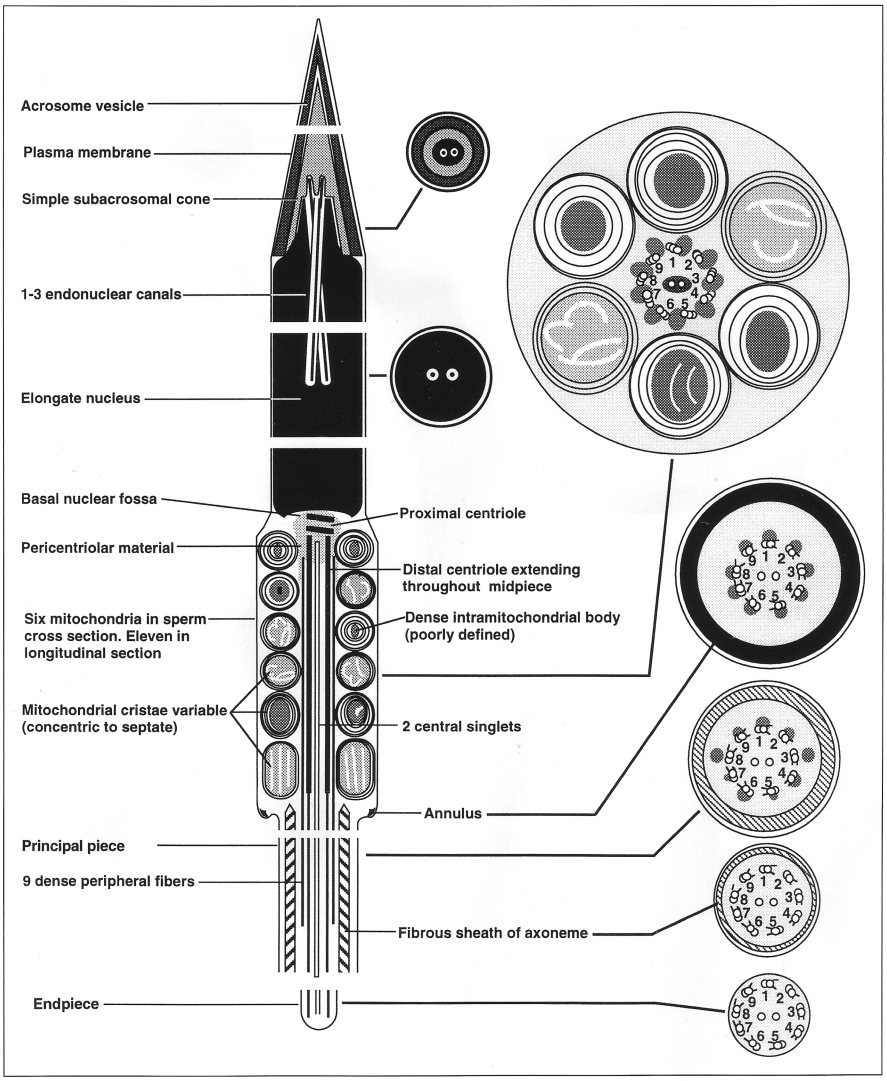

Fig. 8.2 Diagrammatic longitudinal section of the spermatozoon of *Crocodylus johnstoni*. The spermatozoon of the Palaeognathae is closely similar to the crocodile spermatozoon. From Jamieson, B. G. M., Scheltinga, D. M. and Tucker, A. D. 1997. Journal of Submicroscopic Cytology and Pathology 29: 265-274, Fig. 1.

galliforms (Fig. 8.11, 8.14, 8.18), anseriforms (Fig. 8.19, 8.20), gruiforms (reduced, Fig. 8.32), charadriiforms (Fig. 8.34) and in mammals (Jamieson 1995, 1999).

Nine peripheral axonemal fibers. Nine longitudinal dense fibers (coarse fibers) peripheral to the nine axonemal doublets, or to the distal centriole also where this is elongated as in chelonians, *Sphenodon*, crocodiles and paleognath birds,

are a fundamental feature of amniote sperm (Fig. 8.1), being found in all classes (Healy and Jamieson 1992; Jamieson and Scheltinga 1993; Jamieson 1995, 1999). As nine peripheral fibers are seen in lampreys and the fish *Pantodon* (references in Jamieson 1991) but also in heterobranch and cephalopod molluscs (references in Jamieson 1999) it might be considered that nine is the basic sarcopterygian, rather than merely amniote, number and that amphibians have lost all but those represented by the fibers at doublets 3 and 8. However, there is no evidence in extant Lissamphibia for such a reduction and the presence of only two lateral elements in dipnoans and *Latimeria* suggests that nine fibers were an amniote synapomorphy, albeit homoplastic with the other, non-amniote taxa.

Fibers at 3 and 8. It is possible that a further basal amniote apomorphy is enlargement and lateral displacement of two fibers, at doublets 3 and 8, and that all fibers in the centriolar region intruded into the inter-triplet radii, as in 'lower' amniotes (Chelonians, *Sphenodon* and crocodiles) (Jamieson 1995, 1999). This displacement is not retained in birds.

Longitudinal columns. In the principal piece, two longitudinal keel-like outward projections or thickenings (longitudinal columns) of the fibrous sheath opposite doublets 3 and 8, may be present, with or without inward projections to these doublets, as shown for mammalian spermatozoa (Fawcett 1975; Jamieson 1999) and Ostrich sperm (Baccetti *et al.* 1991; Soley 1993).

Retronuclear body transformation. The striated columns of mammalian sperm are possibly derivatives of the tetrapod retronuclear body, present in dipnoans and (as the neck structure) in urodeles (Jamieson 1999). No definite conclusion can be made as to whether structures in bird sperm are homologues, viz. the non-segmented columns in Ostrich sperm and the three projections (stated to be probably equivalent to striated columns) in Crested pigeon (*Ocyphaps lophotes*) (Fig. 8.24C).

8.3 SPERMATOZOA OF CROCODYLIA

As the Crocodylia are traditionally considered to be the extant sister group of the birds a consideration of their sperm ultrastructure provides insights into the evolution of bird spermatozoa (we presumably will never know the sperm of theropods). The ground plan for the Crocodylia, as exemplified by *Crocodylus johnstoni* (Jamieson 1995, 1999; Jamieson *et al.* 1997) (Fig. 8.1), is very similar to that of the Chelonia and *Sphenodon*. All three have two or three endonuclear canals and, though requiring further confirmation for crocodiles, concentric cristae with intramitochondrial bodies. In *Crocodylus johnstoni* the mitochondria are subspheroidal to slightly elongate and possess few septate to (more externally) concentric cristae; a central dense mitochondrial body reported for *Caiman crocodylus* (Saita *et al.* 1987) is questionably present.

Caiman crocodylus, resembles ratites in having only one perforatorium but this may be a reversion from multiple perforatoria homoplastic with the single condition in ratites.

Synapomorphic conditions in the Crocodylia have little relevance to consideration of avian evolution. However, it may be noted that one condition, a dense sheath investing the two central singlets within the elongate distal centriole, previously considered an apomorphy of crocodile sperm (Jamieson 1999) is also seen in Ostrich sperm. It could therefore have been an apomorphic state of a common (theropod?) + crocodile + avian stock.

Restriction of the endonuclear canal in caiman sperm to the anterior region of the nucleus indicated by Saita *et al.* (1987) appears to be apomorphic relative to the longer condition in *Crocodylus johnstoni* but requires confirmation. A similar trend to shortening in ratites and, progressively, in non-passerines presumably occurred in parallel.

8.4 SPERMATOZOA OF AVES—INTRODUCTION

As mentioned in the introduction, the light microscopical works of Ballowitz (1886, 1888, 1913) and of Retzius (1909, 1911, 1912) retain their significance in current avian spermatology. Species which they investigated are tabulated here (Tables 8.1, 8.2).

Table 8.1 Bird species examined by Ballowitz for spermatozoal morphology by light microscopy

Date	*Figure*	*Species*	*Valid name if different*	*Common name*
		Galliformes		
1888	135-147	Haushahns	*Gallus domesticus*	Rooster
1888	128-134	Truthahns	*Meleagris gallopavo*	Turkey
		Anseriformes		
1888	121-127	*Tadorna vulpanser*	*Tadorna tadorna*	Sheld-drake
		Piciformes		Great spotted
1888	98-109	*Picus major*	*Dendrocopos major*	woodpecker
		Cuculiformes		
1888	110	*Cuculus canorus*		Cuckoo
		Charadriiformes		
1888	111-113	*Larus ridibundus*		Black-headed gull
1888	115	*Larus canus*		Common gull
1888	116-120	*Vanellus cristatus*	*Vanellus vanellus*	Lapwing
		Falconiformes		
1888	114	*Milvus ater*		Black Kite
		Caprimulgiformes		
1888	85-90	*Caprimulgus europaeus*		Nightjar
		Columbiformes		
1888	91-97	*Haustaube*	*Columba livia*	Domestic pigeon
		Passeriformes		
		Corvida		
1888	84	*Corvus frugilegus*		Rook
1888	63-75	*Oriolus galbula*	*Oriolus oriolus*	Golden oriole
1888	76-83	*Lanius collurio*		Red-backed shrike

Table 8.1 Contd. ...

Table 8.1 Contd. ...

Date	*Figure*	*Species*	*Valid name if different*	*Common name*
		Passeriformes		
		Passerida		
1888	48-49	*Passer domesticus*		House sparrow
1888	4, 11-15, 25, 38-39	*Muscicapa grisola*	*Muscicapa striata*	Spotted flycatcher
1888	19, 26-28	*Hirundo rustica*		Swallow
1888	5	*Chelidon urbica*		House martin
1888	8, 20-22	*Sylvia nisoria*		Barred warbler
1888	36	*Sylvia atricapilla*		Blackcap
1888	37	*Sylvia cinerea*		Whitethroat
1888	51-52	*Sylvia hortensis*		Garden warbler
1888	16-17	*Rubicilla phoenicura*	*Carpodacus rubicilla*?	Great Rosefinch
1888	18, 50	*Motacilla flava*		Yellow wagtail
1888	6-7	*Phyllopneuste hypolais*	*Hippolais icterina*?	Icterine Warbler
1888	29-34, 40-44	*Phyllopneuste sibilatrix*	*Phylloscopus sibilatrix*	Wood Warbler
1888	35	*Sitta europaea*		Nuthatch
1888	24	*Fringilla canabina*	*Carduelis canabina*	Linnet
1888	1-3, 23, 54-62	*Fringilla caelebs*	*Fringilla coelebs*	Chaffinch
1888	9-10	*Ligurinus chloris*	*Cardeulis chloris*	Greenfinch
1888	45-47, 53	*Emberiza citrinella*		Yellowhammer
1913		*Uria lomvia*		Thick-billed murre (Brünnich's guillemot)

Table 8.2 Bird species examined by Retzius for spermatozoal morphology by light microscopy. (Modified from Afzelius 1995)

Volume (date)	*Species*	*Valid name if different*	*Common name*
	Struthioniformes		
16 (1911)	*Struthio molybdophanes*	*Struthio camelus*	Somalian ostrich
14 (1909)	**Galliformes**		
	Gallus gallus		Domestic rooster
14	**Anseriformes**		
	Anas boschas domestica	*Anas platyrhynchos*	Domestic duck
14	*Fuligula fuligula*	*Aythya fuligula*	Tufted duck
14	**Gruiformes**		
	Fulica atra		Common coot
14	*Rallidae*		
	Crex crex		Corncrake
14	**Charadriiformes**		
	Uria Troile	*Uria aalge*	Common guillemot
14	*Larus fuscus*		Lesser black-backed gull

Table 8.2 Contd. ...

Table 8.2 Contd. ...

Volume (date)	*Species*	*Valid name if different*	*Common name*
14	*Vanellus vanellus*		Lapwing
14	*Tringa alpina*	*Calidris alpina*	Alpine dunlin
14	*Totanus ochropus*	*Tringa ochropus*	Green sandpiper
14	*Scolopax rusticola*		Woodcock
14	*Pavoncella pugnax*	*Philomachus pugnax*	Ruff
14, 16	**Columbiformes**		
	Columba livia domestica		Domestic pigeon
14	**Psittaciformes**		
	Psittacus sp.		A parrot species
14	**Strigiformes**		
	Syrnium aluco	*Strix aluco*	Tawny owl
16	**Apodiformes**		
	Cypselus apus		European swift
14	**Piciformes**		
	Dendrocopos major		Great spotted woodpecker
14	**Passeriformes**		
	Corvida		
	Corvus cornix	*Corvus corone*	Hooded crow
17 (1912)	*Corvus frugilegus*		Rook
17	*Perisoreus infaustus*		Siberian jay
16	*Coloeus monedula*	*Corvus monedula*	Jackdaw
14	*Pica pica*		Magpie
16	*Lanius collurio*		Red-backed shrike
14	**Passerida**		
	Sturnus vulgaris		Starling
14	*Turdus musicus*	*Turdus philomelos*	Song thrush
14	*Aedon luscinia*	*Luscinia luscinia*	Thrush nightingale
14	*Muscicapa atricapilla*	*Ficedula hypoleuca*	Pied flycatcher
14	*Phylloscopus sibilator*	*Phylloscopus sibilatrix*	Wood warbler
14	*Alauda arvensis*		Skylark
14	*Anthus obscurus*	*Anthus spinoletta*	Rock pipit
14	*Fringilla coelebs*		Chaffinch
14	*Chrysomitris spinus*	*Carduelis spinus*	Siskin
14	*Chioris chioris*	*Carduelis chloris*	Greenfinch
14	*Passer domesticus*		House sparrow
14	*Emberiza citrinella*		Yellowhammer

Some of the species of birds for which sperm are described in this chapter are illustrated in Fig. 8.3.

8.5 NEORNITHES

According to Gauthier and de Queiroz (2001) the name "Aves" refers to the crown clade stemming from the most recent common of Ratitae (*Struthio camelus* Linnaeus 1758), Tinamidae (*Tetrao [Tinamus] major* Gmelin 1789) and

Table 8.3 Orders and families with number of genera and species examined by McFarlane (1963)

Non-passerines	*Passeriformes*
Procellarilformes	**Suborder Tyranni**
Hydrobatidae (1 gen., 1 sp.)	**(Suboscines)**
Ciconiiformes	Dendrocolaptidae (2 gen., 3 spp.)
Ardeidae (2 gen., 2 spp.)	Furnariidae (1 gen., 1 sp.)
Anseriformes	Formicariidae (2 gen., 2 spp.)
Anatidae (1 gen., 1 sp.)	Cotingidae (4 gen., 4 spp.)
Falconiformes	Pipridae (2 gen., 3 spp.)
Accipitridae (1 gen., 1 sp.)	Tyrannidae (12 gen., 14 spp.)
Galliformes	**Suborder Passeres**
Tetraonidae (1 gen., 1 sp)	**Corvida**
Phasianidae (1 gen., 1 sp.)	Corvidae (2 gen., 2 spp.)
Gruiformes	Laniidae (1 gen., 1 sp.)
Rallidae (1 gen., 1 sp.)	Vireonidae (2 gen., 4 spp.)
Charadriiformes	**Passerida (Oscines)**
Charadriidae (1 gen., 1 sp.)	Alaudidae (1 gen., 1 sp.)
Scolopacidae (2 gen., 2 spp.)	Hirundinidae (4 gen., 4 spp.)
Recurvirostridae (1 gen., 1 sp.)	Paridae (1 gen., 4 spp.)
	Sittidae (1 gen., 2 spp.)
Laridae (3 gen., 7 spp.)	Troglodytidae (2 gen., 2 spp.)
Rynchopidae (1 gen., 1 sp.)	Mimidae (3 gen., 3 spp.)
Alcidae (1 gen., 1 sp.)	Muscicapidae (=Turdidae) (3
Columbiformes	gen., 7 spp.)
Columbidae (5 gen., 6 spp.)	Sylviidae (2 gen., 2 spp.)
Cuculiformes	Bombycillidae (1 gen., 1 sp.)
Cuculidae (1 gen., 1 sp.)	Sturnidae (1 gen., 1 sp.)
Strigiformes	Coerebidae (1 gen., 1 sp.)
Strigidae (1 gen., 1 sp.)	Parulidae (13 gen., 27 spp.)
Caprimulgiformes	Ploceidae (1 gen., 1 sp.)
Caprimulgidae (1 gen., 1 sp.)	Icteridae (8 gen., 8 spp.)
Apodiformes	Thraupidae (7 gen., 8 spp.)
Apodidae (1 gen, 1 sp.)	Fringillidae (17 gen., 23 spp.)
Trochilidae (5 gen., 5 spp.)	
Trogoniformes	
Trogonidae (1 gen., 1 sp.)	
Piciformes	
Picidae (1 gen., 1 sp.)	
Ramphastidae (1 gen., 1 sp.)	

Neognathae (*Vultur gryphus* Linnaeus 1758). It seems preferable, however, to use the term Neornithes for this assemblage.

8.6 PALAEOGNATHAE

This is the Parvclass Ratitae of Sibley and Ahlquist (1990). According to Gauthier and de Queiroz (2001), *"Palaeognathae"* refers to the crown clade

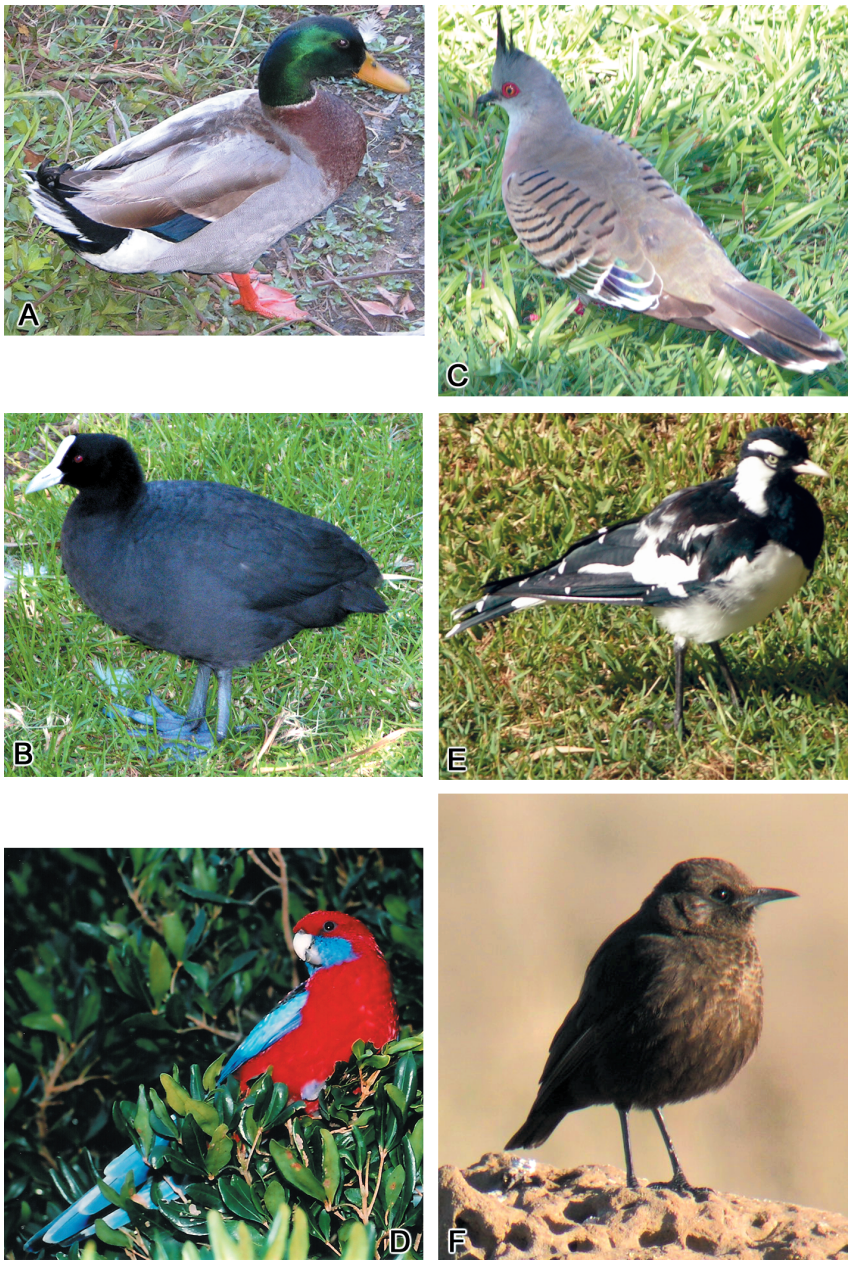

Fig. 8.3 Some bird species examined for spermatozoal structure. **A**. Mallard (*Anas platyrhynchos*) Anseriformes. **B**. Coot (*Fulica atra*) Gruiformes. **C**. Crested pigeon (*Ocyphaps lophotes*) Columbiformes. **D**. Crimson Rosella (*Platycercus elegans*) Psittaciformes. **E**. Magpie lark (*Grallina cyanoleuca*) Corvida, Passeriformes. **F**. Southern ant-eater chat (*Myrmecocichla formicivora*) Passerida, Passeriformes. Photos © A, B, C, E, Barrie Jamieson. D, Christopher Tudge. F, Eric Hermann.

stemming from the most recent common ancestor of Tinamidae (*Tetrao* [*Tinamus*] *major* Gmelin 1789) and Ratitae (*Struthio camelus* Linnaeus 1758).

8.6.1 Order Struthioniformes

The Order Struthioniformes contains the families Apterygidae, Casuariidae, Rheidae and Struthionidae. The Struthionidae is monotypic for the genus *Struthio*; Casuariidae contains the genera *Casuarius* and *Dromaius*; Rheidae contains the genera *Pteroicnemia* and *Rhea*.

8.6.1.1 Taxa examined

The following Struthioniformes have been examined for spermatozoal ultrastructure (see also Table 8.4): Struthionidae, *Struthio camelus*, Ostrich (Soley 1989; Baccetti *et al.* 1991; Soley 1993, 1994; Soley and Roberts 1994; Soley 1994b, 1996; 1999); Rheidae, *Rhea americana albisceus*, Rhea (Asa *et al.* 1986; Phillips and Asa 1989); Casuariidae, *Dromaius novaehollandiae*, Emu (Baccetti *et al.* 1991).

8.6.1.2 Overview of struthioniform spermatozoa

This diagnostic resumé is drawn chiefly from the accounts for Ostrich (*Struthio camelus*) sperm (Baccetti *et al.* 1991; Soley 1993, 1999; Soley and Roberts 1994). Lesser detail has been obtained from the accounts for Rhea (*Rhea americana albisceus*) (Phillips and Asa 1989) and Emu (*Dromaius novaehollandiae*) (Baccetti *et al.* 1991).

The nucleus is an elongate cylinder and extends as a tapering nuclear rostrum far apically into the subacrosomal space (amniote symplesiomorphies). The acrosome vesicle is conical, shorter than the nucleus (amniote symplesiomorphy), about one tenth (*Dromaius*) to one fifth (*Struthio*) its length. The midpiece is shorter than the head (amniote symplesiomorphy), only about a quarter to a third of its length. An annulus is present at the junction of midpiece and principal piece (amniote synapomorphy by reversal?). The principal piece, several times the length of the nucleus, is defined by the presence of an annulated fibrous sheath (amniote synapomorphy) ensheathing the axoneme. There is a short endpiece consisting of axoneme (and plasma membrane) only. A subacrosomal space contains homogeneous material but no subacrosomal cone is present (synapomorphy of Aves). The nucleus is penetrated axially by an endonuclear canal (amniote symplesiomorphy) which contains the putative perforatorium. The perforatorium does not extend apically beyond the tip of the nucleus (Struthioniform + Timamiform synapomorphy), except, at most, for a little filamentous material. The endonuclear canal and enclosed perforatorium are absent in *Dromaius* (autapomorphy of this genus). There is a small basal nuclear fossa behind which is the proximal centriole orientated at right angles to the long axis of the spermatozoon (amniote symplesiomorphy). This is followed by the distal centriole (basal body) in the long axis and continuous with the axoneme. The distal centriole, also with nine triplets, is extremely elongate (amniote synapomorphy, reducing in non-paleognaths) and extends through the length

Table 8.4 Ultrastructural studies on bird spermatozoa*

Taxon	*Reference*
Struthioniformes	
Rhea americanus albisceus, Rhea	Asa *et al.* 1986[1] Phillips and Asa 1989[1]
Struthio camelus, Ostrich	Baccetti *et al.* 1991[1 2]; Soley 1989[1], 1993[1]. 1994a,b[1], 1996[1], 1999[1]; Soley and Roberts 1994[2]
Dromaius novaehollandiae, Emu	Baccetti *et al.* 1991[1 2]
Tinamiformes	
Eudromia elegans, Crested tinamou	Asa *et al.* 1986[1 2]; Asa and Phillips 1987[1]
Anseriformes	
Anas platyrhynchos, Mallard	Humphreys 1972[1]; Maretta 1975a, b[1]
Branta sandvicensis, Hawaiian goose	Humphreys 1972[1]
Craciformes	None
Galliformes	
Gallus gallus/domesticus, Rooster	Grigg and Hodge 1949[1 2]; Bonadona 1954[2]; Nagano 1960[1], 1962[1]; McIntosh and Porter 1967[1]; Nicander and Hillstrom 1967[1]; Krustev and Danov 1968[1]; Lake *et al.* 1968[1]; Nicander 1970b[1]; Tingari 1973[1]; Bakst and Howarth 1975[1]; Gunawardana and Scott 1977[1]; Bakst and Sexton 1979[1]; Bakst 1980[1]; Xia *et al.* 1985[1]; Xia *et al.* 1986[1]; Xia *et al.* 1988[1]; Bae and Kim 1987[1]; Woolley and Brammall 1987[1]; Thurston and Hess 1987[1]; Sprando and Russell 1988[1]; Jamieson 1999[1]
Coturnix japonica, Japanese quail	Saita *et al.*1980[1]; Maretta *et al.* 1982[1]; Lin and Jones 1993[1]; Woolley 1995[1]; Tripepi *et al.* 1991[1]; Vernon and Woolley 1999[1]
Coturnix chinensis, Blue-breasted quail	This study[1]
Meleagris gallopavo, Turkey	Marquez and Ogasawara 1975[2]; Bakst and Sexton 1979[1]; Baccetti *et al.* 1980[1]; Bakst 1980[1]; Bradley *et al.* 1986[1]; Thurston and Hess 1987[1 2]
Tragopan caboti, Cabot's tragopan	Wen *et al.* 1997[1]
Numida meleagris, Guineafowl	Hess *et al.* 1986[1]; Thurston *et al.* 1982[1 2]; Thurston and Hess 1987[2]; Aire and Soley 2003[1]
Turniciformes	None
Piciformes	
Melanerpes carolinus, Red-bellied woodpecker	Henley *et al.* 1978[1]
Galbuliformes	None
Bucerotiformes	None
Upupiformes	None
Coraciiformes	None
Coliiformes	None
Cuculiformes	

Table 8.4 Contd. ...

Table 8.4 Contd. ...

Taxon	*Reference*
Crotophaga ani, Cuckoo	Saita *et al.* 1982; Tripepi *et al.* 1991[1]; this study[1]
Psittaciformes	
Melopsittacus undulatus, Budgerigar	Humphreys 1975[1]; Samour *et al.* 1986[1 2]; Jamieson *et al.* 1995[1].
Agapornis roseicollis, Peach-faced lovebird	Jamieson *et al.* 1995[1]
Platycercus elegans, Crimson Rosella	Jamieson 1999[1]
Nymphicus hollandicus, Cockatiel	Jamieson *et al.* 1995[1]
Apodiformes	
Apus (=Cypselus) apus, Common swift	Tripepi et *al.* 1984[1]; this study[1]; Jamieson and Tripepi 2005[1]
Apus melba, Alpine swift	Tripepi et *al.* 1991[1]
Trochiliformes	None
Musophagiformes	None
Caprimulgiformes	
Caprimulgus europaeus, European nightjar	Tripepi *et al.* 1991[1]; this study[1]
Columbiformes	
'Pigeon'	Fawcett *et al.* 1971[1]
Columba livia, Domestic pigeon	Yasuzumi and Yamaguchi 1977[1] (spermiogenesis only); Vernon and Woolley 1999[1]
Streptopelia roseogrisea, Turtle dove	Mattei *et al.* 1972[1]
Geopelia striata, Peaceful dove	Jamieson *et al.* 1995[1]; 1999[1]; this study[1]
Ocyphaps lophotes, Crested pigeon	Jamieson *et al.* 1995[1]; Jamieson 1999[1]; this study[1]
Gruiformes	
Grus vipio, White-necked crane	Asa and Phillips *1987*[1 2]; Phillips *et al.* 1987[1 2]
The following 7 orders were previously placed in the order **Ciconiiformes** *sensu* Sibley and Ahlquist (1990)	
Charadriiformes	
Jacana jacana	Saita *et al.* 1983[1]; Tripepi *et al.* 1991[1]
Falconiformes	
Falco peregrinus, Peregrine falcon	Wagley 1980[2]
Pelecaniformes	None
Procellariformes	None
Podicepediiformes	None
Sphenisciformes	None
Gaviiformes	None
Passeriformes	
SUBOSCINES	
Tyrannldae	
Tyrannus verticalis, Western kingbird	McFarlane 1971[1 3]
Tyrannus tyrannus, Eastern kingbird	Feduccia 1979[1]
Contopus virens, Eastern wood peewee	Feduccia 1979[1]

Table 8.4 Contd. ...

Table 8.4 Contd. ...

Taxon	*Reference*
Myiarchus crinitus/ M. griseisticta, Great crested flycatcher	McFarlane 1971[1 3] ; Asa and Phillips 1987[1]
OSCINES	
Corvida	
Corvidae	
Cyanocitta cristata, Blue jay	Henley *et al.* 1978[1]
Corvus splendens, Crow	Bawa *et al.* 1990[1 2]
Grallinidae	
Grallina cyanoleuca, Magpie lark	Jamieson 1995[1],1999[1]
Vireonidae	
Vireo olivaceus, Red-eyed vireo	McFarlane 1971[3]; Henley *et al.* 1978[1]
Vireo griseus, White-eyed vireo	Asa and Phillips 1987[1]
Passerida	
MUSCICAPOIDEA	
Muscicapidae (including Turdidae)	
Musclcapidae	
Myrmecocichila formicivora, S. Ant-eater chat	Jamieson *et al.,* unpublished[1]; this study[1]
Turdus greyi, Clay-colored robin	McFarlane 1963[1]
Turdus merula, Blackbird	Furieri 1961[1]
Turdus migratorius, American robin	McFarlane 1971[1 3]; Henley *et al.* 1978[1]
Sturnidae	
Sturnus vulgaris, Starling	Koehler 1995[1 2] ‘ Vernon and Woolley 1999 [1 2]
Sylvioidea	
Paridae	
Parus bicolor, Tufted titmouse	McFarlane 1971[1 3]; Henley *et al.* 1978[1]
Hirundinidae	
Tachycineta thalassina, Violet-green swallow	McFarlane 1971[1 3]
Certhioidea	
Troglodytidae	
Thryothorus ludovicanus, Carolina wren	Asa and Phillips 1987[1]
Troglodytes troglodytes, Wren	Tripepi and Perrotta 1991[1]
Certhiidae	
Certhia brachydactyla	Tripepi and Perrotta 1991[1]
Sittidae	
Sitta europaea	Tripepi and Perrotta 1991[1]
PASSEROIDEA	
Parulidae	
Dendroica pinus, Pine warbler	Asa and Phillips 1987[1]
Dendroica dominica, Yellow-throated warbler	Asa and Phillips 1987[1]
Protonataria citrea, Prothonotory warbler	Asa and Phillips 1987[1]
Fringillidae	
Ammodramus maritimus (=Ammospiza maritima), Seaside sparrow	McFarlane 1963[1]
Fringilla coelebs, Chaffinch	Furieri 1962[1]; Tripepi and Perrotta 1991[1]

Table 8.4 Contd. ...

Table 8.4 Contd. ...

Taxon	*Reference*
Pipilo erythrophthalmus, Rufous-sided towhee	Henley *et al.* 1978[1]
Piranga rubra, Summer tanager	Henley *et al.* 1978[1]
Serinus canaria, Canary	Humphreys 1972[1]
Serinus canaria, Canary × *Cardeulis cardeulis* Goldfinch, hybrid	Swan 1985[1]
Zonotrichia albicollis, White-throated sparrow	Henley *et al.* 1978[1]
Carduelis (=Chloris) chloris	Tripepi and Perrotta 1991[1]
Icteridae	
Agelaius phoeniceus, Redwing	Asa and Phillips 1987[1]; Koehler 1995[2]
Icterus galbula, Northern oriole	Asa and Phillips 1987[1]
Molothrus ater, Brown-headed cowbird	Koehler 1995[1]
Quiscalus quiscula, Grackle	Koehler 1995[1 2]
Emberizldae	
Cardinalis cardinalis, Cardinal	Henley *et al.* 1978[1]; Koehler 1995[1 2]
Emberiza cirlus, Cirl bunting	Tripepi and Perrotta 1991[1]; Tripepi *et al.* 1991[1]
Estrildidae	
Lonchura striata (=Uroloncha striata), Love bird	Yasuzumi and Sugioka 1966[1]; 1971[1]. Yasuzumi 1974[1 2]; Kondo *et* al. 1988[1]
Lonchura castaneothorax × *L. puntulata*	Swan and Christidis 1987[1]
Taeniopygia guttata, Zebra finch	Nicander 1970a[1]; Fawcett *et al.* 1971[1]; Asa and Phillips 1987[1] Vernon and Woolley 1999[1]
Passeridae	Humphreys 1972[1]; Asa and Phillips 1987[1 2]
Passer diffusus, Grey-headed sparrow	This study[1]
Passer domesticus, House sparrow	Koehler 1995[1 2]
Passer italiae, italian sparrow	Furieri 1961[1]
Thraupidae	
Piranga rubra, Summer tananger	McFarlane 1971[1 3]
Pioceidae	
Philetairus socius, Social weaver	Jamieson *et al.,* unpublished[1]; this study[1]
Euplectes orix, Red bishop	This study[2]
Ploceus capensis, Cape weaver	This study[2]
Quelea qualea, Red-billed quelea	This study[2]
Prunellidae	
Prunella collaris, Alpine accentor	Chiba and Nakamura 2001[1 2]

[1]TEM [2]SEM [3]*fide* Koehler (1995)

*For a list of additional species examined by Birkhead *et al.* (2006) see section 8.10.12.17.
For additional species examined by Tripepi and Perrotta (1991) see section 8.10.10.1.

of the midpiece. It is penetrated by the two central singlets of the axoneme (amniote synapomorphy) around which is a dense sheath (crocodile-bird synapomorphy, not described for *Rhea* and *Dromaius* and lost in non-struthioniforms). The midpiece consists of approximately ellipsoid (*Struthio,*

Rhea) or spheroidal (*Dromaius*) mitochondria ensheathing the axoneme (amniote synapomorphy), four (*Struthio*), four to six (*Rhea*), or five to six (*Dromaius*) per transverse section, joined by electron-dense cement, totaling about 20-25 (*Struthio*), 30 (*Rhea*) or 40 (*Dromaius*) in a poorly defined helix, ending posteriorly at the annulus; cristae longitudinal plates, not in the basal amniote concentric arrangement. The fibrous sheath of the principal piece consists of semicircular ribs, enlarging near axonemal doublets 3 and 8 as longitudinal columns (amniote synapomorphies, replaced by amorphous sheath in other nonpasserines). A small dense fiber is attached to each doublet in the principal piece (*Struthio*, *Rhea*, shorter in *Dromaius*) (the fibers are a crocodile-avian symplesiomorphy, though reduced in the latter). At the posterior end of the endpiece, the doublets separate into individual components, and the dense contents of the A microtubules disappear. The resulting disorganized collection of 20 lucent microtubules displays a random decrease in number towards the tip (amniote plesiomorphy?).

8.6.1.3 *Struthio camelus*

The following account of Ostrich sperm is drawn from (Soley 1984b, 1989, 1993, 1999; Soley and Roberts 1994), with some substitution with the terminology of the present author, and from the earlier, succinct account of Baccetti *et al.* (1991). Spermatogenesis (Soley 1994, 1996) is discussed in Chapter **7** of this volume.

General morphology. The mature spermatozoon of Ostrich is illustrated in Figs. 8.4 and 8.5. Examined by scanning electron microscopy (SEM) (Fig. 8.5Q-U) it is a 60 m long (70-80 μm in Soley 1989, 1999), filiform cell with an anterior tapering, cylindrical, 16 μm long head (acrosome + nucleus) (13 μm in Soley 1999) which reaches a diameter of 0.8 μm (Baccetti *et al.* 1991) near its base (greatest width of nucleus 0.5 μm, Soley 1999). A slight circular depression, the posterior ring of Soley (1999), marks the posterior limit of the 2 μm long acrosome. Immediately posterior to the head is the 3 μm long, 0.7 μm wide, midpiece, followed by the 40 μm long, 0.7 μm wide, cylindrical principal piece of the tail, tapering towards its posterior limit and followed in turn by 1 μm long, 0.2 μm wide, endpiece (Baccetti *et al.* 1991). A raised hoop-like ring at the junction of the midpiece and principal piece was identified as the site of the annulus (Soley and Roberts 1994). Soley gives detailed tables of dimensions from a total of 200 sperm from 10 Ostriches, in which mean lengths, in μm, were: acrosome 1.91; nucleus (excluding the portion covered by the acrosome) 10.95; total head 12.86; midpiece 3.16; principal piece 51.18; endpiece 2.39; total tail 56.73; total sperm length 69.59. The mean tail to head ratio was 4.43. Differences in dimensions relative to those given by Baccetti *et al*. (1991) may be real as Soley and Roberts (1994) recognized two populations, possibly subspecies, in their own data, with one bird intermediate between the two.

The head. The head of Ostrich sperm is a slightly curved, cylindrical structure tapering gradually at its most anterior aspect and measures 13 μm in length

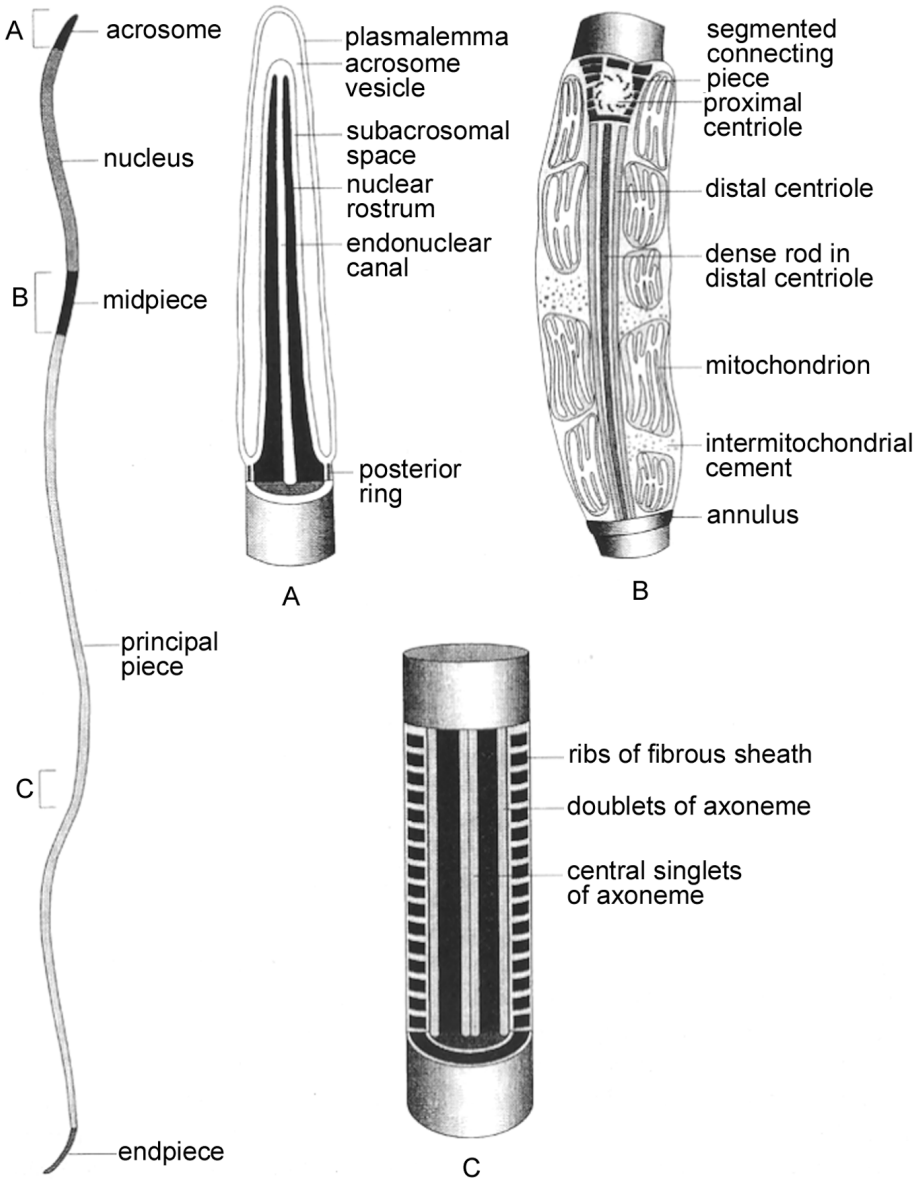

Fig. 8.4 *Struthio camelus*, Ostrich. Schematic representation of a spermatozoon illustrating the various components of the head (acrosome and nucleus) and tail (midpiece, principal piece and endpiece). The three figures on the right show finer details, not drawn to the same scale) of the structures labeled on the whole spermatozoon (left). **A**. Acrosome. **B**. Midpiece. **C**. Principal piece. Relabeled after Soley, J. 1999. Pp. 129-158. In D. C. Deeming (ed). *The Ostrich: Biology, Production and Health*, CAB International, Fig. 6.5.

and 0.5 μm in width at its widest point (dimensions from Soley and Roberts 1994, throughout). The tip of the head is invested by the 2 μm long acrosome vesicle containing fine homogeneous material of moderate electron density (Fig. 8.5A, B) (Baccetti *et al*. 1991; Soley 1993). A subacrosomal space, 30 nm wide, is present between the inner acrosomal and nuclear membranes but this widens to 150 nm at the tip of the head (Baccetti *et al*. 1991). Flocculent material of an electron-density similar to the contents of the acrosome is observed in this space, often in close association with the nuclear membrane (Fig. 8.5A, B). Baccetti *et al*. (1991) state that the apical subacrosomal space contains a bundle of filamentous material which emerges from the endonuclear canal, opening at the nuclear tip; it is correctly regarded as a continuation of the rod (perforatorium) within the endonuclear canal. However, the prenuclear material is minimal and Soley (1993) did not recognize its presence. He regarded the avian perforatorium as merely residual. In contrast, Baccetti *et al*. (1991). considered that the subacrosomal filaments were probably actinic, as in other birds (Campanella *et al*. 1979). An area of close contact existed between the plasmalemma and nuclear membrane at the caudal extremity of the acrosome, forming a structure similar to the posterior ring of mammalian sperm (Fig. 8.5A) (Soley 1993).

Nucleus. The nucleus forms a cylinder except for the part covered by the acrosome where it tapers sharply, as the nuclear rostrum, to end in a fine point beneath the tip of the acrosome. From the tip of the nucleus an axial endonuclear canal, lined by invaginated nuclear membrane, and containing the rod-like perforatorium, extends through the length of the rostrum for roughly 1/4 of the length of the main body of the nucleus (Soley 1993) or for ca 5 mm and about the apical third of the head, being ca 30 nm wide (Baccetti *et al*. 1991) (Fig. 8.5A-C). The chromatin is compact and electron-dense (Fig. 8.5C) excepting small light regions indicative of incomplete condensation throughout the nucleus, particularly in the rostrum. The basal, implantation fossa consists of a small central concavity surrounded by a shallow circular moat or series of depressions running around the perimeter of the nuclear base (Soley 1993). This was interpreted as two parallel implantation fossae, each with an undulating basal lamina by Baccetti *et al*. (1991).

The neck and midpiece. Beneath the base of the nucleus, a short (0.3 μm) proximal centriole displays the characteristic nine sets of triplet microtubules (Baccetti *et al*. 1991; Soley, 1993). These are embedded in a ring of dense amorphous material. The central cavity of the centriole sometimes contains flocculent or granular material similar to that observed between the mitochondria of the midpiece (Fig. 8.5E). Dense material associated with the juxta-nuclear surface of the proximal centriole fills the center of the nuclear fossa, merging with similar peripheral material provided by dense non-segmented columns emanating from the walls of the proximal and distal centrioles (Fig. 8.5D, E). The distal centriole is perpendicular to the proximal centriole, and occupies the entire length of the midpiece. In transverse section

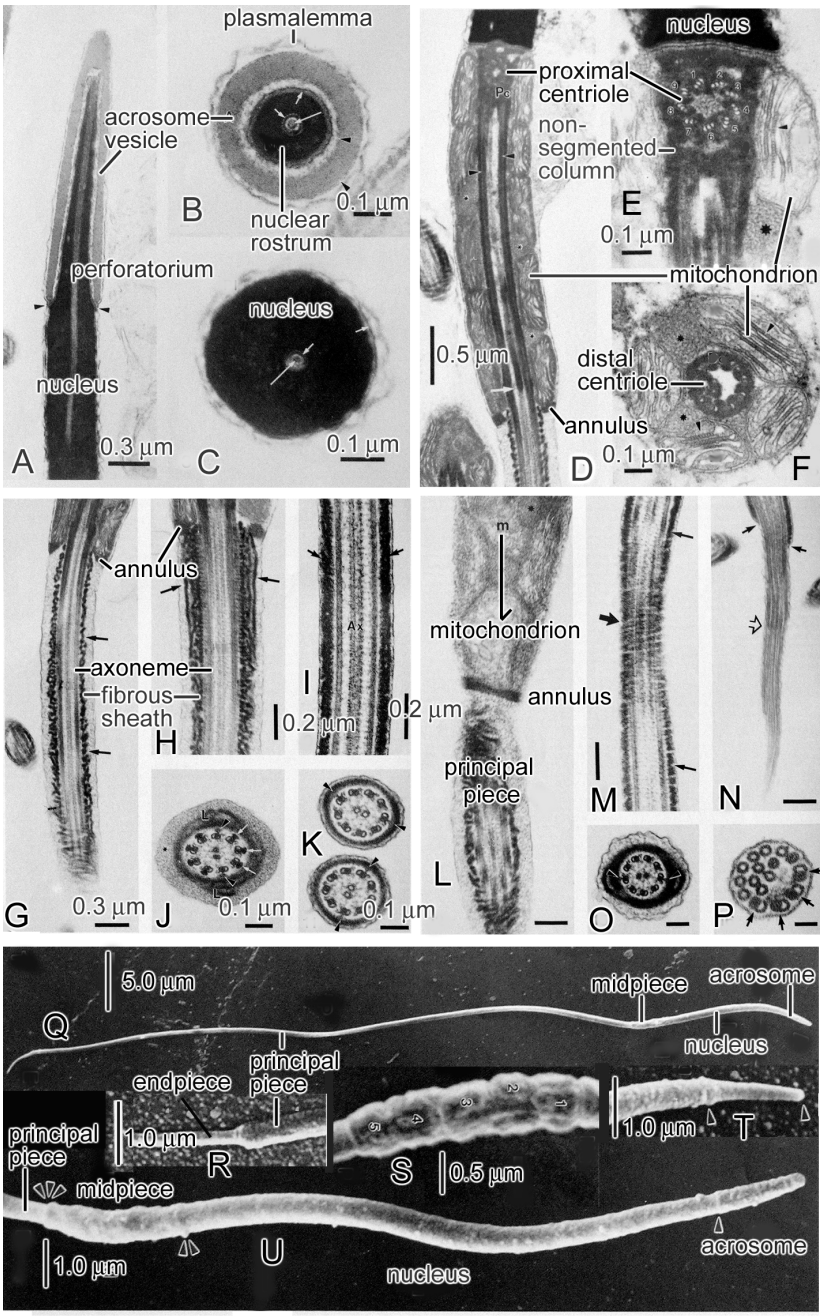

Fig. 8.5 *Struthio camelus*, Ostrich. **A-P**. Transmission electron micrographs. **A**. Longitudinal section (LS) of acrosome and anterior nucleus. **B**. Transverse section (TS) of acrosome vesicle and the enclosed nuclear rostrum with central

Fig. 8.5 Contd. ...

it consists of a narrow ring of electron-dense material from which nine evenly spaced dense projections jut into the centriolar cavity. Nine sets of characteristically arranged triplet microtubules are situated between the projections. Within the centriolar cavity are two singlets (central tubules) (Baccetti *et al*. 1991; Soley 1993, 1999). Around these Soley (1993) describes a rod, sometimes eccentric, of dense material extending posteriorly as far as the annulus and containing a pair of microtubules (Fig. 8.5D, E). The rod is occasionally seen in the form of two closely apposed but separate units, each containing a single microtubule. Posterior to the annulus a typical central pair of microtubules extends throughout the rest of the tail.

The 0.3 μm long midpiece is slightly wider (0.65 μm) than the nucleus and contains about 20 (Soley 199) or 24-25 (Baccetti *et al*. 1991) mitochondria arranged in an helical pattern around the proximal and distal centrioles (Fig. 8.5D, F, S) as a single layer. The mitochondria have flattened, rectangular profiles (Figs. 8.4B, 8.5D), although round or oval forms are sometimes

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Fig. 8.5 Contd. ...

endonuclear canal. **C**. TS nucleus and perforatorium. **D**. LS midpiece. Note the termination of the inner dense rod of the distal centriole in the vicinity of the annulus (white arrow). The dense walls of the distal centriole, which runs the length of the midpiece are indicated by black arrows. **E**. LS of the neck region showing the proximal centriole cut transversely. **F**. TS midpiece and distal centriole. A dense rod of material containing two singlet microtubules is eccentrically situated. Small black arrow heads indicate atypical cristae. **G**. LS proximal region of principal piece, showing annulus at posterior limit of midpiece, and fibrous sheath. Arrows indicate ribs of this sheath. **H**. Similar section but showing (arrows) longitudinal columns of the fibrous sheath. **I**. LS of a more distal region of the principal piece, through longitudinal columns (arrows) of the fibrous sheath. J. TS of the proximal principal piece, showing, above and below, the longitudinal columns. Dense fibers 3 and 8 have been incorporated into the columns. **K**. TS of two sperm tails posterior to the region with dense fibers but still surrounded by the fibrous sheath. **L**. LS at junction of midpiece and principal piece, showing the annulus I tangential section. **M**. LS distal region of principal piece. The ribs of the fibrous sheath are shown in cross section (arrows) and longitudinal profile (squat arrow). **N**. LS endpiece. Note the staggered termination of the fibrous sheath (arrows). **O**. TS intermediate region of the principal piece. The coarse fibers have disappeared. Doublets 3 and 8 are connected to the longitudinal columns. The cytoplasmic layer surrounding the axoneme is thinner than further proximally. P. TS endpiece at disruption of 9+2 pattern, resulting in a collection of doublets (arrows) and dense and translucent singlet microtubules. **Q-U**. Scanning electron micrographs. **Q**. Entire spermatozoon. **R**. Junction of principal piece and endpiece. **S**. Midpiece, with 5 tiers of mitochondria. **T**. Tapered tip of the nucleus (rostrum), between arrows, revealed after loss of the acrosome vesicle. **U**. Detail of the head and midpiece. A-P modified after Soley, J. T. 1993. Onderstepoort Journal of Veterinary Research 60**:** 119-130, Figs. 1-16. Q-U after Soley, J. T. and Roberts, D. C. 1994. Onderstepoort Journal of Veterinary Research 61**:** 239-246, Figs. 1-5.

observed (Fig. 8.5D, S) and in tangential sections they appear as rectangular or polygonal structures (Fig. 8.5L) (Soley 1993) but Baccetti *et al*. (1991) found them to be elliptic in both longitudinal and transverse section; four in each cross section, joined by an electron-dense cement. The cristae are longitudinal, in a dense matrix. Atypical cristae containing paracrystalline inclusions occur in some mitochondria (Figs. 8.5F, arrow heads). The inclusions display two forms depending on the plane of section. Those sectioned longitudinally presented the appearance of tight junctions while oblique sections revealed a pattern of parallel fibers with a regular spacing and direction. Sandwiched between the mitochondria are conspicuous accumulations of granular material (Soley 1993) (Fig. 8.5F).

Annulus. A well developed annulus (Figs. 8.4B, 8.5D, G, H) marks the boundary between the midpiece and the principal piece (Baccetti *et al*. 1991; Soley 1993, 1999), although a retro-annular recess is not apparent. The annulus is situated beneath the last row of midpiece mitochondria in close association with the plasmalemma and is composed of homogeneous, electron-dense material.

Principal piece. The principal piece forms the longest segment of the tail (ca 50 μm) and consists of the 9+2 axoneme surrounded by a ribbed fibrous sheath (Soley 1993, 1999) (Fig. 8.5D-O). The ribs are semicircular and about 50 nm thick, enlarging near the axonemal doublets 3 and 8 (Baccetti *et al*. 1991) as the 'longitudinal columns' described below by Soley (1993). The A microtubule of each doublet is a circular structure filled with dense material whereas the B microtubule formed an incomplete lucent cylinder. Dynein arms project from each A microtubule towards the neighboring doublet and radial links form a connection with the central microtubules (Figs. 8.5K, O). The principal piece is a tapered structure and gradual decreases in diameter along its length, coupled with changes in the composition of the fibrous sheath, allow three regions to be distinguished:

1. The first region lies immediately posterior to the annulus where the tail abruptly narrows to a diameter of 0.5 μm. The axoneme is surrounded by a loosely arranged fibrous sheath consisting of two dense longitudinal columns connected by circumferential bands of dense material. The longitudinal columns and the interconnecting ribs appeared to be composed of alternating layers of electron-dense and loosely packed material, giving both structures a laminated appearance. The columns lie in line with the two central microtubules in the position occupied by coarse fibers 3 and 8 in mammalian sperm. Peculiar to this region is the presence of nine small (rudimentary, Soley 1999), dense, coarse fibers (the accessory fibers, reniform in cross section, noted by Baccetti *et al*. 1991) lying between the fibrous sheath and the axonemal doublets, each in close association with an A microtubule. A prominent cytoplasmic layer, maximally 60-80 nm wide (Baccetti *et al*. 1991) containing fine flocculent material is interposed between the fibrous sheath and the cell membrane (Fig. 8.5G-J, L).

2. In the second region the diameter of the tail narrows to 0.4 μm, the rudimentary coarse fibers disappear, and the ribs of dense material appear as solid structures. The longitudinal columns, however, retain their laminated appearance while the cytoplasmic layer becomes narrower. Septum-like inward extensions of the longitudinal columns make contact with the adjacent microtubular doublets (Fig. 8.5J).
3. The third region of the principal piece is characterized by a progressive decrease in diameter, from 0.3 μm to approximately 0.2 μm. The longitudinal dense columns became solid, less conspicuous, structures which eventually disappear leaving a thin dense band of material surrounding the axoneme. The plasmalemma is closely applied to the layer of dense material (Fig. 8.5I, K, M) (Soley 1993).

Endpiece. The endpiece forms the short, narrow, 2.4 μm long (Soley and Roberts 1994) or 2-3 μm (Soley 1999), termination of the tail and consisted of the axoneme covered only by plasmalemma. Because the transition from principal piece to endpiece is gradual, remnants of the fibrous sheath are sometimes seen around the axoneme (Soley 1993) (Fig. 8.5N). The organized structure of the axoneme is disrupted towards the end of the tail (Baccetti *et al*. 1991; Soley 1993, 1999). The specific orientation of the axonemal microtubules is lost, the dynein arms and radial spokes linking the microtubules have disappeared, the doublets separate into individual components, and the dense contents of the A microtubules disappear (Fig. 8.5P). The resulting disorganized collection of 20 lucent microtubules displays a random decrease in number at the tip of the endpiece (Soley 1993).

The crocodiloid spermatozoon. Ballowitz and Retzius recognized the 'sauropsid' features of non-passerine spermatozoa. However, the ratite and lower non-passerine spermatozoon, especially the former, would more appropriately be termed crocodiloid. Features of Ostrich sperm which are similar to those of crocodiles are: the pointed acrosome vesicle; the perforatorium in a long endonuclear canal; the midpiece with several tiers of mitochondria surrounding an extremely long distal centriole and terminating at an annulus; presence of nine dense fibers; and a fibrous sheath consisting of transverse ribs (Figs. 8.4C, 8.5G). All of these features are also seen in Chelonia (Healy and Jamieson1992) and are basic (symplesiomorphic) to amniotes, only the fibrous sheath and the long distal centriole being amniote synapomorphies. The sole spermatozoal synapomorphy of crocodiles and birds is the dense sheath investing the two central singlets within the elongate distal centriole (Jamieson 1999). In birds this sheath is known only in ratites (Ostrich) and the Galloanserae.

8.6.1.4 *Rhea americana albisceus*

The following account of the sperm of Rhea, *Rhea americana albisceus*, is drawn from Phillips and Asa (1989). Additional data are added from perusal of their illustrations and current terminology is employed.

Head. The head is curved and tapered. A substantial acrosome composed of moderately electron-dense homogeneous material fits over the anterior portion of the nucleus. A narrow cylindrical structure (endonuclear canal) extends from the anterior portion of the acrosome to deep within the nucleus (Fig. 8.6C, D). The center of the cylinder (putative perforatorium) is composed of material that is about the same electron density as the acrosome, which is circumscribed by an electron-lucid region, in turn surrounded by a thin region of moderate electron density (Fig. 8.6C, D). The chromatin is compact but not as condensed as is observed in spermatozoa of insects or mammals (Fig. 8.6A-D). A short midpiece lies between the head and principal piece (Fig. 8.6A). The long principal piece comprises most of the length of the cell.

Neck region and centrioles. As in many other animals, the neck region of Rhea sperm is characterized by a precisely shaped posterior portion of the nucleus. Electron-dense material associated with the proximal centriole fits into the contour of the sperm nucleus and associated nuclear membrane (Fig. 8.6A, D). The proximal centriole is short, only about 0.4 μm long, and is inlaid with electron-dense material (Fig. 8.6D, E). The distal centriole is much longer. It apparently extends the entire length of the midpiece. Both centrioles are embedded in electron-dense material. Although the distal centriole displays the characteristically disposed triplet subtubules, the central tubules of the flagellum extend into the center of the centriole (Fig. 8.6E).

Midpiece. The midpiece contains about 30 mitochondria, four to six in transverse section, and about seven longitudinally, with a very dense matrix typical of spermatozoal mitochondria. In the interior of the mitochondrion distal to the centrioles, there is a complex configurations of mitochondrial membranes (Fig. 8.6A, D, E).

Principal piece. Transverse sections through the principal piece reveal a small fibrous sheath. The sheath is larger opposite doublet tubules 3 and 8 and is connected to these two doublets by a thin band of dense material, as in mammals, but enlargement is only slight, less than in the latter (Fig. 8.6F).

Dense axonemal fibers. There are very tiny dense fibers present only for a short region of the principal piece (see Fig. 8.6F), given (erroneously?) in the original account as the very anterior portion of the "midpiece". They are no larger than microtubules and are associated with the doublet subtubules A and B (Fig. 8.6F).

Remarks. The spermatozoon of Rhea is closely similar to that of Ostrich, the similarities including the deep extension of the endonuclear canal and perforatorium into the nucleus. Soley (1993) considers Ostrich sperm to differ from that of Rhea, however, in that the central tubules are embedded in a core of dense material which only disappears in the vicinity of the annulus. The account of Phillips and Asa (1989) does not refer to this feature and no sheath of dense material is visible in the relevant micrograph (Fig. 8.6E).

In both Ostrich and Rhea, modifications of the mitochondrial membranes have been observed, with those of Rhea (Phillips and Asa 1989) resembling

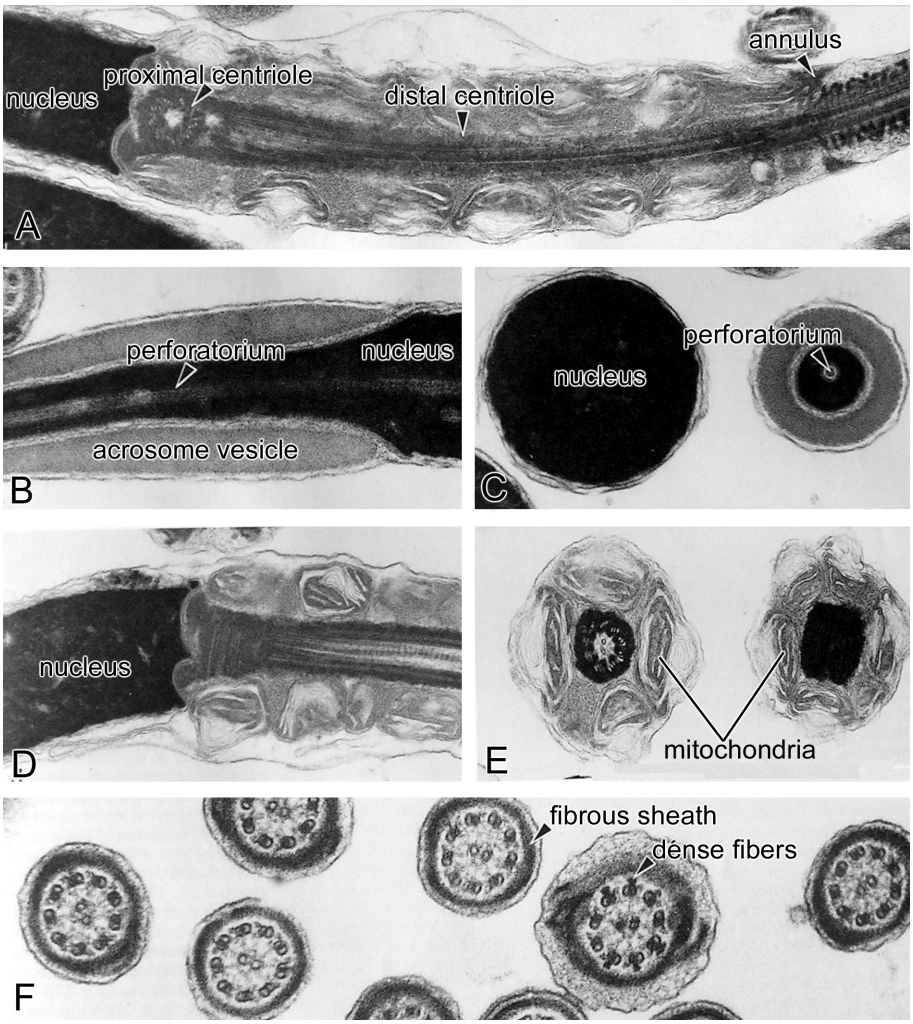

Fig. 8.6 *Rhea americana albisceus.* **A**. Longitudinal section (LS) midpiece, penetrated by the long distal centriole, and terminating at the annulus. **B**. LS basal region of acrosome vesicle enclosing the nuclear rostrum and, at the center of this, the perforatorium. **C**. Transverse section (TS) of a nucleus and (right) the acrosome vesicle and nuclear rostrum. **D**. LS neck region showing short proximal centriole perpendicular to the distal centriole. **E**. TS midpiece; right, through the proximal centriole; left, through the distal centriole, showing two central singlets. **F**. TS through the principal piece of several spermatozoa. Relabeled after Phillips, D. M. and Asa, C. S. 1989. Anatomical Record 223**:** 276-282, Fig. 1. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

myelin figures and those of Ostrich adopting the form of atypical cristae containing paracrystalline material. The significance of these structural modifications is unknown. Although organized and arranged in a fashion

similar to that of mammalian sperm, the ribbed sheath of Ostrich sperm is more flimsy in structure, resembling that described in Rhea (Phillips and Asa 1989) and tinamou (Asa *et al*. 1986; Soley 1993). The latter author correctly observes that the ribbed form of the fibrous sheath seen in ratites does not occur in other non-passerines. We may add that the transverse ribbing is a basic amniote (Fig. 8.1) and crocodile (Fig. 8.2) feature. This unique crocodiloid feature of ratite sperm endorses the basal position of ratites in bird phylogeny.

The proximal segment of the principal piece of Ostrich sperm displays a prominent cytoplasmic layer situated between the axoneme and the plasmalemma. This layer is filled with fine amorphous material and resembles a similar region seen in Rhea sperm (Phillips and Asa 1989). Tinamou sperm are structurally similar in this respect although particulate material which morphologically resembles glycogen is found throughout this region (Asa *et al*. 1986). A similar region is absent in other non-passerine birds (Soley 1993).

As Soley (1993) observes, penetration of the long distal centriole by the central singlets of the axoneme is a feature shared by Rhea and Ostrich but is absent from Tinamou. As noted by the present author, this penetration is a crocodilian (and chelonian) feature and is therefore a symplesiomorphy of Rhea and Ostrich signifying an unchanged basal amniote condition but not necessarily indicating rheid-struthionid monophyly.

8.6.1.5 *Dromaius novaehollandiae*

General morphology. The mature sperm cell of Emu seen by SEM (8.7A-C) is ca 65 μm long. The cylindrical, tapered head measures ca 12 μm in length and 0.8 μm in maximum diameter. The acrosomal region is 1.5 μm long. The midpiece is 3 μm long and not as deeply marked as in Ostrich. The principal piece is ca 47 μm and the endpiece 3 μm long. Compared to Ostrich, the spermatozoon of Emu has a longer tail and a shorter head, while the midpiece (i.e. the distal centriole) is the same length (Baccetti *et al.* 1991). TEM sections of the spermatozoon are shown in Fig. 8.7D-I.

Fig. 8.7 *Dromaius novaehollandiae*, Emu, spermatozoa. **A-C**. Scanning electron micrographs. **D-I**. Transmission electron micrographs. **A** Whole spermatozoon. × 2200. **B**. Enlarged view excepting posterior flagellum. × 4400. The head is shorter, the midpiece equal in length, and the tail longer than in Ostrich. **C**. Posterior end, showing the endpiece. × 19000. **D**. Acrosome vesicle (A) on the anterior tapering rostrum of the nucleus (N). × 49600. **E**. Longitudinal section (LS) of the posterior nucleus and the midpiece. × 63600. **F**. LS midpiece and anterior principal piece. × 33400. **G**. Transverse section (TS) of the acrosome vesicle and nuclear rostrum. × 47700. As in A, note the absence of an endonuclear canal and perforatorium. **H**. TS of the principal piece showing the fibrous sheath. × 44200. **I**. TS endpiece; note absence of fibrous sheath. × 45000. A, acrosome vesicle; AF, accessory fibers; AX, axoneme; dc, distal centriole; FS, fibrous sheath; M, mitochondria; N, nucleus; Modified after Baccetti, B., Burrini, A. G. and Falchetti, E. 1991. Biology of the Cell (Paris) 71(1-2): 209-216. Figs. 3-5, 7, 12, 13, 9, 21, 20.

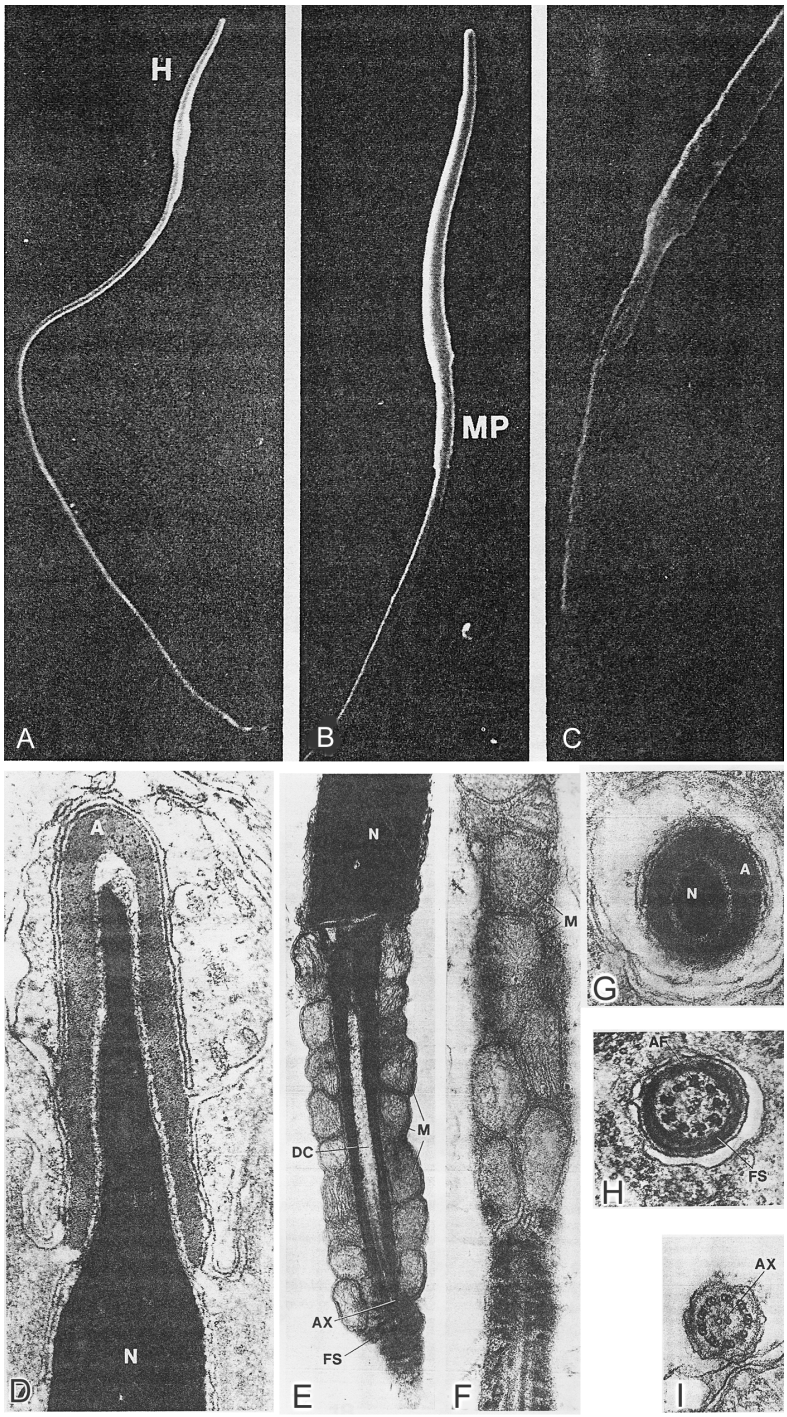

Fig. 8.7

Acrosome. Examined in sections (Fig. 8.7D, G) by transmission electron microscopy (TEM), the acrosomal complex is made up of a truncate-conical, 100 nm thick acrosome vesicle, which holds the apical tapered portion of the nucleus, and of an extremely thin bundle of microfilaments evident in the apical subacrosomal space (Fig. 8.7D). This space is extremely reduced if compared to that of Ostrich, and no endonuclear canal and no rod-like subacrosomal structure are evident. Therefore, the perforatorium, represented only by the sparse extranuclear microfilaments, is almost absent.

Nucleus. The nucleus, which is strongly condensed, is cylindrical and similar to that of Ostrich.

Centrioles and midpiece. The same is true for the centrioles, while the mitochondria are smaller, spheroidal, 5-6 in a cross-section of the midpiece, numbering 8-10 in longitudinal section (Fig. 8.7E) and reach a total number of 40 or more. They are closely juxtaposed, and the intermitochondrial cement is sparse. This organization explains the reduced demarcation of the midpiece, seen from the exterior by SEM. No penetration of the distal centriole by the two axonemal singlets is apparent in the micrograph (Fig. 8.7E) but absence perhaps requires confirmation.

Tail. The axoneme has the same characteristics as in Ostrich, but the region containing the small accessory dense fibers (Fig. 8.7H) is even shorter, and the fibrous sheath (Fig. 8.7H) is progressively thinner toward the end part of the tail and is, by definition, lacking from the endpiece (Fig. 8.7I). The tail is longer than in Ostrich, and contains the basic 9 + 2 axoneme (Baccetti *et al.* 1991).

Remarks. The spermatozoon of Emu resembles that of Ostrich and Rhea in most respects but shows three remarkable departures. Two of these, the absence of an endonuclear canal and contained perforatorium, are clearly correlated. The third is the rounded form of the apex of the acrosome vesicle.

Loss of the perforatorium is enigmatic if, as the author hypothesizes, it contributes to the acrosome reaction at fertilization. Soley (1993) remarks that no definite function has yet been ascribed to the avian perforatorium and states that it would appear merely to represent a residual structure. However, Campanella *et al.* (1979) found that the turkey perforatorium consisted of actin and Baccetti *et al.* (1980) believed that it supported the conical shape of the acrosome.

A role in the acrosome reaction and fertilization has been shown for the perforatorium which lies in an endonuclear canal penetrating most of the nucleus in the lamprey. In *Lampetra fluviatilis,* the central fiber (putative perforatorium) is capable of extrusion as a 50 µm long 'head filament' (Afzelius and Murray 1957; Kille 1960). In the acrosome reaction, the plasma membrane is drawn out into a slender sheath containing the central fiber (Stanley 1967). The putative perforatorium undergoes no observable change on extrusion (Follenius 1965). In *L. planeri* sperm in the egg coatings show a true acrosome reaction in which the central fiber is extended in an acrosomal tubule which penetrates the egg envelopes to reach the egg surface (Nicander

and Sjödén 1968, 1971). In the hagfish *Eptatretus* an acrosome reaction occurs with formation of an acrosomal process with a filamentous core and is deduced to involve polymerization of actin (Morisawa and Cherr 2002). Analysis of proteins in the rat perforatorium, which does not form an acrosome process, failed to detect actin (Oko *et al.* 1990).

It is thus difficult to accept a merely residual status for the avian perforatorium. On the other hand, even if it is involved in an acrosome reaction in Ostrich and Rhea, which has yet to be demonstrated, it may also have a supportive function for the conical acrosome vesicle as Baccetti *et al.* (1980) suggested and its loss in Emu could conceivably be related to the rounded form of the vesicle. It is clear that in Emu, as in many other birds, e.g. pigeons and passerines, similarly lacking a perforatorium, an acrosome reaction must nevertheless occur.

8.6.2 Order Tinamiformes

The Tinamiformes contain only the family Tinamidae, the Tinamous, with 9 genera and 48 species.

8.6.2.1 *Eudromia elegans*

The spermatozoon of Crested tinamou has been described by Asa *et al.* (1986) and Asa and Phillips (1987). Their accounts are paraphrased and augmented here.

The sperm head. The head of Tinamou spermatozoon is cylindrical and slightly curved. The smooth, tapered anterior-most end is characterized by a small bump at the tip (Fig. 8.8A).

Acrosome. Although the form of acrosome vesicle is undescribed, it is seen in the illustration to be a hollow cone, the inner and outer membranes of which are almost in contact at the tip and are more widely separate at about mid-length than elsewhere. The vesicle contents appear homogeneous and moderately electron-dense. In thin sections, a small space is observed between the cell membrane and the outer acrosomal membrane (Fig. 8.8B).

Nucleus. The nucleus (Fig. 8.8A) is a moderately elongate cylinder. In contrast with the acrosomal region, the cell membrane appears to be closely associated with the nuclear membrane in the region immediately posterior to the acrosome. The chromatin rarely appears completely condensed. In oblique or longitudinal sections, at the edge of the nucleus thick strands of chromatin are seen disposed obliquely to the long axis of the nucleus. This arrangement suggests that the chromatin spirals around the endonuclear canal.

Endonuclear canal and perforatorium. Transverse and longitudinal sections of the nucleus reveal a tube-like structure (endonuclear canal) which extends from one end of the nucleus to the other and abuts the tip of the acrosome (Fig. 8.8B).

Neck Region and Midpiece. The posterior edge of the sperm nucleus is indented as a concave disk. A short proximal centriole is situated near the

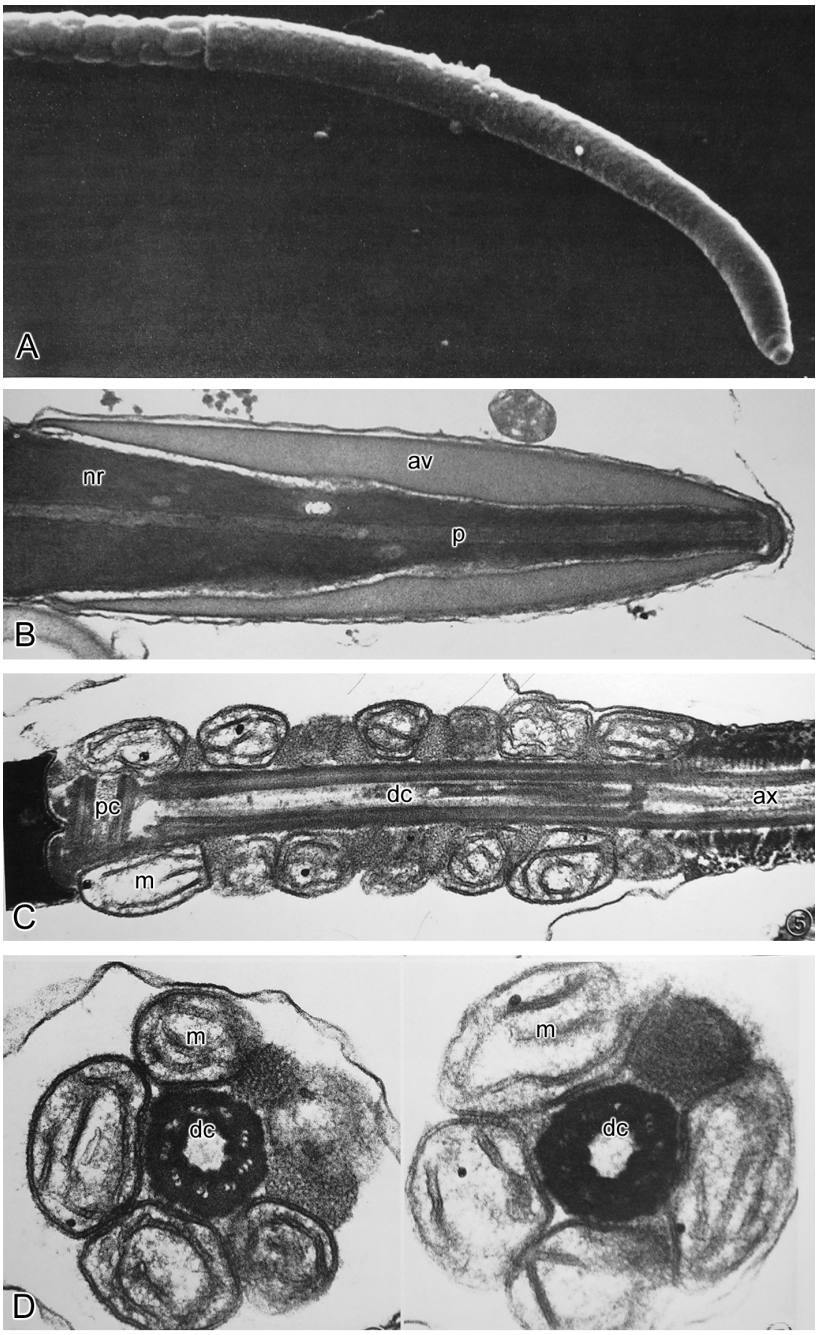

Fig. 8.8 *Eudromia elegans elegans*, Crested tinamou. **A**. Scanning electron micrograph of the anterior portion of a spermatozoon. About 20 mitochondria are

Fig. 8.8 Contd. ...

base of the nucleus (Figs. 8.8C, D, 8.9A). Dense columns of the neck piece surround this centriole and extend into the distal centriole for, apparently, about half of its length. The distal centriole is 3 μm in length, the entire length of the midpiece, and is embedded in dense material (Figs. 8.8C, 8.9A). The mitochondria are roughly spherical. The midpiece contains about 20 mitochondria arranged in seven tiers with about five around the centriole (Figs. 8.8C, D, 8.9A). Flocculent material is observed between the mitochondria (Figs. 8.8C, D, 8.9A) (Asa *et al.* 1986).

Annulus and principal piece. A distinct annulus is situated posterior to the short midpiece (Figs. 8.8C, 8.9), as in mammalian spermatozoa (Fawcett, 1975). Asa *et al.* (1986) state that no dense fibers were observed in tinamou spermatozoa, either in the midpiece or the principal piece. However, they later state (Asa and Phillips 1987) that dense fibers are present in the proximal principal piece to which they are restricted. Periodic structures are observed in longitudinal sections of the fibrous sheath (Figs. 8.8C, 8.9C). In transverse section, the fibrous sheath shows the typical ribs opposite dense fibers 3 and 8. Material which appears morphologically similar to glycogen surrounds the fibrous sheath in the anterior region (Fig. 8.9C) (Asa *et al.* 1986).

Tinamou sperm and ratite phylogeny. Spermatozoal characters have been considered equivocal as to whether the Tinamiformes or Struthioniformes are the most primitive (Soley 1993) or, in other words, if they are sister-groups, which is the apomorph sister-group. Asa *et al.* (1986) observe that Tinamou is exceptional among avian species in the great length of what is here termed the endonuclear canal, extending to the base of the nucleus. They considered it possible that the contents of the canal possess actin which can polymerize to cause the acrosome to be propelled through egg investments during the acrosome reaction as shown for other groups.

Fig. 8.8 Contd. ...

observed in the midpiece. × 6800. **B**. Longitudinal section of the anterior portion of the sperm head. The contents of the acrosome vesicle and moderately electron dense. A perforatorium lying in an endonuclear canal runs through the center of the nucleus where it abuts the tip of the acrosome vesicle. × 32500. **C**. Midsagittal section through the midpiece. The short proximal centriole and the long distal centriole are each seen in longitudinal section. The distal centriole, surrounded by spheroidal mitochondria, extends the entire length of the midpiece as is crocodile and other ratite sperm. The posterior end of the nucleus has a concave circular fossa. × 22000. **D**. Transverse sections through the midpiece. The distal centriole, embedded in electron dense material, is observed throughout the length of the midpiece. Flocculent material is interspersed between some mitochondria. × 53000. Av, acrosome vesicle; ax, axoneme; dc, distal centriole; m, mitochondrion; nr, nuclear rostrum; pc, proximal centriole; Adapted and relabeled after Asa, C., Phillips, D. M. and Stover, J. 1986. Journal of Ultrastructure and Molecular Structure Research. 1986; 94(2): 170-175, Figs. 1, 2, 5-7. With permission from Elsevier.

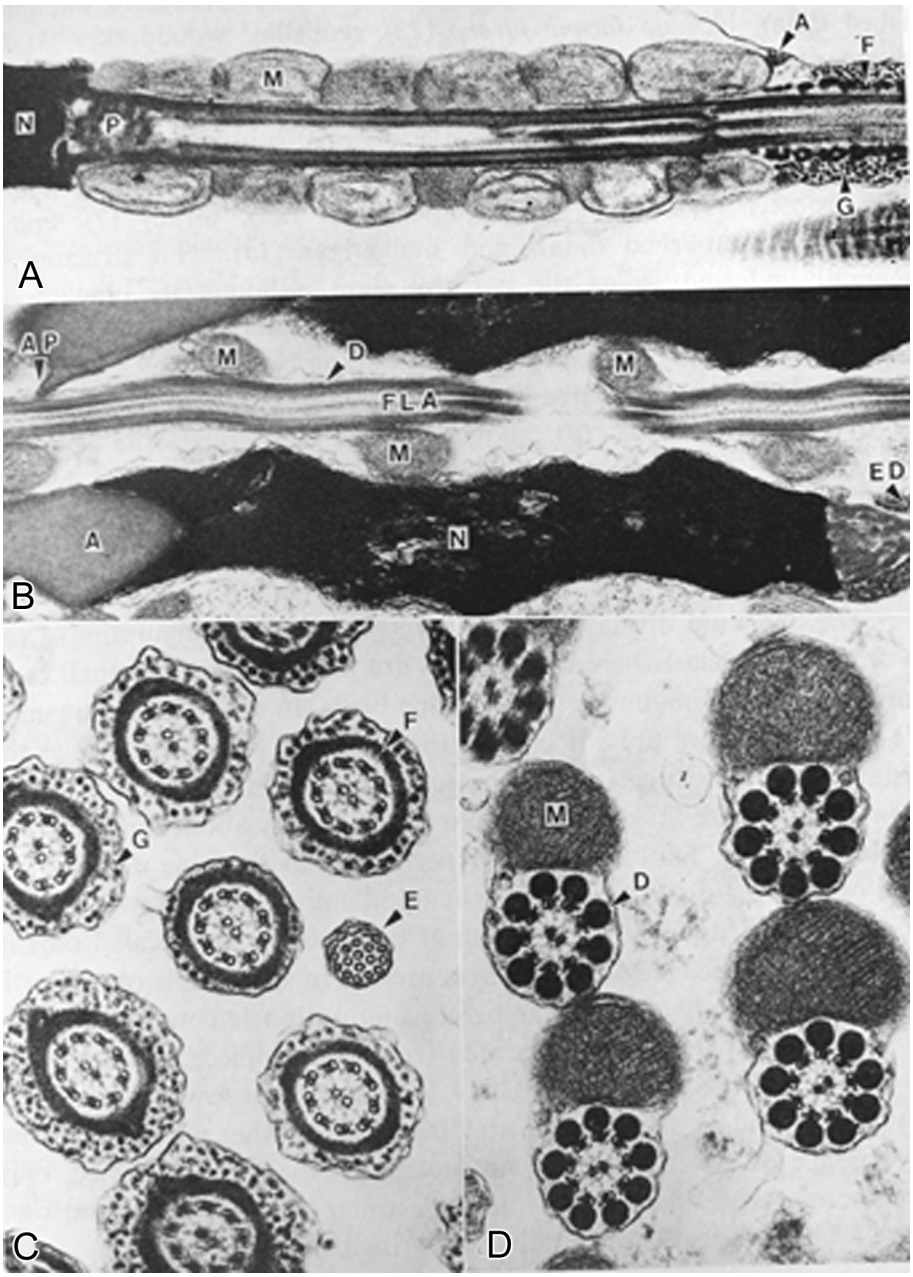

Fig. 8.9 A. Longitudinal section (LS) showing the midpiece of a tinamou spermatozoon. A, annulus; M, mitochondrion; F, fibrous sheath; G, glycogen; p, proximal centriole. × 17400. **B**. LS showing portions of three spermatozoa of *Thryothorus ludovicanus*, the Carolina wren. The mitochondria (M) which spiral around the flagellum (FLA) have been referred to as the undulating membrane.

Fig. 8.9 Contd. ...

They also considered the Tinamou spermatozoon to be unusual in that there is a single distal centriole which extends the entire length of the midpiece. However, this has also been described for Struthioniformes (see Section 8.6.1), and for chelonians, and *Sphenodon* (Healy and Jamieson 1992; Jamieson and Healy 1992) and therefore appears to be a symplesiomorphy of Palaeognathae. Asa *et al.* (1986) observed that other birds such as fowl and ducks also have long distal centrioles, but that the midpiece of spermatozoa of these species is longer and the centriole only occupies the anterior portion of the midpiece. However, it is here noted that in addition to being shorter than the midpiece, the absolute length of the distal centriole in these is less than that in Struthioniformes and Tinamiformes. The presence of glycogen, around the fibrous sheath, appears to be unique to the Tinamou spermatozoon and therefore an autapomorphy insofar as tinamiforms have been studied.

Soley (1993) reasonably considered that there is a trend to enlargement of axonemal dense fibers in birds. He therefore considered Tinamou to be the most primitive ratite in this respect in the erroneous belief that it lacked dense fibers in contrast with the rudimentary dense fibers observed in the proximal segment of the principal piece of Ostrich and Rhea (Phillips and Asa 1989) sperm. Therefore consideration of dense fibers does not contribute to phylogenetic analysis within the ratites.

Furthermore, although (Soley 1993) rightly recognizes a trend to reduction in the length of the endonuclear canal and contained perforatorium in the non-passerines, with their loss in passerines, it is by no means certain that the fact that these structures are longer in tinamou than in struthioniforms indicates that Tinamou is more primitive. Doubt is cast on the latter inference by the fact that the canals and perforatoria are shorter in Crocodylia than in struthioniforms.

As Soley (1993) argues, Ostrich and Rhea sperm appear to be more primitive than those of Tinamou in respect of the structure of the distal centriole. In these birds a central pair of microtubules occupies the centriolar cavity, a feature which is typical of chelonian (Furieri 1970; Hess *et al.* 1991; Healy and Jamieson 1992) and, we may add, crocodilian sperm (Jamieson *et al.* 1997). Tinamou sperm (and, apparently, see Section 8.6.1.5, Emu sperm) are said to lack this microtubular arrangement and to display an empty distal centriole (Asa *et al.* 1986; Asa and Phillips 1987) as in other non-passerine birds. However, central singlets appear to reach almost halfway into the

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Fig. 8.9 Contd. ...

A, acrosome; AP, lateral projection of acrosome; D, dense fibers; ED, electron-dense material of the neck; N, nucleus. × 17400. **C**. Transverse section (TS) through the principal piece of tinamou spermatozoa. E, endpiece; F, fibrous sheath; G, glycogen. × 48700. **D**. TS of spermatozoa from the vas deferens of the Carolina wren. Viewed in cross section the dense fibers are approximately circular and are similar to one another. M, mitochondria. × 45900. After Asa, C. S. and Phillips, D. M. 1987. Pp. 365-373. In H. Mohri (ed). *New horizons in sperm cell research*, Japan Science Society Press, Gordon and Breach Scientific Publications, Tokyo/New York, Figs. 1-4.

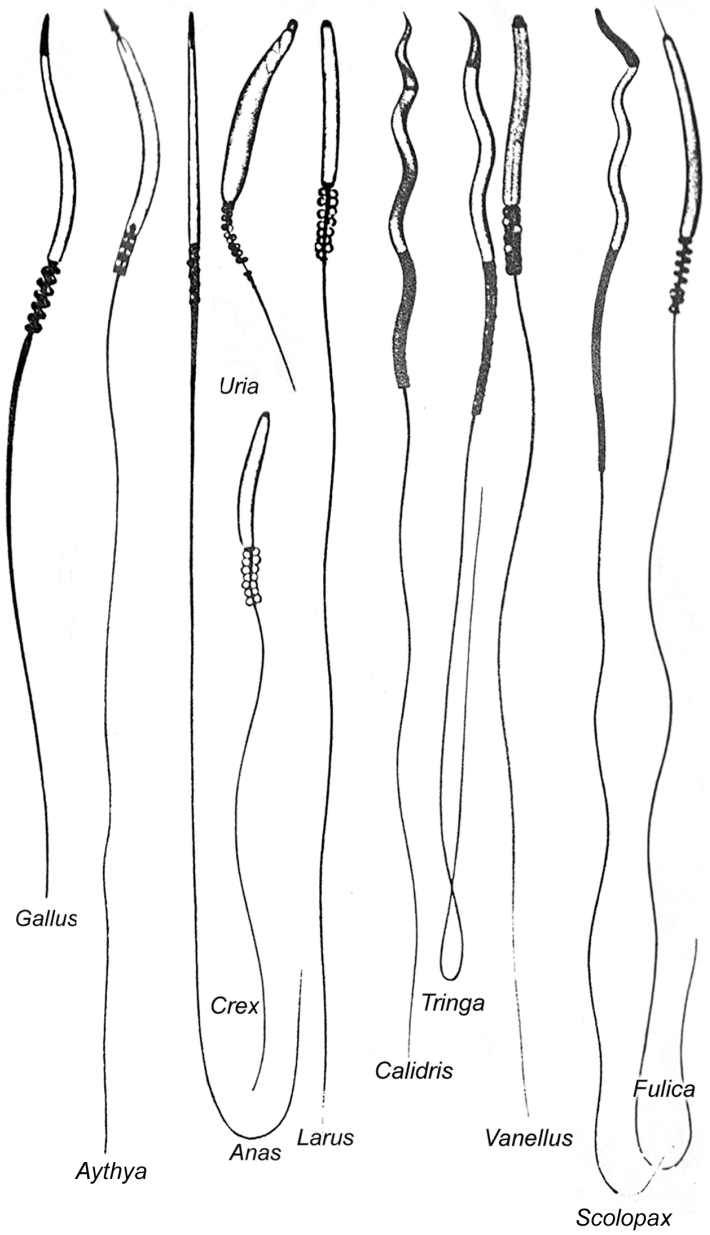

Fig. 8.10 Drawings by light microscopy of spermatozoa of non-passerine birds. Rooster (*Gallus gallus*); Tufted duck (*Aythya fuligula*); Domestic duck (*Anas platyrhynchos*); Common guillemot (*Uria aalge*); Corncrake (*Crex crex*); Lesser black-backed gull (*Larus fuscus*); Alpine dunlin (*Calidris alpine*); Green sandpiper (*Tringa ochropus*); Lapwing (*Vanellus vanellus*); Woodcock (*Scolopax rusticola*); Common coot (*Fulica atra*). After Retzius, G. 1909. Biologische Untersuchungen, Neue Folge 14(10): 89-122 Taf XIX-XXXVII.

centriole in Crested tinamou sperm (see Fig. 8.9A, above, from Asa and Phillips 1987).

The phylogeny of ratites is discussed in Section 8.11.

8.7 NEOGNATHAE

According to Gauthier and de Queiroz (2001) "Neognathae" refers to the crown clade stemming from the last common ancestor of *Charadrius pluvialis (Pluvialis apricari*a) Linnaeus 1758 and all extant birds sharing a more recent common ancestor with that species than with *Struthio camelus* Linnaeus 1758 and *Tetrao (Tinamu*s) *major* Gmelin 1789. Neognathae consists of two primary crown clades, Galloanserae and Neoaves.

8.8 GALLOANSERAE

This is the Parvclass Galloanserae of Sibley and Ahlquist (1990). Its monophyly versus paraphyly are discussed by Harshman in Chapter **1**. Spermatozoal ultrastructure is consistent with monophyly.

The ancestor of the Galliformes and Anseriformes was presumably a generalized form lacking the highly derived filter feeding apparatus of Anseriformes.

8.8.1 Order Galliformes

8.8.1.1 *Gallus gallus (=domesticus)*

The spermatozoon of the rooster, *Gallus gallus* (= *domesticus*), Phasianidae, has been described ultrastructurally (Grigg and Hodge 1949; Bonadona 1954; Nagano 1960, 1962; McIntosh and Porter 1967; Nicander and Hillstrom 1967; Krustev and Danov 1968; Lake *et al.* 1968; Nicander 1970b; Tingari 1973; Bakst and Howarth 1975; Gunawardana and Scott 1977; Bakst and Sexton 1979; Bakst 1980; Xia *et al.* 1985; Xia *et al.* 1986; Bae and Kim 1987; Thurston and Hess 1987; Woolley and Brammall 1987; Sprando and Russell 1988; Xia *et al.* 1988; Jamieson 1999). The following account is based chiefly on a re-examination. Reference is made to other accounts where they are not in agreement or give additional or important supporting information.

General morphology. The *Gallus* spermatozoon (Figs. 8.11, 8.12, 8.16J, P, R) has the usual non-passerine components in anterior-posterior sequence: an acrosome vesicle; perforatorium (apical spine of Grigg and Hodge 1949); acrosome spine of Lake *et al.* 1968; acrosomal spine of Tingari 1973) lying in the subacrosomal space and extending posteriorly into an endonuclear canal, here in the form of a deep anterior nuclear fossa; elongate nucleus; proximal and distal centrioles; midpiece, consisting of mitochondria encircling the 9+2 axoneme and ending posteriorly at the annulus; principal piece, consisting of the axoneme surrounded by a fibrous, here amorphous, sheath; and the endpiece, consisting of the axoneme surrounded by the plasma membrane but lacking a fibrous sheath. The endpiece contains a central dense 'tip granule'

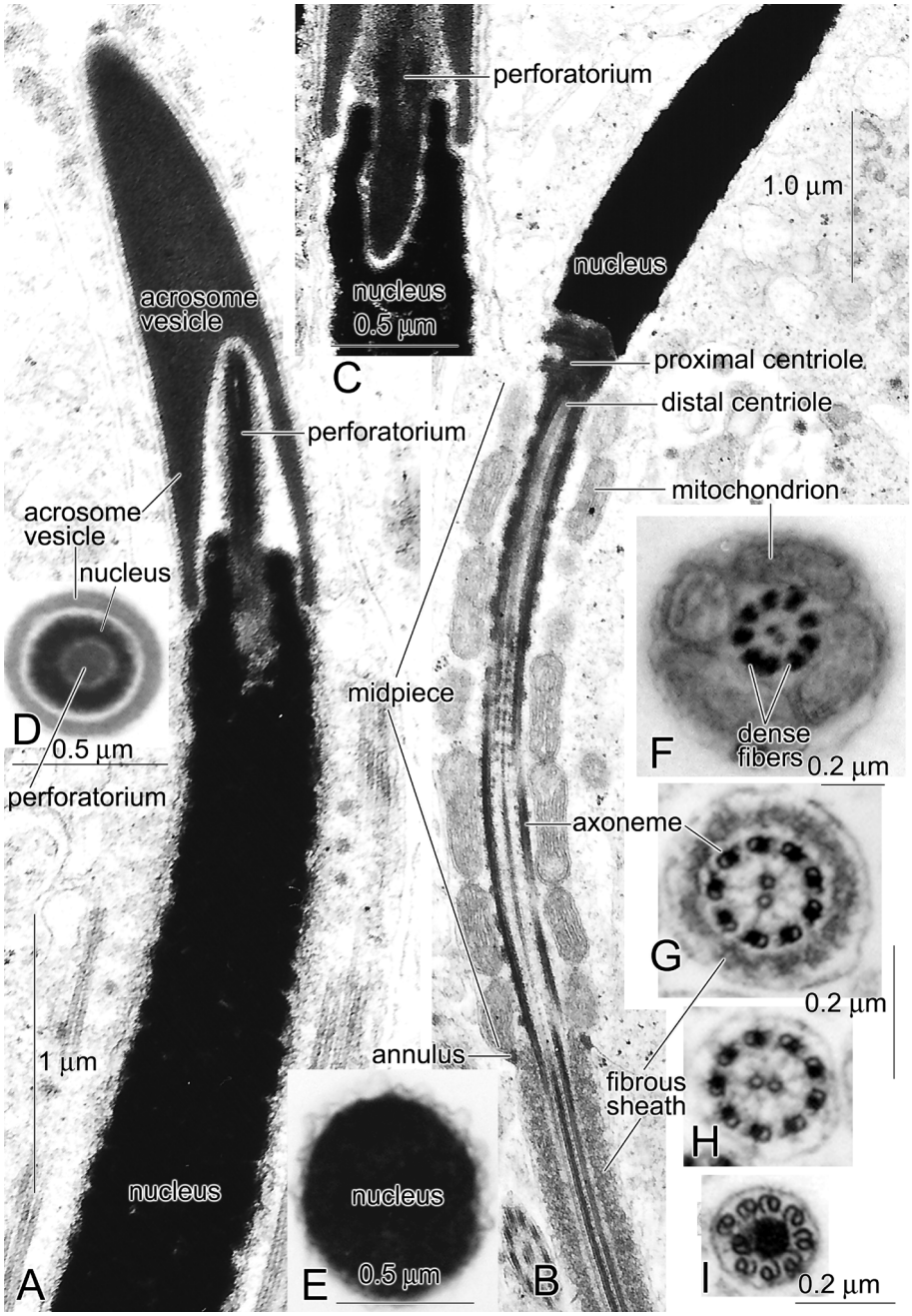

Fig. 8.11 *Gallus gallus*. Transmission electron micrographs. **A**. Longitudinal section (LS) of the acrosome and adjacent nucleus, showing perforatorium in the subacrosomal space and extending into the nuclear fossa (endonuclear canal). **B**. LS basal portion of nucleus, midpiece and anterior portion of principal piece. **C**. LS

Fig. 8.11 Contd. ...

(terminology of Woolley 1995 for *Coturnix*), also observed in *Gallus* by Woolley and Brammall (1987). Grigg and Hodge (1949) give a total length for the sperm "head" (nucleus but contrary to usual definitions apparently excluding the acrosome) of 14 µm and a diameter not exceeding 0.5 µm, and a length for the sperm of more than 100 µm, c.f. 90 µm or more (Thurston and Hess 1987).

Acrosome. The acrosome vesicle is approximately 2.2 µm long (present study), agreeing with 2 µm or greater (Thurston and Hess 1987). It has the form of an elongate cone, asymmetry of which correlates with a slightly curved shape. The base of the vesicle overlaps a short, abruptly narrowed anterior region of the nucleus (Figs. 8.11A, C, 8.12A). In the large, conical subacrosomal space which occupies a little more than the basal third of the acrosome, there lies a dense rod, the perforatorium, ca 1.2 µm long (1.0 µm, Thurston and Hess 1987), the tip of which closely abuts the apex of the subacrosomal space. Approximately the posterior fourth of the perforatorium is contained in, and closely fits, the anterior nuclear fossa which is here considered the homologue of the ratite endonuclear canal.

Amorphous material in the subacrosomal space is said to be continuous with the perforatorium and to be part of it by Xia *et al.* (1988). This condition is seen in the present study in Fig. 8.11C but may represent a dissolution of the perforatorium as the latter is a distinct rod in the absence of amorphous material, except that closely ensheathing the rod, in Fig. 8.11A.

Micrographs of cross sections at the base of the perforatorium of tannic acid-fixed rooster sperm demonstrate the plasmalemma, inner and outer acrosomal, and double nuclear membranes (Thurston and Hess 1987) (Fig. 8.16J).

Nucleus. The nucleus is an elongate cylinder, slightly tapering anteriad, with a slight shoulder supporting the base of the acrosome vesicle (Fig. 8.11A, B, C). It is slightly curved, at least in fixation. The chromatin is strongly condensed and electron-dense. At its base there is a very shallow, slightly asymmetrical basal (implantation) fossa.

Centrioles. The short proximal centriole lies at right angles to the long axis of the spermatozoon, with its anterior region in the shallow implantation fossa (Fig. 8.11B). It consists of 9 triplets embedded in a dense ring. It is linked by an unstriated connecting piece to the base of the nucleus (Bakst and Howarth 1975), termed the non-striated connecting piece and considered to include the proximal centriole by Thurston and Hess (1987). This is termed the capitulum

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Fig. 8.11 Contd. ...

base of acrosome vesicle, perforatorium and anterior nuclear fossa. **D**. Transverse section (TS) through anterior nuclear fossa, showing enclosed perforatorium. **E**. TS nucleus, showing circular profile. **F**. TS midpiece, showing four mitochondria and the axoneme with nine outer dense fibers. **G**. TS principal piece, distinguished by presence of a fibrous (amorphous) sheath around the axoneme. **H**. TS endpiece, distinguished by the axoneme lacking a fibrous sheath. **I**. TS terminal region of endpiece, showing doublets undergoing disruption and central singlets replaced by a dense 'tip granule'. Original.

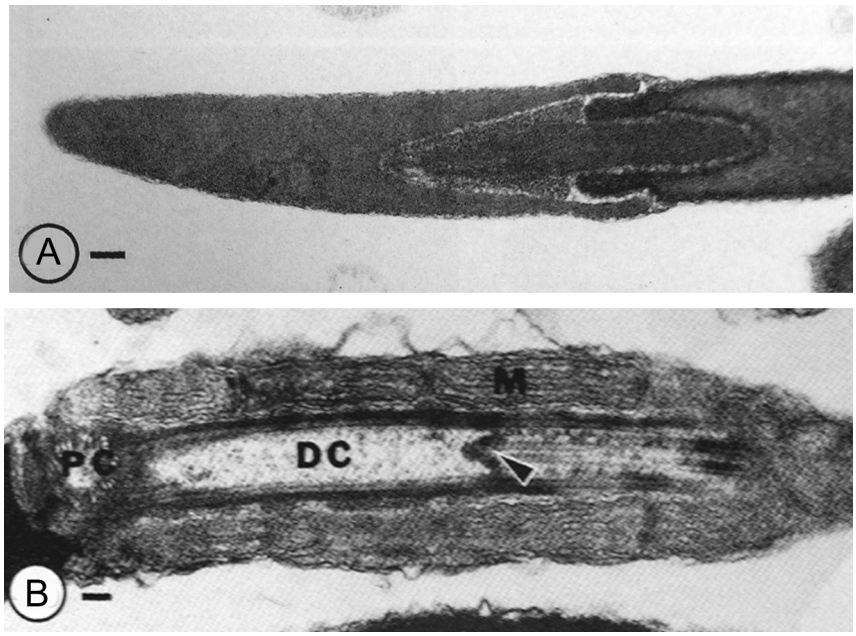

Fig. 8.12 *Gallus domesticus*. **A**. Longitudinal section of the acrosomal region**.** The acrosomal vesicle overlaps a perforatorium which inserts into a nuclear concavity. The perforatorium is not membrane bound and is surrounded by amorphous, granular material. **B**. Longitudinal section of the centrioles and midpiece, the proximal centriole (PC) is orientated at right angles to the distal centriole (DC) which is surrounded by mitochondria (M). Bars: 0.1 μm. From Thurston, R. J. and Hess, R. A. 1987. Scanning Microscopy 1**:** 1829-1839, Figs. 2b, 4b.

by Tingari (1973) who states that it forms in the epididymis by fusion of previously existing dense masses and that in the excurrent ducts the reduction in size of the central cavity of the proximal centriole may be due to deposition of dense matrix.

The distal centriole, contiguous with the proximal centriole, lies in the longitudinal axis of the sperm and is continuous with the axoneme. It is surrounded by the most anterior mitochondria of the midpiece. Unlike the condition in struthioniforms, the central singlets do not extend to the proximal end of the centriole. It is considered that these commence at its posterior end (Nagano 1962; Bakst and Howarth 1975; Gunawardana and Scott 1977) but some micrographs suggest a considerable penetration of the singlets into the distal end of the centriole (Fig. 8.12B). From a micrograph of Bakst and Howarth (1975) the length of the distal centriole is ca 1.8 μm and it extends for ca 0.4 of the length of the midpiece. It has nine triplets embedded in a ring of dense material (Lake *et al.* 1968; Bakst and Howarth 1975; present study). The distance from the proximal end of the distal centriole to the commencement of the inner paired microtubules is 0.9 μm (Thurston and Hess 1987).

Tannic acid fixation reveals for the proximal (Fig. 8.16J) and distal centrioles 13 protofilaments for subtubule A and 10 for each of B and C (Thurston and Hess 1987).

In the round spermatid the two centrioles are said to be of the same size and to lie end to end and almost in a straight line (Nagano 1962), the condition seen in the mature *Coturnix* sperm. However, Xia *et al.* (1986) clearly indicate a small proximal centriole at right angles to a long distal centriole in the round spermatid, as confirmed here for the spermatozoon in Fig. 8.11B.

Midpiece. The midpiece is ca. 3.7 μm long (agreeing with 4 μm, Grigg and Hodge 1949). It shows, in transverse section, four mitochondria encircling the axoneme (Fig. 8.11F) and 7 or 8 along its length (Fig. 8.11B), totaling ca 28-32, agreeing with approximately 30 according to Bakst and Howarth (1975). Their arrangement is helical (Lake *et al.* 1968; Bakst and Howarth 1975; Thurston and Hess 1987). The cristae form stacks of plates orientated longitudinally (Figs. 8.11B, 8.12B). The mitochondria in tangential section appear as closely fitting slightly elongate polygons (usually hexagons) (Bakst and Howarth 1975). The axoneme in the midpiece, posterior to the distal centriole, has nine conspicuous dense fibers (osmiophilic masses of Bakst and Howarth 1975); each fiber being in the radius of its doublet and enveloping the latter in its inner extremity. The dense fibers do not extend into the principal piece (Lake *et al.* 1968; Bakst and Howarth 1975; present study).

Annulus. A small, compact annulus marks the posterior limit of the midpiece (Fig. 8.11B).

Principal piece. This commences at the annulus and is defined by the presence, encircling the axoneme, of a fibrous sheath (Figs. 8.11B, G, 8.16P). This is amorphous in that it does not show the annulation or ribbing seen in ratites.

Endpiece. The endpiece (Figs. 8.11H, 8.16R) consists of the axoneme and plasma membrane and lacks the fibrous sheath. As in the principal piece, the A subtubule of each of the 9 doublets has dense contents, as first noted by Nagano (1962), and the outer dynein arms are more conspicuous than the inner arms. Posteriorly the two central singlets are replaced by a large dense structure, the so-called 'tip granule' (Fig. 8.11I). At this level and posteriorly the doublets are progressively disrupted. Location of the tip granule at the end of the two central singlets and not terminally is deduced from the observations of Woolley (1995) for *Coturnix coturnix*.

8.8.1.2 *Coturnix japonica*

In the present account the valid name *Coturnix japonica* is used in place of *Coturnix coturnix*, *Coturnix coturnix japonica* and *Coturnix coturnix* var. *japonica* employed in the various accounts summarized. The spermatozoon of *C. japonica* (Phasianidae) has been described by Marquez and Ogasawara (1975); Saita *et al.* (1980); Maretta *et al.* (1982); Lin and Jones (1993) and Woolley (1995). The dynamics of spermatozoal motility are described and illustrated

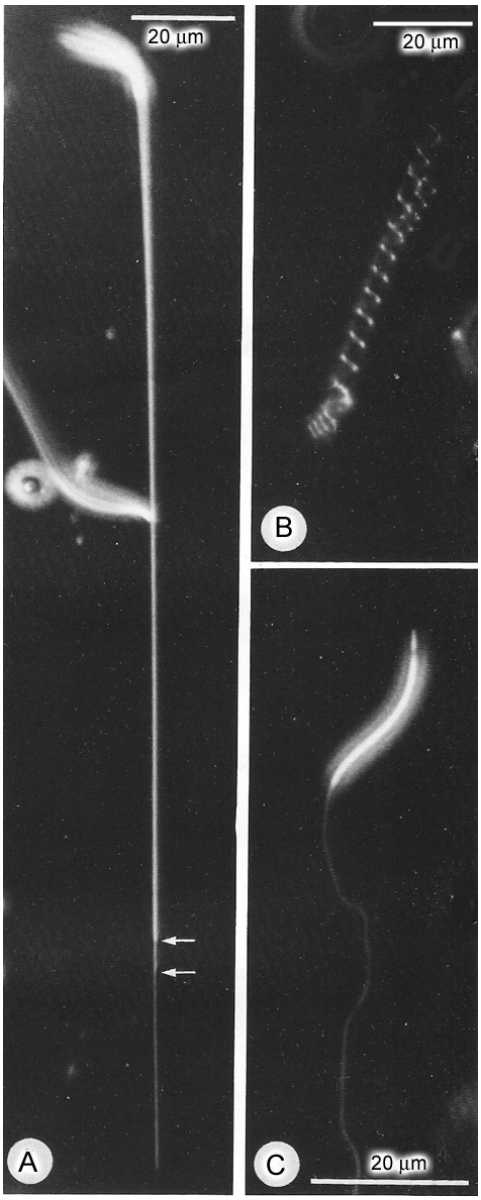

Fig. 8.13 *Coturnix japonica*. Light microscopy of spermatozoon. **A**. Immotile spermatozoon, wet preparation. Upper arrow indicates the midpiece-principal piece boundary. Lower arrow, the distal limit of the principal piece and its sheath. The long unsheathed more posterior region is here termed the endpiece. **B**. Flagellum after trypsin digestion, showing helical midpiece. **C**. The acrosome, nucleus, and proximal flagellum contrasted supravitally with eosin-yellow. After Woolley, D. M. 1995. Acta Zoologica (Copenhagen) 76**:** 45-50, Figs. 1-3.

by Vernon and Woolley (1999) who repeat the ultrastructural data of Woolley (1995). The following account is drawn chiefly from Woolley (1995).

General morphology. Eosin-yellow *in vivo* staining of the spermatozoon reveals a pointed conical acrosome many times shorter than the cylindrical nucleus (Fig. 8.13C). Light micrographs of the flagellum revealed three zones of decreasing thickness, later confirmed to be the midpiece, the proximal (sheathed) principal piece and the "distal principal piece" (endpiece) (Fig. 8.13A). The mean overall length of the flagellum was 207.6 μm. The mean lengths of the three zones (± one standard deviation) were: midpiece 161.4 (± 2.8) μm, proximal (sheathed) principal piece 5.4 (± 0.7) μm and endpiece 40.8 (± 1.7) μm (n = 10, a single bird).

Acrosome. The acrosome is conical, about 2.6 μm (Fig. 8.13C). The mode of attachment of the acrosome (Fig. 8.14A) involves an overlapping joint, as is usual in galliforms, with a perforatorium (length 1.5 μm) engaged in conical depressions in both the acrosome and the nucleus, and connected to each through a granular matrix.

Nucleus. The sperm nucleus is a curved cylinder, 20.6 μm long. The nuclear envelope is thickened where it lies against the inner acrosomal membrane.

Centriolar region. The neck of the spermatozoon contains two separate centrioles lying *almost* on the same axis. The proximal centriole is thickened abaxially to support an implantation plate; distally it ends in an annular thickening into which the base of the longer distal centriole is anchored (Fig. 8.14F-H). The triplet microtubules are not continuous between the two centrioles (Fig. 8.14F). Beyond the distal centriole there is a transition region characterized by the appearance of the central pair, by electron dense material peripheral to the nine doublets and by additional densities associated with the radial spokes, showing their 96 nm periodicity (Fig. 8.14F, I). The transition region is within the mitochondrial sheath.

Midpiece. Each mitochondrion is disc-shaped, diameter about 0.25 μm, and curved against the axoneme, with the packing of adjacent ones often making the profiles slightly hexagonal (Fig. 8.14J). Transverse sections typically show four mitochondria at any level (Fig. 8.14K). The arrangement can be modeled as four parallel 'out-of-register' chains of mitochondria surrounding the axoneme. The total number of mitochondria per sperm is estimated as ca 2,500. The midpiece terminates at a thin annulus (Fig. 8.14M).

Principal piece. The short "proximal principal piece" consists of the axoneme encased in a cylindrical fibrous sheath that tapers distally (Fig. 8.14N). Beyond this sheath, the axoneme is simple (Fig. 8.14O). Although the fibrous sheath is amorphous in the mature sperm, it develops as a series of circumferentially orientated hoops in the stage 2 spermatid (Lin and Jones 1993).

Endpiece. The endpiece exceeds 1.5 μm in length. In it the central pair of microtubules terminates first. Posterior to this, for about 0.4 μm, the center of

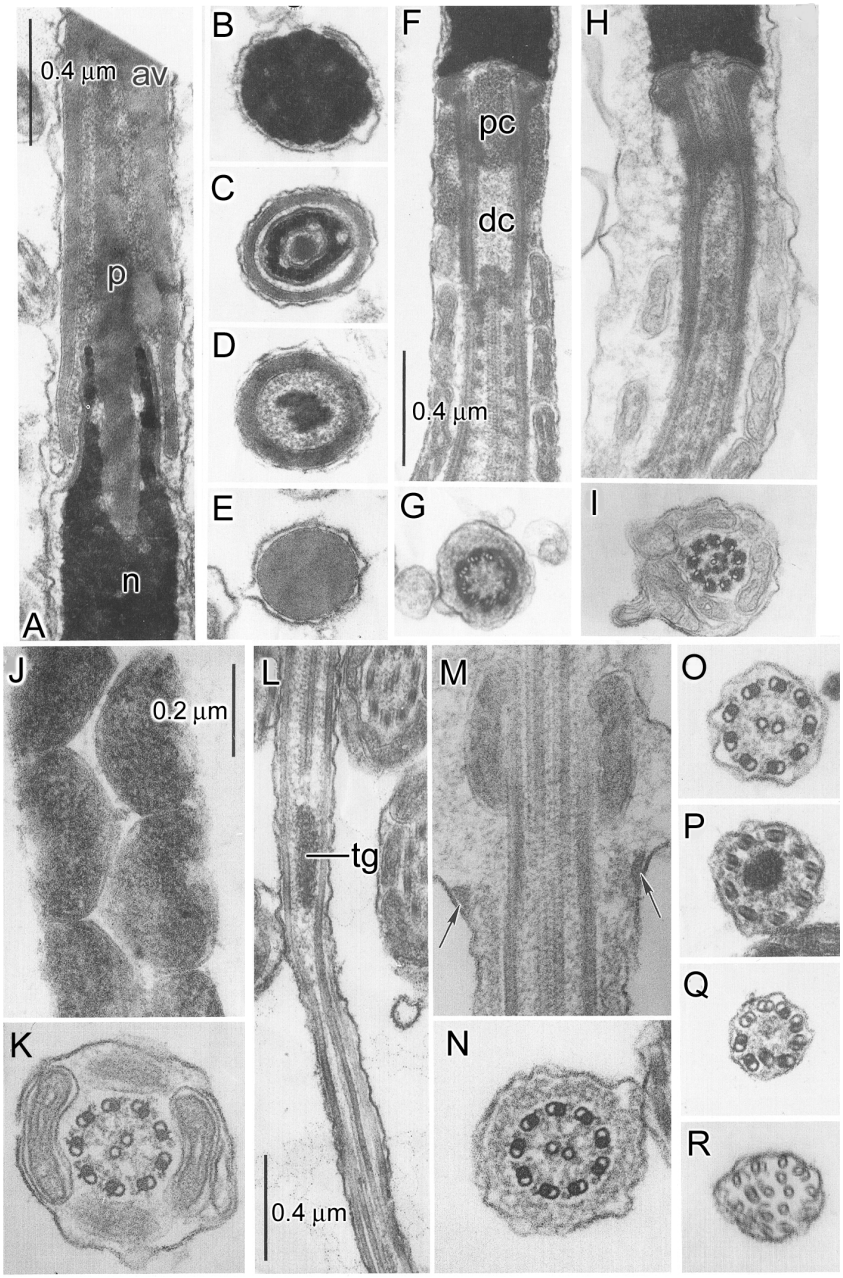

Fig. 8.14 *Coturnix japonica.* TEM of sperm. **A**. Longitudinal section (LS) through the junction between the acrosome vesicle (av) and the sperm nucleus (n). The perforatorium (p) inserts into each structure. **B**. Transverse section (TS) of nucleus,

Fig. 8.14 Contd. ...

the axoneme is occupied, as in *Gallus*, by an electron dense body tip granule (Fig. 8.14L, P). Thereafter the nine doublets become progressively simplified and end as singlets attached to the plasmalemma (Fig. 8.14Q, R) (Woolley 1995).

Remarks. Woolley (1995) gives an interesting discussion of the sperm of *Coturnix coturnix*, some points of which will be discussed here. The morphology of the acrosome and sperm nucleus is typical of galliform birds. Furthermore a conical acrosome, with a perforatorium occurs in most of the other non-passerine orders that have been examined ultrastructurally (reviewed by Asa and Phillips 1987).

As Woolley (1995) notes, the ultrastructure of the sperm neck in *Coturnix* has an unusual feature. In spermatozoa generally, there are two centrioles (at least, during development): a distal centriole that gives rise to the flagellum and a proximal one at right angles to it. This arrangement is also general in birds. In *Coturnix japonica,* however, the two centrioles lie *almost* in line. This condition is also seen in *C. chinensis* (8.8.1.3) and *Numida meleagris* (8.8.1.6).

As also noted by Woolley (1995), the general features of the flagellum in *Coturnix japonica* spermatozoa are qualitatively typical of non-passerine birds. Its dimensions, however, are unusual. First, at 208 μm, the flagellum is more than twice as long as in the other galliforms noted. In passerine bird sperm the flagella range up to 263 μm in length, in *Dendroica petechia,* the yellow warbler (Briskie and Montgomerie 1992). Flagellar length is useful taxonomically within avian genera, as shown for *Dendroica* by McFarlane (1963). The significance, taxonomic or physiological, of the flagellar

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Fig. 8.14 Contd. ...

which has a circular profile at all levels. **C**. TS of the region where acrosome and nucleus are interlocked. **D**. TS of caudal acrosome, showing the perforatorium centrally. **E**. TS of rostral acrosome. **F**. LS through the neck region, which consists of a proximal centriole (pc), a distal centriole (dc) and a transitional region with periodic densities suggestive of mechanical re-enforcement. **G**. TS through one of the centrioles, probably the distal one. **H**. A further LS of the neck to show the separate identity of the centrioles, indicated by their lack of continuity and slightly different axes. **I**. TS of the transition region. The microtubular triplets have been reduced to doublets, a central singlet has appeared and there are extra densities both peripherally and centrally. **J**. Tangential section of the midpiece to show the shape and arrangement of the mitochondria. **K**. TS of midpiece showing the most common arrangement of the mitochondria. **L**. LS of the flagellar tip showing particularly the *tip granule.* **M**. LS showing annulus *(arrows)* at the distal limit of the midpiece. **N**. TS proximal principal piece, where fibrous sheath occurs between the axoneme and the cell membrane. **O**. TS distal principal piece. **P**. TS flagellum at the level just beyond the termination of the central pair, showing the central *tip* granule (tg). **Q, R**. TS showing progressive reduction of the axoneme in the flagellar tip. After Woolley, D. M. 1995. Acta Zoologica (Copenhagen) 76**:** 45-50, Figs. 4-21.

elongation in *Coturnix* is unknown. However, the physiological explanation may lie in the positive correlation shown by Briskie and Montgomerie (1992) between sperm flagellar length in birds and the length of the sperm storage tubules in the female of the species.

The length of the midpiece in *Coturnix japonica* is noteworthy (Woolley 1995) and requires confirmation. Thurston and Hess (1987) estimated that the turkey, the domestic fowl and the Guineafowl all have 25-30 mitochondria in each spermatozoon (confirmed here for *Gallus*). For *Coturnix,* Saita *et al.* (1980), using electron micrographs of testicular tissue, estimated ca 350 mitochondria per sperm. The estimate of midpiece length (161 µm) in *Coturnix japonica* (=*Coturnix coturnix* var. *japonica*) by Woolley (1995) was made from light micrographs, supported by the very high frequency of midpiece profiles seen in the thin sections. This length measurement led to an estimate of mitochondrial number of ca 2500 per sperm. This would suggest a surprising 616 tiers of mitochondria. The only report of a midpiece approaching this length in non-passerine sperm is for the Order Columbiformes. In contrast, in the Passerida the mitochondria make a single spiral thread along much of the axoneme (reviewed by Asa and Phillips 1987, and this chapter).

8.8.1.3 *Coturnix chinensis*

General morphology. The ultrastructure of the spermatozoon of *Coturnix chinensis*, the Blue-breasted quail (Fig. 8.15A-L) is closely similar, excepting some dimensions, to that of *Gallus* and, particularly, of *Coturnix japonica*. It has the usual non-passerine components described above for *Gallus*. By light microscopy, approximate dimensions are: total length of the spermatozoon 91 µm; head (acrosome + nucleus) 9.7 µm; midpiece 24.4 µm (n=1).

Acrosome. The acrosome vesicle, by TEM, is approximately 1.9 µm long (n=2). It has the form of an elongate, straight cone. The base of the vesicle overlaps a short, narrower anterior region of the nucleus (Figs. 8.15C, E). The nuclear shoulders are rounded and not as distinct as in *Gallus*. In the large, conical subacrosomal space which occupies more than half the length of the acrosome vesicle, there lies a dense rod, the perforatorium, ca 1.3 µm long. This agrees with *C. japonica* and *Gallus* in abutting the apex of the subacrosomal space but differs from that of *Gallus* in more closely fitting the sides of the space, there being a relatively thin later of granular material between it and the vesicle laterally. Approximately the posterior third of the perforatorium is contained in, and closely fits, the anterior nuclear fossa (reduced endonuclear canal).

Nucleus. The nucleus is an elongate cylinder, ca 6.4 µm long (cf 20.6 µm for *C. japonica*), circular in cross section (Fig. 8.15B), slightly narrowing anteriad, but conspicuously clubbed basally on one side (Fig. 8.15C). Seen in the plane of the clubbing (Fig. 8.15C, F) the nucleus is tilted at an angle to the long axis of the midpiece and flagellum. In a plane at right angles to this (Fig. 8.15G) clubbing is absent and the nucleus appears to be in the long axis. The chromatin is homogeneous and electron-dense (Figs. 8.15C, F, G, H). Where it

appears granular (Fig. 8.15E) this may be due to incomplete maturity, as a transition from granular to condensed is noted by Maretta *et al.* (1982) for *C. japonica*. At its base there is a very considerable, slightly asymmetrical basal (implantation) fossa (Figs. 8.15F, G, H). As in *C. japonica*, the extreme tip of the nucleus is attenuated around the anterior half of the anterior fossa (Fig. 8.15E), being thinner on each side than in *Gallus*, and is asymmetrical so that in transverse section it may appear interrupted on one side (Fig. 8.15A).

Centriolar region. The neck of the spermatozoon contains two separate centrioles lying *almost* on the same axis (Fig. 8.15G, D), as in *C. japonica*. The triplet microtubules are not continuous between the two centrioles. The posterior limit of the distal centriole is difficult to define but appears to be three or four times the length of the proximal centriole. It is penetrated for about half of its length by a central axonemal element. This consists of a dense sheath around and capping the two central singlets (Fig. 8.15G). At the level of the tip of this sheath, the nine triplets of the centriole lie in a dense ring and their triplet structure is almost obscured though persistent (Fig. 8.15I). The ring is surrounded by the mitochondria of the midpiece.

Midpiece. Transverse sections of the midpiece typically show four mitochondria at any level (Fig. 8.15I, J). The length of the midpiece has not been determined by TEM, nor has its posterior limit been sectioned. Small outer dense fibers, one in the radius of and contiguous with, each doublet are present in the midpiece (Fig. 8.15J); in some sections they are considerably larger than those shown in this figure.

Principal piece. This is defined by the presence, encircling the axoneme, of a fibrous sheath (Fig. 8.15K). This is amorphous in that it does not show the annulation or ribbing seen in ratites.

Endpiece. The endpiece (Fig. 8.15L) consists of the axoneme and plasma membrane and lacks the fibrous sheath. As in the principal piece, the A subtubule of each of the 9 doublets has dense contents. A tip granule has not been demonstrated in the few sections obtained.

Remarks. The difference in length of the nucleus between *Coturnix coturnix* and *C. chinensis*, 20.6 μm versus 6.4 μm is remarkable. Even more disparate is the midpiece length of 161 μm contrasting with approximately 24 μm determined by light microscopy for *C. chinensis*. The two species agree, and differ from *Gallus*, in the much greater length of the midpiece, and in the almost co-linear arrangement of the proximal and distal centrioles, the proximal centriole being at right angles to the distal in *Gallus* as in other birds studied, with the exception of *Numida meleagris*.

The clubbing and angular deflection of the nucleus in *C. chinensis* is echoed in a light micrograph by Woolley (1995) of the sperm of *C. japonica* (Fig. 8.13A) but may reflect a dynamic situation as it is not evident in a second micrograph (Fig. 8.13C).

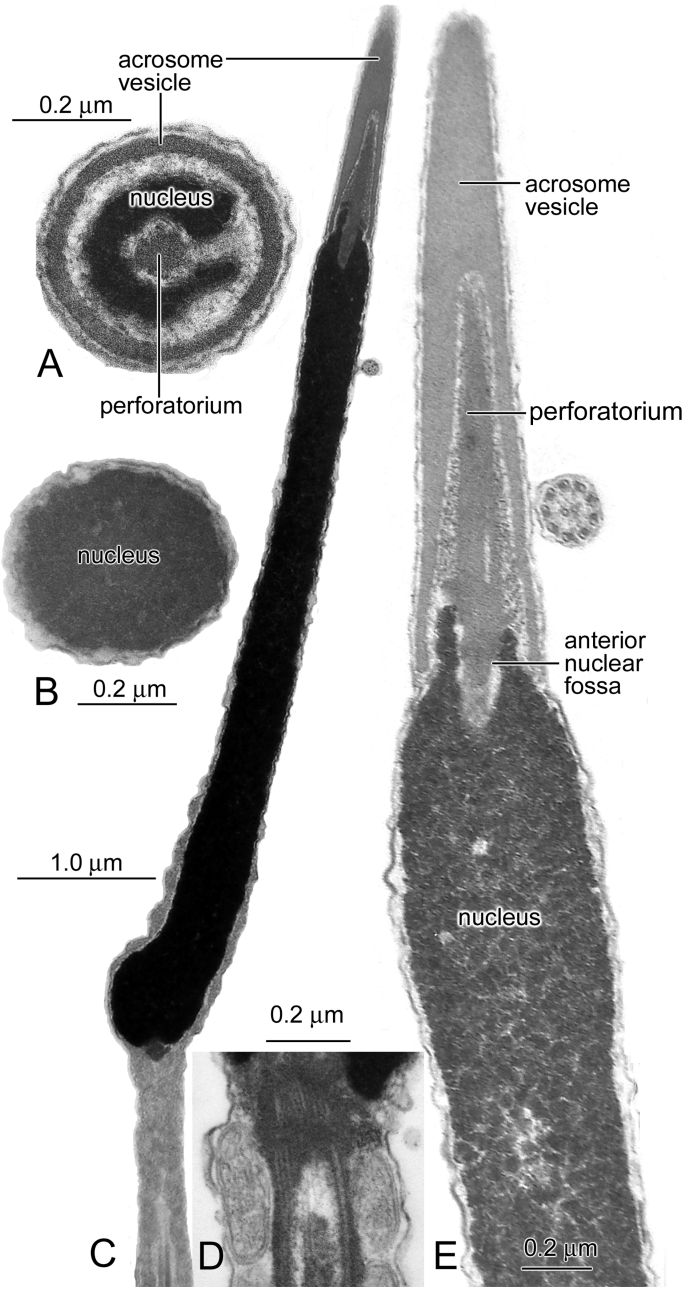

Fig. 8.15 *Coturnix chinensis*. TEM of sperm. **A**. Transverse section (TS) acrosome through the tip of the nucleus, showing asymmetry of the anterior nuclear fossa containing the base of the perforatorium. **B**. TS nucleus showing circular profile.

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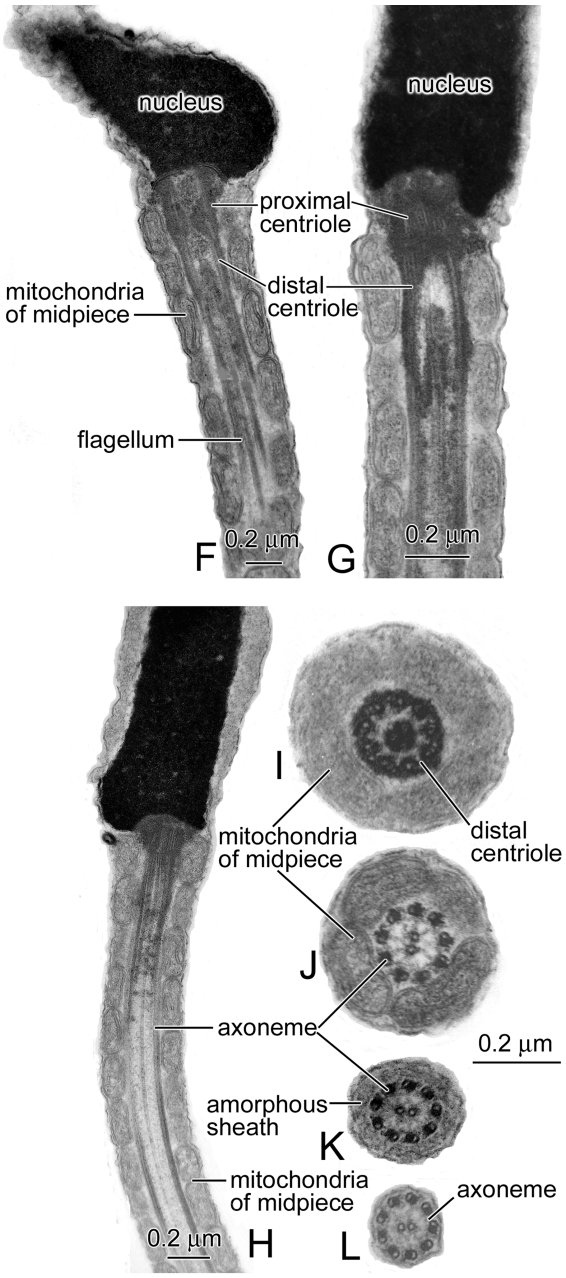

Fig. 8.15

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C. LS of the entire acrosome and nucleus, with centriolar and anterior midpiece. **D**. Detail of centriolar region, showing proximal centriole almost in line with the distal

Fig. 8.15 Contd. ...

8.8.1.4 *Meleagris gallopavo*

The spermatozoon of the turkey, *Meleagris gallapavo* (Phasianidae), has been described ultrastructurally (Marquez and Ogasawara 1975; Bakst and Sexton 1979; Baccetti *et al*. 1980; Bakst 1980; Bradley *et al*. 1986; Thurston and Hess 1987). The account of Thurston and Hess (1987) gives a valuable comparison of the sperm of Turkey, Guineafowl and rooster and is summarized here for the turkey, with some reference to the other species and other accounts.

General morphology. As shown by SEM, the general shape of turkey, Guineafowl ('guinea') and rooster spermatozoa is remarkably similar. As in *Gallus*, the spermatozoa are long and narrow with a vermiform appearance, and an acrosome, nucleus, midpiece (Fig. 8.16A). The nucleus is usually curved. The anterior end of the sperm consists of a conical acrosome which is most prominent in Guineafowl sperm (Fig. 8.16B). The acrosome of the turkey is 1.0-2.6 µm (mean 1.8) µm long (Marquez and Ogasawara 1975), compared to 2 µm or greater (Thurston and Hess 1987), reaching 2.5 µm (Marquez and Ogasawara 1975) for the rooster acrosome. The nucleus gradually increases in diameter from its junction with the acrosome to its distal end at the beginning of the midpiece. The turkey sperm nucleus is said to be shorter (7 to 9 µm) than that of Guineafowl or rooster (10 to 14 µm in length) (Thurston and Hess 1987). However, there is considerable overlap between species as Marquez and Ogasawara (1975) give a length of 7.2-11.0 µm, with a mean width of 0.8, for the turkey. Junction of the nucleus with the midpiece at the neck region is not as conspicuous as in Guineafowl sperm (Thurston and Hess 1987).

The flagellum comprises most of the length of the spermatozoon, although the junction between the principal and end piece could not be discerned with SEM. Turkey and Guineafowl sperm flagella are usually 60-65 µm long (Thurston and Hess 1987) (61 µm, Marquez and Ogasawara 1975), cf often more than 70 µm in rooster spermatozoa. The overall sperm length of 75-80 µm in turkey and Guineafowl sperm is also less than that of rooster sperm (90 µm). For all three species, the spermatozoa increased in width from the acrosome to a maximum of 0.5-0.7 µm at the junction of the nucleus with the midpiece. The width then decreased to 0.1-0.2 µm at the end of the flagellum (Thurston and Hess 1987).

Fig. 8.15 Contd. ...

centriole. **E**. LS acrosome and adjacent nucleus. **F**. LS base of nucleus, centriolar region and anterior midpiece. **G**. Same, in a plane approximately at right angles. **H**. Same but showing some mitochondria around the nucleus. **I**. TS distal centriole, within the midpiece, and through dense sheath at commencement of central singlets. **J**. TS midpiece and contained axoneme showing minute outer dense fibers. K. TS principal piece, showing amorphous sheath surrounding axoneme. L. TS endpiece. Original.

Acrosome. As seen by TEM, the membrane-bound cap-like acrosomal vesicle (Fig. 8.16C, D) contains a granular, amorphous material which surrounds the perforatorium, and adjacent to the perforatorium is fine, granular material of moderate density (more abundant in rooster spermatozoa. At its distal end, the acrosomal cap encircles projections of chromatin from the apical portion of the nucleus. At its posterior end the perforatorium inserts into a concavity of the nucleus and extends obliquely forward approximately half the length of the acrosomal cap in turkey as in rooster, contrasting with nearly the entire length of the cap in Guineafowl sperm. Thus, the perforatorium of the turkey and rooster, at 1.0 μm, is appreciably shorter than that of the Guineafowl (1.9 μm). The base of the perforatorium of turkey is narrower than that of rooster and Guineafowl sperm (Fig. 8.16A).

The substance of the perforatorium is dense and amorphous, and often interrupted by lucent channels which contain granular material similar to that adjacent to the perforatorium (Fig. 8.16D, E).

Nucleus. A longitudinal section of the base of the nucleus and anterior portion of the midpiece is shown in Fig. 8.16F. The nuclear chromatin is dense and granular with occasional small lucent areas giving it a mottled appearance (Fig. 8.16G). The distal end of the nucleus terminated in a concavity, the implantation fossa (Fig. 8.6F, H).

Centrioles and neck. For turkey (Figs. 8.16F, H) and rooster sperm, dense processes extended radially from the proximal centriole wall to abut against the nuclear membrane in the implantation fossa. The centriole complex plus the projections constitute the non-striated connecting piece of the neck of the spermatozoon (Bakst and Howarth 1975). Turkey and rooster have a proximal centriole orientated perpendicular to the distal centrioles (contrast Guineafowl below).

In *Numida*, as for all three species, cross sections of the centrioles have the typical 'pinwheel' arrangement of nine triplet microtubules embedded in a cylindrical, dense wall (Fig. 8.16H). Each projection of the non-striated connecting piece is associated with one set of the triplet microtubules (Fig. 8.16H).

Midpiece. From SEM micrographs of turkey sperm (Fig. 8.16D) and those of midpiece cross sections (Fig. 8.16K) where the mitochondrial length varied progressively from long to short, it is ascertained (Thurston and Hess 1987) that the midpiece had 25-30 mitochondria arranged in an helical pattern. Marquez and Ogasawara (1975) observed lengths for the midpiece of 4.0-6.0 μm; plate-like mitochondria numbering four per turn surround the axoneme; this arrangement is repeated seven times to give approximately 28 mitochondria per midpiece. In surface view (Thurston and Hess 1987), the mitochondria are polygonal with the dimensions of approximately $0.8 \times 0.11 \times 0.3$ μm. In contrast to Guineafowl, cristae of turkey and rooster sperm mitochondria are parallel to the outer membrane (Fig. 8.16F).

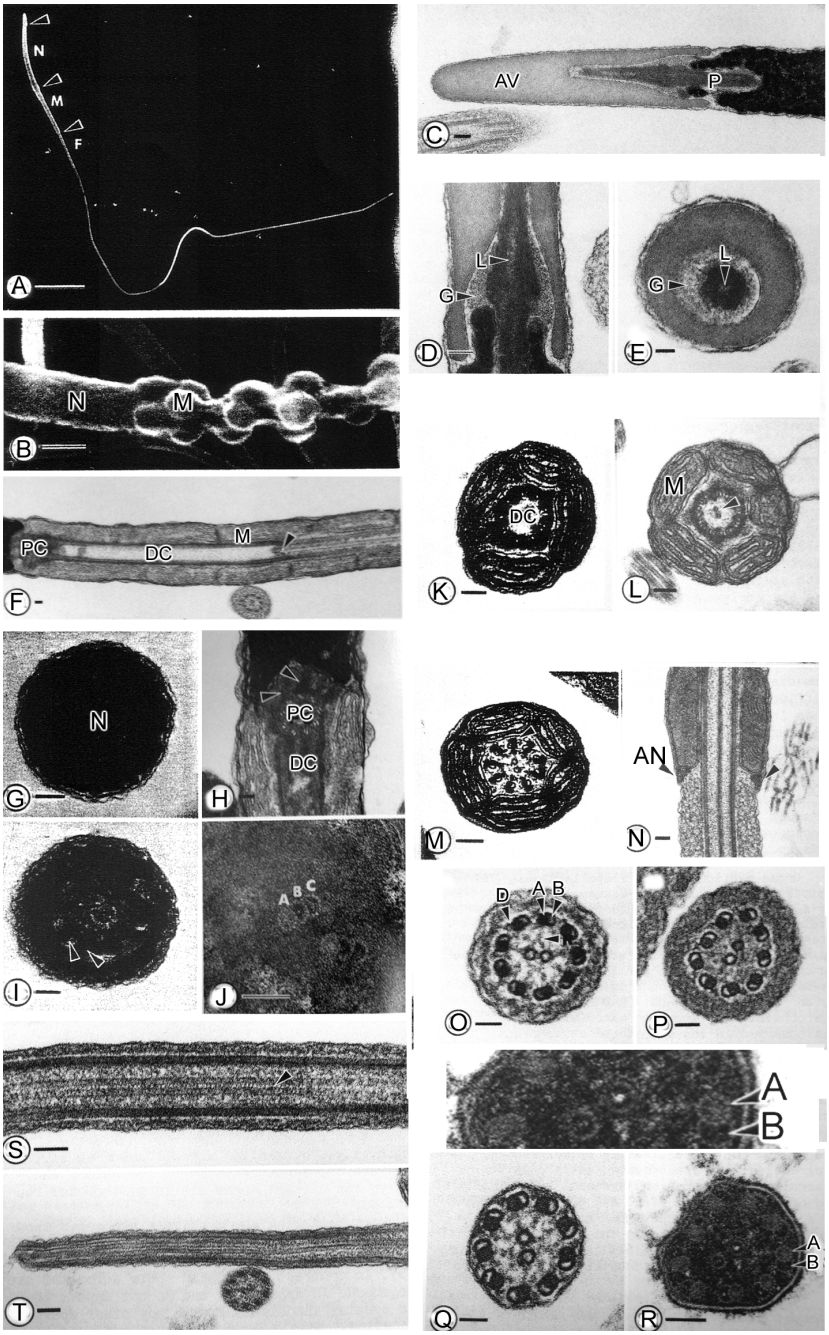

Fig. 8.16 A-H, K-M, O, **Q-T**. *Meleagris gallopavo*. Turkey spermatozoa. **I, N**. *Numida meleagris*, Guineafowl. **J**, **P, R**. *Gallus gallus*, Rooster spermatozoa. **A**, **B**. SEM of turkey sperm. **A**. The narrow, vermiform shape of the turkey spermatozoon is typical

Fig. 8.16 Contd. ...