



STRUCTURAL AND FUNCTIONAL ASPECTS OF SPERM HEAD
MORPHOLOGY IN THE PLAINS MOUSE, Pseudomys australis

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by

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ABSTRACT

The primary aim of this study was to examine the structure and function of the three curved hooks on the apical margin of the sperm head of the plains mouse, Pseudomys australis.

There is a single dorsal hook, and two ventral hooks which are united caudally, and each has a characteristic shape. Electron microscopical studies of late spermatids and spermatozoa revealed that the dorsal hook is structurally similar to the falciform sperm head of the rat. The core of the ventral hooks consists of sub-acrosomal material, and bundles of microfilaments which appear to have a morphogenetic role in shaping the hooks. The peripheral layer is an extension of the post-acrosomal dense lamina, with rows of parallel ridges linking the plasma membrane and lamina. The structure of the sperm head posterior to the hooks follows the general mammalian pattern.

Electrophoretic analysis of sperm head proteins revealed that the major constituent of the sub-acrosomal material in the hooks is one or more 15 kd polypeptides. A 42 kd protein which comigrates with actin is also present, thus confirming the localization of actin filaments in the ventral hooks using NBD-phalloidin.

Epididymal maturation involves a remodelling of the apical segment of the acrosome, migration of the cytoplasmic droplet, acquisition of motility, and stabilization of sperm head and tail structures, including the ventral hooks, by disulphide bonds.

Oocyte-cumulus complexes were studied at the EM level after routine fixation, or staining with ruthenium red, but no unusual structural features were noted in either the cumulus oophorus or zona pellucida. Attempts to study gamete interactions in vitro were hampered by initial difficulties in superovulating female plains mice. Hence, a

heterologous I.V.F. system was used, with plains mouse sperm and zona-free rat and laboratory mouse oocytes, but only low rates of fertilization were obtained.

The functional design of the sperm head is discussed in relation to a possible role for the hooks in assisting penetration of the oocyte investments. The possibility that the ventral hooks arose as a reproductive isolation mechanism is also discussed.

PhD THESIS AMENDMENTS

V

THESIS: STRUCTURAL AND FUNCTIONAL ASPECTS OF SPERM HEAD
MORPHOLOGY IN THE PLAINS MOUSE, *Pseudomys australis*.

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On the recommendations of the examiners, the following changes should be inserted in the text to replace the existing sentence(s):

Page iii, lines 8-10:

" The core of the ventral hooks consists of sub-acrosomal material and bundles of microfilaments. These appear to be actin filaments, and probably assume a role in shaping the hooks."

Page iii, lines 16-18:

" A 42 kd protein which comigrates with skeletal muscle actin is also present in the ventral hooks. The ventral hooks stain strongly with the filamentous actin-specific probe, NBD-phalloidin, suggesting that this protein is actin."

Page iii, last line to page iv, line 3:

"Hence, a heterologous sperm-egg fusion system was used, with plains mouse sperm and zona-free rat and laboratory mouse oocytes. However, only low rates of fertilization were obtained, due to unsuitable culture conditions for inducing the acrosome reaction in plains mouse sperm."

Page 144, lines 27-29:

" Hence, it appears that one of the main functions of these microfilaments could be to shape and curve the ventral hooks. However, this requires experimental verification."

Page 198, lines 12-16:

" However, investigations of sperm-egg interactions in this species have been hampered by difficulties encountered in inducing superovulation, so attempts have been made to investigate the possible role of the ventral hooks in sperm-egg fusion using penetration of zona-free rat and laboratory mouse oocytes by plains mouse sperm."

Page 229, line 4:

Heading 4.3.b needs to be changed to :

Heterologous Insemination of Zona-Free Eggs

Page 229, lines 22,23:

The word "fertilization" should be changed to "penetration".

Page 234, lines 5-9:

" Bundles of 6nm microfilaments, presumably actin based on NBD-phalloidin staining and electrophoretic analysis of hook extracts, occur in the hooks. These may be responsible for the the acquisition and maintenance of the shape of the apical regions of the hooks."

Page 252, lines 1,2:

" Bundles of actin filaments possibly maintain the curvature of the hooks, and the shape of their apical regions."

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SECTION 1 : INTRODUCTION

A. GENERAL INTRODUCTION

Fertilization in mammals involves the union of haploid male and female gametes to produce a diploid zygote. Highly specialized gametes have evolved, as well as modifications of the reproductive tracts for their transport, nourishment and protection.

The male gametes or spermatozoa are formed in the testis by a series of cell divisions and differentiation stages, but undergo further maturation during transit through the epididymis. The female gametes or oocytes are produced in the ovary, and become surrounded by several extra-cellular coats. They are subsequently shed into the oviduct, where fertilization occurs, after which the developing embryo is nourished and protected by the female reproductive tract until birth (Austin and Short, 1982).

It is the purpose of this review to examine the morphological and biochemical features of the mature spermatozoon and oocyte, and the processes leading up to, and including, incorporation of the spermatozoon into the oocyte at fertilization. The material presented will be limited to eutherian mammals, from which most of our knowledge of mammalian fertilization is derived.

B. CHARACTERISTICS OF SPERMATOOZOA

The mature spermatozoon consists of the compact "head", which contains the haploid set of chromosomes, and the "tail", which is a modified flagellum that provides the cell with its locomotory force. Although they are highly differentiated cells, and often vary in size as

well as in the shape of the head, eutherian spermatozoa all conform to a basic design, and have many components and properties in common with somatic cells (Fawcett, 1975).

1. VARIATIONS IN SIZE AND SHAPE

1.1 Size

The total length of the spermatozoon varies from about 41 μm in the sperm whale to 250 μm in the Chinese hamster. The length of rodent sperm is often 100-250 μm , whilst in other eutherian groups, the spermatozoa are usually 50-80 μm in length. These are, however, very broad classifications, and exceptions do occur (Friend, 1936; Bishop and Walton, 1960). In general, there is an inverse correlation between sperm size and body mass (Cummins and Woodall, 1985).

In contrast to the total length, there is considerably less variation in the size of the sperm head. This generally measures about 7-9 μm in length (Bishop and Walton, 1960), but in many primates and cetaceans, it is only 4.5-5.0 μm long (Bishop and Walton, 1960; Gould, 1980; Fleming et al., 1981), whilst the length of the sperm head in the Asiatic musk shrew is 21.5 μm (Green and Dryden, 1976).

The middle piece of the sperm tail usually comprises one quarter to one third of the length of the tail, although in higher primates and the bottlenose dolphin, it is extremely short (Friend, 1936; Gould, 1980; Fleming et al., 1981). In most species, therefore, the variation in total spermatozoon length is largely attributable to variations in the length of the middle and principal piece of the tail (Bishop and Walton, 1960; Cummins and Woodall, 1985).

1.2 Shape of the Sperm Head

The eutherian sperm head is flattened dorso-ventrally, but the shape is highly characteristic of the species (Fawcett, 1970), although slight inter-strain differences may occur (Beatty and Sharma, 1960).

In many non-rodent species, the sperm head is flattened, and spatulate or ovoid in shape. The anterior border is rounded and may be bulbous or slightly thickened. In sagittal section, the sperm head is wedge-shaped, pear-shaped, or shows little change in thickness from base to tip (Nicander and Bane, 1966; Bedford, 1974a; Gould, 1980).

Rodents, on the other hand, show considerable variation in the shape of the sperm head. In hystricomorph rodents, it is usually ovoid or paddle-shaped (Fawcett, 1965; Jones, 1975), but is falciform or hooked in cricetid rodents (Bishop and Austin, 1957). In the Muridae, however, both hooked and ovoid shapes occur (Friend, 1936; Breed, 1983). The length, shape and orientation of the hook varies between species (Friend, 1936; Bishop and Austin, 1957). The two main variations are, (1) the entire sperm head is curved and gently tapers to an anterior tip e.g. rat; and (2) the posterior region of the sperm head is broader, with a thin hook of variable curvature protruding from the apical margin e.g. laboratory mouse (Friend, 1936; Bishop and Austin, 1957).

Another aspect of sperm head shape that merits attention is variation within an ejaculate. Most species show minimal variation, but in man and the gorilla, an extreme degree of pleomorphism is evident (Bedford, 1967; Zamboni et al., 1971; Gould, 1980). Such is the variation in human ejaculates, that it led Zamboni et al. (1971) to state: "The heterogeneity of human spermatozoa observed in this study is so great that it defies any attempt on our part to define the morphological characteristics of the head of a normal spermatozoon".

2. STRUCTURAL AND BIOCHEMICAL FEATURES

2.1 General Organization of the Sperm Head

The sperm head is a highly differentiated and specialized structure. It consists of: (a) the nucleus, which contains the condensed chromatin surrounded by a nuclear envelope; (b) the acrosome, a Golgi-derived organelle that consists of a membrane-bound collection of hydrolytic enzymes; (c) the sub-acrosomal space, that separates the nucleus and acrosome; (d) the post-acrosomal region, consisting of a sub-membranous lamina that overlies the nucleus posterior to the acrosome; and (e) a plasma membrane, which covers the entire head and tail of the spermatozoon (Fawcett, 1970; 1975). Each of these regions will now be discussed in greater detail.

2.2 Nucleus

During spermiogenesis, the nucleus is transformed into a highly condensed and inert structure. It acquires a species-characteristic shape, which is largely responsible for the overall shape of the sperm head, except in species with large acrosomes, such as the guinea pig (Fawcett, 1965; 1975). The compaction and streamlining of the sperm nucleus is thought to protect the male genome, increase the hydrodynamic efficiency of motility, and assist in penetration of the extracellular coats around the oocyte (Bedford, 1974b; Fawcett, 1975).

Ultrastructural studies have shown the nucleus to consist of homogeneous, electron-dense material, with little or no observable fine structure. Small irregular cavities or nuclear vacuoles are found in most species, and are usually 70-100 nm in diameter, although they may be larger in human sperm (Fawcett and Ito, 1965; Saacke and Almquist, 1964a; Fawcett, 1970). The freeze-fracture technique, however, has

revealed a definite sub-structure in the eutherian sperm nucleus. The chromatin is arranged in the form of stacks of lamellae that run in the longitudinal axis of the sperm head (Koehler, 1966, 1970; Plattner, 1971; Mortimer and Thompson, 1976; Pfaller et al., 1976; Koehler et al., 1983; Livolant, 1984). Although some species variation is evident, there generally appears to be about 10-12 lamellae, which are 10-25 nm in thickness, and spaced 20-30 nm apart (Koehler, 1966, 1970; Plattner, 1971; Koehler et al., 1983). In the human sperm nucleus, however, lamellae have not been observed, although some indication of an ordered structure has been obtained (Koehler, 1972). This may be due to the extreme variability in the degree of nuclear condensation which characterizes human sperm (Fawcett, 1975), and is probably the result of incomplete condensation during spermiogenesis (Zamboni et al., 1971; Pedersen, 1974).

A typical nuclear envelope completely surrounds the nucleus, consisting of two membranes which are 7-10 nm apart, and separated from the chromatin by a space of only a few angstroms (Fawcett, 1970; Pedersen, 1974). On the posterior margin of the sperm head, there is a circumferential constriction known as the striated or posterior ring, which represents multiple points of fusion between the nuclear membranes and the plasma membrane. Posterior to this constriction, the nuclear envelope is reflected away from the chromatin to form the posterior nuclear space, which contains less dense nuclear material (Wooding and O'Donnell, 1971). This portion of the nuclear envelope is characterized by tightly packed, hexagonal arrays of nuclear pores, which are absent from the membranes anterior to the posterior ring (Koehler, 1970; Plattner, 1971; Friend and Fawcett, 1974; Stackpole and Devorkin, 1974; Mortimer and Thompson, 1976). It is called the redundant nuclear

envelope, and may extend back into the neck region before returning to a position close to the chromatin (Fawcett, 1970, 1975).

At the caudal margin of the sperm head, there is a recess called the implantation fossa, which is the site of the head-tail junction. It is lined by the nuclear envelope, which is devoid of pores and may contain particle-free zones (Friend and Fawcett, 1974; Stackpole and Devorkin, 1974). The nuclear membranes are linked by periodic fine striations, spaced about 6 nm apart (Pedersen, 1974), and the outer membrane has a dense thickening on its outer aspect. This band of dense material is called the basal plate, and forms the point of articulation with the tail (Nicander and Bane, 1962; Fawcett, 1965, 1970, 1975).

The nucleus contains DNA and protein, in approximately equal quantities (Pogany et al., 1981), but is devoid of RNA, and there is a complete lack of DNA transcription (Bhattacharyya and Zaneveld, 1983). During spermiogenesis, the somatic histones are replaced by a new class of nuclear basic protein called protamines (Balhorn et al., 1977). These are characterized by a lower molecular weight of 5-9 kd, 46-55 amino acid residues, a very high content of arginine (47-61%) and cysteine (7-16%) residues, a well conserved N-terminal sequence, and a middle portion consisting almost entirely of arginine and cysteine residues in positions 16 to 40 (Coelingh et al., 1969; Monfoort et al., 1973; Bellve et al., 1975; Coelingh and Rozijn, 1975; Calvin, 1976a; Balhorn et al., 1977; Bellve and Carraway, 1979; Gaastra et al., 1978). The number of protamines varies from one in the bull, boar, rat, guinea pig, stallion and possibly ram (Coelingh et al., 1969; Monfoort et al., 1973; Calvin, 1975, 1976a; Coelingh and Rozijn, 1975), to two in the mouse, rabbit and man (Bellve et al., 1975; Calvin, 1976a; Balhorn et al., 1977; Bellve and Carraway, 1979; Gaastra et al., 1978). Recent studies suggest that small quantities of other histone-like or non-histone proteins may also

be present in the nucleus of ram, mouse and human sperm (Avramova et al., 1980; Pruslin and Rodman, 1983; Rodman et al., 1983, 1984), and may form a proteinaceous nuclear skeleton (Tsanev and Avramova, 1981; Pruslin and Rodman, 1983). The high cysteine content of protamines is thought to be responsible for the large number of disulphide linkages in mature sperm nuclei, which, in combination with the dense packing of the chromatin, forms a rigid, stable and inert nucleus (Bedford and Calvin, 1974).

Several investigators have suggested that the chromatin is organized into nucleosomes as in somatic cell nuclei (Gusse and Chevalier, 1980; Tsanev and Avramova, 1981). In other studies, however, a "beads-on-string" appearance of the chromatin fibres has not been observed (Young and Sweeney, 1979; Evenson et al., 1978), and Pogany et al., (1981) calculated that sperm chromatin arranged into nucleosomes could not be packaged into the available nuclear volume. Hence, numerous models have appeared, postulating that the chromatin exists as supercoiled DNA (Pogany et al., 1981; Balhorn, 1982), arranged into sheets or lamellae (Sipski and Wagner, 1977; Koehler et al., 1983), and the chromatin may behave as a cholesteric liquid crystal (Sipski and Wagner, 1977; Livolant, 1984).

2.3 Acrosome

The acrosome is a membrane-bound organelle which covers the anterior half to three quarters of the nucleus, and extends for a variable length beyond it (Nicander and Bane, 1966; Fawcett, 1970). It is often divided into three parts: (a) the apical segment, which projects beyond the anterior border of the nucleus; (b) the principal segment, which covers a variable portion of the nuclear surface; and (c) the equatorial segment, in the posterior or caudal region (Fawcett,

1970, 1975). The acrosome is a Golgi-derived structure which forms during spermiogenesis, and is postulated to aid sperm penetration of the extra-cellular coats around the oocyte (Yanagimachi, 1981) and is involved in sperm-egg fusion (Bedford et al., 1979).

The apical segment exhibits considerable variation in size and shape between species. It may extend quite a distance beyond the apical margin of the nucleus and assume a species-characteristic shape as in the guinea pig, ground squirrel, musk shrew, field vole, European mole and some primates (Fawcett, 1965; Fawcett and Phillips, 1969b; Bedford, 1974a; Green and Dryden, 1976; Koehler, 1978a; Castellani-Ceresa et al., 1980). In most species, however, the apical segment is smaller and less bizarre in shape, and usually projects only a short way beyond the nucleus (Nicander and Bane, 1966; Fawcett, 1970, 1975). The anterior margin may be rounded and bulbous, thereby producing a U-shaped marginal thickening (Bedford, 1964; Nicander and Bane, 1966; Jones, 1975; Gould, 1980). In the bottlenose dolphin, armadillo, stallion, elephant and many primates the apical segment is extremely small, and is barely evident (Nicander and Bane, 1966; Nagy and Edmonds, 1973; Bedford, 1974a; Jones et al., 1974; Fleming, et al., 1981).

The principal segment, however, shows little variation between species except in the area of the nucleus it covers (Fawcett, 1975). The only unusual feature of this region is the discoid protrusions which occur in some hystricomorph rodent sperm (Jones, 1975; Berrios et al., 1978).

The junction between the principal and equatorial segments is usually characterized by a decrease in the thickness of the acrosome (Nicander and Bane, 1966). The equatorial segment often appears as a semi-lunar area or narrow circumferential band at the posterior margin of the acrosome, and usually constitutes one fifth to one third of the

acrosome covering the nucleus (Nicander and Bane, 1966; Yanagimachi and Noda, 1970a; Pedersen, 1974; Bustos-Obregon and Flechon, 1975). In the rat and mouse, however, it covers most of the lateral surface of the sperm head, whilst the principal and apical segments are restricted to a narrow band along the convex margin (Phillips, 1977b).

The acrosome consists of moderately electron-dense granular material enclosed within a continuous unit membrane. It is usual to describe both an outer acrosomal membrane (OAM) and inner acrosomal membrane (IAM), although they are continuous at the posterior border of the equatorial segment (Fawcett, 1975). The acrosomal contents generally appear more or less homogeneous, although heterogeneities have been reported in the apical segment of some species (Fawcett, 1965; Fawcett and Phillips, 1969b; Yanagimachi and Noda, 1970a; Wooding and O'Donnell, 1971; Flechon, 1979a). An ordered sub-structure has also been reported in the acrosome of rat and bull sperm (Phillips, 1972; Friend and Fawcett, 1974; Olson and Winfrey, 1985). The contents of the equatorial segment may appear denser (Fawcett, 1970), and periodic striations linking the acrosomal membranes have been observed in this region (Moore and Bedford, 1978a; Russell et al., 1980; Olson and Winfrey, 1985).

The acrosomal membranes differ from the plasma membrane in having a higher content of cholesterol, and are less fluid (Vijayasarathy et al., 1982). They are typically trilaminar, although a pentalaminar appearance has been reported in some species, especially in the equatorial segment (Wooding and O'Donnell, 1971; Pedersen, 1974; Roomans, 1975; Moore and Bedford, 1978a). Linear arrangements of particles have been demonstrated in the OAM and IAM (Koehler, 1972; Friend and Fawcett, 1974; Stackpole and Devorkin, 1974; Friend, 1977; Suzuki, 1981), and platinum/carbon surface replicas have revealed that the OAM in the equatorial segment of a variety of species consists of hexagonally arranged particles spaced

17 nm apart (Phillips, 1977a, 1977b).

The acrosome has been considered to be a modified lysosome (Allison and Hartree, 1970), although more recent work seems to suggest that it may represent a modified zymogen or secretory granule (Friend, 1977; Harrison, 1983). It contains a large number of proteins, and a variety of carbohydrate moieties (Hartree and Srivastava, 1965; Garner et al., 1971). Since initial claims that a variety of lysosomal enzymes are present in the acrosome, considerable interest has been generated in the various acrosomal enzymes, and their possible role in the fertilization process (McRorie and Williams, 1974; Harrison, 1983).

One enzyme in particular, a trypsin-like protease called acrosin (Zaneveld et al., 1971), has been studied extensively, due mainly to the finding that fertilization is inhibited by trypsin inhibitors (Stambaugh et al., 1969; Zaneveld et al., 1970, 1971), and acrosomal extracts containing this enzyme can solubilize the zona pellucida (Srivastava et al., 1965; Stambaugh and Buckley, 1968; Meizel and Mukerji, 1976). Acrosin is a serine protease that shares many properties with trypsin, but not chymotrypsin (Morton, 1976; 1977). It exists almost entirely in the form of an inactive precursor called proacrosin (Huang-Yang and Meizel, 1975), which has a molecular weight of 53-73 kd (Huang-Yang and Meizel, 1975; Meizel and Mukerji, 1975, 1976; Polakoski and Parrish, 1977). At the time of the acrosome reaction, proacrosin is converted to multiple forms of active acrosin, of molecular weights of 30-41 kd (Meizel and Mukerji, 1975, 1976; Polakoski and Parrish, 1977; Brown and Harrison, 1978; Goodpasture et al., 1981). This conversion occurs at neutral pH by way of an autocatalytic process (Meizel and Huang-Yang, 1973), although it may also involve other enzymes (McRorie et al., 1976; Srivastava and Ninjoor, 1982), or uterine glycosaminoglycans (Wincek et al., 1979). Acrosin is optimally active at pH 8-9, requires Ca^{++} for

maximal activity, and is inhibited by natural as well as synthetic trypsin inhibitors (Huang-Yang and Meizel, 1975; Meizel and Mukerji, 1975, 1976). It is generally accepted that acrosin is located in all regions of the acrosome except the equatorial segment (see reviews by Morton, 1976, 1977), although a recent report by de Vries et al. (1985) suggests that acrosin is present in the equatorial segment of boar sperm.

Some of the other enzymes thought to be found in the acrosome include hyaluronidase, which has a similar localization to acrosin (see reviews by McRorie and Williams, 1974; Morton, 1976, 1977); acid proteases (Polakoski et al., 1973); alkaline proteases (Ninjoor and Srivastava, 1985); neuraminidase (Srivastava et al., 1970), esterases (Zaneveld and Williams, 1970; Bradford et al., 1976), phospholipase C (Srivastava et al., 1982), arylsulfatases (Allison and Hartree, 1970; Yang and Srivastava, 1974b; Dudkiewicz, 1984) and β -N-acetylhexose aminidase (Rodger and Young, 1981). With the exception of acrosin and hyaluronidase, however, little or nothing is known about the localization of these enzymes in the acrosome, or their exact function (Morton, 1976; Harrison, 1983).

2.4 Sub-acrosomal Space

The IAM is closely applied to the outer nuclear membrane, but is separated from it by a narrow space called the sub-acrosomal space (Fawcett, 1965; Nicander and Bane, 1966). The size of this space, and the amount of material contained within it, varies considerably between species. The presence of sub-acrosomal material (SAM) has been denied for a few species (Fawcett, 1965; Fawcett and Ito, 1965; Pedersen, 1974), but in most, moderately electron-dense material has been found in the sub-acrosomal space. There is usually an accumulation of SAM on the

anterior border of the nucleus, and it may project into the acrosome as a thin, finger-like process bounded by the IAM (Bedford, 1964; Nicander and Bane, 1966; Jones, 1970, 1971; Wooding and O'Donnell, 1971; Courtens et al., 1976). Beneath the equatorial segment, there is sometimes a greater accumulation of SAM, which, in the rabbit and hare, forms sub-acrosomal blisters (Nicander and Bane, 1966; Jones, 1970; Wooding and O'Donnell, 1971; Bernstein and Teichman, 1972).

In many rodents, in which the sperm head is falciform in shape, the sub-acrosomal space on the anterior border of the nucleus is enlarged, and filled with dense, granular material. This structure is called the perforatorium, although it should, perhaps, be called a "pseudoperforatorium" until its exact function can be ascertained (Baccetti, 1979). It occurs in many species of rodents such as the rat, mouse, golden and Chinese hamsters, cotton rat, field mouse and deer mouse (Friend, 1936; Austin and Bishop, 1958a; Marston and Chang, 1966). It does not occur, however, in microtine rodents such as the field vole, in which the apical segment of the acrosome constitutes the hook (Friend, 1936; Koehler, 1978a). In the rat, the perforatorium is a triangular-shaped rod that projects from the apical tip of the nucleus, and has three prongs, one dorsal and two ventral, which extend caudally over the nucleus (Clermont et al., 1955). Ultrastructural studies have shown that the perforatorium consists of moderately electron-dense granular material, without any definite sub-structure. It is closely bounded by the nuclear envelope and IAM, but may be free and in contact with the plasma membrane along its ventral margin (Piko, 1969; Franklin et al., 1970; Yanagimachi and Noda, 1970a). In the golden hamster, however, it consists of two layers of differing electron density, with a more electron-dense core and a less-dense peripheral component (Franklin et al., 1970).

There is little known about the chemical composition of the sub-acrosomal space and its contents. Cytochemical studies have revealed the presence of phospholipids (Teichman et al., 1972, 1974), lysine-rich proteins (Courstens et al., 1976), and acid phosphatase activity (Teichman and Bernstein, 1971). In the rat, the SAM consists of a 13 kd, cysteine-rich protein (Olson et al., 1976a). Disulphide cross-linking between such molecules probably accounts for the rigidity and resilience of this structure (Bedford and Calvin, 1974a). Similarly, studies in the mouse suggest that one or more proteins of 14-15 kd may constitute this material (O'Brien and Bellve, 1980a; Bellve and O'Brien, 1983; Pruslin and Rodman, 1983).

The exact function of the SAM remains unclear (Fawcett, 1975). When present in meagre quantities, it may serve to bind the IAM to the nuclear envelope (Fawcett, 1970; Courstens et al., 1976), but the apical accumulation of SAM, and the rodent perforatorium, have been implicated in penetration of the tough zona pellucida which surrounds the oocyte (Yanagimachi and Noda, 1970b; Bedford and Calvin, 1974; Moore and Bedford, 1983).

2.5 Post-acrosomal Region

The area overlying the nucleus posterior to the equatorial segment is referred to as the post-acrosomal region (Fawcett, 1975). In most species, it is present as a circumferential band across the posterior margin of the sperm head, but in the rat and mouse, it also extends along the ventral or concave aspect of the sperm head (Bishop and Austin, 1957; Bishop and Walton, 1960).

This region is considered to be a specialization of the plasma membrane (Fawcett, 1970). It consists of a 5-20 nm thick layer of amorphous material, interposed between the nuclear envelope and plasma

membrane, but separated from the nuclear envelope by a continuation of the sub-acrosomal space (Nicander and Bane, 1966; Fawcett, 1975). The plasma membrane closely parallels this dense lamina, and in favourable sections, it can be seen that periodic striations spaced 10-20 nm apart link the membrane and dense lamina (Fawcett and Ito, 1965; Fawcett, 1970; Wooding and O'Donnell, 1971; Gordon, 1972; Pedersen, 1972; Olson et al., 1983a; Toyama and Nagano, 1983). These periodicities are thought to represent linear striations or ridges, oriented parallel to the longitudinal axis of the sperm head (Olson et al., 1983a).

Several variations of this general pattern have been noted. In the bottlenose dolphin, for example, the post-acrosomal dense lamina is not continuous, but consists of 14-16 parallel laminae, oriented in the longitudinal axis of the sperm head, with periodic striations linking the plasma membrane to each lamina (Fleming et al., 1981). In human sperm, the striations or ridges are only present in the anterior 1/4 to 3/4 of the post-acrosomal region, and are oriented circumferentially, and not in the longitudinal axis (Pedersen, 1972).

Owing to difficulties in its recovery, little is known about the composition of the post-acrosomal dense lamina (Fawcett, 1975). It is strongly argyrophilic (Hancock and Trevan, 1957; Bishop and Walton, 1960; Elder and Hsu, 1981), and is stainable with phosphotungstic acid (PTA) at low pH (Bane and Nicander, 1963). It has also been shown to be stabilized to some degree by disulphide bonds (Bedford and Calvin, 1974). In the mouse, Bellve and O'Brien (1983) suggest that the SAM and dense lamina are composed of multiple proteins of 8-80 kd, but no distinction between the two regions is given. Several studies have localized actin in the acrosomal or post-acrosomal region (Talbot and Kleve, 1978; Clarke and Yanagimachi, 1978; Tamblyn, 1980; Greenberg and Tamblyn, 1981; Clarke et al., 1982; Virtanen et al., 1984; Welch and

O'Rand, 1985), although it appears to be in the non-polymerized or monomeric form (Tamblyn, 1980; Ochs, 1984, Virtanen et al., 1984; Welch and O'Rand, 1985) and is not present in all species (Clarke and Yanagimachi, 1978; Halenda et al., 1984). Virtanen et al. (1984) could not demonstrate myosin in the post-acrosomal region, so the function of non-polymerized actin is obscure. It has been suggested that microtubule-like structures are present in this region (Koehler, 1978a; Stambaugh and Smith, 1978), although more recent studies have failed to find microtubules (Olson et al., 1983a), or tubulin in this region (Virtanen et al., 1984). Hence, both the presence and function of cytoskeletal elements in the post-acrosomal region remains obscure.

2.6 Plasma Membrane

The entire spermatozoon is enclosed within the plasma membrane (Fawcett, 1970). It is a highly regionalized structure, over both the head and tail, and consists of many domains and sub-domains which often differ structurally, and in chemical composition (Friend and Fawcett, 1974; Friend, 1977; Holt, 1982; Primakoff and Myles, 1983). For this reason, the sperm plasma membrane will be dealt with as a single entity, and its properties over the head and tail will be examined concurrently in this section.

The plasma membrane (PM) is closely applied to the post-acrosomal dense lamina, but is found at varying distances from the OAM and tail structures, which may be an artifact of fixation (Fawcett, 1970; Jones, 1971). It is a typical trilaminar unit membrane, and a glycocalyx of variable thickness and distribution has been demonstrated using cytochemical techniques (Friend and Fawcett, 1974; Flechon and Morstein, 1975; Friend, 1977; Flechon, 1979b). It is most evident on the guinea pig sperm head, where it resembles a honeycomb in grazing sections

(Friend, 1977).

The surface of the spermatozoon is characterized by a series of landmarks. Triangular serrations are often observed at the junction of the equatorial segment and post-acrosomal region (Bustos-Obregon and Flechon, 1975), whilst the posterior ring is represented by a narrow groove (Woolley, 1970a; Bustos-Obregon and Flechon, 1975). Tubular structures have been described on the surface of Chinese hamster and mouse sperm, and parallel ridges occur on the peri-acrosomal PM of rat and guinea pig sperm (Phillips, 1972, 1977b, 1977c).

Studies employing the freeze-fracture technique have provided a great deal of information on the complexity of the sperm surface. In most species, the peri-acrosomal PM shows a fairly random distribution of intra-membranous particles (Koehler, 1966, 1970; Plattner, 1971; Stackpole and Devorkin, 1974; Pfaller et al., 1976; Suzuki, 1981). Hexagonal arrays of particles are found on the anterior tip of the rat sperm head (Friend and Fawcett, 1974; Suzuki and Nagano, 1980a), and the guinea pig sperm glycocalyx is represented by plaques of geometric arrays in a quilted pattern (Friend and Fawcett, 1974; Friend, 1977; Friend and Heuser, 1981). The post-acrosomal region, however, shows a number of specializations in non-rodent sperm. Serrations, pits or ridges often characterize the anterior margin of this region, at the border with the equatorial segment (Koehler, 1970; Friend and Fawcett, 1974). Cords of particles or basal striations often radiate from near the base of the sperm head, coursing in differing directions, and exhibit variations in the length of the striations and size of the particles between species (Koehler, 1966, 1970, 1972; Pfaller et al., 1976; Suzuki, 1981). Hexagonal crystalline arrays have been described in the post-acrosomal region of boar sperm (Suzuki, 1981). The posterior ring, which marks the end of this region, often shows fine striations

(Koehler, 1966, 1972; Friend and Fawcett, 1974; Suzuki, 1981).

On the sperm tail, hexagonal arrays of particles have been localized over the mouse and boar sperm middle pieces (Stackpole and Devorkin, 1974; Toyama and Nagano, 1983), whilst circumferential strands of small particles often course around the middle piece, overlying the mitochondria (Friend and Fawcett, 1974; Stackpole and Devorkin, 1974). These features cease at the annulus, which has a very high density of particles (Friend and Fawcett, 1974; Stackpole and Devorkin, 1974; Suzuki, 1981). In most species, the PM overlying the principal piece of the tail is characterized by a structure called the "zipper", which runs for variable lengths and consists of one or more double rows of particles overlying outer dense fibre number 1 (Friend and Fawcett, 1974; Stackpole and Devorkin, 1974; Friend, 1977; Suzuki, 1981).

The heterogeneity and regionalization of the sperm PM has also been examined using a variety of biochemical and cytochemical techniques for lipids, membrane proteins, and exposed ligands (Holt, 1982).

Plasma membrane lipids occur in localized regions, with suppression of lateral diffusion (Friend, 1982). In guinea pig and ram sperm, anionic phospholipids occur in the PM over the acrosome and middle piece, but not in the post-acrosomal region (Bearer and Friend, 1980; Bradley *et al.*, 1980). They are only found, however, in the interstices of the guinea pig sperm glycocalyx (Bearer and Friend, 1980). β -hydroxy sterols occur throughout the guinea pig sperm PM (Friend, 1981), and are only absent from the post-acrosomal region of ram sperm (Bradley *et al.*, 1980). In guinea pig sperm, there is also a heterogeneous distribution of cholesterol. It is distributed over most of the cell surface, but is absent from the quilted plaques, annulus and zipper, and cholesterol levels are low in the post-acrosomal region (Elias *et al.*, 1979; Friend and Bearer, 1981).

Sperm PM proteins may be important for events that occur during fertilization (Friend, 1977, 1982). The number of proteins, their relative molecular weights and characteristics have been found to vary between species (Esbenshade and Clegg, 1976; Vierula and Rajaniemi, 1980; Young and Goodman, 1980, 1982; Ji et al., 1981a; Olson and Gould, 1981; Noland et al., 1983; Russell et al., 1983b). Some are distributed evenly throughout the PM, whilst others are localized in certain regions where they may perform special functions (Ji et al., 1981b; Russell et al., 1983).

The localization of sperm surface antigens, many of which are probably peripheral or integral membrane proteins, has further attested to the extreme heterogeneity of the sperm surface (Koehler, 1978b). Antigens are localized either throughout the membrane, or to certain regions which often correspond to structural entities such as the equatorial segment, post-acrosomal region, middle piece and so forth (Brogaard-Hansen and Hjort, 1971; Koehler and Perkins, 1974; Tung, 1975, 1976; Herr and Eddy, 1980; Tung et al., 1982). Some are mobile, whilst the lateral mobility of others is restricted (Koehler, 1975; O'Rand, 1978).

With the recent development of the monoclonal antibody technique, it has become possible to accurately map the sperm surface with antisera of high specificity (Eddy and Koehler, 1982). In the guinea pig, at least 5 distinct domains can be recognized, corresponding to discrete morphological zones, although several different antigens may be found in a particular zone (Primakoff and Myles, 1983). This technique has also been applied to studies of the sperm surface in man (Isahakia and Alexander, 1984), mouse (Schmell et al., 1982), rat (Gaunt et al., 1983), hamster (Moore and Hartman, 1984), and boar (Saxena et al., 1984). Similar antigenic determinants may be present on surface antigens

of a range of species (Isahakia and Alexander, 1984), although limited cross-reactivity has been reported in other cases (Primakoff et al., 1983).

Since the initial finding by Kashiwabara et al. (1965) that soybean phytoagglutinins caused tail-to-tail agglutination of bull sperm, there have been many studies using lectins to probe for exposed carbohydrates on the sperm surface (Koehler, 1981). Marked species differences have been observed in the degree and site of agglutination using different lectins (Edelman and Millette, 1971; Nicolson and Yanagimachi, 1972). Labelling studies at the light and electron microscope levels have revealed that different sugar moieties are localized in different regions of the sperm surface, often corresponding to structural zones, and that marked species differences are apparent (Edelman and Millette, 1971; Kinsey and Koehler, 1976; Russel et al., 1983b; Sinowatz and Friess, 1983; Lee and Damjanov, 1985). It should be noted, however, that the distribution of carbohydrates is complex, for lectins with the same sugar affinity often give different labelling patterns (Edelman and Millette, 1975). It has also been shown that the number of binding sites differ for various lectins, and marked inter-specific differences occur in the number of sites for a particular lectin (Nicolson et al., 1972; Edelman and Millette, 1975).

Early studies of the electrophoretic mobility of bull and rabbit sperm clearly showed that there was a higher density of negative charges on the tail (Bangham, 1961; Nevo et al., 1961). The distribution of negative surface charges has since been studied using the colloidal iron hydroxide (CIH) technique, and a wide variation has been found between species (Eddy and Koehler, 1982). In bull and human sperm, there is an even distribution of negative charges (Yanagimachi et al., 1972; Bedford, 1974a), whereas in the rabbit, mongoose, rat, guinea pig,

hamster, boar and macaque, there is stronger labelling over the sperm tail, with some label bound either over the acrosome or post-acrosomal region (Yanagimachi et al., 1972; Flechon and Morstin, 1975; Flechon, 1979b; Fain-Maurel et al., 1984). The ligands responsible for these negative charges remain largely unknown. Sialic acid has been implicated in somatic cells, but neuraminidase has no effect on the labelling of sperm, suggesting they are insensitive sialic acid residues, or other charged moieties such as sulphates (Yanagimachi et al., 1972; Fain-Maurel et al., 1984). In addition, sialic acid is found exclusively on the human sperm head, whereas the entire sperm surface exhibits negatively charged groups (Levinsky et al., 1983).

2.7 General Organization of the Sperm Tail

The sperm tail is a specialized flagellum that provides the spermatozoon with the motility necessary to ascend to the site of fertilization, and penetrate the extra-cellular coats around the oocyte (Yanagimachi, 1981). It is divided into the neck, middle piece, principal piece and end piece. The neck region contains the connecting piece, which is the structural base of the tail and forms the surface for articulation with the head. The motor apparatus or axoneme extends the length of the tail. In the middle piece and part of the principal piece, it is surrounded by 9 outer dense fibres (ODF), which terminate at various points along the principal piece. In the middle piece, there is also a helically-arranged mitochondrial sheath, whilst in the principal piece, the fibrous sheath encloses the axonemal complex. This general organization occurs in all species, with differences in the size and shape of some elements (Fawcett, 1970, 1975). Each of the structural elements of the sperm tail will now be discussed in greater detail.

2.8 Neck Region

The neck is a constricted region at the base of the tail, extending from the implantation fossa to the commencement of the mitochondrial sheath (Fawcett, 1965; Zamboni and Stefanini, 1971). Its constituent elements include the connecting piece, proximal centriole, redundant nuclear envelope and a few mitochondria (Fawcett, 1975).

The main structural element is the connecting piece, which is composed of 9 segmented columns which have fused anteriorly to form the capitulum, a large articular structure that conforms to the shape of the implantation fossa. At the distal end of the neck, the segmented columns are continuous with the outer dense fibres, although they are of different origins (Fawcett and Phillips, 1969a). Anteriorly, the columns fuse to form two major columns, and four (Fawcett and Ito, 1965; Zamboni and Stefanini, 1971) or five (Fawcett, 1965) minor columns. One or both of the major columns form the capitulum, to which the minor columns are attached on its dorsal and ventral aspects. The capitulum is usually elongated in the transverse axis of the sperm head, with a triangular-shaped extension (Fawcett, 1970, 1975). The segmented columns consists of fibrous material with a period of 50-66.5 nm, and 10 or 11 intra-period bands (Fawcett and Ito, 1965; Fawcett and Phillips, 1969a; Fawcett, 1970; Pedersen, 1974).

In all species studied except the rat, the interior of the connecting piece contains the proximal centriole, which is oriented at an angle of $45-90^{\circ}$ with respect to the axoneme, and is composed of 9 triplets of microtubules (Nicander and Bane, 1962; Fawcett, 1965; Fawcett and Ito, 1965; Zamboni and Stefanini, 1971; Pedersen, 1974). Remnants of the centriolar adjunct sometimes appear at the distal end of the centriole in human sperm (Zamboni and Stefanini, 1971). The distal centriole is lost during spermiogenesis (Fawcett and Phillips, 1969a),

although its remnants are sometimes found near the proximal end of the axoneme (Fawcett, 1965).

A few elongated mitochondria are often present in the neck region (Fawcett, 1975), and are flattened and triangular in shape (Fawcett and Ito, 1965). Another feature of this region is the extension of the redundant nuclear envelope (Fawcett, 1970), which varies from simple folds (Nicander and Bane, 1962; Fawcett, 1965; Bedford, 1974a), to complex arrays or membranous scrolls (Fawcett and Ito, 1965; Bedford, 1967; Gordon, 1972).

2.9 Mitochondrial Sheath

The middle piece is the thickest part of the sperm tail, and extends from the neck to the annulus at the junction with the principal piece. The main feature of this region is a sheath of mitochondria arranged helically around the axonemal complex (Fawcett, 1975).

The length of the middle piece and the number of gyres of mitochondria varies considerably between species (Fawcett, 1970). The number of gyres is 3-4 in the dolphin (Fleming *et al.*, 1981), 10-12 in man (Zamboni and Stefanini, 1971), 40-43 in the guinea pig, rhesus monkey and macaque (Fawcett, 1965; Phillips, 1977c), 60-75 in the bull (Phillips, 1977c), 87-89 in the mouse (Woolley, 1970b), 195 in the golden hamster, and 362 in the rat (Phillips, 1977c). The pitch of the spiral is about $20-25^{\circ}$ (Phillips, 1977c), and in the mouse, at least, the direction of the helix is left-handed (Woolley, 1970b). The number of helices in the sheath varies from one to five (Woolley, 1970b; Phillips, 1977c).

There is also variation in the arrangement and characteristics of the mitochondria (Phillips, 1977c). In many species, the mitochondria abut end-to-end randomly in the helix, whilst in others, there is a

regular pattern (Fawcett and Ito, 1965; Phillips, 1977c). They are usually elongate and cylindrical, but vary in thickness between species (Fawcett, 1970; Phillips, 1977c). Two distinct types of mitochondria occur in dolphin sperm (Fleming et al., 1981), whereas the size of individual mitochondria can vary in human sperm (Zamboni and Stefanini, 1971; Pedersen, 1974).

In mature sperm, the mitochondrial cristae are arranged concentrically, parallel to the outer mitochondrial membrane (OMM), and the matrix is very dense (Nicander and Bane, 1962; Fawcett and Ito, 1965; Fawcett, 1970). The OMM is thicker than the inner membrane (Elfvin, 1968; Friend and Heuser, 1981), and the PM is closely adherent to it (Fawcett, 1970). Fine periodicities link the PM and OMM in the region of the circumferential particle strands (Friend and Fawcett, 1974).

At the distal end of the middle piece, immediately caudal to the last mitochondrial gyre, is the annulus, which consists of closely packed filamentous subunits about 3-4 nm in diameter, oriented circumferentially around the tail (Fawcett, 1970; Pedersen, 1974). In cross-section, it appears either triangular or semi-circular in shape and the PM is closely applied to it (Fawcett and Ito, 1965; Fawcett, 1970; Pedersen, 1974; Stackpole and Devorkin, 1974).

2.10 Axoneme

The axoneme or axial filament complex is the motor apparatus of the sperm tail, and exhibits the ubiquitous 9 + 2 arrangement of microtubules that characterizes cilia and flagella (Fawcett, 1975).

It consists of nine outer doublets of microtubules radially arranged about a central pair. Each outer doublet consists of a complete, round subfibre A composed of 13 protofilaments, and an

incomplete, crescentic subfibre B made up of 10 protofilaments. Subfibre A is 26 nm in diameter, often appears uniformly dense, and lies slightly closer to the central pair of microtubules. Two dynein arms project clockwise from subfibre A of one doublet to subfibre B of the next doublet. The outer dynein arm has an appendage, whilst the inner one is connected to the subfibre B by a peripheral link. Nexin bridges also connect the doublets (Telka et al., 1961; Nicander and Bane, 1962; Fawcett and Ito, 1965; Fawcett, 1970, 1975). A scheme for numbering the outer doublets has now been adopted (Afzelius, 1959). The plane that divides the tail into two equally large areas and is perpendicular to a line through the central pair, transects doublet number one. The others are numbered increasingly in a clockwise direction.

The central pair of microtubules are separate, and each consists of 13 protofilaments. They are enclosed within a central sheath, with radial spokes extending from this sheath to subfibre A of each doublet (Fawcett, 1975). Fine ribs spaced 13.5-16.0 nm apart join the two central microtubules (Pedersen, 1974; Olson and Linck, 1977). In human sperm, the sheath is composed of two evenly spaced, 6 nm thick threads, which are arranged spirally around the central pair, with a pitch of $10-15^{\circ}$ and a 13.5 nm periodicity. The radial spokes are spaced 27 nm apart, with spokes in one half of the axoneme arising from one thread, and in the other half from the other thread (Pedersen, 1974). In rat sperm, however, the spokes are arranged in groups of three, with a 24 or 32 nm periodicity (Olson and Linck, 1977).

This organization is maintained until the endpiece of the tail, where it becomes disrupted. The dynein arms are absent, the doublets dissociate into single tubules, and the number of tubules gradually decreases as the endpiece tapers down near its tip (Nicander and Bane,

1962; Fawcett, 1965; Pedersen, 1974; Suzuki and Nagano, 1980b; Woolley and Nickels, 1985).

2.11 Outer Dense Fibres

Nine outer dense fibres (ODF) lie peripheral to the axonemal doublets for a variable length of the sperm tail, creating a $9 + 9 + 2$ arrangement. They are numbered according to the doublet with which they are associated (Fawcett, 1970). There are significant differences in the size, shape and cross-sectional area of the fibres, and the order in which they terminate. Differences in size may not be apparent at the anterior end, but appear in the middle piece (Nicander and Bane, 1962; Fawcett, 1965). In most species, fibres 1, 5 and 6 are larger, with number 9 being of intermediate size (Nicander and Bane, 1962; Saacke and Almquist, 1964b). The fibres usually have a convex outer surface, and either a concave or tapering inner surface (Telka et al., 1961; Nicander and Bane, 1962; Fawcett, 1965; Pedersen, 1974). They are thickest at their anterior end, and taper posteriorly until they disappear. The smallest terminate first, and the largest terminate last, although the actual order varies between species (Telka et al., 1961; Saacke and Almquist, 1964b; Fawcett and Ito, 1965; Fawcett, 1970; Pedersen, 1974). Fibres 3 and 8, however, always terminate at the anterior end of the principal piece, and are replaced by the longitudinal columns of the fibrous sheath. If the fibres are small initially, as in man, macaque and bat, they terminate in the anterior half of the principal piece. If the converse applies, however, as in most rodents, they extend the entire length of the principal piece (Fawcett, 1970). As the fibres taper posteriorly, they move closer to the outer doublets, and become joined just prior to their termination (Fawcett and Ito, 1965).

Although the ODFs are extremely dense structures, they are not entirely homogenous, but have a thin cortical region which is present on the convex outer surface, and sometimes on the inner surface (Telka et al., 1961; Fawcett, 1965; Phillips and Olson, 1975). In rat sperm, the cortical zone is composed of 6-7 nm globular sub-units, showing a 40 nm periodicity at a 60-80° angle to the axis of the fibre (Phillips and Olson, 1975; Olson and Sammons, 1980). Similarly, a 40 nm periodicity has been described in ODFs from human and mouse sperm (Woolley, 1971; Pedersen, 1974), but the periodicity is 50-51 nm in the bull (Baccetti et al., 1976).

In some species, fibrillar structures about 15-20 nm in diameter are found around the outer dense fibres. These satellite fibrils only occur in the middle piece, where they run along the inner aspect of the ODFs. They are most extensive in species with thick middle pieces, such as the ground squirrel (Fawcett, 1965; Fawcett and Ito, 1965; Fawcett, 1970; Ploen et al., 1979).

2.12 Fibrous Sheath

The principal piece of the sperm tail is encased in another accessory structure called the fibrous sheath, which consists of circumferential, crescentic ribs that attach at their dorsal and ventral margins to two longitudinal columns. The plane through the two columns passes through the central pair of microtubules. Both the ribs and columns become thinner posteriorly as the tail narrows (Fawcett, 1970, 1975). In rodent sperm, the columns are triangular in outline and are well developed. The ribs bifurcate where they join the columns, thus giving the principal piece an elliptical outline (Telka et al., 1961; Fawcett, 1970). In human, monkey, bull and boar sperm, the columns are poorly developed and become inconspicuous posteriorly, and the ribs do

not bifurcate (Telka et al., 1961; Nicander and Bane, 1962; Saacke and Almquist, 1964b; Pedersen, 1974). The columns are composed of 5-6 nm thick filaments arranged in parallel (Olson et al., 1976b), whilst the ribs consist of thin fibrous material embedded in a dense matrix (Fawcett, 1965).

In the initial portion of the sheath, the adaxial surface of the columns is attached to fibres 3 and 8 (Fawcett, 1975). After these fibres terminate, thin laminae extend in from the two columns and attach to small ridges radiating from doublets 3 and 8. This persists until the fibrous sheath terminates (Pedersen, 1974). Another feature which warrants comment is the "zipper", which appears to attach the PM firmly to the fibrous sheath opposite fibre 1 (Friend and Fawcett, 1974).

2.13 Chemical Composition of Tail Accessory Structures

Several studies have shown that the accessory structures of the sperm tail, namely the ODFs, fibrous sheath, connecting piece, and OMM, have a high degree of disulphide crosslinking, bestowing on them rigidity and resilience (Bedford and Calvin, 1974b; Calvin, 1975). This may, for instance, explain the elastic role in flagellar wave propagation that has been postulated for the ODFs and fibrous sheath (Phillips and Olson, 1975; Olson and Sammons, 1980).

There is little known about the composition of the bonds which link the capitulum and basal plate, except that in some species, they are susceptible to proteolytic cleavage (Edelman and Millettee, 1971; Calvin, 1976b). It has been postulated that the connecting piece is composed of proteins, but they have not been isolated (Calvin et al., 1975; O'Brien and Bellve, 1980a). The fibrous sheath of rat sperm, however, has been isolated, and consists of a single 80 kd polypeptide (Olson et al., 1976b).

The outer dense fibres from bull, rat and human sperm have been isolated and their protein composition determined. In all three species, the amino acid content of the ODFs was similar, with a high content of proline and cysteine. SDS-PAGE revealed two proteins of 72-75 and 54-57 kd which were low in cysteine, and two proteins of 29 and 30 kd which contained large amounts of cysteine (Baccetti et al., 1976). In another study by Olson and Sammons (1980), however, rat ODFs were found to contain an 87 kd protein which was low in cysteine and found in the cortex of the fibres, and three proteins of 12, 19 and 25 kd, which resided in the medulla, and contained 10-12% cysteine. Proteins of similar molecular weights have been found in the mouse sperm tail (O'Brien and Bellve, 1980a).

Bull sperm mitochondria have been isolated and found to have a completely different complement of proteins from those of somatic cell mitochondria. The OMM is cross-linked by disulphide bonds and forms a tough capsule around the mitoplast (Pallini, 1979). This membranous shell consists of proteins of 20, 29 and 30 kd, which contain 18% cysteine (Pallini, 1979; Pallini et al., 1979).

2.14 Cytoplasmic Droplet

The cytoplasmic droplet is an expanded portion of the cytoplasm that is located in the neck region or middle piece (Fawcett, 1965). Its size and shape, the organization of its organelles, and the density of the ground substance varies between species (Bloom and Nicander, 1961). It contains tubular or lamellar structures, sometimes curved or concentrically arranged, which probably represent remnants of the Golgi or endoplasmic reticulum (Bloom and Nicander, 1961; Nicander and Bane, 1962; Dott and Dingle, 1968; Garbers et al., 1970). Crystalline inclusions have been described in droplets from bull sperm (Olson

et al., 1983b). The PM lacks the circumferential particle strands that sometimes characterize the middle piece (Friend and Fawcett, 1974), but may contain rectilinear particle arrays or lattices (Friend and Heuser, 1981; Suzuki, 1981).

The cytoplasmic droplet contains a variety of lysosomal enzymes which operate over a wide pH range, but appear to be metabolically inert (Roberts et al., 1976). These include acid and alkaline proteases, acid and alkaline phosphatases, arylsulphatase, hyaluronidase, β -glucuronidase and 5'-nucleotidase (Dott and Dingle, 1968; Roberts et al., 1976).

C. EPIDIDYMAL MATURATION OF SPERMATOZOA

Since the initial discovery by Young (1931) that guinea pig sperm from the distal regions of the epididymis gave higher rates of fertilization than those from the proximal regions, it has become increasingly obvious that mammalian sperm are infertile when they leave the testis, but gradually acquire the ability to fertilize during epididymal transit (Bedford, 1975). The epididymis consists of a highly convoluted tubule, which can be divided into caput, corpus and cauda regions, each of which may be subdivided into proximal, mid and distal parts (Glover and Nicander, 1971; Hamilton, 1975). The average time taken for sperm to pass through the epididymis is about 9-14 days (Rowley et al., 1970; Bedford, 1975). During this period, spermatozoa undergo morphological, biochemical and physiological changes that culminate in the acquisition of the ability to fertilize an oocyte (Bedford, 1975).

1. MORPHOLOGICAL CHANGES

There do not appear to be any changes in the structure of the sperm

tail during epididymal transit, but significant changes in the plasma membrane, acrosome and cytoplasmic droplet have been reported (Bedford, 1975).

1.1 Plasma Membrane

During epididymal maturation, the peri-acrosomal PM becomes more susceptible to ballooning (Bedford, 1965; Fawcett and Phillips, 1969b; Bedford and Nicander, 1971), which, in the rat at least, is associated with the loss of flocculent material from the peri-acrosomal space (Suzuki and Nagano, 1980a). This may simply represent an artifact of fixation (Jones, 1971), but even so, it must reflect a change in the properties of the PM (Bedford, 1975).

In addition, freeze-fracture studies have revealed a loss of crystalline arrays from the peri-acrosomal PM of rat sperm (Suzuki and Nagano, 1980a), rearrangement of particle strands on the guinea pig sperm middle piece (Olson and Orgebin-Crist, 1982), and changes in the distribution of PM particles over the acrosome, post-acrosomal region, and anterior middle piece of boar sperm (Suzuki, 1981).

1.2 Acrosome

One of the most characteristic features of epididymal maturation is a remodelling and reduction in size of the acrosome (Bedford, 1975), although this does not occur in some species such as man (Bedford, et al., 1973). In the rat, rabbit, boar, elephant and some primates, it has been found that the apical segment undergoes a reduction in size and may become more rounded (Bedford, 1965; Bedford and Nicander, 1971; Jones, 1971; Jones et al., 1974; Suzuki and Nagano, 1980a). The most significant changes, however, occur in species in which the apical segment is large or elongated (Bedford, 1975). The acrosomal hook of

bushbaby sperm becomes condensed (Bedford, 1974a), and in the chinchilla, the odd-shaped apical segment is moulded into its mature form (Fawcett and Phillips, 1969b). During epididymal maturation of guinea pig sperm, the heterogeneities appear in the acrosomal matrix, the acrosome becomes repositioned at an angle of 135° to the long axis of the sperm head, the U-shaped ridge appears, and the tip of the acrosome curves ventrally (Fawcett and Hollenberg, 1963).

1.3 Cytoplasmic Droplet

At spermiation, the cytoplasmic droplet forms in the neck region of the spermatozoon. As sperm progress through the epididymis, it moves distally along the middle piece (Bedford, 1975), and is either lost before ejaculation (Paufler and Foote, 1968; Bedford et al., 1973; Dott, 1975), or is maintained at the distal end of the middle piece at least until ejaculation (Hancock, 1957; Jones et al., 1974; Suzuki and Nagano, 1980a). Droplet migration occurs in the caput epididymis in the rat, stallion, bull and ram (Bedford, 1975; Suzuki and Nagano, 1980a; Amann et al., 1982), or distal to the caput as in the boar and rabbit (Hancock, 1957; Bedford, 1975). Changes in the shape, quantity and orientation of the droplet organelles may also occur during its migration (Bloom and Nicander, 1961; Bedford, 1965).

2. CHANGES IN THE STRUCTURAL COMPONENTS OF SPERM

In addition to morphological changes, there are changes in the structural integrity of the nucleus, sub-acrosomal material, and accessory tail structures. Calvin and Bedford (1971) showed that during transit from the caput to caudal regions, there is a progressive stabilization of sperm structures by disulphide bonds. This occurs in a

wide variety of species including the rat, rabbit, mouse, guinea pig, bull, boar, cat, ferret, shrew and primates (Calvin and Bedford, 1971; Bedford et al., 1973; Bedford, 1974a). A similar affect can be induced by maintaining sperm in the caput region, suggesting that development of these bonds is time-dependent (Calvin and Bedford, 1971). Disulphide bonds form more quickly in the anterior part of the nucleus, which is also more stable in mature sperm (Calvin and Bedford, 1971). Sperm nuclei also become more resistant to decondensation by heparin and trypsin during maturation (Meistrich et al., 1976; Carranco et al., 1983). In addition to the nucleus, the SAM, post-acrosomal dense lamina, connecting piece, ODFs, fibrous sheath, and OMM become stabilized by disulphide bonds (Calvin and Bedford, 1971; Bedford and Calvin, 1974b; Olson et al., 1976b). The increase in disulphide bonding is paralleled by a decrease in the free sulphhydryl content of the sperm head and tail (Calvin et al., 1973). The increased stability of the head structures is viewed as an adaptation to penetration of the extracellular coats around the oocyte (Bedford, 1983a; 1983b), whilst changes in the tail structures may be associated with the changing pattern of motility (Bedford and Calvin, 1974b).

3. CHANGES IN THE SURFACE PROPERTIES OF SPERM

The environment which surrounds the maturing spermatozoon changes considerably from the testis to the distal regions of the epididymis. The interaction of the sperm surface with this environment leads to a variety of changes in the sperm PM and glycocalyx, which contribute to the acquisition of fertility (Orgebin-Crist et al., 1975).

3.1 Lipid Composition

Changes occur in the lipid composition of the sperm membranes during epididymal maturation which may be important in relation to later events which occur during the fertilization process (Holt, 1982).

Several studies have reported decreases in the total phospholipid content, or of various phospholipid classes, during transit of the epididymis (Dawson and Scott, 1964; Quinn and White, 1967; Scott et al., 1967; Nikolopoulou et al., 1985; Parks and Hammerstedt, 1985), although rat sperm may be an exception (Dawson and Scott, 1964). The cholesterol content has also been shown to decrease in epididymal sperm (Quinn and White, 1967; Scott et al., 1967; Legault et al., 1979a). It has therefore been postulated that the decrease in cholesterol, coupled with an increase in the ratio of unsaturated to saturated fatty acids would produce a more fluid membrane (Voglmayr, 1975). Recent studies, however, do not seem to support this contention. In hamster and boar epididymal sperm, although the cholesterol level decreases, there is a significant increase in the desmosterol content, which would stabilize, not destabilize the membrane (Legault et al., 1979a, 1979b; Nikolopoulou et al., 1985). Additional evidence is provided by the finding that a barrier to lipid diffusion is set up in ram spermatozoa during maturation (Wolf and Voglmayr, 1984). Hence, it would appear that the sperm plasma membrane lipid bilayer is progressively stabilized during epididymal transit.

3.2 Changes in Exposed Surface Ligands

As mentioned in Section B.2.6, negative surface charges may be exposed either on the entire sperm surface, or predominantly on the tail. It now appears that these charges are not well developed on testicular sperm, but are largely acquired or exposed during epididymal

maturation (Bedford, 1963b; Cooper and Bedford, 1971a; Yanagimachi et al., 1972; Bedford et al., 1973; Bedford, 1974a; Courtens and Fournier-Delpech, 1979; Fain-Maurel et al., 1984). The exception appears to be the Asiatic musk shrew, in which there is no change during passage through the epididymis (Cooper and Bedford, 1976). The addition of sialic acid residues has been implicated in this acquisition of negative charge (Moore, 1979; Holt, 1980). Recent studies on the rat, however, suggest the involvement of charged groups other than sialic acid, since the total number of anionic sites increases during maturation, but the amount of sialic acid bound to the sperm decreases (Toowicharanont and Chuvalatnatol, 1983a, 1983b).

Changes in the carbohydrate groups on the sperm surface have been examined using lectins (Eddy and Koehler, 1982). As with negative charges, Cooper and Bedford (1976) found little or no change in the pattern of binding of Con A or WGA to musk shrew sperm. In other species, however, there are often extensive modifications to the labelling pattern with some lectins, but no change with others. For instance, the binding of Con A to the peri-acrosomal PM increases during epididymal maturation in the rabbit, mouse, rat and macaque (Gordon et al., 1975; Lewin et al., 1979; Dooher, 1981; Fain-Maurel et al., 1984), but no changes occur in the bull or marmoset (Friess and Sinowatz, 1984; Moore et al., 1984). There are also marked inter-specific differences in changes to the distribution of receptors for other lectins such as RCA and WGA (Nicolson et al., 1977; Fain-Maurel et al., 1984; Friess and Sinowatz, 1984; Moore et al., 1984). Hence, significant alterations in the type and pattern of distribution of carbohydrate moieties on the sperm surface do occur during epididymal maturation, but a unifying concept has been difficult to formulate.

3.3 Plasma Membrane Polypeptides

The localization of sperm surface antigens has been found to change during epididymal maturation (Willson and Katsh, 1965; Killian and Amann, 1973). The use of monoclonal antibodies has revealed that in the rat, mouse, boar and guinea pig, some sperm surface antigens are deposited in the testis, others are acquired in the epididymis, and the distribution of some changes during epididymal maturation (Feuchter et al., 1981; Jones et al., 1983; Myles and Primakoff, 1983; Saxena et al., 1984).

Although there are only minor quantitative and qualitative changes in the integral proteins of the sperm PM during maturation (Voglmayr et al., 1980; Russell et al., 1984), radioiodination of surface glycoproteins containing tyrosine, sialic acid or galactose residues, has revealed significant modifications to exposed surface glycoproteins (Nicolson et al., 1979; Voglmayr et al., 1980, 1983; Olson and Danzo, 1981; Brown et al., 1983; Dacheux and Voglmayr, 1983). It would appear, therefore, that the previously mentioned changes in negative charges, carbohydrates and surface antigens may be the result of changes in the type, quantity or distribution of exposed glycoproteins on the sperm surface.

There is an increasing body of evidence to suggest that the mammalian epididymis is involved in the synthesis, secretion and absorption of proteins at various levels of the tract (Barker and Amann, 1971; Brooks and Higgins, 1980; Jones et al., 1980; Kohane et al., 1980b; Moore, 1980; Dacheux and Voglmayr, 1983; Turner, 1984). The synthesis and secretion of some, but not all, of these proteins is androgen-dependent (Brooks and Higgins, 1980; Jones et al., 1980; Brooks, 1981, 1983a), and some bind to different parts of the sperm surface, often at different levels of the epididymis (Kohane et al., 1980a; Moore, 1981a;

Courtens et al., 1982; Brooks, 1983b; Brooks and Tiver, 1983). These and other studies have lead to the concept that many of the changes occurring at the sperm surface during epididymal maturation are the result of adsorption of epididymal secretory proteins or elution of surface proteins (Killian and Amann, 1973; Moore, 1980; Voglmayr et al., 1980; Moore, 1981a; Dacheux and Voglmayr, 1983), internalization of surface components (Voglmayr et al., 1982), or enzymatic modification of exposed carbohydrate moieties by epididymal galactosyl transferases (Hamilton, 1980; Hamilton and Gould, 1982) or sialyl transferases (Holt, 1982).

4. ACQUISITION OF MOTILITY

Sperm motility is not considered important for sperm transport through the epididymis (Bedford, 1975), since, in their native fluids, epididymal sperm of the rat, mouse, guinea pig, hamster and bull are immotile (Wyker and Howards, 1977; Morton et al., 1978; Acott and Carr, 1984; Turner and Reich, 1985), whilst rabbit, dog and human sperm show variable, but low, degrees of motility (Morton et al., 1978; Turner and Reich, 1985). The visco-elasticity of the cauda epididymal fluid, or the presence of a pH-dependent quiescence factor has been implicated in maintaining this immobile state (Acott and Carr, 1984; Usselman et al., 1984).

Motility is initiated at ejaculation, or upon dilution into a simple salt solution (Turner and Howards, 1978; Acott and Carr, 1984). Sperm from different regions of the epididymis, however, differ in their capacity for motility upon dilution. Testicular sperm are usually immotile (Gaddum, 1968; Walt and Hedinger, 1983), and the percentage of sperm capable of initiating motility increases from the proximal to

distal regions of the epididymis (Gaddum, 1968; Bedford et al., 1973; Hinton et al., 1979; Amann et al., 1982; Moore et al., 1984). Hence it appears that spermatozoa also acquire the capacity for motility during passage through the epididymis. However, there is also a change in the pattern of motility (Bedford, 1975). Testicular sperm are usually immotile, but may exhibit weak vibratile tail movements or low amplitude beats, but never forward progression (Gaddum, 1968; Lambaise and Amann, 1973; Mohri and Yanagimachi, 1980; Mohri et al., 1983). In the caput region, however, the flagellar beat frequency increases, and a non-progressive type of motility develops, which is characterized by sperm swimming in tight circles. The middle piece is curved and rigid, whilst the principal piece exhibits high amplitude beats (Blandau and Rumery, 1964; Gaddum, 1968; Hinton et al., 1979; Mohri et al., 1983; Moore et al., 1984). During transit through the corpus and proximal cauda regions, spermatozoa acquire the mature pattern of motility exhibited by distal cauda sperm. This is characterized by progressive forward motility, with flagellar waves of moderate amplitude travelling the length of the tail, and the plane of bending is asymmetrical and three-dimensional, which leads to rotation of the sperm about its longitudinal axis (Gaddum, 1968; Fray et al., 1972; Woolley, 1977, 1979).

It has been suggested that changes in the sperm metabolism (Voglmayr, 1975), and responsiveness to changes in cAMP and carnitine levels (Hinton et al., 1981; Amann et al., 1982) may be involved in the initiation of motility in epididymal sperm. Hoskins et al. (1978) postulated that two separate processes, which act synergistically, are involved in the bull, but may be common to many species (Acott et al., 1979). These are an increased intra-cellular cAMP level, and binding of a forward motility protein (FMP). Bovine FMP is a 37.5 kd protein that is secreted by the epididymis, and binds to sperm in the caput region

(Acott and Hoskins, 1978, 1981). Its principal effect is on the flagellar beat, and in caput sperm, it induces a similar but not quite identical pattern of motility to that observed in mature sperm (Acott et al., 1983).

Mohri and Yanagimachi (1980) found that maturation of the axoneme takes place during sperm maturation. This could involve phosphorylation of the microtubules (Tongkao and Chuvalatnatol, 1979). The increase in disulphide bonding of tail accessory structures could affect the pattern of motility (Bedford and Calvin, 1974b), and direct evidence for this has been provided by the finding that treatment of hamster caput sperm with a sulphydryl oxidizing reagent resulted in a mature pattern of motility (Chang et al., 1983).

5. ACQUISITION OF FERTILIZING ABILITY

The most important or cumulative feature of sperm maturation in the epididymis is the acquisition of fertilizing ability (Bedford, 1975). Testicular sperm are completely infertile (Lambaise and Amann, 1973), but the rate of fertilization increases as sperm from more distal regions of the epididymis are used for insemination (Blandau and Rumery, 1964). The region in which sperm first acquire the ability to fertilize varies between species, but there is usually a sharp rise in the fertility of sperm from the distal corpus or proximal cauda (Bedford, 1966; Horan and Bedford, 1972; Dyson and Orgebin-Crist, 1973; Holtz and Smidt, 1976; Fournier-Delpech et al., 1979). This has also been confirmed using in vitro fertilization techniques, in which sperm from regions proximal to the cauda have been found to be unable to fertilize oocytes with zonae, although some zona-free oocytes are penetrated by corpus sperm (Pavlok, 1974; Moore, 1981b; Moore et al., 1983). There is

also some evidence that even though rabbit sperm from the distal corpus can fertilize oocytes in vivo, there is a delay in fertilization and cleavage, and an increased incidence of embryonic mortality (Orgebin-Crist, 1967b), although this has not been substantiated by the findings of Overstreet and Bedford (1974, 1976).

In an early study on the function of the epididymis, Young (1931) concluded that maturation only requires time for sperm to complete the changes begun in the testis. Ligation studies, however, have indicated that time alone is not sufficient for the acquisition of fertilizing ability (Orgebin-Crist, 1967a; Horan and Bedford, 1972), and it has become apparent that to acquire full fertilizing ability, the maturing spermatozoon must come in contact with the environment provided by a certain region(s) of the epididymis (Orgebin-Crist, 1967a; Dyson and Orgebin-Crist, 1973). This process is under androgenic control, but the androgens exert a direct effect on the epididymal tissue, not on the sperm (Blaquier et al., 1972; Dyson and Orgebin-Crist, 1973; Orgebin-Crist, 1973). Hence, it is interesting that there is a close parallel between the acquisition of fertilizing ability and the binding of epididymal proteins to the sperm surface (Moore, 1980, 1981a). This may be mediated through changes which permit sperm to undergo the acrosome reaction or bind to the zona pellucida, both of which develop during sperm maturation (Cummins, 1976; Fournier-Delpech et al., 1982; Saling, 1982; Fournier-Delpech et al., 1984). The structural integrity of the sperm head, and motility are also important for fertilization (Yanagimachi, 1981; Bedford, 1983b). It appears, therefore, that the acquisition of fertilizing ability is a complex process that involves a close interaction of the spermatozoon with the epididymal environment, and necessitates changes in the morphology, structural integrity, motility and surface properties of sperm.

D. THE EUTHERIAN OOCYTE

The female gamete or oocyte is a very large, highly specialized cell, which varies in diameter from 60-120 μm , and thus, is many times larger than the spermatozoon. The spherical vitellus is surrounded by two extra-cellular coats, the zona pellucida and cumulus oophorus. The zona pellucida consists of a thick layer of glycoproteins, and is separated from the vitellus by a narrow, fluid-filled space called the peri-vitelline space. The cumulus oophorus surrounds the entire oocyte, and is comprised of a large number of granulosa cells from the ovarian follicle, embedded in an extracellular matrix (Austin, 1961; Wolf, 1983a).

1. VITELLUS

1.1 Meiotic Spindle

During the pre-ovulatory maturation of the oocyte, the first meiotic division is completed and the first polar body ejected, but the second meiotic division is arrested at metaphase. Thus, in all species except the dog, ferret and possibly horse, the oocyte is ovulated at this arrested stage (Austin, 1961). The meiotic spindle and chromosomes are located in the peripheral cytoplasm, in a zone devoid of most organelles (Zamboni and Mastroianni, 1966; Zamboni, 1970; Szollosi, 1971; Cran et al., 1980; Kruip et al., 1983). Neither centrioles nor asters have been observed in association with the spindle, the axis of which is oriented tangentially to the egg surface (Zamboni and Mastroianni, 1966; Szollosi, 1971; Zamboni, 1972; Longo, 1974). In the mouse, the elongated chromosomes assume the typical dyad form shortly after ovulation (Zamboni, 1970).

1.2 Organelles

The cytoplasm contains the usual array of cellular organelles, although their distribution varies between species. Golgi are present in the cortical zone of mouse oocytes (Zamboni, 1970), throughout the rabbit oocyte (Zamboni and Mastroianni, 1966), but are not usually detected in hamster or bovine oocytes (Weakley, 1966; Kruip et al., 1983). Large numbers of mitochondria are scattered randomly throughout the cytoplasm (Weakley, 1966; Fleming and Saacke, 1972; Longo, 1974a; Cran et al., 1980), and contain cristae which run parallel to the outer membrane, and a very dense matrix (Merchant and Chang, 1971; Szollosi, 1972). In sheep and cow oocytes, the mitochondria often assume a "hooded" appearance, with a curved process protruding from one end (Senger and Saacke, 1970; Cran et al., 1980). Smooth endoplasmic reticulum, ribosomes and lipid droplets are abundant, especially in association with mitochondria, but rough endoplasmic reticulum is uncommon (Weakley, 1966, 1968; Fleming and Saacke, 1972; Zamboni, 1972; Cran et al., 1980). Multivesicular bodies are common in hamster and mouse oocytes (Weakley, 1968; Longo, 1974a). Mouse, rat and hamster oocytes also contain additional structures called cytoplasmic non-membranous lamellae (Weakley, 1968). They consist of stacks of thin sheets, the size, width and thickness of which vary between species (Weakley, 1968). Each sheet consists of repeating rhombohedral units (Green, 1985).

1.3 Cortical Granules

Austin (1956) observed that unfertilized hamster oocytes refracted light under darkfield illumination, whereas fertilized eggs did not. Phase contrast microscopical observations revealed the presence of evenly distributed cortical granules just beneath the oolemma.

Subsequent studies have demonstrated the presence of cortical granules in the unfertilized oocyte of all species (Szollosi, 1962, 1967; Cran et al., 1980; Sathananthan and Trownson, 1982; Kruip et al., 1983).

Cortical granules are spherical or slightly ovoid organelles which usually lie immediately below the oolemma, separated from it by a space of a few hundred angstroms or less (Gulyas, 1980). They are enclosed in a single membrane, and vary in size from 80-600 nm in diameter, although most are in the range 200-500 nm (Austin, 1956; Szollosi, 1962, 1967; Nicosia et al., 1977; Gulyas, 1980). The density of the contents varies between species, as well as between different granules of the same oocyte, probably reflecting differences in maturity or function (Gulyas, 1980). The contents range from uniformly light to uniformly dark (Nicosia et al., 1977), and usually consist of homogeneous material (Szollosi, 1967), although a crystalline structure has been reported in mouse cortical granules (Nicosia et al., 1977). In human and sheep oocytes, the cortical granules typically possess an electron-dense core, with a thin halo beneath the membrane (Cran et al., 1980; Sathananthan and Trownson, 1982).

Most cortical granules occur in fairly uniform single or multiple layers beneath the oolemma, but may also occur in small clusters. Some may be found deeper in the cytoplasm, but this is uncommon (Zamboni and Mastroianni, 1966; Nicosia et al., 1977; Gulyas, 1980). They are absent, however, from the region overlying the meiotic spindle, which in the mouse comprises 17-24% of the surface area (Szollosi, 1967; Nicosia et al., 1977; Sathananthan and Trownson, 1982). Austin (1956) estimated that the hamster oocyte contained about 8,000-15,000 cortical granules. The density is about 33-95 per 100 μm of membrane (Nicosia et al., 1977; Gulyas, 1980).

Knowledge of the chemical composition of the granule contents is sparse. Several studies have suggested the presence of both proteins and carbohydrates (Szollosi, 1967; Guraya, 1969). In addition, a trypsin-like protease has been described from the cortical granules of hamster and mouse oocytes (Gwatkin et al., 1973; Wolf and Hamada, 1977), whilst Gulyas and Schmall (1980) reported ovoperoxidase activity in mouse cortical granules.

1.4 Surface Specializations

The surface of the oocyte is characterized by a relatively dense covering of microvilli of similar size (Phillips and Shalgi, 1980a; Ebensperger and Barros, 1984). They are not present, however, on the surface of the mouse oocyte overlying the spindle, which is relatively smooth and comprises about 20% of the surface area (Nicosia et al., 1977, 1978). This cortical polarity forms during oocyte maturation, and relates to movement of the meiotic spindle to the cortical cytoplasm (Longo and Chen, 1985). There is dispute as to whether a similar region occurs in the hamster oocyte (Longo, 1974a; Phillips and Shalgi, 1980a; Ebensperger and Barros, 1984).

A freeze-fracture study by Suzuki and Yanagimachi (1983) revealed a random distribution of 10 nm particles in the hamster oolemma, although smaller particles were found in the membrane adjacent to cortical granules. There is a moderate but random distribution of negatively charged groups on the oocyte surface (Cooper and Bedford, 1971b; Yanagimachi et al., 1973). Using a variety of lectins, significant differences in the exposed sugar moieties have been detected (Nicolson et al., 1975; Yanagimachi and Nicolson, 1976; Wu et al., 1984). In the mouse oocyte, the smooth area over the spindle does not bind Con A, unlike the remainder of the surface (Johnson et al., 1975; Nicolson,

et al., 1975), and it is interesting to note that this region also exhibits greater mobility of membrane proteins (Wolf and Ziomek, 1983).

2. PERI-VITELLINE SPACE AND FIRST POLAR BODY

Just prior to ovulation, the follicle cell processes are retracted from the zona pellucida, and a narrow, fluid-filled peri-vitelline space forms between the vitellus and zona pellucida (Odor, 1960). Microvilli extend into the peri-vitelline space, but it contains little else other than fine flocculent material that stains with ruthenium red (Sathananthan and Trounson, 1982; Talbot and DiCarlantonio, 1984b). Membrane whorls, vesicles and organelles may also be found, but probably originate from breakdown of the first polar body (Odor, 1960; Nicosia et al., 1977).

Located within the peri-vitelline space, and overlying the spindle, is the first polar body, which is extruded upon completion of the first meiotic division (Austin, 1961). It is usually flattened and elliptical in shape, and contains cortical granules, vesicles, mitochondria, and an irregular mass of condensed chromosomes, which are not surrounded by a nuclear envelope (Baca and Zamboni, 1967; Zamboni, 1970; Longo, 1974a).

3. ZONA PELLUCIDA

The zona pellucida is a 3-15 μ m thick transparent glycoprotein coat that is deposited around the oocyte during oogenesis (Austin, 1961; Piko, 1969; Wolf, 1983a). Early ultrastructural studies suggested it was a product of either the follicle cells (Chiquoine, 1960), oocyte (Kang, 1974), or both (Odor, 1960; Stegner and Wartenberg, 1961). Recent biochemical and cytochemical studies indicate that the zona pellucida is

synthesized primarily by the oocyte (Haddad and Nagai, 1977; Bleil and Wassarman, 1980b; Bousquet et al., 1981; Greve et al., 1982), although the follicle cells may participate in some stages (Tesoriero, 1984; Wolgemuth et al., 1984). It is postulated to function in the prevention of polyspermy, holding the blastomeres together until tight junctions form at the 8-cell stage, ensuring normal transport of the embryo to the uterus, and providing physical protection for the embryo (Gwatkin, 1982).

3.1 Structural Features

The zona pellucida consists of homogenous, finely granular or filamentous material of moderate electron density (Zamboni and Mastroianni, 1966; Longo, 1974a; Baranska et al., 1975; Dudkiewicz et al., 1976; Talbot, 1984). In the human, pig and hamster zonae, there is a gradient of decreasing density from the inner to outer surface (Stegner and Wartenberg, 1961; Dudkiewicz et al., 1976; Dietl and Czuppon, 1984). Baranska et al. (1975), however, claim the mouse zona is most dense on its outer surface.

Scanning electron microscopical studies reveal that in a wide variety of species, the zona pellucida appears as a fenestrated or sponge-like structure, with a lattice-work of fibrous material interspersed with pores of variable diameter (Dudkiewicz and Williams, 1977; Meyenhoffer et al., 1977; Phillips and Shalgi, 1980a, 1980b; Tesoriero, 1984). The basic appearance of the outer surface of the zona is similar in all species, although there are differences in the pore diameter and thickness of the fibrous material. The distribution of pores is usually random, except in the cat, where they are arranged concentrically. The largest pores are found in the rabbit and cat zonae, and the smallest in the cow zona (Dudkiewicz and Williams, 1977). The

pores may represent the paths left after retraction of follicle cell processes (Dunbar, 1983), and may be responsible for its permeability properties (Hastings et al., 1972; Sellens and Jenkinson, 1975). The inner surface of the zona, however, is morphologically dissimilar, and either contains pores of greatly reduced size (Dudkiewicz and Williams, 1977; Dunbar, 1983), or lacks them completely (Phillips and Shalgi, 1980b).

3.2 Chemical Composition

Histochemical studies have demonstrated that the zona pellucida consists mainly of neutral glycoproteins and sulphated glycosaminoglycans, and also contains sialic acid and hyaluronic acid (Braden, 1952; Da Silva Sasso, 1959; Stegner and Wartenberg, 1961; Soupart and Noyes, 1964; Tadano and Yamada, 1978). The hyaluronic acid is probably from the matrix of the cumulus oophorus, which extends into the porous outer layer (Talbot, 1984; Talbot and DiCarlantonio, 1984b). The presence of negatively charged groups has been found using CIH labelling (Yanagimachi et al., 1973), and a variety of glycosidic groups localized using a variety of lectins (Nicolson et al., 1975; Oikawa et al., 1975; Yanagimachi and Nicolson, 1976; Dunbar et al., 1980; Watanabe et al., 1981; Wu et al., 1984).

The mouse and rat zonae contain about 4-5 ng of protein, which constitutes about 80% of its dry mass (Repin and Akimova, 1976; Bleil and Wassarman, 1980a; Wassarman, 1983). The pig and bull zonae each contain 33-36 ng of protein (Dunbar et al., 1980; Gwatkin et al., 1980). Recent biochemical studies have isolated and characterized the zona glycoproteins in several species.

The mouse zona is composed of three sulphated glycoproteins called ZP1 (200 kd), ZP2 (120 kd) and ZP3 (83 kd), which constitute 36,47 and

17% respectively. Each protein has a unique polypeptide sequence, but they also vary in sugar composition (Bleil and Wassarman, 1980a; Shimizu et al., 1982). No function has been attributed to ZP1. The major glycoprotein, ZP2, is evenly distributed throughout the zona (East and Dean, 1984). ZP3 functions as the sperm binding protein, but also induces the acrosome reaction in vitro (Bleil and Wassarman, 1980c, 1983). The binding activity is dependent on the carbohydrate portion, whilst the ability to induce the acrosome reaction also depends on the polypeptide sequence (Florman et al., 1984).

The porcine zona pellucida also consists of three major families of glycoproteins, which are called ZP1 (90 kd), ZP2 (65-70 kd), and ZP3 (55-60 kd). The main family is ZP3. Each glycoprotein family has a distinct polypeptide chain, but contains up to 30 members, each differing in size and charge due to heterogeneity of the carbohydrate groups (Dunbar et al., 1980, 1981; Sacco et al., 1981).

A study by Sacco et al. (1981) of the zonae of human, mouse, rabbit and squirrel monkey oocytes, revealed that the zona in each species is composed of 2-4 families of glycoproteins (43-132 kd), each family exhibiting considerable size and charge heterogeneity.

The zona pellucida is highly antigenic, and zona antibodies result in inhibition of fertilization (reviewed by Dunbar, 1983). In the mouse and pig zonae, the principal antigen is the major glycoprotein, the receptor molecules are not very antigenic, and the inhibition of zona binding appears to be due to steric hindrance (Ahuja and Tzartos, 1981; Aitken et al., 1982; Sacco et al., 1983; East and Dean, 1984). ZP3, the major porcine glycoprotein family, exhibits multiple antigenic determinants (Yurewicz et al., 1984). Studies of the inter-specific cross-reactivity between zona antigens has revealed a variable array of species-specific and shared antigenic determinants (Gwatkin et al.,

1980; Sacco et al., 1981; Wood and Dunbar, 1981; Drell and Dunbar, 1984).

One final point worth discussing is the heterogeneity of the inner and outer surfaces of the zona. Dietl and Czippon (1984) suggest this may be due to a difference in the spatial arrangement of the components, rather than differences in chemical composition. This view is strengthened by the finding that ZP2, the major glycoprotein of the mouse zona, is distributed evenly throughout the zona (East and Dean, 1984). Nevertheless, real differences in the chemical nature of the zona substance have been demonstrated. A differential binding of lectins has been demonstrated in several species (Nicolson et al., 1975; Yanagimachi and Nicolson, 1976), and Tadano and Yamada (1978) localized acidic complex carbohydrates only on the outer surface. Fab antibodies to the hamster zona do not bind uniformly, suggesting an immunological dissimilarity (Ahuja and Bolwell, 1983). This slightly heterogeneous distribution of some components may affect the physiological properties of the zona, such as the greater capacity of the outer surface of hamster zonae to bind sperm (Phillips and Shalgi, 1980b; Ahuja and Bolwell, 1983).

4. CUMULUS OOPHORUS

During the final stages of oocyte maturation, the cluster of follicle cells around the oocyte undergoes a series of changes which includes expansion, deposition of an extra-cellular matrix, changes in the shape of the cells, and cessation of inter-cellular coupling between cumulus cells and the oocyte (Andersen and Albertini, 1976; Gilula et al., 1978; Phillips et al., 1978, Moor et al., 1980). The mature cumulus oophorus consists of a large number of cells which radiate away

from the oocyte in a three-dimensional array, embedded in an extra-cellular matrix (Austin, 1961; Ball et al., 1982). The inner layer of cells is closely adherant to the zona and tightly packed in some species, and is known as the corona radiata (Austin, 1961). In the hamster cumulus, it has been estimated there are about 5,700 cells, which are spaced 14 μm apart in the corona layer, but 50 μm apart elsewhere (Talbot and DiCarlantonio, 1984a). The cumulus cells have a blebbed surface (Phillips et al., 1978), but have few other unusual features (Zamboni, 1970; Tesarik and Dvorak, 1982).

The matrix appears to consist of fine flocculent material with routine fixation procedures (Zamboni, 1970; Tesarik and Dvorak, 1982). Stabilization of the matrix with ruthenium red, however, reveals that in the hamster it consists of 37-38 nm diameter granules, interconnected by 5 nm thick filaments. The filaments are abundant near the corona cells, but are absent in the outer regions of the cumulus, where the granules often aggregate. The matrix is similar in the mouse cumulus, but the granules are slightly larger (Talbot and DiCarlantonio, 1984b).

Hyaluronidase has been found to dissolve the cumulus matrix, suggesting the presence of hyaluronic acid (McClellan and Rowlands, 1942). This has since been confirmed (Braden, 1952; Tadano and Yamada, 1978), and hyaluronic acid constitutes 95% of the matrix secreted in bovine pre-ovulatory follicles (Ball et al., 1982). However, it has also been demonstrated histochemically that the matrix contains neutral glycoproteins, sulphated glycosaminoglycans and mucopolysaccharides, but not sialic acid (Tadano and Yamada, 1978; Talbot and DiCarlantonio, 1984b).

E. GAMETE TRANSPORT IN THE FEMALE TRACT

The fertilization process is complicated, and comprises a number of

stages which lead to the final union of sperm and egg. The first stage is the transport of spermatozoa and oocytes to the site of fertilization in the ampulla of the oviduct.

1. SPERM TRANSPORT

A number of studies have revealed that, of the millions of sperm deposited in the vagina or uterus at ejaculation, relatively few ever reach the ampulla at the time of fertilization. The number of sperm in the ampulla when the oocytes arrive is about 10-17 in the ferret (Hammond and Walton, 1934), 10-24 in the rat (Blandau and Odor, 1949; Braden and Austin, 1954a), 17-19 in the mouse (Braden and Austin, 1954a), 250 in the rabbit (Braden, 1953), and about 673 in the sheep (Braden and Austin, 1954a). The significance of this is probably several fold. Firstly, it may be a mechanism to provide just enough sperm for maximal fertilization rates, whilst preventing polyspermy (Braden and Austin, 1954a). Secondly, it may constitute selection of a population of spermatozoa by the female tract (Cohen and McNaughton, 1974), although evidence exists both for and against this postulate (Overstreet and Katz, 1977; Cohen, 1983). Thirdly, the millions of sperm that do not reach the ampulla may serve physiological functions beyond fertilization, such as fostering embryonic development (Chaykin and Watson, 1983). Nevertheless, it is obvious that definite mechanisms must operate to limit the transport of all but a few spermatozoa to the site of fertilization.

In the rabbit, within minutes of insemination, there is a rapid transport of mainly immotile and degenerate sperm to the ampulla. This rapid phase of transport lasts only a few minutes, and may occur in other species (Overstreet and Katz, 1977). It is a passive mode of

transport, mediated by muscular contractions of the uterus and oviduct, and the sperm involved appear to play no role in fertilization. It may, however, act as a messenger to prime the female tract for receiving the sperm that will fertilize the oocytes (Overstreet, 1983).

Shortly after the rapid phase, a second population of sperm becomes established, which will participate in the events of fertilization. Transport of these sperm to the oviduct depends on muscular contractions and ciliary currents, as well as sperm motility. The number of sperm reaching successive regions of the female tract decreases, and sperm may be sequestered at the cervix, utero-tubal junction, or isthmus of the oviduct (Overstreet, 1983).

After vaginal insemination, the cervix acts to a variable degree as an anatomical block to sperm passage. In the rat, large numbers of sperm reach the uterus within 6-10 minutes (Matthews and Adler, 1977), but in other species, sperm may be sequestered in the cervical canals, where motility is depressed. The exact functional significance of this is unclear (see review by Overstreet, 1983). After intra-uterine insemination, it is believed that sperm quickly migrate to the utero-tubal junction. It seems doubtful that this acts as any more than a temporary sperm reservoir (Overstreet, 1983). Sperm motility appears to be an important factor for traversal of this barrier (Gaddum-Rosse, 1981). The isthmus of the oviduct, once reached, appears to act as a major reservoir of sperm (Yanagimachi and Mahi, 1976; Shalgi and Kraicer, 1978; Overstreet, 1983; Hunter, 1981; Hunter and Nichol, 1983). Sperm begin to enter the isthmus soon after coitus, and sufficient numbers for fertilization are present by well before ovulation (Shalgi and Kraicer, 1978; Hunter, 1981). The reservoir is situated in the caudal isthmus region, near the utero-tubal junction, and few sperm are found above this region (Hunter, 1981; Hunter and Nichol, 1983). Sperm

are sequestered in the narrow epithelial folds with their motility greatly reduced (Overstreet and Cooper, 1975; Flechon and Hunter, 1981; Overstreet, 1983).

Few, if any, sperm are present in the ampulla until about the time of ovulation, when they ascend from the isthmus in small numbers (Braden, 1953; Yanagimachi and Mahi, 1976; Shalgi and Kraicer, 1978; Overstreet and Cooper, 1979b; Hunter, 1981, 1984; Hunter and Nichol, 1983). This is an active process, and does not simply reflect the time required for sperm to reach the ampulla (Hunter, 1981). It may be the result of increased oviductal contractions which occur at this time (Battalia and Yanagimachi, 1979), or adovarian ciliary currents (Gaddum-Rosse and Blandau, 1973b, 1976; Hunter, 1981). Sperm also exhibit an altered pattern of motility called "hyperactivation" (Yanagimachi, 1981), which may contribute to their release from the isthmus and transport to the ampulla (Overstreet and Cooper, 1979).

2. OOCYTE TRANSPORT

After ovulation, the oocytes enter the oviduct and are transported to the ampulla. The ovary-ostium arrangement seems to dictate the method of ovum pickup. In rodents, where the ovary is surrounded by a bursa, oocytes are probably swept into the infundibulum by ciliary currents arising from the fimbria (Overstreet, 1983). This is a rather complicated process, however, involving interaction of the cilia with a component of the cumulus matrix, and may rely on other factors as well (Mahi-Brown and Yanagimachi, 1983). In species where a bursa is absent, the ostium usually overlies the ovarian surface at the time of ovulation (Overstreet, 1983).

The passage of the cumulus-oocyte complex from the infundibulum to the distal ampulla is a very rapid process (Braden, 1953), and probably involves ciliary currents which are directed towards the uterus (Gaddum-Rosse and Blandau, 1973a, 1973b, 1976). Muscular contractions of the oviduct may also be important, although an integrated description of the process is unavailable at present (Overstreet, 1983).

F. CAPACITATION

Independent observations by Austin (1951a) and Chang (1951) revealed that sperm were incapable of fertilizing immediately after ejaculation, and needed to reside in the female tract for a certain period of time before they could penetrate the oocyte. This lead Austin (1952) to coin the term "capacitation", to denote the physiological changes that accompanied this residence. The need for sperm capacitation has since been demonstrated in many species (Bedford, 1970), although it can also be achieved in vitro using suitable culture conditions (Rogers, 1978). The time required varies from less than one hour in the mouse (Bryan, 1974), to 8-12 hours in the guinea pig and deermouse (Yanagimachi, 1972; Hanada and Chang, 1978). The region of the female tract in which capacitation occurs may vary between species (Yanagimachi, 1981). It can be achieved in either the uterus or oviduct, although the most efficient form involves a synergistic effect of both environments (Austin, 1951a; Chang, 1951; Bedford, 1969; Hunter and Hall, 1974). It seems that much of the capacitation process is completed before sperm leave the isthmus reservoir (Yanagimachi, 1981), although the final changes may occur during transit to the ampulla (Hunter and Nichol, 1983).

The original definition of capacitation denoted those physiological changes that enabled the spermatozoon to penetrate the zona pellucida (Austin, 1952). It has since become apparent that capacitation is a multi-step process, involving modifications of the sperm surface, which subsequently enables sperm to undergo the acrosome reaction, as well as a change in the pattern of motility (Yanagimachi, 1981). Hence, the term capacitation is often used only to refer to those events which precede the acrosome reaction (Austin, 1967; Bedford, 1983a). The acrosome reaction is, however, an absolute prerequisite for penetration of the zona pellucida (Yanagimachi, 1981), and as such, is included in the original definition (Chang, 1984). Therefore, in accord with the view expressed recently by Chang (1984), the acrosome reaction will be included in this discussion as the second phase of capacitation. It is, however, fully appreciated that the surface changes and the acrosome reaction are distinct phases, and can be separated under some circumstances (Yanagimachi, 1975; Barros and Berrios, 1977; Yanagimachi, 1981).

1. MODIFICATIONS OF THE SPERM SURFACE

1.1 Removal or Alteration of Surface Components

One of the principal changes that occurs during capacitation is the removal of coating substances from the sperm surface, which are adsorbed during epididymal maturation or at ejaculation (Yanagimachi, 1981). A large glycoprotein called "decapacitation factor", which is present in epididymal fluid and seminal plasma, has been found to reversibly inhibit fertilization (Chang, 1957; Bedford and Chang, 1962; Weinman and Williams, 1964; Dukelow et al., 1966, 1967), and is postulated to mask binding sites or stabilize the plasma membrane, thus preventing the

acrosome reaction (Bedford, 1970; Eng and Oliphant, 1978). Removal of this factor probably constitutes part of the capacitation process (Bedford and Chang, 1962). In addition, other changes that have been demonstrated include loss of sperm-coating antigens (Hunter and Nornes, 1969; Oliphant and Brackett, 1973; Koehler, 1976), redistribution of surface antigens during capacitation of guinea pig sperm in vitro (Myles and Primakoff, 1984), and removal or alteration of peripheral membrane polypeptides (Esbenshade and Clegg, 1980; Voglmayr and Sawyer, 1984).

A decrease in the negative surface charge following intra-uterine incubation has also been reported (Vaidya et al., 1971; Courtens and Fournier-Delpech, 1979), although Moore (1979) was unable to confirm these findings. Areas that are free of certain lectin-binding sites, appear on the rabbit and hamster sperm head surfaces during capacitation, which has been interpreted as loss of surface components (Gordon et al., 1975; Kinsey and Koehler, 1978). Loss of certain carbohydrate groups has been confirmed in other species (Schwarz and Koehler, 1979; Lewin et al., 1979), but it has also been shown that there may be an increase in lectin labelling on some regions of the sperm, which is possibly due to exposure of cryptic binding sites after removal of coating substances (Talbot and Franklin, 1978; Ahuja, 1984).

Koehler (1981) suggests that this phase of capacitation may involve an indiscriminate sweeping of bound glycoproteins from the sperm surface, which then exposes sites that are predisposed for fusion during the acrosome reaction. The removal or alteration of surface components may be achieved by simple elution (Yanagimachi, 1981), phosphorylation of proteins (Huacuja et al., 1977), or enzymatic modification (Kirton and Hafs, 1965).

1.2 Changes to the Plasma Membrane

There are no observable ultrastructural changes in the sperm PM other than a loosening or ballooning away from the OAM (Bedford, 1964, 1970; Koehler, 1976; Kinsey and Koehler, 1978). Freeze-fracture studies, however, have revealed that the characteristic glycocalyx on the guinea pig sperm PM is lost during capacitation, and the quilt pattern is replaced by a random array of particles (Friend and Fawcett, 1974; Friend et al., 1977). The circumferential strands of particles on the middle piece also become disorganized (Koehler and Gaddum-Rosse, 1975; Friend, 1977). In the guinea pig and hamster, particle-free regions appear on the peri-acrosomal PM, and have been suggested as possible sites of membrane fusion during the acrosome reaction (Friend et al., 1977; Kinsey and Koehler, 1978; Friend, 1980).

Considerable changes also occur in the lipid bilayer during capacitation. The proportions of various phospholipid classes in the membrane may change (Clegg et al., 1975), there may be a rearrangement of amino phospholipids (Elliott and Higgins, 1983), or an increased methylation of phospholipids, which would tend to increase the membrane fluidity (Llanos and Meizel, 1983). Evidence has also been presented that there may be transfer of phospholipids from albumin to the sperm PM, and of cholesterol from sperm to the protein, which would decrease the microviscosity of the bilayer and increase its fluidity, thereby facilitating the acrosome reaction (Davis et al., 1979, 1980). Another stabilizing influence, cholesteryl sulphate, is also removed from the sperm PM (Langlais et al., 1981). The importance of membrane fluidity is further illustrated by studies in which bat sperm were cooled to 4°C, which prevents capacitation and the acrosome reaction, and then rewarmed to 32°C, which initiated these changes (Lambert, 1981).

During capacitation of guinea pig sperm in vitro, there is a redistribution of lipid moieties in the plasma membrane. After the quilted glycocalyx is lost, the level of anionic phospholipids and cholesterol increases in the peri-acrosomal PM, and there is a flip-flop of phospholipids to the external leaflet of the bilayer (Bearer and Friend, 1982). When the particle-free zones appear, however, cholesterol is excluded from these regions, which is interpreted as an adaptation for membrane fusion with the OAM (Friend, 1980).

2. MOTILITY CHANGES ACCOMPANYING CAPACITATION

2.1 Hyperactivation

Sperm gradually acquire the capacity for active movement during epididymal maturation. It has been found that there is a change in this pattern of motility during capacitation (Yanagimachi, 1981). Whilst studying hamster sperm capacitation, Yanagimachi (1970) described a different form of motility, which was called "activation", although it has since been changed to "hyperactivation" (Yanagimachi, 1981). A similar type of hyperactivated motility has now been found to accompany capacitation in the mouse (Fraser, 1977a), rabbit (Cooper et al., 1979), guinea pig (Yanagimachi, 1972), dog (Mahi and Yanagimachi, 1976), dolphin (Fleming et al., 1981), ram (Cummins, 1982), Chinese hamster (Yanagimachi et al., 1983a), man (Mortimer et al., 1984), and primates (Moore, 1981b).

The geometrical details of hyperactivation vary between species, but the basic features are an increased amplitude of flagellar bending, a decreased beat frequency, wide arcs of movement of the sperm head sometimes tracing figure-of-eight patterns, and often no net forward

progression (Yanagimachi, 1970; Fraser, 1977; Woolley, 1979; Cummins, 1982).

Hyperactivation is an inherent property of capacitation, and sperm exhibit this pattern of movement after capacitation has proceeded to a certain extent. This is often by the time sperm are ready to acrosome react, but there is no definite relationship between the two events (Barros and Berrios, 1977; Yanagimachi, 1981; Cummins, 1982; Suarez et al., 1984). In vivo, hyperactivated motility usually appears at about the time of ovulation, when sperm leave the isthmus and move towards the ampulla, but it may be interspersed with short bursts of non-activated progressive motility (Yanagimachi, 1970; Cooper et al., 1979; Katz and Yanagimachi, 1980; Johnson et al., 1981; Cummins, 1982; Cummins and Yanagimachi, 1982).

Several functions have been ascribed to hyperactivation, such as escape from the isthmus reservoir (Overstreet and Cooper, 1979), transport to the ampulla, primarily through interaction of sperm with the epithelial surface (Katz and Yanagimachi, 1980), interaction with the cumulus in the lumen of the oviduct (Katz et al., 1978), stirring of the fluid around the sperm head to facilitate transfer of ions, nutrients and enzymes (Dresdner and Katz, 1981), and penetration of the cumulus oophorus and zona pellucida (Katz and Yanagimachi, 1981; Bedford, 1983a, 1983b).

2.2 Mechanism of Hyperactivation

Mohri and Yanagimachi (1980) failed to find any structural changes in the axoneme that could account for hyperactivation, so it has been suggested that some other feature(s) of the sperm tail must prevent its occurrence until capacitation. In the guinea pig, a close correlation has been found between hyperactivation and the loss or disorganization

of the particle strands on the middle piece, which may exert a biomechanical force that restricts the flexion of this part of the sperm tail (Koehler and Gaddum-Rosse, 1975). An increased stabilization of the head-tail junction may also affect the pattern of movement (Young and Cooper, 1983b).

The biochemical basis for the initiation of hyperactivation is rather obscure, but it has an absolute requirement for calcium (Yanagimachi and Usui, 1974; Fraser, 1977; Yanagimachi, 1982). The removal of coating substances may allow calcium access to binding sites on the sperm PM, or the membrane may become more permeable to calcium, thus increasing the level of calcium in the flagellum (Babcock et al., 1978; Triana et al., 1980; Yanagimachi, 1982; Cooper, 1984). The metabolic state of the spermatozoon is also important (Yanagimachi, 1975; Fraser and Quinn, 1981). Evidence has been presented of a role for catecholamines and a β -amino acid called taurine, both of which are present in the oviduct, and sustain motility in vitro (Cornett and Meizel, 1978; Meizel et al., 1980). These substances may act by triggering an increased intra-cellular cAMP level, which may be linked to the metabolism of the cell (Fraser, 1981; Tash and Means, 1983; Cooper, 1984).

3. THE ACROSOME REACTION

The first phase of capacitation is generally viewed as a preparation for the sperm to undergo the acrosome reaction, whereby the contents of the acrosome are released. This is an absolute requirement for fertilization, for without it, sperm can neither penetrate the zona pellucida, nor fuse with the oocyte (Yanagimachi, 1981).

3.1 Morphological Features

The mammalian acrosome reaction is characterized by a series of morphological changes which involve an initial swelling of the acrosome (Jones, 1973; Yanagimachi and Usui, 1974), followed by multiple point fusions between the outer acrosomal membrane and plasma membrane, at points previously cleared of membrane particles and sterols (Friend, 1980; Bearer and Friend, 1982; Yanagimachi and Suzuki, 1985). This results in fenestrations through which the contents of the acrosome escape (Barros et al., 1967). The fenestrated membrane complex adheres to the sperm head via fibrous remnants of the acrosomal matrix (Barros et al., 1967; Russell et al., 1980), and takes on the form of an array of hybrid membranous vesicles, each consisting of roughly equal portions of OAM and PM (Russell et al., 1979). It is unclear as to whether the fusion occurs initially at particular regions of the sperm head, or simultaneously throughout the acrosome (Yanagimachi, 1981). The area of vesiculation includes the entire acrosome except for the equatorial segment (Bedford et al., 1979). Microtubules and/or microfilaments have been found in the region of vesiculation following the acrosome reaction in boar and monkey sperm (Peterson et al., 1978; Stambaugh and Smith, 1978).

This sequence of events has been shown to occur in many species (Bedford, 1968, 1972; Yanagimachi and Noda, 1970b; Jones, 1973; Yanagimachi and Usui, 1974; Szollosi and Hunter, 1978; Shams-Borhan and Harrison, 1981; Crozet, 1984). The time required for completion of the acrosome reaction probably varies between species, although nothing is known about the time required in vivo (Yanagimachi, 1981). Studies in vitro, however, indicate that it requires 3-6 minutes in the guinea pig (Yanagimachi and Usui, 1974), and 20 minutes in the hamster (Talbot and Franklin, 1976).

3.2 Initiation of the Acrosome Reaction

Initiation of the acrosome reaction is strictly dependent on adequate extracellular calcium levels (Yanagimachi and Usui, 1974; Talbot et al., 1976; Yanagimachi, 1982). Due to increased membrane permeability, an influx of Ca^{++} down an electrochemical gradient may constitute the first step of the acrosome reaction (Green, 1978a). The calcium regulatory protein calmodulin may be involved, as it has been localized in the acrosomal region (Jones et al., 1980; Peterson et al., 1983).

The acrosome reaction is a complex process, and may require the action of several compounds for its initiation (Yanagimachi, 1981). Glycosaminoglycans (GAG), which occur in the cumulus matrix, as well as in follicular fluid, can induce the acrosome reaction in bull, boar, and rabbit sperm. The active component is the sulphate moiety, and the most effective GAG is chondroitin-sulphate A (Lenz et al., 1982, 1983a, 1983b). Cabot and Oliphant (1978) suggested a possible role for immunological complement, but Bedford and Witkin (1983) failed to find evidence in support of this. Serum albumin (Lui et al., 1977), catecholamines (Cornett and Meizel, 1978; Meizel and Working, 1980), intracellular levels of inorganic ions (Fraser, 1984), and the availability of metabolic substrates have also been implicated (Rogers and Yanagimachi, 1975b; Fraser and Quinn, 1981).

3.3 Mechanisms of the Acrosome Reaction

A unifying concept for the molecular mechanism of the acrosome reaction has not been forthcoming, and different mechanisms have been proposed by researchers, based mainly on results obtained in their own laboratories (Yanagimachi, 1981). Many features of these concepts are similar, but some of the details, and the interpretations, often vary.

Each of these proposals will now be briefly outlined.

- (1) A mechanism has been proposed by Gordon (1973) and Gordon et al. (1978), based on cytochemical evidence of ATPases in the OAM and PM. Removal of a seminal plasma factor activates a Ca^{++} -independent ATPase on the PM, which transports Ca^{++} into the peri-acrosomal space. Calcium neutralizes phospholipids in the two membranes, and is also transported into the acrosome by a Ca^{++} -dependent ATPase, where it activates proacrosin. After fusion, acrosin digests away the membranes, causing vesiculation and release of the acrosomal contents.
- (2) Yanagimachi and Usui (1974) suggested that calcium enters the peri-acrosomal space and inhibits Mg^{++} -dependent ATPases on both membranes. This depletes water and ion pumping mechanisms, causing the acrosome to swell due to water influx. The membranes come together, and disintegrate due to the action of enzymes.
- (3) Green (1978a, 1978b) disagreed with (2), and proposed that removal of a receptor antagonist on the sperm PM allowed an influx of Ca^{++} into the cytoplasm. No mechanism for fusion was suggested. He suggests that calcium enters the acrosome, and activates the conversion of proacrosin to acrosin, which solubilizes the matrix, thereby increasing the colloid osmotic pressure, and causing the contents to cavitate with their resultant release.
- (4) Davis (1981) suggests that during capacitation, cholesterol is transferred from the PM to uterine fluid proteins, which destabilizes the membrane. Fusion is induced by calcium binding to anionic phospholipids in the PM and OAM, which weakens the bilayer by causing condensation or aggregation of phospholipids.
- (5) Another model proposed by Fleming and Yanagimachi (1981) and Yanagimachi (1981), suggests that removal of coating substances

facilitates calcium influx into the peri-acrosomal space. This stimulates production of lysophospholipids by phospholipases in both membranes. At sufficient concentrations, these induce a phase transition in the lipid bilayers which facilitates fusion. Calcium may also directly induce fusion by neutralizing electrostatic repulsion between the two membranes, and by causing the formation of crystalline phospholipid domains.

- (6) Meizel (1978, 1984) postulates that there is a calcium influx into the peri-acrosomal space. Calcium is transported into the acrosome, where it inhibits a Mg^{++} -dependent ATPase (H^+ pump), which combined with high K^+ levels, causes an efflux of H^+ and a rise in the intra-acrosomal pH. This activates acrosin, which then either acts on OAM proteins, or activates a phospholipase which produces lysophospholipids that destabilize the membranes for fusion. No role is postulated for acrosin in dispersal of the acrosomal matrix.

3.4 Site of the Acrosome Reaction

Studies in vitro of hamster sperm-egg interactions suggest that only acrosome-intact sperm can bind to the zona pellucida (Phillips and Shalgi, 1980b). In the mouse, the acrosome reaction can occur either before or after zona binding, but only acrosome-intact sperm can bind to the zona, penetrate it, and fertilize the oocyte (Saling and Storey, 1979; Saling et al., 1979; Florman and Storey, 1982). This occurs whether the cumulus oophorus is present or absent (Storey et al., 1984). In the guinea pig, however, only acrosome-reacted sperm are able to bind to, and penetrate, the zona pellucida (Huang et al., 1981).

A large number of studies suggest that the situation in vivo may be different. Austin and Bishop (1958b) found that, in five species of

rodents, the acrosome reaction occurred whilst sperm were penetrating the cumulus. This has since been confirmed in the rabbit (Bedford, 1968, 1972), pig (Szollosi and Hunter, 1978), guinea pig (Yanagimachi and Mahi, 1976), golden hamster (Cummins and Yanagimachi, 1982; Yanagimachi and Phillips, 1984), and Chinese hamster (Yanagimachi et al., 1983a). Some confusion still exists as to the site of the acrosome reaction during fertilization in the mouse and rat (Austin and Bishop, 1958b; Stefanini et al., 1969; Toshimori, 1982). Recent studies of in vivo fertilization in the sheep and cow suggest that the acrosome reaction occurs at the zona surface (Crozet, 1984; Crozet and Dumont, 1984). This should be viewed cautiously, however, as the criterion employed was only the presence of acrosomal ghosts on the zona surface, which also occurs in the rabbit and hamster, where the acrosome reaction occurs during passage of the spermatozoon through the cumulus (Bedford, 1972; Yanagimachi and Phillips, 1984).

In conclusion, therefore, there is good evidence that in vitro, the zona pellucida is the site of the functional acrosome reaction in the mouse, but it is doubtful this occurs in most species in vivo, where it is most likely initiated during sperm passage through the cumulus oophorus.

G. SPERM PENETRATION OF THE CUMULUS AND ZONA PELLUCIDA

Once it reaches the site of fertilization, the spermatozoon is faced with two further obstacles, the cumulus oophorus and zona pellucida, which it must negotiate before it can fertilize the oocyte.

1. PENETRATION OF THE CUMULUS OOPHORUS

Several functions in the fertilization process have been ascribed

to the cumulus oophorus. Firstly, glycosaminoglycans from the matrix, or follicular fluid, may be involved in induction of the acrosome reaction (Lenz et al., 1982), although this view is not universally accepted (Bedford, 1983a). Secondly, the cumulus may help prevent polyspermy (Austin, 1961), even though there are usually insufficient sperm present for this to occur (Braden and Austin, 1954a; Cummins and Yanagimachi, 1982). Thirdly, it may act as a barrier to penetration by mis-shapen sperm (Krzanowska and Lorenc, 1983). And lastly, the disposition of the cumulus components may direct sperm towards the zona pellucida (Austin and Braden, 1954), or affect the angle at which sperm penetrate the zona (Shalgi and Phillips, 1983). Nevertheless, the cumulus is not a prerequisite for fertilization, for studies both in vivo and in vitro have shown that fertilization can occur in its absence (Miyamoto and Chang, 1972; Moore and Bedford, 1978b; Yanagimachi, 1981).

It has been postulated that a chemotactic stimulus may draw sperm towards the cumulus (Piko, 1969). It is generally agreed, however, that chemotaxis does not occur in mammalian fertilization (Yanagimachi, 1981; Bedford, 1983a), and that hyper-activation may aid localization of the cumulus by spermatozoa (Katz et al., 1978). Enzymes which are released during the acrosome reaction, especially hyaluronidase, may effect sperm penetration by dissolving the matrix and decreasing its viscosity, thereby permitting sperm to "glide" between the cumulus cells (Austin, 1948; Piko, 1969; Cummins, 1982). It has been suggested, however, that the layout of the cumulus and corona cells would tend to suppress the flagellar waveform of hyperactivated sperm (Talbot and DiCarlantonio, 1984a). This is supported by the finding that hamster sperm within the cumulus exhibit a more progressive, low amplitude beat (Cummins and Yanagimachi, 1982). Nevertheless, hyperactivated sperm move efficiently in confined spaces, and require only a few minutes to penetrate the

cumulus, suggesting that hyperactivation is an important part of the process (Yanagimachi, 1981; Cummins, 1982; Cummins and Yanagimachi, 1982).

Since the initial discovery that hyaluronidase can disperse the cumulus matrix (McLean and Rowlands, 1942), it has become recognized as an important factor in penetration of the cumulus (Yanagimachi, 1981). Further evidence for such a role comes from its localization in the principal segment of the acrosome, which is lost during the acrosome reaction (Barros et al., 1967; Morton, 1977), its acid pH optimum (approximately 4), which corresponds to the acidic environment of the cumulus matrix (Yang and Srivastava, 1974a; Rodger and Young, 1981; Lewin et al., 1982), and studies showing that antibodies to hyaluronidase or inhibitors of this enzyme will inhibit cumulus dispersion and/or fertilization (Metz et al., 1972; Yanagimachi, 1981). Hyaluronidase which is adsorbed to the sperm plasma membrane has been found to be released during capacitation, prior to the acrosome reaction, and thus may assist penetration of the cumulus prior to occurrence of the acrosome reaction (Talbot and Franklin, 1974; Talbot, 1985; Zao et al., 1985). However, recent studies have indicated that peri-vitelline rabbit sperm (Kuzan et al., 1984), and motile cells from other phylogenetic groups which lack hyaluronidase (Talbot et al., 1985) can also penetrate through the hamster OOC to the zona pellucida. This has prompted Talbot (1985) to suggest that hyaluronidase may not be necessary for penetration of the cumulus, but instead may only facilitate this process, and thus its role in fertilization should be re-evaluated.

It should also be noted, however, that hyaluronidase does not disperse the bovine cumulus, suggesting that other enzymes may be involved in cumulus penetration (Lorton and First, 1979). These may

include the so called corona-penetrating esterase (CPE), which is thought to aid penetration of the rabbit corona radiata (Zaneveld and Williams, 1970; Bradford et al., 1976), and enzymes such as arylsulphatase and β -N-acetylhexosaminidase, either alone or in conjunction with hyaluronidase (Allison and Hartree, 1970; Farooqui and Srivastava, 1979, 1980; Dudkiewicz, 1984). Rodger and Young (1981) have postulated that hyaluronidase is the rate limiting step, but that β -N-acetylhexosaminidase and arylsulphatase are also required.

2. PENETRATION OF THE ZONA PELLUCIDA

The second barrier to sperm passage is the tough, elastic zona pellucida. Spermatozoa must first bind to the zona, and then penetrate it to reach the vitellus (Yanagimachi, 1981).

2.1 Sperm Binding

Most of the studies of zona binding have used in vitro fertilization techniques. In the mouse, only acrosome-intact sperm can bind to the zona pellucida (Saling and Storey, 1979). Binding involves the PM on the apical and lateral surfaces of the sperm head, takes about 15 minutes, requires calcium, and initially is reversible (Saling et al., 1978, 1979). The activity of a trypsin-like enzyme (Saling, 1981), galactosyl transferases (Shur and Hall, 1982), or sialylated glycoproteins on the sperm surface (Lambert and Van Le, 1984), have also been implicated in this process. Specific polypeptides on the sperm surface which have an affinity for the zona pellucida, i.e. zona binding proteins, have recently been characterized in the mouse, hamster, rabbit, boar and man (O'Rand et al., 1985; Peterson et al., 1985; Saling and Lakoski, 1985; Sullivan and Bleau, 1985).

A complex series of interactions occur between the hamster zona and sperm in vitro. Capacitated, but acrosome-intact, sperm first loosely attach to the zona. This stage is called "attachment". Several minutes later, sperm become tightly bound during the B₁ phase. This is rapidly terminated after 2 minutes by the synergistic effect of the vitellus and an S₁ factor released from the sperm-zona interaction, probably by a trypsin-like protease. The sperm remain attached to the zona for 30-35 minutes, after which they bind firmly again in the B₂ phase, then penetrate the zona (see review by Hartmann, 1983). The involvement of fucose, galactose, glucosamine and galactosamine residues on the sperm surface has been demonstrated (Ahuja, 1982).

The situation may not apply to other species, and does not necessarily represent the sequence of events in vivo (Yanagimachi, 1981). In the guinea pig, the IAM is the principal region involved in sperm-zona binding, which is calcium-dependent and may involve fucose residues (Huang et al., 1982; Huang and Yanagimachi, 1984). Studies of rabbit and hamster fertilization in vivo reveal that spermatozoa bind to the zona with the vesiculated acrosomal ghost or the IAM (Bedford, 1972; Moore and Bedford, 1983; Yanagimachi and Phillips, 1984), although the equatorial segment or remnants of the acrosomal matrix may also be involved (Yanagimachi, 1981; Yanagimachi and Phillips, 1984).

The species-specificity of sperm-zona binding has also been studied. In man and the guinea pig, sperm show limited or no affinity for heterologous zonae (Bedford, 1977; Yanagimachi, 1981), whereas in other species such as the rat, mouse and hamster, sperm often exhibit affinity for heterologous zonae (Bedford, 1977; Schmell and Gulyas, 1980a; Yanagimachi, 1981; Swenson and Dunbar, 1982; Lambert, 1984). In the hamster, the "attachment" phase is not species-specific, but the "binding" phase is highly species-specific (Hartmann, 1983; Jedlicki and

Barros, 1985). The exact meaning, however, of such interspecific zona binding is unclear at present (Hartmann, 1983), especially owing to the high degree of specificity of zona penetration (Yanagimachi, 1977; Fukuda et al., 1979).

2.2 Zona Penetration

Sperm penetration of the zona pellucida takes 3-7 minutes in the hamster (Yanagimachi, 1966; Yang et al., 1972), and about 20 minutes in the mouse (Sato and Blandau, 1979a). During penetration, the sperm head does not move through the zona smoothly, but often shows erratic movements back and forth, progressing forward a little at a time, accompanied by vigorous tail movements (Yanagimachi, 1966; Sato and Blandau, 1979a; Gaddum-Rosse et al., 1984). The path taken is usually oblique to the zona surface, and often becomes steeper in the inner regions of the zona. A narrow penetration slit remains after penetration (Austin and Bishop, 1958b; Dickmann, 1964; Dziuk and Dickmann, 1965; Marston and Chang, 1966; Yanagimachi, 1966). During some in vitro studies, however, sperm have been observed to pass perpendicularly through the zona, although this has not been observed in vivo (Sato and Blandau, 1979a; Yanagimachi, 1981; Gaddum-Rosse et al., 1984).

The vesiculated acrosomal ghost is left on the zona surface (Bedford, 1972; Crozet, 1984; Yanagimachi and Phillips, 1984), and hence the IAM is directly and closely apposed to the zona substance (Yanagimachi and Noda, 1970c; Bedford, 1972). Although some studies suggest that the equatorial segment vesiculates and is lost during zona penetration (Stefanini et al., 1969; Yanagimachi and Noda, 1970c; Szollosi and Hunter, 1978), it is generally agreed that this probably occurs only in supplementary sperm, and that in physiologically-competent sperm, the equatorial segment remains intact (Barros et al.,

1967; Yanagimachi and Noda, 1970c; Bedford, 1972; Toshimori, 1982; Crozet, 1984). Similarly, the sub-acrosomal material, inner acrosomal membrane, and post-acrosomal region remain intact during zona penetration (Yanagimachi and Noda, 1970c; Bedford, 1972).

Sperm bound to or penetrating the zona pellucida exhibit hyperactivated motility, although the flagellar beat shape is planar (Katz and Yanagimachi, 1981). This pattern of motility, which may or may not involve an increase in power output (Katz et al., 1978; Johnson et al., 1981), has been postulated to be well suited for maximizing the force applied to the zona pellucida by the sperm head (Katz and Yanagimachi, 1981). The bilaterally flattened, and hooked or wedge shaped sperm head has been implicated as an adaptation to penetration of the zona pellucida (Fawcett, 1975; Moore and Bedford, 1983). A similar hypothesis has been proposed to explain the extensive disulphide bonding of the nuclear and sub-acrosomal material, especially in the anterior regions of the sperm head (Bedford and Calvin, 1974a; Bedford, 1983a, 1983b). The perforatorium may act as a mechanical tool for cutting through the zona substance (Yanagimachi and Noda, 1970c). It would appear, therefore, that the thrust developed by hyperactivation, combined with the design of the sperm head and its structural integrity, would greatly facilitate penetration of the zona pellucida (Bedford, 1983a, 1983b). Indeed, it has recently been calculated that a purely mechanical mode of penetration is theoretically possible (Green and Purves, 1984).

Austin and Bishop (1958b) suggested that the so-called "zona lysin", which is supposed to enzymatically digest a path through the zona, may reside in the perforatorium, although it is now accepted that if such an enzyme exists, it is probably one or more of the acrosomal enzymes (McRorie and Williams, 1974). One of these, acrosin, has been

studied intensively as a possible candidate for this role (McRorie and Williams, 1974; Morton, 1976, 1977). The assumption that acrosin is involved in zona penetration is based on a number of observations. Firstly, fertilization in vivo and in vitro is inhibited by natural and synthetic trypsin inhibitors (Stambaugh et al., 1969; Zaneveld et al., 1970, 1971). Secondly, the zona pellucida is solubilized by acrosomal extracts containing acrosin, and by purified acrosin (Srivastava et al., 1965; Stambaugh and Buckley, 1968, 1969; Polakoski and McRorie, 1973; Meizel and Mukerji, 1976; Brown, 1983). And thirdly, it has been found that acrosin is localized on the IAM after the acrosome reaction (Morton, 1975; Green and Hockaday, 1978; Bradford et al., 1981; Johnson et al., 1983; Berruti and Martegani, 1984).

Although this evidence is compelling, it is not conclusive, and much evidence has been compiled against it (Morton, 1977). Firstly, the inhibition of fertilization by trypsin inhibitors is possibly due to inhibition of other sperm enzymes (Morton, 1977), or to prevention of vesiculation, or dispersion of the matrix, during the acrosome reaction (Green, 1978c; Shams-Borhan and Harrison, 1981; Fraser, 1982; Perreault et al., 1982; Meizel, 1984; Huang et al., 1985), or to inhibition of sperm binding to the zona pellucida (Saling, 1981). Secondly, solubilization of the zona by acrosomal extracts has been widely criticized. For instance, crude extracts are often more effective than purified acrosin (Hartree, 1977), and are more effective in the absence of calcium, whereas acrosin requires extremely high calcium levels for maximal activity (Brown et al., 1975; Bhattacharyya et al., 1979; Srivastava et al., 1979). In addition, sheep acrosin is ineffective in solubilizing homologous zonae, even after incubation for 20 hours at a concentration of 20 mg/ml (Brown, 1982). Rabbit sperm can penetrate zonae which have been made resistant to proteolysis by trypsin and

acrosin (Bedford and Cross, 1978), and peri-vitelline (acrosome-reacted) rabbit sperm can fertilize zona-intact rabbit oocytes in vitro (Kuzan et al., 1984). Thirdly, there has been much debate over the localization of acrosin on the IAM (Huang and Yanagimachi, 1985). With the exception of Morton (1975), and Johnson et al. (1983), all the other studies which localized acrosin on the IAM used tagged trypsin inhibitors, which were not shown to bind specifically to acrosin (Morton, 1977). In the study of Morton (1975), the acrosome of uncapacitated sperm was mechanically disrupted, which may be different from a "true" acrosome reaction. In the study of Johnson et al. (1983), although a specific antiserum was used to localize acrosin on the IAM, degenerate sperm were studied, and their results were inconsistent. In addition, several studies using highly specific antisera to purified proacrosin or acrosin, have now shown that acrosin is localized in the acrosomal matrix, and is not bound to the IAM (Garner et al., 1977; Harrison et al., 1982; Huneau et al., 1984; de Vries et al., 1985).

Hence, the evidence now seems to suggest that acrosin may not have a central role, if any, in penetration of the zona pellucida. A redeeming feature, however, could be that there are two populations of acrosin, one which is lost after the acrosome reaction, and another that remains bound to the sperm head (Goodpasture et al., 1981; Huang et al., 1985). It has also been found that boar sperm acrosin exhibits substrate specificity for the porcine zona glycoproteins, and differentially hydrolyzes the three glycoprotein families (Brown and Cheng, 1985; Dunbar et al., 1985; Urch et al., 1985a, 1985b). Hence, the inability of purified boar acrosin to solubilize the porcine zona pellucida (Brown, 1982) does not negate a possible role for acrosin in sperm penetration of the zona pellucida. Rather, these results suggest that the specific proteolytic action of acrosin on the zona glycoproteins may serve to

weaken the zona sub-structure, enabling sperm penetration without compromising the other functions of this structure (Dunbar et al., 1985; Urch et al., 1985b).

As well as acrosin, there are a number of other proteases in the acrosome, some of which have properties similar to acrosin, and may function during zona penetration (Morton, 1976; Hartree, 1977; Ninjoor and Srivastava, 1985). Alternatively, arylsulphatase, disulphide-reductases, β -N-acetylhexosaminidase or hyaluronidase may be involved (Bedford, 1974b; Farooqui and Srivastava, 1980; Perreault et al., 1980). Hyaluronidase may effect penetration of the porous, outer zone, since hyaluronic acid is found in this region of the zona pellucida (Talbot, 1984). A possible synergistic effect of some or all of these enzymes has been suggested (Morton, 1977; Srivastava et al., 1979; Farooqui and Srivastava, 1980; Ninjoor and Srivastava, 1985). The presence and action of these enzymes may explain the discrepancies mentioned earlier. Nothing is known, however, about the localization of such enzymes in the acrosome, except hyaluronidase, so it is not known if they are present on the IAM. If they are not, then it is still plausible that they, and acrosin, may become secondarily adsorbed to the IAM after the acrosome reaction (Morton, 1977).

The current evidence, therefore, suggests that zona penetration requires mechanical and enzymatic processes, but that acrosin may not participate as much as previously thought.

H. SPERM-EGG FUSION AND LATER EVENTS

The final stage in the fertilization process is sperm-egg fusion, incorporation of the spermatozoon, and formation of the diploid zygote.

1. SPERM-EGG FUSION

Fusion of the spermatozoon and oocyte can occur at any place on the microvillous portion of the egg surface, but does not occur in the vicinity of the spindle, where the surface is smooth (Johnson et al., 1975; Nicosia et al., 1977; Shalgi and Phillips, 1982).

There has been much debate as to which region of the sperm head first fuses with the oolemma. Some studies have implicated the post-acrosomal region (Piko, 1969; Stefanini et al., 1969; Yanagimachi and Noda, 1970c; Thompson et al., 1974; Barros and Herrera, 1977), whilst other workers claim it is the equatorial segment (Barros et al., 1967; Barros and Franklin, 1968; Bedford, 1972; Moore and Bedford, 1978a; Shalgi and Phillips, 1980a, 1980b; Phillips and Shalgi, 1982). In the most recent studies, it is usually quoted as being the equatorial segment, although the anterior third of the post-acrosomal region may also be involved (Koehler et al., 1982; Phillips and Yanagimachi, 1982; Talbot and Chacon, 1982; Crozet and Dumont, 1984). Bedford and co-workers suggest that the equatorial segment preserves a discrete region of labile membrane for fusion. It is further suggested that the post-acrosomal region appears to be involved because fusion spreads rapidly from the equatorial segment to this region (Bedford et al., 1979). Hence, it would seem that the equatorial segment, and possibly the anterior part of the post-acrosomal region, is the initial site of fusion (Bedford et al., 1979; Talbot, 1985).

The acrosome reaction is an absolute requirement for fusion, for although acrosome-intact sperm can bind to the oolemma, they cannot fuse with it (Yanagimachi and Noda, 1970d; Wolf et al., 1976; Hanada and Chang, 1978; Phillips and Yanagimachi, 1982; Koehler et al., 1984). It has thus been postulated that, as a result of the acrosome reaction,

there is a change in the properties of the plasma membrane in the equatorial segment and post-acrosomal region (Yanagimachi and Noda, 1970d; Phillips and Yanagimachi, 1982). Freeze-fracture studies of hamster and guinea pig sperm suggest that this change may be reflected in a slight redistribution of membrane particles in the equatorial segment (Friend et al., 1977; Kinsey and Koehler, 1978).

During fertilization in vivo, the sperm head usually lies flat on the surface of the oocyte (Yanagimachi, 1981), although in the rat, it may attach by the convex margin, then rotate to lie flat on the surface at a later stage (Phillips and Shalgi, 1982). In vitro, however, there is often considerable variation in the manner of association of sperm with the vitellus (Shalgi and Phillips, 1980b, 1982). Egg microvilli seem to play an important role in these events, although it is not clear whether the tips of the microvilli, or the inter-villous regions, are involved in fusion (Yanagimachi, 1981; Moore and Bedford, 1983). Numerous microvilli surround the sperm head, initially over the anterior region, but later over the equatorial segment and post-acrosomal region (Yanagimachi and Noda, 1970c; Koehler et al., 1982; Talbot and Chacon, 1982). Fusion then occurs between the oolemma and the sperm PM in the equatorial segment, and spreads posteriorly along the post-acrosomal region (Bedford, 1972; Bedford et al., 1979; Shalgi and Phillips, 1980a; Phillips and Shalgi, 1982).

The possible role of receptors or enzymes in the attachment and binding of sperm to the oolemma has been investigated in several studies. Proteins have been implicated in the mouse because proteases prevent sperm-egg fusion (Wolf et al., 1976). However, treatment of hamster oocytes with proteases or glycosidases does not affect fusion (Hirao and Yanagimachi, 1978a). Treatment of rabbit and hamster oocytes with lectins failed to inhibit fertilization, suggesting that

carbohydrate groups on the oolemma are not important (Gordon and Dandekar, 1976; Yanagimachi, 1977). Wolf et al. (1977) implicated trypsin-like enzyme activity in the binding of mouse sperm to zona-free oocytes, although other studies have failed to confirm this result (Yanagimachi, 1981; Fraser, 1982; Moore and Bedford, 1983).

As soon as sperm-egg fusion commences, there is a sudden reduction or cessation of sperm motility (Yanagimachi, 1966; Yang et al., 1972; Wolf and Armstrong, 1978; Sato and Blandau, 1979a; Gaddum-Rosse et al., 1984). Hence, although sperm motility may be involved in attachment of the sperm to the vitellus, it does not appear to be necessary for fusion (Yanagimachi, 1981). Similarly, neither cytoskeletal elements nor phospholipases are involved (Longo, 1978; Yanagimachi, 1981). The exact mechanism of fusion of the two membranes has not been elucidated, but is thought to be comparable to somatic cell fusion (Moore and Bedford, 1983). Of utmost importance to fusion appears to be the integrity of the sperm plasma membrane, the presence of specific polypeptides in the equatorial segment which play a role in this process (Saling et al., 1985), and the fluidity and lipid phase of the oolemma (Hirao and Yanagimachi, 1978a, 1978b; Fleming and Yanagimachi, 1981). Other requirements include calcium, which may contribute to destabilization of the membranes, and potassium ions (Yanagimachi, 1978; Rogers et al., 1981; Yanagimachi, 1982).

Sperm-egg fusion is markedly less species-specific than sperm penetration of the zona pellucida (Yanagimachi, 1977), although in most species, there is still only a limited degree of fusion with heterologous sperm. The hamster oocyte is an exception, however, and has been found to fuse with sperm from at least eleven species including man (see review by Yanagimachi, 1981), and thus has become a useful diagnostic tool for the study of human male fertility (Yanagimachi

et al., 1976).

2. INCORPORATION OF SPERM

Once fusion is initiated, the entire spermatozoon is drawn into the oocyte (Austin, 1961), except in the vole, Chinese hamster and deermouse, in which the tail is often left in the perivitelline space (Austin, 1957; Marston and Chang, 1966; Pickworth et al., 1968). It takes about 5-12 minutes for incorporation of the sperm head (Wolf and Armstrong, 1978), and 3-6 hours for incorporation of the entire spermatozoon (Gaddum-Rosse et al., 1982, 1984). The mid-posterior region of the sperm head is incorporated first, followed by the anterior region, and then the sperm tail (Barros and Franklin, 1968; Noda and Yanagimachi, 1976; Moore and Bedford, 1978a; Shalgi and Phillips, 1980a). This process seems to be primarily a function of the vitellus (Austin, 1951a; Yanagimachi, 1966; Yanagimachi and Noda, 1970c; Gaddum-Rosse et al., 1982), although several studies have implicated a variable degree of sperm motility (Blandau and Odor, 1952; Wolf and Armstrong, 1978).

Almost immediately after fusion, decondensation of the sperm nucleus commences in the mid-posterior region, and progresses anteriorly, as well as inwards from the nuclear periphery. The nuclear membranes disappear, and dense particles may appear in the post-acrosomal region (Stefanini et al., 1969; Yanagimachi and Noda, 1970c; Bedford, 1972; Thompson et al., 1974; Hunter, 1976). This may involve a change in the shape of the sperm head, and an increase of up to five times in its size (Chang and Hunt, 1962; Stefanini et al., 1969; Wolf and Armstrong, 1978). During this process, sperm protamines dissociate from the chromatin almost as soon as decondensation begins, and the

chromatin is "naked" for a short time until newly synthesized histones are incorporated (Kopecny and Pavlok, 1975a, 1975b, 1984; Rodman et al., 1981).

Nuclear decondensation is not simply due to hydration of the nucleus, but involves a sperm nucleus decondensation factor (SNDF), which is present in the oocyte cytoplasm in greatest amounts just before ovulation (Usui and Yanagimachi, 1976; Yanagimachi, 1977, 1981). This factor may involve disulphide reductase-like activity (Mahi and Yanagimachi, 1975), but does not involve acrosin-like proteases as was once thought (Young, 1979). It is also possible that endogenous egg proteinases may function in this process (Perreault and Zirkin, 1982; Rodman et al., 1982). This subject has recently been reviewed by Zirkin et al. (1985).

Changes also occur in other sperm head structures at this time. The IAM is incorporated, but enclosed in a vacuole, since it does not fuse with the oolemma, and separates from the nucleus (Huang and Yanagimachi, 1985). The equatorial segment and post-acrosomal dense lamina also separate from the nucleus as they are incorporated. These structures may persist in the oocyte cytoplasm until decondensation is complete (Stefanini et al., 1969; Yanagimachi and Noda, 1970c; Bedford, 1972). On the other hand, the perforatorium may persist even beyond the first cleavage division (Blandau and Odor, 1952; Chang and Hunt, 1962). The various tail elements often show signs of degeneration soon after fertilization, but usually persist as long, if not longer, than sperm head structures (Blandau and Odor, 1952; Bedford, 1972).

At the time of sperm-egg fusion, a distinct intermixing occurs between membrane components of the two cells (Yanagimachi et al., 1973; Gaunt, 1983). During sperm incorporation in the mouse, and hamster, a microvillous-free area forms over the incorporated sperm head, and often

enlarges in size concomittant with bulging of the ooplasm, thus forming an incorporation cone (Stefanini et al., 1969; Shalgi et al., 1978; Phillips and Shalgi, 1980a; Shalgi and Phillips, 1980a; 1980b). In the rat, the sperm nucleus lies at the apex of the cone, which reaches its maximum size by 2.5 hours post-fusion, then gradually disappears (Shalgi et al., 1978). However, the formation of the cone is not a ubiquitous event (Battaglia and Gaddum-Rosse, 1984).

3. FORMATION AND CONJUGATION OF PRONUCLEI

Once the sperm nucleus has fully decondensed, the chromatin becomes organized into the male pronucleus (Austin, 1961), a process which takes about 3-4 hours (Oh and Brackett, 1976). A nuclear envelope forms around the dispersed chromatin, then many small, round nucleoli form, which gradually coalesce to form larger nucleoli (Austin, 1961; Bedford, 1972; Hunter, 1976). There are species differences in both the number and size of these nucleoli (Marston and Chang, 1966).

After fusion, the oocyte is activated to complete the second meiotic division and form the female pronucleus (Austin, 1961). The male and female pronuclei form and grow in a similar manner, and at about the same time, although the female pronucleus is usually slightly smaller (Austin, 1951b, 1957; Marston and Chang, 1966; Oh and Brackett, 1976). Upon completion of the second meiotic division, the second polar body is ejected (Austin, 1951b; Sato and Blandau, 1979b), which differs from the first polar body only in its shape, and the chromatin, which is not dispersed (Zamboni, 1970).

The pronuclei migrate from the cortex towards the centre of the oocyte (Austin, 1961). During migration, the pronuclei enlarge, and the nucleoli coalesce and decrease in number (Austin, 1951b). They come to

lie very close together in the centre of the oocyte, then the pronuclear envelopes break down, the chromosomes condense, a mitotic spindle forms and the first cleavage ensues (Austin, 1951b, 1961; Marston and Chang, 1966; Zamboni, 1972).

4. MECHANISMS FOR THE PREVENTION OF POLYSPERMY

The entry of more than one spermatozoon into the oocyte usually results in embryonic death. The low numbers of sperm in the ampulla and the extra-cellular coats around the oocyte minimize the chances of this occurring, but the oocyte has developed two extra mechanisms, collectively called the block to polyspermy. This consists of blocks to sperm penetration at the level of the zona pellucida, and the vitelline membrane (Wolf, 1983b). Each species utilizes one or both of these mechanisms. For instance, in the hamster, dog, pig and sheep, there is a strong zona block, but a weak or absent vitelline block. The mouse, rat, guinea pig, ferret, Chinese hamster and cat use both mechanisms, whereas in the rabbit, pocket gopher and mole, there is only a functional vitelline block (Braden et al., 1954; Austin and Braden, 1956; Wolf et al., 1977; Yanagimachi et al., 1983).

4.1 Zona Reaction

The zona reaction is the first block to polyspermy, and prevents supplementary sperm from binding to, or penetrating, the zona pellucida (Wolf, 1983b). After sperm-egg fusion, the cortical granules are released into the peri-vitelline space, which in some way alters the properties of the zona (Yanagimachi, 1977). Direct evidence for this has been obtained in the mouse and hamster, by using cortical granule exudates to induce a functional zona reaction in unfertilized eggs

(Barros and Yanagimachi, 1971; Gwatkin et al., 1973; Wolf and Hamada, 1977). The zona block is established in the hamster in 8-15 minutes (Barros and Yanagimachi, 1972; Gwatkin et al., 1973), but requires up to 2 hours in the mouse and rat (Braden et al., 1954; Wolf et al., 1977).

Shortly after sperm-egg fusion, the cortical granules fuse with the oolemma, and release their contents into the peri-vitelline space. Several granules may fuse together before fusion with the oolemma. The electron-dense contents often coat the surface of the oocyte, and interact with the substance of the inner layer of the zona pellucida (Austin, 1956; Szollosi, 1962, 1967; Baranska et al., 1975; Nicosia et al., 1977; Santhananthan and Trownson, 1982).

As a result of cortical granule dehiscence, there are a number of changes in the properties of the zona pellucida. There appear to be no distinguishable changes in the morphology of the zona (Piko, 1969; Meyenhoffer et al., 1977; Phillips and Shalgi, 1980a), although it becomes more resistant to proteolytic enzymes, reducing agents, and heat (Chang and Hunt, 1956; Gwatkin, 1964; Cholewa-Stewart and Massaro, 1972; Inoue and Wolf, 1974, 1975a, 1975b). The functional significance of this "zona hardening" is obscure, however, since it occurs in the rabbit, which does not exhibit a functional zona block (Fraser and Dandekar, 1975; Bedford and Cross, 1978), but doesn't occur in the hamster, which has a very strong zona block (Chang and Hunt, 1956; Inoue and Wolf, 1975b). One of the most significant changes is the prevention of sperm binding to the zona pellucida (Barros and Yanagimachi, 1971; Inoue and Wolf, 1975; Gwatkin and Williams, 1977; Meyenhoffer et al., 1977), which may be due to changes in the properties of the zona glycoproteins (Bleil et al., 1981; Bleil and Wassarman, 1983).

It has been suggested that a trypsin-like enzyme present in mouse cortical granules is responsible for at least part of the mechanism of

action of the contents of the cortical granules (Gwatkin et al., 1973; Wolf and Hamada, 1977). This would be consistent with the proteolytic modification of zona glycoproteins as described by Bleil et al. (1981). It has also been suggested that ovoperoxidase activity in the cortical granules may be involved in the zona reaction (Gulyas and Schmell, 1980; Schmell and Gulyas, 1983). The exact mechanism of the zona block, however, is still unclear and remains to be elucidated.

4.2 Vitelline Block

The vitelline block to polyspermy operates at the level of the oolemma to prevent fusion of supplementary sperm (Wolf, 1983b). It is established in about 40 minutes in the mouse (Wolf and Soper, 1978), although Sato (1979) claims it takes less than one minute. A functional vitelline block does not occur in the hamster oocyte (Usui and Yanagimachi, 1976), although evidence has been obtained that it may become established about 3.5 hours after fertilization (Barros and Yanagimachi, 1972).

The mechanism of the vitelline block is poorly understood (Wolf, 1983b). It has been suggested that it may involve the cortical granule exudate (Fraser and Dandekar, 1975; Yanagimachi, 1977), although such a mechanism has been shown not to operate in the mouse (Wolf et al., 1979). It has also been postulated to be mediated through reduced mobility of membrane components (Johnson and Edidin, 1978), or an increase in negative surface charge (Cooper and Bedford, 1971b). The best evidence to date, however, seems to suggest that the vitelline block may be mediated through a change in the electrical potential of the oolemma (Cuthbertson et al., 1981; McCulloch et al., 1981; Wolf, 1983b).

I. AIMS OF THIS PROJECT

1. SPERM MORPHOLOGY OF AUSTRALIAN NATIVE RODENTS

In Australia, there are about 61 species of rodents, which all belong to the family Muridae. There are 53 endemic species, of which 7 belong to the subfamily Murinae and the genus Rattus, whilst the remaining 46 species belong to the subfamily Hydromyinae, and are grouped into 12 genera (Watts and Aslin, 1981).

To date, the sperm morphology of five Rattus species, and about 30 hydromyine species has been studied (Breed and Sarafis, 1979; Breed, 1980, 1983, 1984). The Rattus sperm possess a long, attenuated, curved sperm head (Breed and Sarafis, 1979). However, 25 of the 30 species of hydromyine rodents exhibit a most unusual sperm head morphology, which has not been described in any other mammals, and consists of three curved hooks projecting from the apical margin of the sperm head. There is a single dorsal hook, and two ventral hooks which are united caudally (Breed and Sarafis, 1979; Breed, 1980, 1983, 1984).

An early ultrastructural study of the sperm morphology of the plains mouse, Pseudomys australis, which has a three-hooked sperm head, suggested that the dorsal hook contained nuclear material, which was covered by the acrosome, whilst the two ventral hooks consisted mainly of acrosomal material (Sarafis et al., 1981). It has since been shown conclusively, however, that the ventral hooks are not acrosomal in nature, but consist almost entirely of electron-dense sub-acrosomal material, which is confluent with the perforatorium in the dorsal hook (Flaherty, 1981; Flaherty and Breed, 1983a). The ventral hooks have also been found to contain filamentous actin, using the fluorescent dye NBD-phalloidin (Flaherty et al., 1983). These structural features have

recently been shown to be a characteristic feature of the hooks on the sperm head of the other species, although there are variations in the length, shape and orientation of the hooks (Breed, 1983, 1984).

2. AIMS OF THIS PROJECT

This project represents an extension of the work of Flaherty (1981) on the sperm head morphology of the plains mouse, P. australis. This species was chosen as it has a characteristic three-hooked sperm head, and is available from a breeding colony maintained in the Medical School animal house, University of Adelaide.

Following the discovery of two layers of material in the ventral hooks (Flaherty, 1981), attempts have been made to further define the structural features of the sperm head, especially the hooks, using scanning and transmission electron microscopy, and platinum-carbon replicas. To this end, an ultrastructural study of the formation of the hooks during spermiogenesis has also been undertaken. The ventral hooks are believed to consist largely of proteinaceous material (Flaherty and Breed, 1983a), so the hook proteins have been analyzed by selective solubilization and SDS polyacrylamide gel electrophoresis, to determine which proteins constitute the ventral hooks, and to confirm the presence of actin. The changes that occur during epididymal maturation have also been investigated, with particular emphasis on the morphology and motility of the spermatozoa, and the structural integrity of the nucleus and sub-acrosomal material.

The ultrastructure of the oocyte, especially the zona pellucida and cumulus oophorus, has also been studied to determine if there are any unusual features which could account for the development of the three-hooked sperm head. It was originally intended to study gamete

interactions during fertilization in this species, to gain insight into the fate of the ventral hooks during the acrosome reaction, zona penetration and sperm-egg fusion. However, for reasons that will be explained later, this has not been achieved. Instead, attempts have been made to study sperm-egg fusion during heterologous insemination in vitro using zona-free mouse and rat oocytes.

Portions of this work have already been published (Flaherty and Breed, 1983a, 1983b).

SECTION 2 : ULTRASTRUCTURE OF THE SPERM HEAD

1. INTRODUCTION

In the previous section, a detailed outline has been presented of the structural and biochemical features of eutherian spermatozoa. Although there are inter-specific differences, spermatozoa from all species conform to a general structural pattern (Fawcett, 1975). Nevertheless, some of the variations are highly unusual, such as the three hooks on the sperm head of many of the Australian hydromyine rodents (Breed and Sarafis, 1979; Breed, 1980). In the plains mouse, the two ventral hooks have been shown to consist largely of an extension of the sub-acrosomal material (Flaherty, 1981; Flaherty and Breed, 1983a), a feature which is evident in most of the other species (Breed, 1983, 1984). However, there are a number of aspects of the structure of the ventral hooks which remain obscure, such as the identity of the core and peripheral layers (Flaherty and Breed, 1983a), and the localization of filamentous actin (Flaherty *et al.*, 1983). Hence, it is the aim of this section to address these and other aspects of sperm head morphology in the plains mouse, and to provide, for the first time, a comprehensive study of the ultrastructure of the sperm head. A variety of techniques have been utilized, including scanning and transmission electron microscopy, platinum/carbon surface replicas, silver staining, and localization of actin using NBD-Phalloidin.

2. MATERIALS AND METHODS

2.1 Animals

The animals used in this and subsequent sections were adult male

plains mice, Pseudomys australis, which were 5-13 months old, and had body weights of 40-80 g (see Appendix I).

2.2 Preparation of Sperm Suspensions

Animals were killed by cervical dislocation or an overdose of ether, and the caudae epididymides quickly removed and dissected free of fat and large blood vessels. The large distal tubules were pricked with a 23G needle, and sperm extruded into warm Hank's balanced salt solution (HBSS) or phosphate buffered saline (PBS-8.00 g NaCl, 1.15 g Na_2HPO_4 , 0.20 g KH_2PO_4 , 0.2 g KCL per litre, pH 7.4). After allowing 10 minutes for dispersal, the resultant sperm suspensions were used unwashed, or after washing twice by resuspension and centrifugation at 500 g for 10 minutes. Only suspensions exhibiting rapid dispersion and initial motility of > 70% were used.

2.3 Preparation for Transmission Electron Microscopy (TEM)

Aliquots of washed or unwashed sperm were fixed for 2-4 hours at 4°C in 2.5% glutaraldehyde/2% formaldehyde in 0.1 or 0.15 M cacodylate buffer (pH 7.2), and pelleted in small microcentrifuge tubes at 1500 g for 10 minutes. The pellets were removed, and rinsed in three changes of buffer. Some were then rinsed twice in 0.05 M maleate buffer (pH 5.2), and en bloc stained in the dark for 2 hours in 1% uranyl acetate in maleate buffer (Karnovsky, 1967). The remaining pellets were post-fixed for 1-2 hours in 1% OsO_4 in cacodylate buffer, and either en bloc stained in uranyl acetate, or in some instances, treated with tannic acid according to the method of Simionescu and Simionescu (1976). This involved incubation for 30 minutes in 1% tannic acid (AR code no. 1764, Mallinckrodt Inc.) in cacodylate buffer, followed by a rinse in buffer containing 1% sodium sulphate. All the samples were then dehydrated

through a graded series of alcohols (70,80,85,90,95,100,100/CuSO₄), and embedded in Spurr's epoxy resin.

Ultrathin sections of grey to silver-gold interference colours were cut using a Sorvall Porter-Blum Mt-2B or Reichert Ultracut ultramicrotome, and mounted on uncoated 200 mesh grids. Sections were subsequently stained with saturated uranyl acetate in 70% alcohol, followed by lead citrate (Reynolds, 1963), except those from pellets treated with tannic acid, which were examined unstained. The sections were examined at 80 kV in a Philips EM300, and micrographs recorded on Kodak 4489 film.

2.4 Preparation for Scanning Electron Microscopy (SEM)

Washed sperm were allowed to settle onto round coverslips previously soaked overnight in 0.1% poly-L-lysine, and the excess washed off. All solutions were millipore filtered (0.22 μ m) before use. Sperm were fixed for up to 24 hours in the glutaraldehyde/formaldehyde fixative, rinsed several times in cacodylate buffer, and in some cases, post-fixed in OsO₄. The coverslips were dehydrated through a graded series of acetones (70,75,80,85,90,95,100%), and critical point dried from liquid carbon dioxide. After mounting on stubs, the coverslips were coated with Au/Pd (10-15 nm) and sometimes also with carbon (10-15 nm), and examined at 30 kV in a Siemens-Etec Autoscan or Philips 505 scanning electron microscope. Images were recorded on Kodak Panatomic x film.

2.5 Preparation of Pt/C Surface Replicas

Surface replicas of sperm were prepared according to the method of Phillips (1977a). Washed sperm suspensions were processed as outlined in 2.4, with the omission of post-fixation in OsO₄. In addition, some sperm were incubated in 1% Triton X-100 $\pm$ 1 mM dithiothreitol (DTT) for 60

minutes at 4°C to remove the plasma membrane, then washed twice in 0.05 M tris-HCl buffer (pH 7.4) prior to fixation.

Replicas were prepared in a Denton DV-502 vacuum evaporator at a vacuum of $0.3 - 1.0 \times 10^{-5}$ TORR. The coverslips were shadowed with platinum at an angle of 45°, followed by carbon at 90°. The replicas were removed in 48% hydrofluoric acid, washed in distilled water, cleaned in 5% aqueous sodium hypochlorite, and picked up on uncoated 200 mesh grids. They were examined at 80 kV in a Philips EM 300.

2.6 Measurements

TEM micrographs were recorded at initial magnifications of 27 - 115,000 x, and photographically enlarged 4-5 times. Measurements were made directly from prints using vernier calipers. Magnifications were calibrated using replica line gratings (2160 lines/mm) or catalase crystals. Measurements are shown as the mean $\pm$ standard error of the mean (n = number of observations).

2.7 Phase Contrast Microscopy

Drops of fixed sperm, either washed or unwashed, were placed on a clean slide, a coverslip added, and examined with an Olympus Vanox microscope equipped with phase contrast optics. Micrographs were taken with a x100 (NA 1.30) objective and recorded on Ilford Pan F film.

2.8 NBD-Phalloidin Staining

Actin was localized using a slight modification of Flaherty et al. (1983). NBD-Phalloidin (7-nitrobenz-2-oxa-1, 3-diazole-phalloidin) was obtained from Molecular Probes Inc., Plano, Texas. A stock solution was prepared in absolute methanol (3.3×10^{-6} M) and stored at -20°C. The working solution (3.3×10^{-7} M) was prepared fresh before use, by drying

down 100 μ l of the stock solution, and resuspending in 1.0 ml of 1% formaldehyde in PBS (pH 7.4) containing 100 μ g/ml of L- α -lyso phosphatidylcholine palmitoyl.

Washed sperm suspensions were prepared in PBS and attached to coverslips, then fixed for 15 minutes in 1% formaldehyde in PBS (pH 7.4), extracted with 0.1% Triton X-100 for 2 minutes, and rinsed thoroughly in PBS. The coverslips were incubated with the working solution for 60 minutes in the dark at 37°C in a humidified chamber, rinsed five times in PBS and twice in distilled water, and air dried. They were mounted on slides using Olympus fluorescence immersion oil, and the edges sealed with D.P.X. Slides were examined using an Olympus BHF microscope, fitted with an IF 490 exciter filter, B dichroic mirror, O-515 barrier filter, and a x100 (NA 1.30) phase contrast objective. Paired phase contrast and fluorescence images were recorded on Ilford HP-5 film.

Control preparations were incubated under identical conditions with a working solution devoid of NBD-phalloidin.

2.9 Silver Staining

The distribution of the post-acrosomal region was examined by silver staining sperm according to the procedure outlined by Elder and Hsu (1981). This involved the preparation of a washed sperm suspension, to which formalin (3%) was added to a final concentration of 0.1%, and the suspension fixed for 30 minutes. Sperm smears were prepared on alcohol cleaned slides, rinsed in 70% and 95% alcohol, and air dried. The slides were flooded with 50% aqueous AgNO_3 containing 0.03% formalin, a coverslip added, and incubated in a humidified chamber at 60-65°C for 30-120 minutes. When staining was deemed adequate, the slides were rinsed in three changes of distilled water, dehydrated,

cleared and mounted in P.I.X. The slides were examined with brightfield optics and photographed on Ilford Pan F.

3. RESULTS

Some parts of this section have been previously published (Flaherty and Breed, 1983a).

3.1 Terminology

The following description of sperm head morphology utilizes the terminology of Holt (1979), as applied by Sarafis et al. (1981) and Flaherty and Breed (1983a). Thus, the convex surface of the sperm head is considered as dorsal, and the concave surface as ventral. Apical or anterior is towards the head, whilst caudal or posterior is towards the tail.

The terminology used for sperm structures will be in accordance with Fawcett (1970, 1975), except that the so-called "perforatorium" will be referred to as the pseudoperforatorium (Baccetti, 1979).

3.2 General Features of Sperm Head Morphology

The sperm head of the plains mouse was falciform in shape, with three curved hooks projecting from its apical margin. The maximum length of the head was about 9 μm , and the dorso-ventral width about 2 μm . The hooks curved ventrad from their points of origin, and eventually subtended an angle of 90-100° with respect to the long axis of the sperm head. There was a single dorsal hook, about 7 μm in length, and two ventral hooks about 5.5-6 μm long, which were united caudally (Figs. 1-3). The middle hook was slightly larger than the ventral hook, and also sometimes marginally longer than the other two hooks (Figs. 3,5).

The sperm head was bilaterally flattened and slightly thickened posteriorly, with a curved caudal margin. The tail inserted on the ventral surface, about 3 μm from the caudal margin of the sperm head. Immediately anterior to this was the ventral spur, which was about 0.3 μm long and was sometimes angled slightly towards the hooks (Figs. 1-3).

3.3 Orientation and Shape of the Hooks

3.3.a Orientation

The hooks originated from the anterior margin of the sperm head, and, at their base, there was a deep depression on either side of the head (Figs. 3,5a). The dorsal hook curved gradually, whilst the curvature of the ventral hooks occurred mainly in the region of their bifurcation, about one third of the way along their length (Figs. 1,3).

The orientation of the hooks relative to one another varied, depending on the method of preparation. In unwashed sperm, the three hooks lay in close proximity to each other, and formed an overlapping or stacked array (Figs. 2,5h). In washed sperm, however, this pattern was rarely observed, and the hooks were generally separated by greater distances (Figs. 1,4).

3.3.b Shape of the Dorsal Hook

The dorsal hook varied considerably in shape along its length (Figs. 5a-h). Basally, it was asymmetrical, with one side rounded, and the other forming a blunt edge (Fig. 5b). After about 2 μm , a thin ventro-lateral shelf extended from this blunt edge (Fig. 5c), and further apically, another formed on the rounded side of the hook (Figs. 5d-f). The hook became dorso-ventrally flattened, and crescent-shaped towards its tip (Figs. 5f-h). However, the ventro-lateral shelf remained longer on the side from which it first originated, giving the hook a

slightly asymmetrical crescentic shape, which persisted until near the tip of the hook (Figs. 5f-h). This asymmetry was a constant feature, always occurring on the same side of the sperm head. When viewed as in Figs. 3 and 4, the rounded side faces the observer, whilst the blunt edge faces into the page.

3.3.c Shape of the Ventral Hooks

Initially, there was only a single ovoid hook, which bifurcated to produce two round or ovoid hooks (Figs. 5b-d). Apically, however, the ventral hooks became bilaterally flattened, and their dorsal and ventral margins became constricted (Figs. 5e-h). This was more pronounced on the dorsal margin of the hooks, and only developed on the ventral margin of the most ventral hook near its tip (Figs. 5g-h). At the tip of the hooks, they were thin and blade-like, and often appeared to be pointed (Fig. 5h). The overall shape of the apical part of each hook resembled that of an arrow head (Fig. 4).

3.4 Ultrastructure of the Hooks

3.4.a Dorsal Hook

The caudal two thirds of the dorsal hook contained nuclear material, enclosed in a double nuclear envelope (Fig. 6). Basally, the nucleus was asymmetrical in shape (Figs. 5b, 5c), but decreased in diameter apically, and became round or ovoid (Fig. 5f). The nuclear material was very electron-dense and homogeneous, and there was no evidence of nuclear vacuoles (Figs. 5,6).

For its entire length, the nucleus was enclosed by a thick layer of sub-acrosomal material (SAM), which was confluent with the material in caudal regions of the sperm head, and was most prominent on the dorsal and lateral margins of the hook (Fig. 5). The SAM projected beyond the

termination of the nucleus as a crescent-shaped rod or pseudo-perforatorium (Pp) (Fig. 5g). And along the dorsal margin of the hook, two finger-like projections or dorsal ridges (DR) indented the principal segment of the acrosome, although these may be reduced to only one near the end of the nucleus (Figs. 5b-e). The SAM consisted of moderately electron-dense granular material, which was homogeneous in appearance, and had no definite sub-structure or filaments (Fig. 7). At the lateral edges of the hook, a thin layer of material curled around the acrosome, and had a periodic or banded structure (Figs. 7,8).

The acrosome covered much of the surface of the dorsal hook for its entire length. Basally, it almost completely enclosed the hook, whereas in the apical regions, it covered the dorsal surface and was only present on the ventral surface as a 0.2-0.3 μm wide ventral acrosomal plate (VAP), which seemed to bud off from the rounded side of the hook, but terminated shortly before the tip of the hook. The VAP was not completely enclosed by SAM, and part of its ventral surface was apposed to the plasma membrane (Fig. 5). It was constricted in its central region, with slightly expanded lateral edges (Fig. 7).

The principal segment of the acrosome covered the dorsolateral (Figs. 5b,5c) or dorsal surface (Figs. 5d-e) of the hook, but terminated caudal to the tip of the nucleus (Fig. 5f). The thickness of this segment was $67.2 \pm 0.6 \text{ nm}$ ($n = 8$), whilst the remaining portions of the acrosome were considerably thinner at only $28.3 \pm 0.7 \text{ nm}$ ($n = 18$) and appeared to be an extension of the equatorial segment on the lateral surfaces of the sperm head (Fig. 5). The contents of the acrosome were granular and of moderate electron density, and heterogeneous regions have not been observed. The contents of the equatorial segment were more electron-dense than the principal segment. The inner acrosomal membrane (IAM) seemed thicker due to a denser inner leaflet (Figs. 7,8).

The trilaminar plasma membrane (PM) surrounded the entire hook, and was loosely applied over the acrosome and on the lateral edges over the periodic structures. However, it was firmly attached to the SAM on the ventral surface of the hook (Figs. 5-8).

3.4.b Ventral Hooks

Nuclear material was only present at the base of the ventral hooks, after which they were composed mainly of homogeneous, moderately electron-dense material of similar appearance to the SAM in the dorsal hook (Fig. 5). Longitudinal sections revealed that the material in the ventral hooks was confluent with the SAM in caudal regions of the sperm head (Figs. 9,10). No evidence could be found for the presence of microfilaments in the hooks (Figs. 11,18).

In favourable sections, it could also be seen that there was a less homogeneous zone surrounding the core of the hooks, which represented the line of demarcation between the core and peripheral layers. The peripheral layer was 30.5 ± 1.4 nm ($n = 7$) thick, and represented a continuation of the post-acrosomal dense lamina (PADL) along the ventral surface of the sperm head (Figs. 11,12). Periodic structures linked the PADL and PM, with a centre-centre spacing of 14.6 ± 0.1 nm ($n = 35$), and a height of 11.1 ± 0.2 nm ($n = 20$). They were triangular or square in outline, and appeared to be fused with the inner leaflet of the PM. These structures occurred around the entire periphery of the ventral hooks, and along their entire length (Figs. 13-17). The PM was 7.6 ± 0.1 nm ($n = 11$) thick, and very closely adherant to the hooks. The inner leaflet of the membrane was of increased density, and appeared to be fused with the projections (Figs. 5,13-17).

Two thin, round acrosomal projections extended into the base of the ventral hook for about $0.8 - 1.0$ μ m, before terminating in a slightly

bulbous region. They arose from the equatorial segment, but enlarged to a diameter of 65-80 nm, and ran fairly straight, about 40 nm from the surface of the hook (Figs. 5b,5c,9,10,18). A consistent feature, however, was that on the side with the round lateral surface of the dorsal hook, the projection entered the hook further apically than on the other side, and also terminated further apically (Figs. 9,10).

3.5 Structure of the Sperm Head Posterior to the Hooks

The sperm head was bilaterally flattened, with rounded dorsal, ventral and caudal margins. Anterior to the point of tail insertion, the sperm head was 0.5-0.6 μm thick, but thickened posteriorly, especially on the ventral surface, to about 0.8-1.0 μm . As previously mentioned, a deep depression lay at the base of the hooks on either side of the head. This persisted posteriorly as a slight depression on the lateral surface of the head, which travelled almost to the caudal margin of the sperm head (Figs. 19,20).

A description will now be presented of the structural features of the various components of the sperm head.

3.5.a Nucleus

The nucleus occupied much of the volume of the sperm head, and appeared to be the main determinant of its shape (Fig. 20). It consisted of homogeneous, electron-dense material, with no evidence of lamellae. Nuclear vacuoles were rare, and, when present were very small, although small nuclear inconsistencies were frequent (Figs. 20-23). In the ventral nuclear extension which was the site of tail insertion, the chromatin was more electron dense (Fig. 21). The nucleus was completely surrounded by a double nuclear envelope, which had a wavy outline and lay in close proximity to the chromatin (Figs. 23,24,27). It extended

caudally into the neck region as a membranous scroll.

3.5.b Sub-acrosomal Space

A narrow sub-acrosomal space (SAS) separated the nuclear membrane from the IAM and PADL. It was 10-30 nm wide, and contained a variable amount of SAM (Figs. 21-23). Along the dorsal margin of the sperm head, there was an accumulation of SAM, which formed two dorsal ridges which indent the principal segment of the acrosome (Figs. 20-23). These ridges were continuous with those in the dorsal hook (Figs. 5a-c). There was also a localized accumulation of SAM at the junction of the equatorial segment and the PADL (Figs. 21, 26-30). Filamentous structures have not been observed in the SAS in any region of the sperm head.

3.5.c Acrosome

The acrosome covered about two thirds of the dorsal and lateral surface of the sperm head (Fig. 19). An apical segment was not distinguishable, and the 67 nm thick principal segment capped the dorsal surface, extending ventrally to the indentation (Figs. 19, 20). The equatorial segment was 30.6 ± 0.4 nm ($n = 28$) thick and existed as a narrow band on the lateral surface of the sperm head (Figs. 19, 20). There was, however, a distinct asymmetrical distribution of these two segments of the acrosome. On the side with the rounded surface of the dorsal hook, the acrosome covered a greater area of the lateral surface of the sperm head. The extent of the principal segment was usually greater, and consequently, the dorso-ventral width of the post-acrosomal region was less (Figs. 5a, 20-22). This was consistently observed in all sections, and was also apparent on scanning electron micrographs.

The acrosomal contents were finely granular and of moderate electron density, although the contents of the equatorial segment were

of increased density (Fig. 23). There was a heterogeneous distribution of the acrosomal matrix in the principal segment. This was most evident in specimens which were not post-fixed in OsO_4 , in which more electron-dense regions occurred near the IAM (Figs. 20-23). The inner acrosomal membrane (IAM) was 7.1 ± 0.1 nm ($n = 14$) thick, and had a denser inner leaflet, whilst the outer acrosomal membrane (OAM) was 6.7 ± 0.1 nm ($n = 13$) thick.

3.5.d Post-acrosomal Region

The post-acrosomal region (PAR) covered the ventro-lateral and caudal margins of the sperm head (Fig. 3). The characteristic feature of this region was a 14.4 ± 0.4 nm ($n = 19$) thick dense lamina (PADL) which was interspersed between the nuclear membrane and the plasma membrane, but separated from the nuclear envelope by a continuation of the SAS (Figs. 26-30). Near the base of the ventral hooks, the thickness of the PADL increased to 20-40 nm (Figs. 32,33). The PM and PADL were linked by parallel ridges or periodic projections with a centre-centre spacing of 13.6 ± 0.3 nm ($n = 18$) (Figs. 31-33). The projections were similar in outline to those in the ventral hooks, but did not appear to be as closely associated with the PM (Figs. 23,33). In the caudal region, they were only observed in transverse sections of the sperm head, indicating that the ridges were oriented in the long axis of the sperm head (Fig. 31). Along the ventro-lateral margin, however, the ridges were oriented in the dorso-ventral axis, as the projections were only apparent in longitudinal sections (Figs. 32, 32).

At the junction of this region with the equatorial segment, there was an accumulation of SAM on the ventral border of the equatorial segment, attached to the inner surface of the adjacent PADL (Figs. 26-30). The lamina bulged out slightly, giving rise to the ridge on the

sperm surface (Figs. 3,19,26-30). Near this junctional area, there was often a dense structure in the underlying SAS, which had a definite sub-structure and was closely associated with the nuclear envelope (Figs. 26,27,29).

3.5.e Other Sperm Head Structures

The trilaminar plasma membrane was closely applied to the PAR (Figs. 27,31-33), but existed at a greater distance from the OAM. In the principal segment, it was smooth in contour and located at a uniform but close distance from the OAM, whereas in the equatorial segment, it lay at a variable distance from the acrosome, and had a ruffled or wavy appearance (Figs. 20,23).

The ventral spur was a small, triangular or pyramidal protrusion on the ventral surface of the sperm head, and consisted of a core of SAM, surrounded by an extension of the PADL (Figs. 3,19).

The basal plate was the point of articulation of the sperm head with the connecting piece in the neck region of the tail. It was a 31.5 ± 1.1 nm ($n = 4$) thick electron-dense plate, intimately associated with the outer nuclear membrane in the implantation fossa (Fig. 34). The posterior or striated ring encircled this nuclear extension, and was distinguished as a narrow groove on the sperm surface at the posterior border of the PAR (Fig. 3), and represented a zone of close contact or fusion between the nuclear envelope and plasma membrane (Figs. 20b,21,27).

3.6 Surface Features of the Sperm Head

Treatment of spermatozoa with tannic acid revealed a thick glycocalyx over the entire sperm surface, which had no regular sub-structure and was of uniform thickness over the entire surface

(Figs. 35,36).

Scanning electron micrographs revealed some of the distinct surface features of the sperm head, such as the depression at the base of the hooks, the ridge at the junction of the PAR and equatorial segment, the posterior ring, and the point of entry of the acrosomal projections into the ventral hooks (Figs. 37,38). Surface replicas, however, revealed definite features of the sperm surface which corresponded to underlying structural entities such as the principal and equatorial segments, and the PAR (Fig. 39).

3.6.a Plasma Membrane

The plasma membrane overlying the principal segment of the acrosome featured a ruffled surface, with little observable structure (Fig. 40). The surface of the equatorial segment, however, presented a different appearance, the surface being characterized by short blebs or rod-like structures, and 16.4 ± 0.2 nm ($n = 26$) diameter particles spaced 17.3 ± 0.5 nm ($n = 12$) apart and organized with a hexagonal packing (Figs. 40,41). These surface features of the equatorial segment extended onto the lateral surfaces of the dorsal hook (Figs. 42,43).

The surface of the ventral hooks and PAR was completely different, and appeared as a rather random array of furrows and ridges, which coursed in all directions. Although a similar pattern was found on the ventral hooks and PAR, it was not identical, for the furrows and ridges were smaller and more closely associated on the ventral hooks (Figs. 44-46).

3.6.b Outer Acrosomal Membrane

The PM was sometimes lost during specimen preparation, thus revealing the surface characteristics of the OAM. In the equatorial

segment, the OAM exhibited a distinct periodicity, with linear rows of 17.0 ± 0.2 nm ($n = 20$) particles with a centre-centre spacing of 19.3 ± 0.6 nm ($n = 13$). At high magnification, a hexagonal packing of these particles was evident (Figs. 47,48). Particles of similar size and spacing occurred on the OAM on the lateral surfaces of the dorsal hook, although the linear particle strands were aligned at an angle of about 55° to the long axis of the hook (Fig. 49). No periodic structures were found in the OAM of the principal segment.

3.6.c Post-acrosomal Dense Lamina

After partial removal of the PM using Triton X-100 and DTT, the surface of the PADL was represented by parallel ridges, which were spaced about 16.0 ± 0.5 nm ($n = 11$) apart. The ridges were oriented in the dorso-ventral plane on the ventral surface of the sperm head, but in line with the longitudinal axis on the caudal margin (Figs. 50-52). At the base of the ventral hooks, the direction of the ridges remained the same, such that they ran along the length of the ventral hooks (Fig. 54). However, the ridges were difficult to visualize in the ventral hooks, due to incomplete dissolution of the membrane (Fig. 55). The bulging ridge at the junction of the equatorial segment and PAR sometimes exhibited a periodic sub-structure, but the lamina appeared broken up (Fig. 53).

3.7 Extent of the PAR as Determined by Silver Staining

The regions of the sperm head in which silver was deposited included the caudal and ventral margins of the sperm head, the two ventral hooks, and the ventral spur. The principal segment of the acrosome, ventral nuclear extension, and the two lateral edges of the dorsal hook displayed a less intense reaction. The equatorial segment

was consistently negative. In the sperm tail, the mitochondrial sheath was removed during processing, but the principal piece and annulus were argyrophilic (Figs. 56,57).

3.8 Localization of Actin Using NBD-Phalloidin

As previously reported filamentous actin has been found to occur in the ventral hooks using NBD-Phalloidin. The fluorescence was more intense in the middle hook, and comparatively weak at the base of the hooks, caudal to the point of bifurcation. In addition, two bands or prongs of fluorescence emanated from the base of the hooks. The most intense was directed dorsally into the concave region between the hooks, whilst the other, of much lower intensity, extended caudally along the ventro-lateral margin of the sperm head for about 5 μ m. The dorsal hook and all other regions of the sperm head were consistently negative (Figs. 58-63). Control sperm exhibited a comparatively weak fluorescence of the middle piece of the tail, but no fluorescence of the sperm head (Figs. 64,65).

4. DISCUSSION

There have been several previous studies of the sperm head morphology of the plains mouse, Pseudomys australis (Lambert, 1979; Sarafis et al., 1981; Flaherty, 1981). The present study, however, represents the first complete and detailed ultrastructural study of the entire sperm head, with particular emphasis on the three hooks. As a result, several important new features have been elucidated, which were not covered in our previous report (Flaherty and Breed, 1983a). These, and other features will now be discussed. (For a discussion of the functional significance of sperm head structures, see Section 7).

4.1 Variations in Sperm Head Morphology

A three-hooked sperm head is a characteristic feature of many species of non-Rattus Australian rodents (Breed and Sarafis, 1979; Breed, 1980). Although the morphological features of plains mouse sperm are representative of this group, there are significant inter-specific differences in the total length, relative lengths, degree of curvature, and orientation of the three hooks (Breed and Sarafis, 1979; Breed, 1980, 1983, 1984).

A characteristic feature of eutherian sperm is a bilateral flattening of the sperm head, and a slight apical decrease in its thickness (Nicander and Bane, 1966; Fawcett, 1970). The falciform, three-hooked sperm head is distinctly different from the ovoid or spatulate shape so characteristic of most non-rodent sperm (Bishop and Austin, 1957; Bustos-Obregon and Flechon, 1975). However, a falciform or hooked sperm head is a characteristic of many murid and cricetid (Friend, 1936; Bishop and Austin, 1957), but not hystricomorph sperm (Fawcett, 1965; Jones, 1975). In the rat and Chinese hamster, the sperm head is sickle-shaped, whereas in other murid rodents, the posterior margin of the sperm head is broader, and a thin hook projects from its apical margin, the orientation of which is somewhat variable (Friend, 1936; Bishop and Austin, 1957). With the exception of the two ventral hooks, therefore, the sperm head of the plains mouse is of typical murid form, with similarities in shape with both the albino rat and laboratory mouse. It most closely resembles, however, the sperm head of the barbary striped mouse, the only apparent difference being the presence of two ventral hooks (Bishop and Austin, 1957).

4.2 Analysis of Hook Shape and Orientation

The dorsal hook has a characteristic shape, which changes markedly

from the base of the hook to its tip. The basal asymmetry is a puzzling feature, since it does not occur in other hooked rodent sperm (Piko, 1969; Franklin et al., 1970; Lalli and Clermont, 1981), although it seems to be a feature of the dorsal hook of the Hydromyinae (Breed, 1983, 1984). It may be a direct consequence of the different extent of the acrosome on the two sides of the sperm head, since the principal segment of the acrosome extends further ventrally on the side of the sperm head which has the rounded lateral surface at the base of the dorsal hook. The possible functional implications of this asymmetry will be dealt with in Section 7. Apart from the hook asymmetry, its basic shape in the apical regions is triangular or crescentic, with a concave ventral surface. The hook on the rat sperm head is also triangular, although it does not become dorso-ventrally flattened as in the plains mouse (Piko, 1969; Suzuki and Nagano, 1980a; Lalli and Clermont, 1981). The shape of the dorsal hook is, however, quite different from the ovoid hook on the sperm head of the golden and Chinese hamster (Franklin et al., 1970; Yanagimachi and Noda, 1970a; Yanagimachi et al., 1985).

Unlike the dorsal hook, the two ventral hooks are bilaterally symmetrical, and change in shape from being ovoid at the base, to spear-shaped and flattened near the tip. These features are well conserved throughout the Hydromyinae (Breed, 1983, 1984). Although the two ventral hooks are similar in shape, there are a number of differences. Firstly, the middle hook is larger than the ventral one, thus providing a gradation in size from the dorsal to ventral hook. Secondly, it is usually longer, and may even be of greater length than the dorsal hook. And thirdly, the thin dorsal and ventral margins of the hooks, which give the hooks their blade-like appearance, are not identical in size or extent on the two hooks. On both hooks, the extent of the dorsal plate is greater than the ventral one, whilst on the ventral hook, the ventral

margin only becomes thinner near the tip of the hook.

The combined orientation of the three hooks is also rather unusual. In freshly extruded cauda epididymal or vas sperm, the three hooks lie very close together, and are not spread apart. The dorsal hook is tilted to one side, and the middle hook comes to lie in the ventral concavity of the dorsal hook, with the ventral hook below it, but slightly off line. This arrangement is only noticed in the apical half of the hooks. The decrease in size of the three hooks, and the narrow dorsal and/or ventral margins of the two ventral hooks may facilitate such a stacked arrangement. The end result is that, as a group, the three hooks do not exhibit any bilateral symmetry. This appears to occur in other Australian murid rodent sperm, although it has not been discussed (Breed, 1983, 1984). After sperm have been washed by centrifugation, this orientation often becomes disrupted, and the hooks become separated. This is probably due to mechanical or shearing forces generating during this preparative procedure, and it will therefore be assumed, that the in vivo situation more likely corresponds to the former description, in which the three hooks lie close together.

4.3 Structure of the Dorsal Hook

The general organization of the structural components of the dorsal hook is similar to that in rat and laboratory mouse sperm (Illisson, 1966, Piko, 1969; Lalli and Clermont, 1981; Flaherty and Breed, 1983a).

The nucleus is present in the basal two thirds of the hook, and probably acts as a skeletal framework, since it is a rigid and inert structure in eutherian spermatozoa (Bedford and Calvin, 1974a). It exhibits no unusual features, other than an asymmetrical shape and angled orientation, which also occurs to a lesser degree in rat sperm (Lalli and Clermont, 1981).

A definitive characteristic of the dorsal hook is the expansive sub-acrosomal space, which contains large amounts of homogeneous, densely-packed sub-acrosomal material (Flaherty and Breed, 1983a). Unlike the ventral hooks, however, the dorsal hook does not appear to contain filamentous actin (Flaherty et al., 1983). The SAM is also homogeneous, and does not contain two layers as in the golden hamster (Franklin et al., 1970). Along the dorsal margin of the hook, two dorsal ridges indent the principal segment of the acrosome, and continue posteriorly along the dorsal surface of the sperm head. Similar structures occur in the rat, mouse and hamster, although there is only a single ridge (Piko, 1969; Franklin et al., 1970), and not two, as occur in sperm of the Hydromyinae (Breed, 1983, 1984; Flaherty and Breed, 1983a).

As the diameter of the nucleus diminishes, the relative thickness of the SAM increases, and it is particularly abundant on the ventro-lateral and dorsal margins of the hook. Beyond the termination of the nucleus, the SAM continues to the tip of hook as a crescent-shaped rod or pseudoperforatorium, which is capped by the acrosome (Flaherty and Breed, 1983a). Such a structure is a characteristic feature of many rodent sperm, although its size and shape varies between species (Friend, 1936; Austin and Bishop, 1958a; Breed, 1983, 1984). Clermont et al. (1955) described three prongs, one dorsal and two ventral, which anchor the rat pseudoperforatorium onto the sperm head. Although not directly observed in this study, a similar system probably exists in plains mouse sperm, since the structure of the hook is similar to the rat, and there is a dorsal, and two ventro-lateral accumulations of SAM around the nucleus.

A considerable portion of the surface of the dorsal hook is enclosed by the acrosome. The apical segment is reduced or non-existent,

unlike that of golden or Chinese hamster sperm (Franklin et al., 1970; Yanagimachi et al., 1985). Instead, part of the dorsal surface of the hook is capped by the principal segment, but only as far as the end of the nucleus. Several lines of evidence have been presented which show that the acrosome on the lateral surface of the hook, as well as on the dorsal surface of the pseudoperforatorium, and the ventral acrosomal plate (VAP) are an extension of the equatorial segment. Firstly, the equatorial segment is present near the base of the dorsal hook, and these regions of the acrosome are of similar thickness and density, although the equatorial segment is slightly thinner on the dorsal hook. Secondly, the plasma membrane over the dorso-lateral surfaces of the dorsal hook exhibits an identical pattern of ridges and particles to that seen on the equatorial segment. And thirdly, the surface features of the OAM are also the same. Therefore, the VAP is also an extension of the equatorial segment, as it arises as an offshoot of this segment on the ventral surface of the hook.

The plasma membrane was found to be closely associated with the SAM on the ventral surface of the dorsal hook, suggesting that this region may be an extension of the PAR. However, periodic projections or ridges were not observed. Nevertheless, two bands of argyrophilic material were present on the ventral or lateral surfaces of the hook, although this reaction was not as intense as on the ventral surface of the hook on rat or mouse sperm (Bishop and Walton, 1960; Elder and Hsu, 1981).

4.4 Structural Features of the Ventral Hooks

Early studies of sperm head morphology in the plains mouse concluded that the two ventral hooks consisted largely of acrosomal material (Sarafis et al., 1981). It was subsequently shown, however, that they represent an extension of the sub-acrosomal material

(Flaherty, 1981; Flaherty and Breed, 1983a), which has since been verified in other hydromyine rodents (Breed, 1983, 1984).

Flaherty and Breed (1983a), however, described two layers of material in the ventral hooks, a core of SAM, and a thin peripheral layer of unknown origin. The most significant finding of the present study is that the peripheral layer of the ventral hooks appears to be an extension of the post-acrosomal dense lamina. In longitudinal sections, the dense lamina on the ventral surface of the sperm head could be observed extending into the base of the ventral hooks. Additional evidence was provided by the intensely argyrophilic nature of the ventral hooks, which is a characteristic feature of the PAR (Hancock and Trevan, 1957; Elder and Hsu, 1981). The structure of the peripheral layer of the hooks was similar to the PADL, with parallel ridges connecting the dense lamina and the plasma membrane. Only transverse sections of the ventral hooks revealed the projections, indicating that the ridges are oriented along the hooks. Surface replicas revealed a change in the orientation of the ridges at the base of the ventral hooks, such that they become oriented along the axis of the hooks. This proved difficult to demonstrate in more apical regions of the hooks, due to incomplete solubilization of the PM. The inability to completely remove the PM attests to the tight association of the ridges and the PM, which seems to be mediated through fusion of the ridges with the inner leaflet of the membrane.

It has previously been shown that the ventral hooks contain filamentous actin (Flaherty et al., 1983). In this study, however, no ultrastructural evidence was obtained for the presence of 5-7 nm microfilaments which would correspond to the actin filaments. This is probably due to the highly condensed and granular nature of the material in the hooks. Nevertheless, the localization of actin in the ventral

hooks was re-examined using NBD-Phalloidin, and several new features have been demonstrated. Firstly, the pattern of fluorescence indicated that the distribution of filamentous actin in the hooks is heterogeneous, with weak fluorescence at the base of the hooks, but intense fluorescence apical to the point of bifurcation. And secondly, it appears that two bands or prongs of actin filaments emanate from the base of the hooks. One is short, and of intense fluorescence, and is directed dorsally into the concave region between the dorsal and ventral hooks. The other band is less intense, and extends caudally along the ventral surface of the sperm head, as previously reported (Flaherty *et al.*, 1983). However, TEM failed to reveal microfilaments in these regions, presumably due to the density of the SAM or the paucity of filaments. Nevertheless, these two bands may function in anchoring the ventral hooks to the sperm head, in a manner similar to that proposed for the three prongs which attach the pseudoperforatorium to the rat sperm head (Clermont *et al.*, 1955). Possible evidence for such a role has been obtained previously, in which treatment of plains mouse sperm with NaOH, which appears to solubilize some components of the ventral hooks, caused a gap to appear between the nucleus and the ventral hooks. The extraction of actin may have caused a loosening of the association of the hooks with the sperm head, causing the appearance of a gap. However, at present this is purely speculative.

4.5 Structural Features of the Sperm Head Caudal to the Hooks

The structure and organization of the various sperm head components has been outlined by Flaherty and Breed (1983a), and follows the general eutherian pattern (Fawcett, 1970, 1975). Hence, only selected aspects will be discussed in this section.

4.5.a Nucleus

The nucleus consists of highly condensed chromatin, with infrequent nuclear vacuoles, which may indicate a high degree of order during the condensation process. This is a feature of most eutherian sperm (Fawcett, 1975), except those of man, in which sperm are released in various stages of nuclear condensation (Zamboni et al., 1971; Pedersen, 1974). It has previously been reported that the nucleus is stabilized by disulphide bonds, and the degree of stabilization is greater in the dorsal hook and anterior regions of the sperm head (Flaherty and Breed, 1983a). This is a characteristic feature of eutherian sperm, and may relate to penetration of the extra-cellular coats around the oocyte (Bedford and Calvin, 1974a).

4.5.b Sub-acrosomal Space

A narrow sub-acrosomal space surrounds the entire nucleus, but contains little material except along the dorsal margin of the sperm head, and at the junction of the equatorial segment and PAR. A dorsal accumulation of SAM occurs in a wide variety of species, and often indents the acrosome as a short, finger-like process (Nicander and Bane, 1966; Franklin et al., 1970; Wooding and O'Donnell, 1971; Courtens et al., 1976), although this process is of considerable length in insectivore sperm (Ploen et al., 1979; Castellani-Ceresa et al., 1980). An unusual feature of the dorsal accumulation of SAM in plains mouse sperm is the presence of two dorsal ridges or processes which indent the acrosome (Flaherty and Breed, 1983a), which appears to be a common feature of hydromyine sperm (Breed, 1983, 1984).

There is also an accumulation of SAM at the junction of the equatorial segment and PAR, which could stabilize this region and maintain its continuity. This would be of great importance for

maintaining cell integrity during penetration of the zona pellucida. A similar accumulation has been reported in boar, ram, rabbit and bull spermatozoa by Courtens et al. (1976), who suggested that a major function of SAM may be to cement the acrosome to underlying structures.

4.5.c Acrosome

In plains mouse sperm, the apical segment of the acrosome is virtually non-existent, the principal segment caps the dorsal margin of the sperm head, and the equatorial segment covers part of the lateral surface of the sperm head (Flaherty and Breed, 1983a). This distribution of the acrosomal segments is similar to the rat and laboratory mouse (Piko, 1969; Stackpole and Devorkin, 1974; Phillips, 1977a), but is different from other rodent sperm, such as those of the guinea pig (Fawcett, 1965), chinchilla (Fawcett and Phillips, 1969b), or golden hamster (Franklin et al., 1970).

There is also a heterogeneous distribution of the contents in the principal segment, although a periodic or crystalline sub-structure has not been observed (Friend and Fawcett, 1974; Olson and Winfrey, 1985). At its junction with the equatorial segment, the thickness of the acrosome decreases, and there is an increase in the density of the matrix, which has been shown to be a feature of this segment (Fawcett, 1970). Another feature noted in the present study, was an asymmetrical distribution of the principal and equatorial segments on the two sides of the sperm head. A similar asymmetry is often visible in the micrographs of other studies and therefore may be a feature of mammalian sperm, but does not seem to have been discussed by other authors. (Nicander and Bane, 1966; Wooding and O'Donnell, 1971; Lalli and Clermont, 1981).

In the equatorial segment of a wide variety of species, the surface of the outer acrosomal membrane exhibits a characteristic pattern of linear rows of particles (Phillips, 1977a, 1977b). In this study, a similar pattern was observed, although the spacing of the particles was found to be marginally greater. This is possibly due to technical differences, such as the degree of shrinkage of specimens during preparation. Caution is required, however, in the interpretation of this pattern of linear or hexagonally arranged particles. It may be an artifact resulting from lipid extraction or protein aggregation (Friend, 1977), although Olson and Winfrey (1985) have shown that it seems to be a structural correlate of the cross-bridges between the IAM and OAM in the equatorial segment (Moore and Bedford, 1978a; Russell et al., 1980).

4.5.d Post-acrosomal Region

The PAR is located on the caudal and ventro-lateral surfaces of the plains mouse sperm head, and as previously mentioned, also constitutes the peripheral layer of the ventral hooks. The use of routine thin sectioning and surface replicas revealed that the structural features of this region are a dense lamina, with parallel ridges connecting it to the plasma membrane. The spacing of the ridges is about 13-14 nm, which is similar to reports of 12-15 nm in a variety of other species (Fawcett and Ito, 1965; Pedersen, 1972; Phillips, 1977a, 1977b; Olson et al., 1983a; Toyama and Nagano, 1983).

In bull and monkey sperm, the ridges are oriented in the long axis of the sperm head (Pedersen, 1972; Olson et al., 1983a), whereas in human sperm they circumnavigate the posterior part of the sperm head (Pedersen, 1972). In the plains mouse, however, the orientation of the ridges is different in the three major zones in which the PADL occurs. They run along the axis of the head in the caudal regions, in the dorso-

ventral axis on the ventral surface of the sperm head, and along the length of the two ventral hooks. In addition, the plasma membrane appears to be more firmly adherant to the PADL of the ventral hooks, which may be of functional significance (see Section 7).

4.6 Concluding Remarks

It would appear, therefore, that the structural features of the sperm head of the plains mouse follow the typical mammalian pattern. There are a number of noteworthy features of the three hooks, such as their shape and specific orientation. It has also been shown for the first time, that the peripheral layer of the ventral hooks is an extension of the post-acrosomal dense lamina, and overlies a core of sub-acrosomal material.

SECTION 3 : MORPHOGENESIS OF THE DORSAL AND VENTRAL HOOKS

1. INTRODUCTION

In the previous section, a detailed study of the sperm head morphology of the plains mouse has been presented, with particular emphasis on the structural features of the three hooks. However, although actin filaments have been reported to occur in the two ventral hooks (Flaherty et al., 1983a), ultrastructural evidence has not yet been obtained for the presence of microfilaments in these structures. Failure to observe these filaments may be due to the compact and dense nature of the material in the ventral hooks of epididymal spermatozoa. Hence, in order to clarify this point and provide further information on the ultrastructure of the hooks, the morphogenesis of the hooks has been examined in this section. Spermiogenesis has been briefly examined in a previous study, although relatively little information was obtained on formation of the hooks (Lambert, 1979). It should be noted that this does not represent an attempt to study all the steps of spermiogenesis, nor the kinetics of the cycle of the seminiferous epithelium, rather to probe the structure of the hooks by studying their formation in late spermatids.

2. MATERIALS AND METHODS

2.1 Tissue Preparation for Transmission Electron Microscopy (TEM)

The testicular tissue examined in this study was fixed by vascular perfusion using a modification of the procedure outlined by Forssmann et al. (1977). Instead of cannulation of the abdominal aorta, solutions were introduced into the aorta via the left ventricle, and the right

atrium was severed to allow for the effluent flow. A blunt 21G needle was used for this purpose. All solutions were millipore filtered (0.45 μm).

Adult male plains mice were anaesthetized with Nembutal, and then perfused with Rinse solution for 3-4 minutes (see Appendix 2 for the composition of solutions). Two different fixation protocols were utilized to overcome the problems of obtaining adequate fixation of the adluminal compartment of the seminiferous tubules:

- A. The two-step method of Forssmann et al. (1977) was employed, whereby animals were perfused with Fix 1 for 3-5 minutes, followed by Fix 2 for 5-15 minutes.
- B. Animals were perfused for 15 minutes with 5% glutaraldehyde in phosphate buffer, containing 0.1% NaN_3 to inhibit anoxic degenerative effects on cellular structure due to the slow penetration of the fixative (Minassian and Huang, 1979) (See Appendix 2).

After perfusion, the testes were removed, and sliced transversely or into small cubes (1 mm^3), and fixed for 2-3 hours at 4°C . The tissue was rinsed in three changes of 0.1 M phosphate buffer, pH 7.4 (containing 2.5% PVP and 1 mM CaCl_2), and post-fixed for 2 hours in 1% OsO_4 in 0.1 M phosphate buffer. After thorough rinsing in phosphate buffer, followed by 0.05 M maleate buffer (pH 5.2), the tissue pieces were en bloc stained in 1% uranyl acetate in maleate buffer for 2 hours in the dark (Karnovsky, 1967), dehydrated through a graded series of alcohols, and embedded in Spurr's epoxy resin. Thick sections were stained with 1% toluidine blue in borax buffer, whilst ultrathin sections were stained with uranyl acetate and lead citrate, and examined at 80 kV in a Philips EM 300. Measurements were made as outlined in

Section 2, and are expressed as mean $\pm$ S.E.M. (n = number of observations).

2.2 Determination of Cellular Associations

The cellular associations of the seminiferous epithelium were determined to correlate formation of the hooks with the stage of spermatid development. Thick (0.5-1.0 μ m) plastic sections were stained with toluidine blue, and three hundred transversely sectioned tubules were photographed at a magnification of 132x. After printing, the various cell associations were determined using spermatid morphology as the principal criterion.

2.3 Localization of Actin During Spermiogenesis

Filamentous actin was localized in developing spermatids and adjacent Sertoli cell ectoplasmic specializations using NBD-Phalloidin (Flaherty et al., 1983).

Spermatids were isolated mechanically or after enzymatic digestion:

A. Mechanical isolation (after Lam et al. (1970)).

The testes were decapsulated, and the tubules minced in warm PBSG (PBS containing glucose (1 g/litre), pH 7.4). After 5 minutes, the supernatant was removed, and the isolated cells washed once by centrifugation at 400 g for 10 minutes, and resuspended in warm PBSG.

B. Enzymatic isolation (after Romrell and Ross (1979)).

The testes were decapsulated, and the tubules minced gently with a razor blade in warm PBSG. Trypsin and collagenase were added to a final concentration of 1 mg/ml (collagenase) and 2 mg/ml (trypsin), and the suspensions were incubated in a water bath at 30°C for 30-45 minutes with gentle agitation. They were then pipetted gently five times, and

the isolated spermatids washed once by centrifugation and resuspended in warm PBSG.

Samples of isolated cells were prepared for TEM as outlined in Section 2.

Aliquots of isolated cells were attached to poly-L-lysine coated coverslips, and stained with NBD-Phalloidin, using the same controls, as outlined in Section 2.

2.4 Silver Staining of Isolated Spermatids

The formation of the ventral hooks and PAR was examined by silver staining spermatids after mechanical isolation as outlined in 2.3. Smears were prepared and stained with AgNO_3 according to Elder and Hsu (1981) as described in Section 2.

3. RESULTS

3.1 Cycle of the Seminiferous Epithelium

The cycle of the seminiferous epithelium was arbitrarily divided into ten stages (I-X), and spermiogenesis was comprised of thirteen steps (1-13). The principal criterion used was spermatid morphology, and the stages were classified according to Leblond and Clermont (1952a, 1952b). Hence, stage I was represented by the first appearance of round spermatids. The cell associations are shown in Figs. 62-71, and in Text Fig. 1.

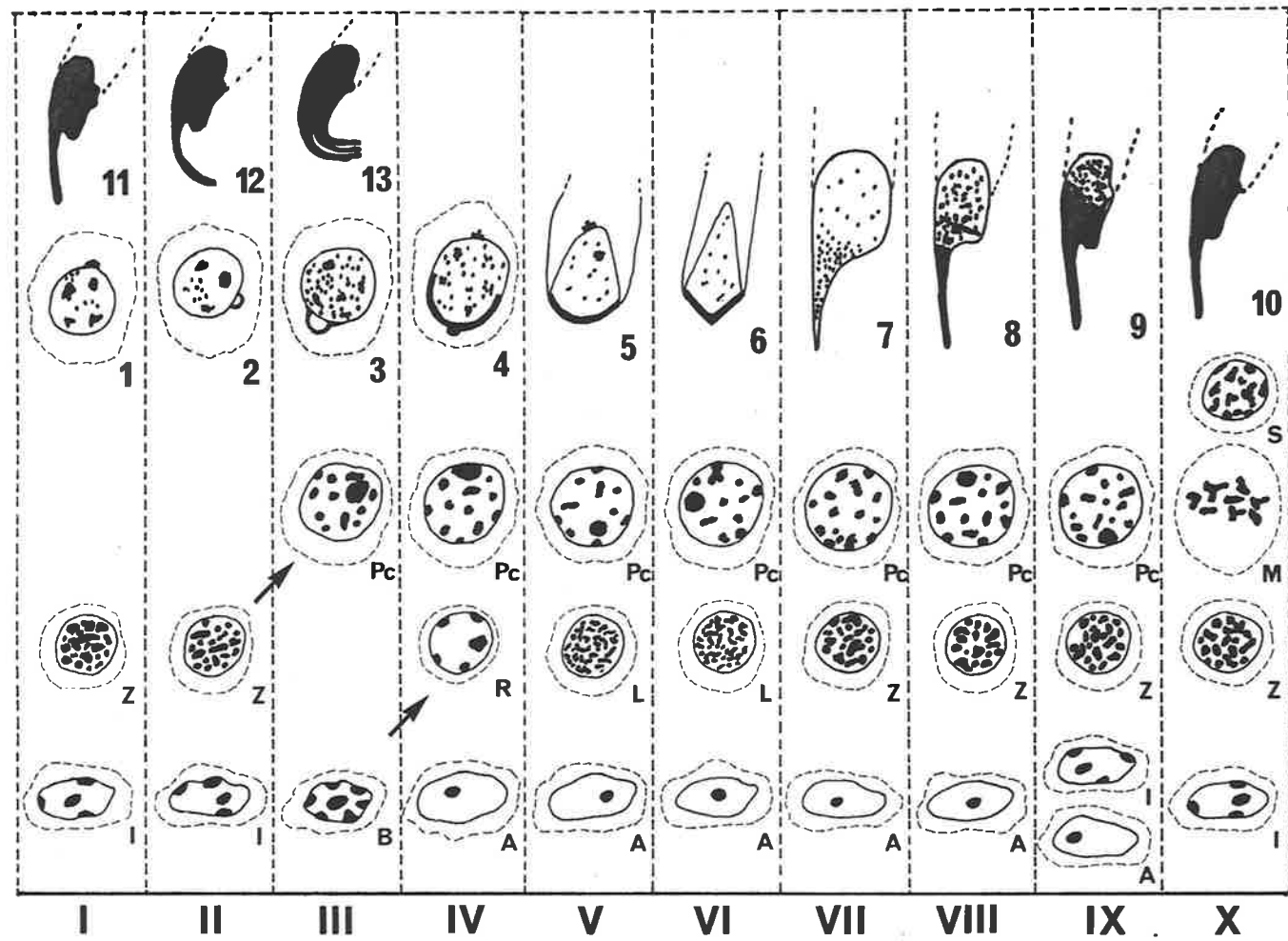
Stage I -

Intermediate spermatogonia and zygotene primary spermatocytes occupied the basal layer of the seminiferous epithelium, whilst the middle layer contained round spermatids. Clusters of maturing spermatids were found amongst the round spermatids, as well as in the luminal

TEXT FIGURE 1

A diagrammatical representation of the 10 cell associations which characterize the cycle of the seminiferous epithelium of the plains mouse. The 13 spermiogenic steps are shown in the upper part of this figure, whilst the letters describing the different types of spermatogonia and spermatocytes are:

A	Type A spermatogonium
B	Type B spermatogonium
I	Intermediate spermatogonium
L	Leptotene primary spermatocyte
M	Spermatocyte at metaphase of the first meiotic division
Pc	Pachytene primary spermatocyte
R	Resting primary spermatocyte
S	Secondary spermatocyte
Z	Zygotene primary spermatocyte



portion, and appeared to have completed nuclear condensation, although the dorsal hook was straight, not curved (Fig. 62).

Stage II -

Similar to stage I, except that the round spermatids often exhibited an acrosomal vesicle, the maturing spermatids were located further adluminally, and were less clustered (Fig. 63).

Stage III -

This stage was rather variable, but has a number of general features. Type B spermatogonia lined the base of the tubule, along with early pachytene primary spermatocytes. Round spermatids occupied most of the epithelium, and possessed a well developed acrosomal vesicle. Maturing spermatids were only found near the tubule lumen, and had three curved hooks on the apical margin of the sperm head (Fig. 64).

Stage IV -

Mature spermatozoa were being released into the tubule lumen, and only residual bodies remained in the Sertoli cell processes. Type A spermatogonia, as well as resting and pachytene primary spermatocytes occupied the abluminal compartment. The anterior surface of round spermatids, capped by the developing acrosome, became oriented towards the base of the tubule, and the connecting piece could sometimes be observed at the opposite pole of the nucleus (Fig. 65).

Stage V -

In this stage, type A spermatogonia, leptotene and pachytene primary spermatocytes were found in the basal compartment, while the anterior margin of developing spermatids was becoming angular, and capped by the acrosome (Fig. 66).

Stage VI -

The same cell types were present as in stage V, but the spermatid nucleus was undergoing elongation, and the acrosome spread posteriorly

along the sperm head (Fig. 67).

Stage VII -

The basal half of the seminiferous epithelium contained type A spermatogonia, zygotene and pachytene primary spermatocytes. The nucleus of maturing spermatids was elongate and constricted in its apical margin (Fig. 68).

Stage VIII -

The same cell types were present as in the previous stage, but nuclear condensation was underway in the spermatids, especially in the dorsal hook and apical regions of the sperm head (Fig. 69).

Stage IX -

In this association, both type A and intermediate spermatogonia were found, along with zygotene and pachytene primary spermatocytes. As in stage VIII, the maturing spermatids were clustered in the luminal half of the epithelium, but nuclear condensation had reached an advanced stage, except in the caudal margin of the nucleus (Fig. 70).

Stage X -

Intermediate spermatogonia and zygotene primary spermatocytes occupied the basal region of the epithelium. Spermatocytes at metaphase of the first meiotic division were observed, as well as secondary spermatocytes, the product of this division. The latter cell type was distinguished from round spermatids by a greater nuclear diameter and more clumped chromatin. Nuclear condensation was almost complete in maturing spermatids (Fig. 71).

3.2 Ultrastructural Features of Spermiogenesis

Spermiogenesis has been classified into thirteen steps. However, since the emphasis of this study was on the morphogenesis and structure

of the hooks, only steps 7-13 have been studied at the ultrastructural level. The two perfusion methods both produced good tissue preservation, and therefore the results have been combined.

Step 7 -

This was the first step in which the dorsal hook was observed. Anteriorly, the sperm head was elongate and thin, whilst posteriorly, it was broad, but becoming bilaterally flattened. The acrosome covered the rounded dorsal margin of the sperm head, separated from the nucleus by a narrow sub-acrosomal space (SAS). The nucleus had a fine, granular texture, although areas of chromatin aggregation were present in its central and peripheral regions. The manchette extended caudally from the border of the acrosome (Fig. 72).

The dorsal hook was bilaterally flattened, symmetrical, and roughly pear-shaped, except apically where it was irregularly ovoid in shape (Figs. 73-75). The nucleus was tear drop-shaped, although it became rounded near its termination. A double nuclear envelope was present, and it extended ventrally into the narrow channel of the SAS as a straight plate (Figs. 73,74). The SAS only contained small amounts of fine, flocculent material, except adjacent to the inner acrosomal membrane (IAM) ventral to the nucleus, where the acrosomal zonule was situated. It consisted of a dense plaque attached to the IAM, and 15.0 ± 0.4 nm ($n = 33$) diameter filaments, spaced 24.5 ± 0.9 nm ($n = 44$) apart.

The acrosome almost totally enclosed the hook, except for a narrow gap along its ventral margin. Apically, however, the hook was totally enclosed, and the apical segment projected beyond the tip of the nucleus for a short distance. At this point, however, there was no expanded SAS representing the pseudoperforatorium. The apical segment was extensive, though irregular in shape, and capped the dorsal margin of the hook, as well as projecting beyond the tip of the nucleus. Its contents were

heterogeneous, with a dense core, and electron-lucent lateral zones. On the lateral surfaces of the hook, the acrosome was about 54.2 ± 1.9 nm ($n = 24$) thick, and contained moderately electron-dense granular material (Figs. 73-75).

The dorsal hook, as well as the peri-acrosomal region of the remainder of the sperm head, was closely surrounded by Sertoli cell ectoplasmic specializations. These consisted of bundles of parallel microfilaments and a cisternum of endoplasmic reticulum. The filaments were 7.4 ± 0.1 nm ($n = 28$) in diameter, and hexagonally arranged with a centre-centre spacing of 11.1 ± 0.2 nm ($n = 38$) and were oriented in the long axis of the sperm head (Figs. 73-75).

Step 8 -

The characteristic feature of this step was a marked increase in the degree of nuclear condensation. The chromatin exhibited a coarse, granular appearance, quite distinct from the finer chromatin in step 7 (Fig. 76). There was also an aggregation of chromatin near the nuclear envelope underlying the acrosome. The sperm head had become more bilaterally flattened, especially along its anterior and dorsal margins (Fig. 76). The dorsal hook was highly flattened, and elongated dorso-ventrally. The nucleus was partially condensed, and greatly reduced in size, but retained the same shape. The acrosome and SAS appeared unchanged, but the plate of excess nuclear membrane was more extensive (Figs. 77,78).

There were two structural features not evident in step 7. Firstly, a dense lamina had formed adjacent to the PM on the ventral margin of the hook which was not enclosed by the acrosome, and projections spaced 14.4 ± 0.7 nm ($n = 9$) apart linked the lamina and PM (Figs. 77,78). Secondly, a narrow space formed between the apical tip of the nucleus and the

apical segment of the acrosome, and the ventral cleft where the lamina was forming, extended to this region (Fig. 79).

Step 9 -

Nuclear condensation was virtually complete in the dorsal hook and anterior region of the sperm head. Posteriorly, however, the chromatin was electron-dense and clumped, with many small vacuoles (Fig. 80).

A number of subtle changes in the dorsal hook structure were found to occur in this step. Firstly, there were a number of changes to the acrosome. The acrosome on the lateral surface of the hook was reduced in thickness to 44.5 ± 1.5 nm ($n = 8$) and the contents appeared slightly more electron-dense (Fig. 81). In the apical regions of the hook, the acrosome extended further ventrally on one side, giving the hook an asymmetrical appearance, and the apical segment no longer capped the end of the hook (Fig. 82). Secondly, nuclear condensation was almost complete (Fig. 82). And thirdly, near the base of the hook, the SAS on the ventral aspect often bulged out, capped by the dense lamina (Fig. 82). The structure of the dense lamina, and the acrosomal zonule is shown in Fig. 83.

Steps 10 and 11 -

Step 10 was rarely encountered in thick sections, presumably since stage X was short lived, and has not been studied at the ultrastructural level. By step 11, nuclear condensation was nearing completion, although many nuclear vacuoles were still present, particularly in the caudal regions. The apical segment on the dorsal margin of the sperm head was more condensed, and there appeared to have been a redistribution of some of the dense contents into the lateral regions of the acrosome, where they lined the IAM. The acrosome on the lateral surfaces of the sperm head was now differentiated into a 72.8 ± 2.2 nm ($n = 11$) thick principal segment, and a 46.0 ± 2.2 nm ($n = 10$) thick equatorial

segment. The PAR had formed on the caudal and ventral margins of the sperm head, and consisted of a dense lamina (PADL) underlying the closely adherent PM. In the SAS on the dorsal margin of the sperm head, there was an accumulation of material, and two indentations of the acrosome formed, although they were widely spaced (Fig. 84).

The dorsal hook was long and straight, but had undergone considerable modification in its shape and the distribution of the acrosomal contents since step 9. Basally, the hook was shortened dorso-ventrally, and the apical segment of the acrosome had become reduced and oriented to one side, such that the hook became asymmetrical, with a rounded side, and an elongated one (Figs. 85,86). Apically, the hook was bilaterally flattened, and an expanded apical segment was absent. However, the hook did not exhibit bilateral symmetry, for a separate plate of acrosome had formed on one side. The dorsal and ventral margins were round and capped by the acrosome (Figs. 87-89). The excess nuclear membrane was reduced basally, but present in large amounts in the middle and apical regions of the hook (Figs. 85-88), and there was often an accumulation of dense material on its ventral aspect (Figs. 86,87). The extent of the acrosomal zonule in the SAS was greater than in step 9, and it underlay most of the acrosome, except the apical segment and the side opposite to the acrosomal plate (Figs. 85-87,89). The dense lamina filled both gaps between the acrosomal segments (Figs. 87-89).

The Sertoli cell ectoplasmic specializations surrounded most of the dorsal hook (Figs. 85,89), and for the first time, almost covered the entire sperm head (Fig. 84). However, the cisternum of endoplasmic reticulum had become divided into multiple cisternae (Fig. 89). Microtubules were common in the Sertoli cell cytoplasm (Fig. 88).

Step 12 -

The structure of the sperm head was similar to the last step, but nuclear condensation was almost complete in the caudal regions, the apical segment of the acrosome was reduced in size, and the asymmetrical distribution of the acrosome on the two sides of the sperm head was apparent (Fig. 90).

The dorsal hook was almost symmetrical at its point of origin, but further apically it became asymmetrical (Figs. 91,92). It was no longer straight, but curved ventrally. In the apical regions, it had become almost semi-circular, with the acrosomal plate on the ventral surface (Fig. 93). There was little or no excess nuclear membrane, and only a small amount of flocculent material in the SAS, although the acrosomal zonule was still present, especially in the apical regions of the hook. The apical segment of the acrosome was restricted to the dorsal margin near the base of the hooks, whilst the acrosome in other parts of the hook was of similar thickness to the equatorial segment (Figs. 91-93).

The ventral hooks first appeared during the later part of step 12. At the base of the hooks on the ventral margin of the sperm head, the SAS became expanded and bulbous (Figs. 94,95). It contained a continuation of the PADL on the ventral surface of the sperm head, and extensive membrane whorls were sometimes found (Fig. 96). At the base of the hook, there was nuclear material and two finger-like acrosomal projections (Fig. 91). After termination of the nucleus, however, the hook was virtually devoid of contents, except for the dense lamina associated with the PM, and some flocculent material (Fig. 97). The extent of the ventral hook(s) was difficult to ascertain, and it seems doubtful that they extended beyond half their full length, for only rarely have sections through two hooks been obtained in step 12 spermatids (Fig. 98).

The Sertoli cell ectoplasmic specializations (EPS) enclosed the dorsal hook and most of the sperm head, but not the ventral hook during its initial formation (Fig. 97). Tubulobulbar complexes were occasionally observed emanating from the dorsal hook, and extended into the concave region ventral to the hook (Fig. 98).

Step 13 -

This step occurred in stage III, when the maturing spermatids were located at the luminal border of the seminiferous epithelium. It was a diverse stage, however, and hence step 13 has been sub-divided into 13a-c to facilitate the ultrastructural description.

Step 13a -

The nucleus appeared to be fully condensed throughout the sperm head, although a few small vacuoles or inconsistencies still occurred. Similarly, the acrosome was fully shaped, and its contents were fairly homogeneous, although the contents of the equatorial segment were often more electron-dense. The SAS was slightly expanded, and contained some flocculent material, but the dorsal ridges were still widely separated (Fig. 99).

The dorsal hook curved ventrally, and had attained the final shape seen in mature epididymal spermatozoa (Figs. 5,106,107). Hence, it was asymmetrical basally, and the shape of the apical regions had changed from semi-circular to dorso-ventrally flattened and crescent-shaped. The acrosome was condensed, and the acrosomal plate (VAP) was situated on the ventral aspect of the hook. The SAS was largely devoid of contents, except for some wispy material which was often attached to the IAM, and the acrosomal zonule was absent. The SAS was in direct contact with the PM on the ventral surface of the hook, except on either side of the VAP, where there was usually a short segment of PADL (Fig. 106).

During this step, the two ventral hooks formed close together, and in close proximity to the dorsal hook, and extended from their base into the concavity ventral to the dorsal hook (Fig. 100). The middle hook developed before the ventral hook, and the hooks formed as round or ovoid structures, of roughly the same size (Fig. 102). They appeared to form as empty, membrane-bound spaces, virtually devoid of contents, although membrane whorls were often found near the anterior end (Figs. 101,102). A dense lamina formed adjacent to the PM as the hooks elongated. Initially it developed as discrete sub-units, each consisting of three projections or ridges, which eventually became joined to produce a continuous lamina. The sub-units appeared on the dorsal margin of the hooks before the ventral margins (Figs. 103-105). There was also a dense band of material on the ventral border of the ventral hook, but it was only observed in sections cut through the hooks at a level caudal to the termination of the nucleus in the dorsal hook (Fig. 105). When hook formation and elongation was complete, the ventral hooks were round or ovoid in outline, with a continuous PADL, and contained clumps of dense material, which were more commonly observed in the basal regions (Fig. 106,107). The structure of the PADL was the same as in the PAR on the caudal and ventral margins of the sperm head (Figs. 108,109).

The Sertoli cell EPS surrounded the entire sperm head, including the three hooks, but the bundles of microfilaments were usually absent from the region between the two ventral hooks. The filaments were oriented along the axis of the hooks (Figs. 99,106). Tubulobulbar complexes extended from the lateral tip of the dorsal hook, but only from the pointed and elongated side (Figs. 101,102,106,107).

Step 13b -

By this step, the thickness of the principal segment of the acrosome had decreased to 67.6 ± 1.1 nm ($n = 5$) and the thickness of the

equatorial segment was reduced to 31.9 ± 0.5 nm ($n = 21$) whilst the apical segment became extended dorsally. The SAS was reduced in volume, and contained a variable amount of fine, granular material but no microfilaments. The two dorsal ridges had formed, but were devoid of SAM (Figs. 110,111). The structure of the dorsal hook remained unchanged (Figs. 112,114,122).

The two ventral hooks underwent a discrete differentiation. Bundles of microfilaments formed in the dorsal and/or ventral regions of each hook, especially in their apical half (Figs. 112-115). The filaments were 6.3 ± 0.2 nm ($n = 36$) in diameter, and hexagonally packed with a centre-centre spacing of 12.1 ± 0.2 nm ($n = 51$) (Figs. 116,117). Some bundles contained more than 100 individual filaments (Fig. 116). The filamentous nature could be seen clearly in longitudinal sections of the hooks (Fig. 118). Bundles of filaments were not evident in the basal region of the hook(s), caudal to the point of bifurcation, although individual filament profiles were sometimes visible. The mode of attachment of the filaments to the PADL was unclear, but some also appeared to embed in the clumped core material at the base of the hooks (Fig. 118). Instead, the basal region was filled with clumps of moderately electron-dense material, which continued into the apical regions of the hooks, though only in the centre of each hook, and also accumulated in the SAS of the dorsal hook (Figs. 114,115,118,119). The PADL in the ventral hooks consisted of a 7.5 ± 0.3 nm ($n = 24$) thick lamina, linked to the dense inner leaflet of the PM by projections spaced 14.2 ± 0.1 nm ($n = 72$) apart (Fig. 120). In oblique sections, the projections appeared to represent parallel oriented ridges (Fig. 121).

Associated with the appearance of the filament bundles, the ventral hooks curved ventrally, especially in the region of the bifurcation (Fig. 118), and the asymmetrical orientation of the three hooks

developed (Figs. 115,125). There was also a change in the shape of the two ventral hooks, but only in the region anterior to the point of bifurcation. The hooks became bilaterally flattened, and elongated in the dorso-ventral plane (Figs. 112-115,122). The dorsal and/or ventral margins became constricted and flattened, producing thin plates, although this only occurred in the vicinity of the filament bundles (Figs. 124,125).

Tubulobulbar complexes arose from the lateral tip of the dorsal hook (Figs. 119,122), and were occasionally observed emanating from the region between the base of the hooks (Fig. 111). Multiple complexes were located in the concavity ventral to the hooks (Fig. 118). During this step, the two ventral hooks came to lie in a single recess in the Sertoli cell, rather than in separate ones as in step 13a (Figs. 113-115,122-125). The endoplasmic reticulum element usually consisted of a single, continuous cisternum, which connected with the cisternum around the dorsal hook (Figs. 122-125).

Step 13c -

The major feature of this step was the formation of electron-dense material in the SAS. This occurred concurrently throughout the sperm head (Figs. 126,129). In the ventral hooks, the material was laid down in a centrifugal manner, from the core towards the PADL, and gradually obscured the bundles of microfilaments (Figs. 127-131). The inner aspect of the lamina became thickened, thus producing the peripheral layer, and although the orientation, size and spacing of the ridges remained unchanged, there appeared to be a closer association between the ridges and PM (Figs. 132,133).

Released Sperm -

Spermatozoa in the tubule lumen in stage IV exhibited similar characteristics to late spermatids (Fig. 134). The ventral hooks were

filled with moderately electron-dense material, although a slightly vacuolated region delineated the core and peripheral layers (Fig. 135). The acrosome was 71.0 ± 1.1 nm ($n = 5$) thick in the principal segment, and 31.9 ± 0.8 nm ($n = 5$) thick in the equatorial segment.

3.3 Silver Staining of Isolated Spermatids

Seven different staining patterns were observed, and have been tentatively assigned to particular steps of spermiogenesis based on morphological comparisons with the ultrastructural features outlined in 3.2.

The first pattern appeared in step 7 or 8 spermatids, in which nuclear condensation was at an early stage. A thin, dense band on the ventro-lateral surface of the sperm head stained with silver nitrate. It extended from the caudal margin of the sperm head along the dorsal hook, but terminated before the tip (Fig. 136).

In step 11 spermatids, the nucleus was considerably reduced in size, and displayed affinity for silver. A thin band of stain was located along the caudal and ventral margins of the sperm head, and continued along the ventral and/or lateral surface of the dorsal hook, although two bands were present near the tip of the hook (Fig. 137).

The expanse of the PAR increased considerably in step 12. There was a broad argyrophilic band on the caudal and ventral aspects of the sperm head, and two narrow bands on the dorsal hook, one present along the entire hook, the other only in the apical two thirds. At the base of the ventral hooks, there appeared to be an expanded area of the PAR, from which a single ventral hook could sometimes be observed extending ventrally (Figs. 138-140).

In step 13 spermatids, the extent of the PAR became reduced on the caudal and ventro-lateral margins, and two ventral hooks, of variable

length and curvature, were apparent. The staining of the ventral hooks was identical to the PAR (Figs 141,142).

3.4 Localization of Filamentous Actin During Spermiogenesis

3.4.a Mechanically-Isolated Spermatids

Transmission electron microscopy revealed that the majority of late spermatids remained intact after this isolation procedure. The Sertoli cell EPS remained firmly adherent to the acrosomal region of the spermatids, and the three hooks.

Early spermatids, corresponding to steps 7-11, showed a similar pattern of staining, which was entirely due to the adherant Sertoli cell EPS. Fluorescence was restricted to the peri-acrosomal region and the dorsal hook, but was not found over the PAR, although this region sometimes exhibited weak fluorescence (Figs. 143,145).

In step 12 and 13a spermatids, the entire sperm head was fluorescent. During their initial formation, however, there was no fluorescence in the vicinity of the ventral hooks, although this situation changed as the hooks elongated (Figs. 146-148). The concave region ventral to the dorsal hook often displayed intense patches of fluorescence, sometimes in the form of ventrally-oriented bands (Fig. 148). The middle piece of the sperm tail exhibited weak fluorescence (Figs. 146-148). In step 13b spermatids, the ventral hooks fluoresced intensely (Fig. 149). The Sertoli cell EPS sometimes became dislodged during specimen preparation, and in such cases, this clearly demonstrated the fluorescence arising from the ventral hooks (Fig. 150).

The pattern of fluorescence of testicular sperm was the same as in cauda epididymal spermatozoa (see Section 2). Fluorescence originated in the two ventral hooks, but did not occur in the dorsal hook, or any

other parts of the sperm head, except for the two bands which arose from the base of the ventral hooks (Fig. 151).

In control preparations, no specific fluorescence was observed over the ventral hooks, and the middle piece of the tail, and sometimes the posterior region of the sperm head, exhibited a weak fluorescence (Figs. 152,153).

3.4.b Enzymatically-Isolated Spermatids

During enzymatic isolation of spermatids, the Sertoli cell EPS were completely removed, and most cells appeared undamaged, although the sperm heads and tails were sometimes separated.

In step 7-12 spermatids, only weak fluorescence was found over the sperm head, and midpiece of the tail. However, in step 13b spermatids, the two ventral hooks fluoresced intensely, and the two bands were visible extending from the base of the hooks. At no stage did the pseudoperforatorium in the dorsal hook exhibit fluorescence (Figs. 154,155). The same pattern of fluorescence occurred in mature testicular spermatozoa (Fig. 156).

In control preparations, the sperm head and middle piece exhibited a weak form of autofluorescence, but fluorescence was never observed in the region of the ventral hooks (Figs. 157,158).

4. DISCUSSION

4.1 Cycle of the Seminiferous Epithelium

In this study, the cycle of the seminiferous epithelium has been divided into 10 stages. Spermiogenesis consists of 13 steps, although step 13 has been further sub-divided into 13a-c to facilitate the ultrastructural study of formation of the hooks. The number of cell

associations varies between species (Leblond and Clermont, 1952a, 1952b; Oakberg, 1956), but will not be discussed in this section since the main aim was to elucidate the structural features of the three hooks during their morphogenesis. The kinetics of the cycle of the seminiferous epithelium of the plains mouse is currently being studied in our laboratory (E. Peirce, personal communication).

4.2 Morphogenesis of the Dorsal Hook

This represents the first detailed study of the formation of the dorsal hook in this species. The dorsal hook first appears during elongation of the sperm head in step 7, and is symmetrical and almost totally enclosed by the acrosome. During steps 8 and 9, the nucleus condenses, and the hook becomes bilaterally flattened, and an expanded space (SAS) forms at the apex of the nucleus. In the ensuing steps (11-13a), however, the hook undergoes extensive reorganization and orientation of its structural components. The contents of the acrosome are redistributed posteriorly, and the apical segment becomes reduced in size, although not on the dorsal margin of the sperm head posterior to the hook. In the apical half of the hook, the acrosome is subdivided into two segments, thereby forming the ventral acrosomal plate (VAP). The SAS enlarges in the region of the pseudoperforatorium, and the plate of excess nuclear membrane disappears. However, the shape of the hook also changes. The basal asymmetry forms, whilst in the apical regions, the VAP comes to lie on the ventral margin of the hook, and the crescentic shape of the hook develops. During step 13c, electron-dense sub-acrosomal material (SAM) accumulates in the SAS, and then the sperm are released.

Apart from species-specific differences in the shape of the hook, the formation of the dorsal hook closely parallels the development of

the anterior part of the falciform rat sperm head during steps 15-19 of spermiogenesis (Lalli and Clermont, 1981). The similarities include:

- (1) Nuclear condensation is not completed until just before spermiation.
- (2) Sub-acrosomal material fills the SAS late in spermiogenesis.
- (3) The curvature of the hook becomes more pronounced during the final steps.
- (4) The contents of the acrosome undergo a caudal translocation, the VAP forms, and there is a reorientation of the acrosomal segments.

During spermiogenesis in the laboratory mouse and hamster, nuclear condensation and curvature of the hook are also completed just prior to spermiation (Leblond and Clermont, 1952b; Oakberg, 1956; Franklin and Fussell, 1972; Doohar and Bennett, 1973). In hamster spermatids, the sub-acrosomal material accumulates in the pseudoperforatorium at the end of spermiogenesis, although in contrast to the plains mouse and rat, two different layers of material appear (Franklin and Fussell, 1972; Lalli and Clermont, 1981). The SAM also appears in rabbit and monkey spermatids shortly before spermiation (Bedford and Nicander, 1971; Franklin and Fussell, 1972).

4.3 Morphogenetic Factors Involved in Dorsal Hook Formation

4.3.a Nuclear Reorganization

The nucleus appears to be one of the main determinants of sperm head shape in eutherian mammals (Fawcett et al., 1971). However, there is still controversy over the factors involved in nuclear shaping during spermiogenesis. Some studies have suggested that the manchette may be an active component in this process, although after careful consideration of the structure of this organelle, Fawcett et al. (1971) concluded that

it could not be a major determinant of nuclear morphology, but probably assisted the posterior movement of the spermatid cytoplasm. Instead, they suggested that the shape of the nucleus was determined by a genetically-controlled pattern of chromatin condensation. This contention has been supported by the discovery that the sperm nucleus has an organized sub-structure consisting of parallel lamellae (Koehler, 1966, 1970; Plattner, 1971; Koehler et al., 1983). Nevertheless, other researchers still envisage a function for the manchette in guiding nuclear condensation, aligning the chromatin, reinforcing the nucleus, or possibly in the process of elongation of the spermatid (Kessel and Spaziani, 1969; Rattner and Brinkley, 1972; Courtens et al., 1983).

Sperm-specific nucleoproteins or protamines have been implicated in the development of nuclear shape (Fawcett et al., 1971; Coelingh and Rozijn, 1975). Support for this suggestion is provided by the incorporation of protamines late in spermiogenesis during the period of maximal nuclear condensation. However, as pointed out by Bellve and O'Brien (1983), there appears to be no correlation between the number or type of protamines present, and the final shape of the nucleus. Instead, they speculate that the peri-nuclear theca may act as a determinant of nuclear shape. However, they provided no substantial evidence, and it is difficult to envisage such a mechanism, especially since the SAM does not form until after the nucleus has assumed its species-specific shape (Franklin and Fussell, 1972; Lalli and Clermont, 1981).

Although there is indecision regarding the factors involved in nuclear morphogenesis, it would appear that the manchette could not participate directly in shaping the nucleus in the dorsal hook of plains mouse spermatids, as it is only found caudal to the hooks. However, if the manchette is involved in nuclear elongation, then it may participate in determining the tear-drop shape of the nucleus, since this occurs in

step 7 when the hook forms, and is maintained throughout later steps. The sperm head assumes its characteristic shape during the condensation phase, and it is only during the later steps that the basal asymmetry of the hook develops, and it curves ventrally. During this period, the manchette is absent, and the sub-acrosomal material has not formed, suggesting that an organized pattern of nuclear organization and condensation is responsible for the shape of the nucleus in the dorsal hook.

4.3.b Remodelling of the Acrosome

The mechanisms involved in acrosomal remodelling and condensation of the matrix are unclear. Some authors have suggested that this may involve a specific condensation of the acrosomal contents, and does not require external forces applied by the Sertoli cell (Fawcett et al., 1971). This view is supported by the discovery of an ordered sub-structure in the acrosomal matrix of some species (Phillips, 1972; Friend and Fawcett, 1974; Olson and Winfrey, 1985). However, in at least one species, the ground squirrel, there is considerable evidence to suggest that microtubules in the adjacent Sertoli cell cytoplasm may be involved in the acrosomal modifications which occur during the final steps of spermiogenesis (Vogl et al., 1983a, 1983b), although this may be necessitated by the extreme size and shape of the acrosome in this species.

The acrosome on the dorsal hook of plains mouse spermatids undergoes considerable changes in its shape, orientation and the distribution of its contents, especially during steps 11 and 12. An interesting feature of this process is the reorientation of the apical acrosomal regions, formation of the VAP, and the caudal translocation of the contents of the apical segment. These changes may be directly linked

to the reorientation of the nucleus at the base of the hooks, and the acquisition of its curvature. The nucleus becomes tilted to one side, which would direct the lateral surface of the hook on which the VAP forms, onto the ventral surface of the hook, where it is located in spermatozoa. The ensuing dorso-ventral flattening of the apical parts of the hook, and the change in shape from semi-circular to crescentic, could be mediated through a specific pattern of condensation of the acrosomal contents.

The contents of the SAS may also be involved in this process of acrosomal reorganization. Although it contains very little material during steps 11 and 12, there is another structure present in the SAS which could be involved, the so-called acrosomal zonule (Maxwell, 1982). It is found in late spermatids of many species, and consists of a dense plaque attached to the IAM on the ventral or posterior border of the acrosome, and adjacent parallel rows of 15 nm diameter filaments (Maxwell, 1982; Russell et al., 1983a; Czaker, 1985). In the plains mouse, the acrosomal zonule is found in association with the IAM along the ventral aspect of the dorsal hook during steps 7-9, but its extent increases in step 11 and 12 spermatids, such that it is found throughout most of the SAS, but disappears at the end of step 12. Hence, although it is generally regarded as adhering the posterior margin of the acrosome to the nucleus prior to formation of the PAR (Maxwell, 1982; Russell et al., 1983a), in the dorsal hook the acrosomal zonule could act as a temporary mold or skeletal support for the reorganization of the acrosome in the apical half of the hook. Support for this contention includes the transient nature of this organelle, and its prominence in the SAS during steps 11 and 12, in which the major acrosomal changes occur. The biochemical composition of this structure is unknown, and therefore a direct contractile nature cannot be assumed.

It is also worthwhile considering the possible involvement of the Sertoli cell in these changes. Sertoli cell ectoplasmic specializations (EPS) surround the acrosomal region of developing spermatids, and consist of bundles of microfilaments which are interposed between the Sertoli cell PM and a cisternum of endoplasmic reticulum (Flickinger and Fawcett, 1967; Ross, 1976; Russell, 1977, 1980). These structures have been postulated to anchor late spermatids in Sertoli cell processes, either by gripping the spermatids, stiffening the Sertoli cell recesses, or via direct membrane-membrane adhesion (Ross, 1976; Toyama, 1976; Russell, 1977, 1980). The microfilaments contain actin (Toyama, 1976; Franke et al., 1978), although a contractile nature has not been shown conclusively (Franke et al., 1978; Vogl and Soucy, 1985; Vogl et al., 1985). In this study, the ultrastructural features of the EPS appear to be identical to those reported for other species (Russell, 1980), and stained with NBD-Phalloidin in mechanically isolated spermatids, suggesting that the microfilaments contain actin. These structures could be involved in remodelling of the acrosome, by acting as an external skeleton or mold, as proposed by Vogl and Soucy (1985). Changes in the orientation of the EPS may facilitate the shaping of the apical segment, and the apical regions of the hook. This does not necessitate contractility of the filament bundles, however, as previous studies have shown connections between microtubules in the Sertoli cell cytoplasm and the EPS, and the microtubules may mediate any changes in the orientation of the EPS (Russell, 1977, 1980).

4.3.c Development of the Pseudoperforatorium

The expanded SAS beyond the apical tip of the nucleus is not present in step 7 spermatids, and only appears in steps 9-11. This is associated with nuclear condensation, and may develop as the result of

nuclear retraction posteriorly during condensation, a view supported by the extensive accumulations of excess nuclear membrane in this region, presumably present as a result of the decrease in nuclear volume. The shape of the pseudoperforatorium seems to be governed by the reorganization of the acrosome in the apical half of the hook, since the SAS is virtually devoid of contents until very late in spermiogenesis, after the hook has attained its final shape. A similar pattern of morphogenesis of the rat pseudoperforatorium has been described (Lalli and Clermont, 1981).

Franklin and Fussell (1972) suggested that in the golden hamster, there is a close association between the nuclear envelope and the accumulation of sub-acrosomal material (SAM) in the pseudoperforatorium. Such an association has not been found in rat spermatids (Lalli and Clermont, 1981), nor in the present study. Although large amounts of nuclear membrane are present in the SAS in step 11 spermatids, these are lost by step 12, well before the SAM forms in step 13b,c. Hence, it would appear that the proteins which comprise the SAM must either be formed earlier in spermiogenesis and stored until step 13, or mRNA must be stored and translated late in spermiogenesis (O'Brien and Bellve, 1980b; Bellve and O'Brien, 1983).

4.4 Formation of the Ventral Hooks

The ventral hooks originate as an outpushing of the SAS on the anterior border of the nucleus during steps 12 and 13. As the hook elongates and bifurcates, a continuation of the post-acrosomal dense lamina (PADL) forms around the periphery of each hook, initially as a series of sub-units, which coalesce to form a complete lamina. These events occur after nuclear condensation is complete, and the formation of the dorsal hook is almost complete. Shortly after, bundles of

microfilaments appear in the dorsal and ventral margins of the hooks, whilst electron-dense material accumulates in the basal regions, and in the core of the apical half of the hooks. At this point, the hooks curve ventrally, and become bilaterally flattened in their apical regions, with constricted dorsal and ventral margins. Just prior to spermiation, moderately electron-dense SAM accumulates in the ventral hooks and in the SAS of the dorsal hook, and the thickness of the PADL increases (Flaherty and Breed, 1983b). A similar sequence of events appears to occur in several other hydromyine rodents (Breed, 1984).

4.5 Morphogenetic Factors Involved in Ventral Hook Formation

4.5.a Initial Formation and Elongation

The ventral hooks form during steps 12 and 13a as an expansion of the SAS. At this time, however, the SAS is empty, and no evidence could be found for the presence of cytoskeletal structures in the hooks which could participate in their formation and elongation. At present, based on the morphological data obtained in this study, there appear to be three possible mechanisms involved in this process:

- (1) Hydrodynamic pressure may force the hooks to bellow out and expand, whilst the formation of the PADL, and the rigid Sertoli cell recesses may prevent the hooks expanding in directions other than along their axes. It should be noted that the entire SAS throughout the sperm head is expanded during step 12, when the ventral hooks first appear, and this may be indicative of fluid accumulation. Such a process would require the formation of additional membrane as the hooks elongated, which may account for the membrane whorls found at the apex of the hooks during their formation. The influx of water may be an osmotic event, and could be mediated by the

action of ion pumps in the PM, as has been postulated to occur during the acrosome reaction (Yanagimachi and Usui, 1974; Yanagimachi, 1981).

- (2) Utilizing both TEM and silver staining, it has been found that the PADL is laid down around the periphery of the hooks as they elongate, and this occurs quite close to the tip. The lamina initially forms as longitudinally oriented bands, which in transverse sections appear as sub-unit laminae, each possessing three ridges which project to the PM. The sub-units coalesce to form a continuous lamina. It is plausible that elongation of these laminae could be responsible for elongation of the ventral hooks. The separate sub-units or bands would be more flexible than a continuous lamina during this process. It should also be noted that at this stage, the tight association between the ridges and the PM has not formed, and this only appears late in step 13b after the hooks are elongated, curved, and have been sculptured. Such a loose association may permit movement of the lamina as the hooks lengthen and change shape. It is also possible that the direction of the ridges, which is along the axis of the hooks, could be involved in formation of the hooks by directing the formation of the laminae, and hence the hooks. The accumulation of dense material along the ventral border of the ventral hook may also be a structural feature involved in guiding the formation of the hooks.
- (3) The third mechanism involves the active or passive participation of the Sertoli cell. Vogl and Soucy (1985) have postulated that the EPS may form a skeletal framework for spermiogenic modifications. In view of this, and the suggestion by Russell (1977) that the EPS stiffens the recesses in which the spermatids reside, it seems possible that the EPS could guide formation of the hooks, and may

even mold the shape of the hooks as they form. However, the EPS are not present apical to the hooks during their elongation, and appear to extend as the hooks elongate, suggesting that molded or pre-formed recesses into which the hooks extend do not occur.

Russell (1977, 1980) has described linkages between the components of the EPS and adjacent microtubules, and postulated that such associations may be involved in the adluminal migration of spermatids. However, such associations could also participate in the formation of the ventral hooks. Contraction of microtubules in the Sertoli cell cytoplasm could extend the EPS away from the base of the hooks, which in turn might extend the ventral hooks due to the tight association between the EPS and the sperm head, shown in the present study by retention by the EPS around the spermatid after mechanical isolation. Such a mechanism is, however, purely speculative, and would require experimental verification.

4.5.b Function of Microfilaments in the Ventral Hooks

During step 13b, bundles of microfilaments appear in the ventral hooks, and are presumably actin filaments on the basis of staining with NBD-Phalloidin, a fluorescent dye which is specific for F-actin (Barak et al., 1980). Even though nothing is known about the kinetics of actin polymerization in the hooks, it probably follows the general pattern exhibited by non-muscle actins from other diverse phylogenetic groups. Such polymerization requires a critical concentration of G-actin monomers, the presence of ATP, and a suitable high ionic strength environment. Once these conditions are established, actin polymerization occurs rapidly until the concentration of G-actin falls below the critical level (Uyemura and Spudich, 1980; Korn, 1982). In the ventral hooks, the bundles of microfilaments form in an orderly fashion, which

implies a regular pattern of polymerization, cross-linking, and attachment to other sub-cellular structures. At present, the biochemical features of this process are unknown, as is the polarity of the filaments, which would determine the end from which they elongate (Tilney *et al.*, 1981; Korn, 1982).

At the end of step 13a, the ventral hooks are round structures which only contain small quantities of dense material. During step 13b, however, they undergo a substantial morphogenesis which includes curvature, bilateral flattening, and shaping of the apical margins of the hooks. The results of this study suggest that these changes may be brought about by the bundles of microfilaments which form in the hooks at this time. The following features are indicative of such a role:

- (1) The shape changes occur in conjunction with the formation of the microfilaments bundles.
- (2) The ventral curvature of the hooks is accentuated during this step.
- (3) At the base of the hooks, where the shape does not change, the fluorescence is weaker after NBD-Phalloidin staining, and few filaments are observed, although there is a substantial accumulation of core material.
- (4) The bundles of filaments are greatest in the apical half of the hooks, whilst the amount of core material decreases, and it is these regions which undergo the most pronounced shape changes during step 13b.
- (5) The microfilament bundles only occur in the dorsal and ventral margins of the hooks, which become constricted and highly flattened.

Hence, it appears that one of the main functions of these microfilaments, presumably actin filaments, is the shaping and curvature of the ventral hooks. There are, however, a number of unanswered

questions concerning this role. Firstly, the mode of attachment of the filaments to the hooks is unclear, as are the exact points of attachment. The bundles of filaments appear to run the entire length of the two hooks, and may be embedded in the dense core material near the point of bifurcation. Such an arrangement would greatly facilitate the ventral curvature of the hooks, as contraction of the filaments would cause bending in the region of the bifurcation, where the curvature is greatest in spermatozoa. At this stage, however, it is not known how contraction of longitudinally oriented filaments could cause the bilateral flattening of the apical regions of the hooks.

The second problem concerns the actual mechanism of contraction, assuming that actin filament contraction is involved in the remodelling of the hooks. This would require the presence of myosin-ATPase, but myosin filaments were not observed, and mature spermatozoa do not seem to contain myosin (see Section 4). However, after completion of contraction, the myosin-ATPase could be removed from the ventral hooks and SAS via the tubulobulbar complexes, which would account for its absence from epididymal sperm.

4.5.c Condensation of Sub-acrosomal Material

In the plain mouse, the electron-dense SAM accumulates in the SAS during step 13c, immediately prior to spermiation. It appears suddenly in the ventral hooks, and accumulates around the core material and the filaments, totally obscuring the internal organization of the hooks. It may also participate in the thickening of the inner aspect of the PADL which occurs at the same time, thus forming the peripheral layer of the hooks.

Although nothing is known about the origin or mode of synthesis of these proteins, it is unlikely that haploid gene expression would occur,

necessitating transcription and possibly translation of these proteins during meiotic prophase or early spermiogenesis (see Bellve and O'Brien, 1983). The proteins which constitute the SAM might enter the SAS during steps 13a or 13b, and condense during step 13c as a result of a change in the properties of the cytoplasm. Tubulobulbar complexes (TBC) have been implicated in the elimination of cytoplasmic fluids during late spermiogenesis (Russell and Clermont, 1976; Russell, 1979a, 1979b; Russell and Malone, 1980). In this study, TBC were found emanating from the lateral tip of the dorsal hook at various points along its length. They were present in step 12 and 13 spermatids, which correlates with reduction in the size of the SAS, condensation of the SAM, and formation of the residual body. Hence, removal of the "watery" components of the cytoplasm (Russell, 1979a, 1979b), may concentrate the SAM, and initiate its aggregation and/or condensation.

It is also possible, however, that the appearance of SAM in the dorsal and ventral hooks during step 13c is a continuation of the deposition of the core material which appears in steps 13a and 13b. Nevertheless, this seems unlikely, since the SAM appears suddenly in step 13c, and studies to be reported in Section 4 show that a disulphide bonded remnant remains after extraction of most of the material in the ventral hooks. Hence, the core layer of the ventral hooks may contain two different types of material.

4.6 Concluding Remarks

This ultrastructural study of the morphogenesis of the hooks has provided valuable new findings on their structure. The ventral hooks have been shown to form as an expansion of the SAS, and are enclosed in an extension of the PADL. The most significant finding, however, is that regularly arranged bundles of 6-7 nm microfilaments appear in the

ventral hooks during step 13. This represents the first morphological evidence for the presence of filamentous actin in these structures, as proposed by Flaherty et al. (1983), and in accord with that study, filaments have not been localized in the pseudoperforatorium of the dorsal hook. It also appears that the core layer of the ventral hooks may consist of two different types of material, which form at different times during step 13 of spermiogenesis.

The asymmetrical disposition of the segments of the acrosome, as well as the asymmetrical shape of the dorsal hook, and the orientation of the three hooks, develop during spermiogenesis. This study has also revealed the presence of two bands of PADL on the ventral surface of the dorsal hook, which probably function to attach the PM tightly to the hook. These undoubtedly correspond to the thin argyrophilic bands on the dorsal hook of mature spermatozoa (see Section 2).

SECTION 4 : PROTEIN COMPOSITION OF THE PERI-NUCLEAR THECA

1. INTRODUCTION

The peri-nuclear theca (PNT) surrounds the nuclear material in the sperm head, and consists of the sub-acrosomal material (SAM) and post-acrosomal dense lamina (PADL) (Bellve and O'Brien, (1983). The sub-acrosomal material has been shown to contain lysine-rich proteins (Courtens et al., 1976), and several electrophoretic studies have indicated that its major component is one or more proteins of 13-15 kd (Olson et al., 1976a; O'Brien and Bellve, 1980a; Pruslin and Rodman, 1983). In the rat at least, there is a single cysteine-rich protein in the pseudoperforatorium, which becomes stabilized by disulphide bonds during epididymal maturation (Calvin and Bedford, 1971; Olson et al., 1976a).

Cytochemical studies have revealed that the PADL has an affinity for phosphotungstic acid (Nicander and Bane, 1966), and is argyrophilic (Elder and Hsu, 1981; Czaker, 1985), suggesting the presence of basic proteins or ribonucleoprotein (Czaker, 1985). Several studies have also claimed to localize monomeric actin in this region (Clarke and Yanagimachi, 1978; Tamblyn, 1980; Greenberg and Tamblyn, 1981; Clarke et al., 1982; Welch and O'Rand, 1985), although little else is known about the chemical composition of this structure (Fawcett, 1975; Bellve and O'Brien, 1983).

In Sections 2 and 3, it has been shown that the core layer of the ventral hooks consists of an extension of the sub-acrosomal material, and also contains bundles of microfilaments, whilst the peripheral layer represents a continuation of the PADL. However, a chemical analysis of these structures has not yet been performed, and, as they are a

characteristic feature of the sperm of many hydromyine rodents (Breed, 1983, 1984), it was decided to determine what proteins are present in the ventral hooks and other peri-nuclear structures. Hence, the aims of this section were:

- (1) To develop a procedure for the isolation and solubilization of the various sperm head structures, notably the PNT, which in the plains mouse includes the SAM, PADL, pseudoperforatorium, and ventral hooks.
- (2) To analyze sperm extracts using one-dimensional SDS polyacrylamide gel electrophoresis, to determine the main protein constituents present in these structures, and to verify the presence of actin and/or other cytoskeletal proteins.

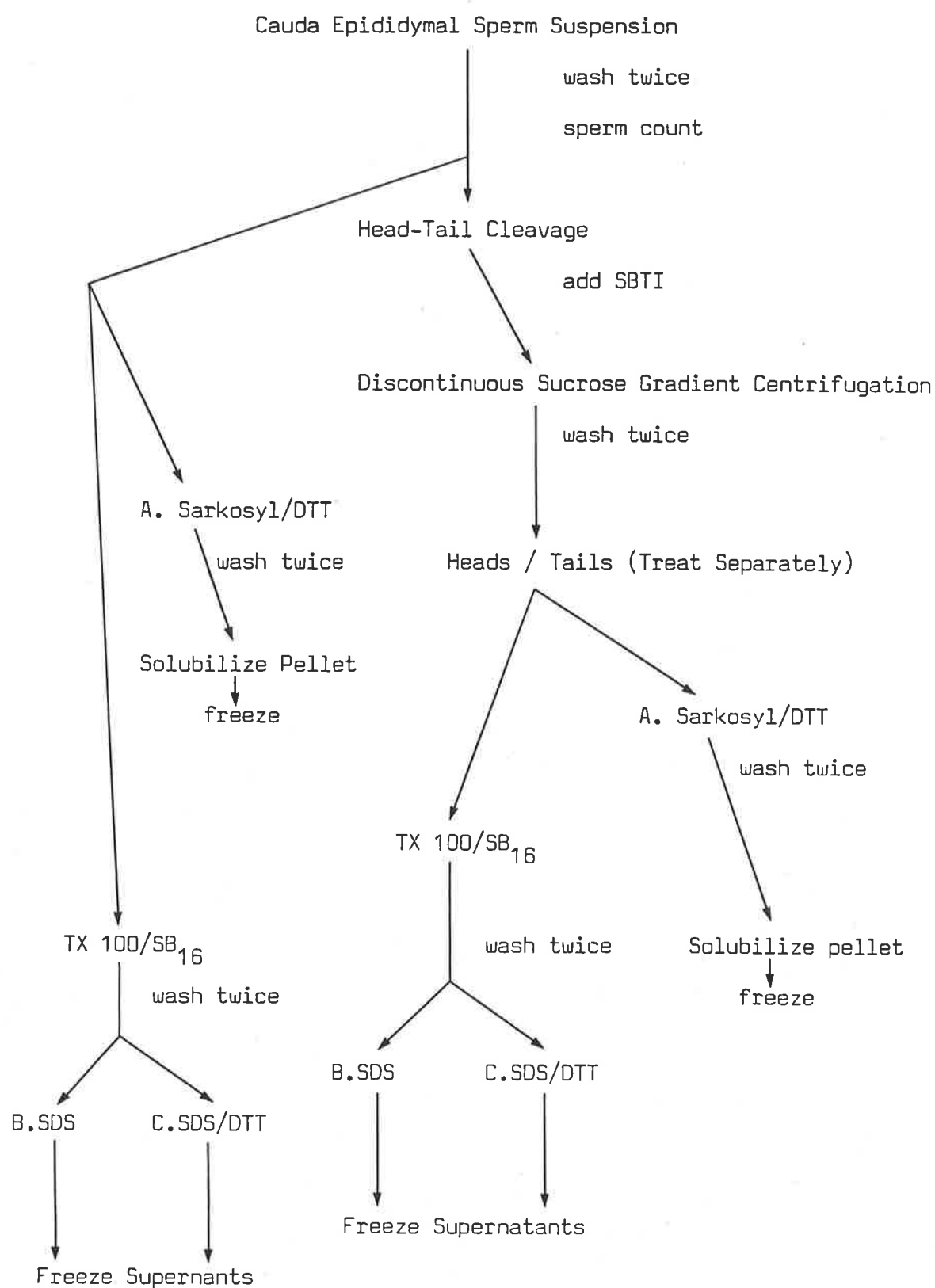
2. MATERIALS AND METHODS

2.1 Isolation of Sperm Heads and Tails

An overview of the procedure for the isolation of sperm heads and tails, and the extraction of proteins, is given in Text Fig. 2.

Four to six adult male plains mice were used per experiment. They were killed by cervical dislocation, and the caudae epididymides carefully dissected free of fat and major blood vessels. Sperm were extruded into warm PBS (pH 7.4) by pricking the large distal tubules with a 23G needle. After 5-10 minutes, the sperm were collected, and washed twice by centrifugation (500 g, 10 minutes), and resuspended in PBS. A sperm count was performed (see Appendix 3), and the concentration adjusted to 2×10^7 sperm/ml.

Cleavage of sperm heads from tails was carried out by incubating sperm at 1×10^7 sp/ml with 1mg/ml trypsin (Sigma, type III) in PBS (pH 7.4) for 5-15 minutes at 21°C, with two inversions of the tube every



TEXT FIG. 2 - Diagrammatic outline of the procedures used for the separation of sperm heads and tails, and the solubilization of proteins from intact sperm, or heads and tails.

minute, followed by vortexing for 3-5 seconds at full speed using a Scientific Products mixer. The degree of cleavage was monitored using phase contrast optics. Soybean trypsin inhibitor (SBTI) (Sigma, type IIs) was then added to a final concentration of 3 mg/ml, and, after 10 minutes, the sperm were pelleted by centrifugation (500 g, 10 minutes), and resuspended in cold buffer P (0.02 M Na_2HPO_4 , pH 6.0). Separation of heads and tails was performed by discontinuous sucrose gradient centrifugation (Calvin, 1976b). The sperm in buffer P were mixed with 2.20 M sucrose to give $1-3 \times 10^7$ sp/ml in 1.80 M sucrose, and 10 ml was layered onto a gradient consisting of 10 ml each of 2.05 M and 2.20 M sucrose in 40 ml nitrocellulose tubes. The tubes were spun at 15-20,000 g_{max} for 60-90 minutes at 4°C in an I.E.C. B20A high speed centrifuge. The 1.80 M layer containing tails was carefully removed, as was the pellet containing isolated heads, and both fractions were washed twice in cold buffer P, a count performed, and the purity assessed microscopically.

2.2 Extraction of Proteins

Three procedures were used to solubilize proteins from intact washed sperm, and isolated sperm heads and tails. These were:

A. Treatment with 250-500 μl of 0.5% sarkosyl (N-lauroyl sarcosine) and 1mM DTT (dithiothreitol) in tris-HCL buffer (pH 9.0) for 20-30 minutes at 37°C, followed by dilution to 10 ml with buffer, centrifugation at 2000 g for 5 minutes, and another wash in buffer. The pellet was solubilized in 1% SDS (sodium dodecyl sulphate) and 10mM DTT in tris-HCL buffer, and stored frozen at -20°C.

B. and C. Sperm fractions were treated with 2% Triton X-100 or 2% SB₁₆ (Calbiochem, zwittergent 3-16) in tris-HCL (pH 7.4) for 2-3 hours at 37°C, then washed twice in buffer. The pellets were then incubated in:

- B. 2% SDS in tris-HCL (pH 9.0) for 2-4 hours at 37°C.
- C. 1% SDS and 1mM DTT in tris-HCL (pH 9.0) for 10-20 minutes at 37°C, until examination with phase contrast optics revealed that the ventral hooks were absent.

The samples were centrifuged at 12,000 g for 2 minutes in 1.5 ml conical tubes, the supernatants removed, and frozen at -20°C.

2.3 Light and Electron Microscopy

The effects of the various stages of these procedures on sperm ultrastructure was monitored by removing samples at different stages, and examination with phase contrast optics or transmission electron microscopy (TEM). Samples were prepared for TEM as outlined in Section 2, and the sections examined at varying magnifications. The purity of the head and tail fractions was assessed by counting five fields of view on slide preparations at a magnification of 100x.

2.4 SDS - Polyacrylamide Gel Electrophoresis

One dimensional SDS-PAGE was carried out according to the method of Laemmli (1970) (see Appendix 4).

Samples were prepared by acetone precipitation in 4 vols of acetone at -20°C for 24-36 hours. After centrifugation at 12,000g for 10 minutes, the pellets were dried under vacuum in a dessicator, then resuspended in 40 µl of loading buffer. To each was then added 2 µl of 1% bromophenol blue, and the samples were boiled for 2 minutes, cooled, then spun at 12,000 g for 4 minutes.

In addition to the sperm samples, two types of protein standards were prepared in loading buffer. These were purified rabbit skeletal muscle actin (Sigma, >90% pure), and molecular weight standards

consisting of an equal mixture of lysozyme, β -lactoglobulin, trypsinogen, pepsin, ovalbumin, and bovine plasma albumin.

The gels used were 1.5 mm thick slab gels, consisting of a 4.2% stacking gel, and a 5-15% gradient separating gel. About 35-40 μ l of each sample was loaded, such that individual tracks contained either 5 μ g of actin, 25 μ g of standards, or protein from $1-3 \times 10^7$ sperm or isolated heads or tails. The gels were run at 30 mAmp for 5 hours, fixed for 1-2 hours in 30% methanol/10% acetic acid, stained overnight in 0.1% coomassie blue R250, and destained over a 3-5 day period in 5 changes of fixer. They were then photographed on Kodak TP2415 film using an orange filter.

Molecular weights were determined by plotting the relative mobility of the molecular weight standards against the natural logarithm of their molecular weight, and a line of best fit determined by linear regression. The mobility of sperm proteins was inserted into the equation, and the anti-natural logarithms calculated to give the molecular weights.

3. RESULTS

3.1 Head-Tail Cleavage

Incubation of sperm with trypsin for 20 minutes resulted in about 64% head-tail cleavage. However, if the suspensions were vortexed for 3-5 seconds after incubation, >97% cleavage was routinely obtained (Fig. 159).

Although the sperm heads appeared intact when visualized with phase contrast optics, TEM revealed that many sperm heads exhibited a variable degree of damage, which included loss of the plasma membrane (PM), swelling or loss of contents from the principal segment of the

acrosome, and occasionally, loss of the post-acrosomal dense lamina (PADL) and swelling of the sub-acrosomal space (SAS) (Figs. 160,161). The point of cleavage occurred at the junction of the capitulum and basal plate, the basal plate remaining firmly attached to the sperm head (Fig. 161). The nucleus and sub-acrosomal material (SAM) in the dorsal hook, and the two ventral hooks, appeared largely unaffected by this treatment, although the PM often became less tightly adherant to the ventral hooks (Fig. 162).

The middle piece of the tails often exhibited displacement of the mitochondria, and sometimes swelling or loss of the matrix, as well as disorganization of the axoneme and outer dense fibres. The effect on the principal piece was variable, with some tails broken at the level of the annulus, and the internal organization disrupted, whilst other tails showed no visible damage (Fig. 163).

3.2 Separation of Heads and Tails

The discontinuous sucrose gradient centrifugation routinely produced tail fractions of >98% purity (Fig. 164), and head fractions of about 95% purity (Fig. 165).

The sperm heads showed no visible changes with phase contrast optics (Fig. 166), but ultrastructural studies revealed loss of the PM and extensive damage to the acrosome (Figs. 167,168). The peri-nuclear theca, including the pseudoperforatorium and ventral hooks showed no signs of damage (Figs. 167,168). Isolated sperm tails, which were often coiled, exhibited a variable degree of erosion of the mitochondrial sheath, and breakage at the annulus (Figs. 164,169). Both the middle piece and principal piece showed loss of the PM and disorganization of the axoneme, although this was more pronounced in the middle piece (Fig. 170).

3.3 Solubilization of Sperm Structures

The treatment of isolated sperm heads with Triton X-100 (TX100) or SB₁₆ resulted in complete solubilization of the remaining PM, except for some remnants on the ventral hooks (Figs. 171,172). The acrosome was also largely extracted, though some remnants of the IAM and/or matrix remained, especially in the equatorial segment on the dorsal hook (Fig. 171). Fewer remnants remained after treatment with SB₁₆ than TX100 (compare Figs. 171,172). The nucleus, basal plate and peri-nuclear theca remained intact (Fig. 173), and the ridges were clearly evident in the post-acrosomal region and in the peripheral layer of the ventral hooks (Figs 171,172,174). The only changes to the sperm tails as a result of treatment with these detergents concerned loss of the PM, and most of the mitochondria except the outer mitochondrial membrane (Fig.175).

After incubation in either TX100 or SB₁₆, treatment of sperm heads with SDS caused substantial, but incomplete, solubilization of the sub-acrosomal material in the dorsal and ventral hooks. The ventral hooks became swollen, elongated, and less dense (Fig. 176). The sub-acrosomal material became very granular, and the ventral hooks displayed a loss of organization (Fig. 177). The anterior regions of the nucleus were unaffected, but posteriorly, the nucleus was less condensed and more vacuolated. The basal plate, PADL and remainder of the SAM were solubilized by this treatment (Fig. 178). The sperm tails were badly damaged by SDS, and the only structures remaining were the connecting piece, outer dense fibres (ODFs), fibrous sheath and outer mitochondrial membrane (OMM). However, the fibrous sheath appeared more granular and less compact, and the cortex of the ODFs was wispy, with an indistinct border (Fig. 179).

Incubation of sperm heads in SDS and DTT for 10-20 minutes resulted in complete extraction of all sperm head structures except the nucleus, which was partially decondensed, especially in its posterior margin (Fig. 180). The entire peri-nuclear theca, including the ventral hooks and pseudoperforatorium, was solubilized by this treatment (Figs. 181,182). The ODFs were largely unaffected by this short incubation in SDS and DTT, but the OMM and much of the fibrous sheath were solubilized (Fig. 183).

Treatment of isolated sperm heads with sarkosyl and DTT for 20-30 minutes produced complete decondensation of the nucleus, leaving only the ventral hooks and pseudoperforatorium (Fig. 184). Ultrastructural observations revealed that the PM and acrosome were completely solubilized, and the only remnants of the nucleus were a few granules and fibrils, which tended to adhere to the hooks (Fig. 185). The orientation, shape and structural integrity of the ventral hooks was unaffected by this incubation, and the SAM in the dorsal hook showed no visible changes (Fig. 184,185). In sperm tails, the mitochondrial sheath, dynein arms and radial spokes were absent after incubation with sarkosyl and DTT, but the ODFs, connecting piece and fibrous sheath were resistant to this treatment (Figs. 186,187). However, the fibrous sheath was noticeably more granular and less electron-dense (Fig. 187).

3.4 Electrophoretic Analysis of Sperm Proteins

3.4.a. Sperm Head Proteins

Although three different methods were used to isolate and solubilize sperm head structures, and the degree of solubilization varied, a similar electrophoretic profile was observed for all extracts (Fig. 190). One dimensional SDS-PAGE revealed three major polypeptide

bands of molecular weights of 14-16 (S_1), 41-42 (S_2) and 44 (S_3) kd. It is obvious from the gels that the most intensely stained band is 14-16 kd, and this protein (S_1) is present in quantities far in excess of the other two. When less protein was loaded onto the gels, S_1 banded distinctly at about 15 kd. Band S_2 (41-42 kd) had a similar electrophoretic mobility to rabbit skeletal muscle actin, but was of marginally greater molecular weight (Figs. 188-190).

In addition to the three major bands, up to 17 minor bands were present, of which only 5 did not comigrate with proteins in the tail fractions. The molecular weights of these were 12.5 (S_4), 46 (S_5), 86.5 (S_6) and 90 (S_7) kd, although there may also have been another band at 13.5 kd (S_8), which was often obscured by the 15 kd band. The other minor bands which had the same mobilities as tail proteins are considered to have been contaminants. At no stage were any polypeptides of >90 kd observed in the sperm head extracts (Figs. 188-190).

3.4.b. Sperm Tail Proteins

Treatment of isolated sperm tails with TX100 or SB₁₆, followed by SDS, revealed 6 major bands in the SDS-soluble fraction. The molecular weights of these bands were 25,31,32,34,48 and 52 kd. A large number of minor bands were also present, the molecular weights of which were 20.5,22,23,24,27,29,30,36,37,38,44,67,72 and 78 kd. In addition other minor bands of 15,41 and 43.5 kd had the same electrophoretic mobilities as the major head proteins. A similar electrophoretic profile was obtained from extracts of sperm tails treated with sarkosyl and DTT.

Solubilization of tail proteins by SDS and DTT produced the same major bands as SDS alone, but in addition, a further 4 major bands of 19,29,31 and 66 kd were present, as well as minor bands of 57.5,60 and 63 kd (Fig. 190).

4. DISCUSSION

A procedure has been developed for the separation of plains mouse sperm heads and tails, and the solubilization of specific sperm head structures, notably the peri-nuclear theca, which includes the SAM, ventral hooks and PADL. This has permitted an investigation of the protein composition of the PNT in this species using one-dimensional SDS-PAGE. No attempt will be made to discuss the protein composition of the intact sperm or isolated tail fractions, as these were only included to assist in the determination of which bands in the head fractions were tail contaminants.

4.1 Trypsin-mediated Head-Tail Cleavage

The majority of studies of the protein composition of sperm head structures have used sonication to cleave the sperm heads from tails (Calvin, 1976b; Olson et al., 1976a). For the present study, a sonicator was unavailable, so trypsin was used. In contrast to sperm from the rat and laboratory mouse, however, which only require a brief treatment with a low concentration of this enzyme (Edelman and Millette, 1971), it was found that a higher concentration of trypsin was necessary in the present study, as well as a 3-5 second burst of violent agitation to produce >97% cleavage. This treatment caused loss of the PM and acrosomal matrix of many sperm, and disorganization of the mitochondrial sheath and axoneme in the middle piece of the tails. The peri-nuclear theca, however, appeared unaffected by this treatment, except for loosening of the PM over the ventral hooks of some sperm. This may indicate that the tight association between the PM and PADL ridges is mediated via peptide linkages. Although the PNT showed no visible signs of proteolysis, it is still possible that trypsin may have acted upon

the proteins in some unknown manner. The point of cleavage by trypsin, between the basal plate and capitulum, was the same as in previous studies of mouse sperm (Millette et al., 1973). Other treatments that have been used to separate sperm heads and tails, such as acid/alkaline pH, primary amines, aldehydes or sulphydryl reagents were not used in this study due to the wide species variability in their effects, and the severe damage incurred to some sperm structures (Millette et al., 1973; Young and Cooper, 1983a).

4.2 Separation of Heads and Tails

A variety of centrifugation techniques have been used in other studies for the recovery of pure populations of sperm heads, such as discontinuous sucrose gradients (Calvin, 1976b; Olson et al., 1976a), linear sucrose gradients (Millette et al., 1973), and single sucrose layers (Pruslin and Rodman, 1985). In this study, the method of Calvin (1976b) was used to obtain populations of sperm heads of about 95% purity, and tails of 98% purity. However, poor overall yields of heads were obtained (approx. 25%), which was probably due to incomplete pelleting in the 2.20 M sucrose layer as a result of the low centrifugal force available (15-20,000 g_{max}).

Although the PM, acrosome and middle piece underwent a variable degree of damage, the SAM in the dorsal and ventral hooks, and the PADL, appeared unaltered by this separation process. The loss of the acrosomal contents suggests the possibility of proteolysis of the PNT by acrosomal proteases, although this seems unlikely since the ultrastructural features remained unchanged, and there was little or no smearing of the tracks of sperm head extracts on the gels. Nevertheless, in future studies it would be advisable to include

protease inhibitors such as PMSF to prevent the possibility of proteolysis of sperm structures by acrosomal enzymes.

4.3 Selective Solubilization of Sperm Structures

The three methods utilized in this study to solubilize various sperm structures were modified from those used in an earlier study to probe the nature of the material in the ventral hooks of plains mouse sperm (Flaherty and Breed, 1983a).

The treatment of sperm heads and tails with Triton X-100 produced comparable effects to those observed with bull sperm (Wooding, 1973), such as loss of the PM, acrosome, and mitochondria except for the outer mitochondrial membrane (OMM). In addition to TX100, however, a zwitterionic detergent (SB_{16}) was also used to solubilize these structures. Zwittergents are non-ionic, amphoteric surfactants of the sulfobetaine class, with a variable alkyl chain length (SB_N) (Baron and Thompson, 1975; Gonenne and Ernst, 1978). When $N > 12$, zwittergents are highly effective agents for the solubilization of peripheral and integral membrane proteins, and bring about the rapid emulsification of lipid bilayers (Gonenne and Ernst, 1978). This may explain why SB_{16} ($N=16$) appeared to leave fewer remnants of the acrosome and PM than Triton X-100. However, neither treatment affected the PNT, which was then available for solubilization with SDS $\pm$ DTT.

Following detergent treatment, the sperm heads were exposed to SDS to solubilize the peri-nuclear theca. This treatment caused complete extraction of the PADL, and the SAM in the sperm head posterior to the hooks, but left the nucleus largely unaffected, except for slight vacuolation in the caudal regions. However, in contrast to a previous report from our laboratory (Flaherty and Breed, 1983a), SDS did not completely solubilize the ventral hooks or pseudoperforatorium. The two

ventral hooks became elongated and swollen, and lost their characteristic shape, but a remnant of granular material remained. This discrepancy between the two studies was not due to the different buffers used, as SDS in borate buffer also fails to completely solubilize the ventral hooks (see Section 5). However, it may be due to the fact that during treatment with SDS, the ventral hook remnants sometimes break off, especially during agitation or centrifugation, which gives the appearance of complete solubilization.

The peri-nuclear theca, including the above-mentioned core remnants, was solubilized entirely by SDS and DTT, indicating that, as in other eutherian species, the SAM in the anterior portion of the plains mouse sperm head is stabilized by disulphide bonds (Calvin and Bedford, 1971; Bedford and Calvin, 1974a; Olson et al., 1976a). The last part of the ventral hooks to be solubilized was the basal region, where the clumped material accumulates during step 13b of spermiogenesis (see Section 3). This suggests that the disulphide bonded material in the ventral hooks may be this clumped material which appears before the condensation of the major portion of the SAM. Complete extraction of the hooks did not occur, however, until the nucleus had undergone partial decondensation, which was less pronounced in the anterior regions. This indicates a higher degree of disulphide bonding in the anterior region of the sperm head (Bedford and Calvin, 1974a). Nevertheless, it seems doubtful that any of the polypeptides characterized in the sperm head fractions were either protamines (Calvin, 1976a) or non-protamine nuclear proteins (Avramova et al., 1980; Pruslin and Rodman, 1983), since no proteins of <12 kd were detected, and the same electrophoretic profile was obtained for SDS extracts, in which the nucleus remains essentially intact (see 4.4).

The only tail structures which were solubilized by SDS were the axoneme and mitochondrial matrix. The connecting piece, OMM, fibrous sheath and dense fibres remained intact, though the cortex of the dense fibres, and the fibrous sheath did exhibit some evidence of partial extraction. Hence, it appears that in plains mouse sperm, as in other species, the accessory elements of the tail are stabilized by disulphide bonds (Bedford and Calvin, 1974b; Calvin, 1975). Brief treatment with SDS and DTT solubilized the OMM and part of the fibrous sheath, but did not affect the ODF, which require longer incubation times. Sarkosyl and DTT induced decondensation of the sperm nucleus, but left the perinuclear theca unchanged, as previously reported (Flaherty and Breed, 1983a). Both layers of the ventral hooks, and the pseudoperforatorium of the dorsal hook, were unaffected by this treatment. The rat pseudoperforatorium has also been found to be resistant to this treatment (Olson et al., 1976a). Some residual nuclear granules and fibrils remained after incubation in sarkosyl and DTT, which may have produced the smearing of tracks containing these extracts. In future experiments, therefore, DNAase extraction would be a useful approach to remove these nuclear contaminants.

4.4 Electrophoretic Analysis of Sperm Head Proteins

In this study, proteins isolated from sperm by various procedures have been analyzed using one-dimensional SDS-PAGE (Laemmli, 1970). The material solubilized from the sperm head was principally the perinuclear theca (PNT), which included the SAM in the dorsal and ventral hooks, and the PADL. All three extraction procedures produced a similar electrophoretic profile for proteins from the PNT. This is particularly puzzling, since SDS did not completely solubilize the SAM, although this was accomplished with SDS and DIT, and yet both extracts produced the

same polypeptide bands. A possible explanation is that several polypeptides of similar molecular weight may constitute the SAM, but because of slight differences in their amino acid composition, one or more may be cross-linked by disulphide bonds, while the remaining one(s) is solubilized by SDS.

4.3.a 15 kd Polypeptide

Electrophoresis of sperm extracts revealed that the major component of the peri-nuclear theca is one or more polypeptides with a molecular weight of about 15kd. Unfortunately, a densitometer was unavailable for quantitation of the different polypeptide bands. The exact localization of this band in the sperm head is unknown, although the fact that it constitutes a high proportion of the protein present, and the SAM in the dorsal and ventral hooks represented most of the material solubilized, suggests that this 15 kd polypeptide(s) is the major constituent of the SAM. At this stage, it is not known if there is a single 15 kd polypeptide, or multiple polypeptides differing in their isoelectric points, and whether these exist as monomers, dimers etc. in situ in the sub-acrosomal material, as the gels were only run under reducing conditions.

Several studies have now shown that the major or only protein component of the sub-acrosomal material in the pseudoperforatorium of rat sperm is a 13 kd, cysteine-rich polypeptide (Olson et al., 1976a; Campanella et al., 1979). Rodman et al. (1983) detected a 15 kd protein in the "peri-nuclear space" of human sperm. Electrophoretic analysis of mouse sperm head proteins has also alluded to the presence of one or more 14-15 kd proteins in the sub-acrosomal material of this species (O'Brien and Bellve, 1980a; Pruslin and Rodman, 1983). In a more recent study, Bellve and O'Brien (1983) claim that the major polypeptides of

the peri-nuclear theca of mouse sperm have molecular weights of 13,15,16.5 and 25 kd, although they made no distinction between proteins from the SAM and PADL. Hence, it would seem that 13-15 kd polypeptides may constitute much of the SAM in sperm from a variety of eutherian species, including the plains mouse.

4.3.b Actin

In a previous communication (Flaherty et al., 1983), filamentous actin was localized in the two ventral hooks of plains mouse sperm using the fluorescent dye, NBD-Phalloidin, which has been confirmed in Section 2 of the present study. In addition, it has been shown in Section 3 that bundles of microfilaments, of the same diameter as actin filaments, form in the ventral hooks during step 13b of spermiogenesis, and the distribution of these filaments is similar to the pattern of NBD-Phalloidin fluorescence. The presence of actin in the ventral hooks is further supported in the present section by the characterization of a 42 kd polypeptide, which comigrates closely with rabbit skeletal muscle actin, and is of a similar molecular weight to actin isolated from a variety of other non-muscle cells (Korn, 1982). The 42 kd polypeptide is the second most intensely stained band in the sperm head extracts, which consist of proteins solubilized from the peri-nuclear theca, and therefore probably corresponds to the large bundles of 5-7 nm microfilaments (see Section 3). However, although most of the filamentous actin appears to be localized in the ventral hooks, actin may also be present in other parts of the sperm head in the monomeric form, which is not detected using NBD-Phalloidin (Barak et al., 1980). It is unlikely that this 42 kd polypeptide was a contaminant from epididymal, epithelial or blood cells, as great care was taken during preparation of the sperm suspensions to avoid

contamination with these cell types, and they were rarely observed in the sperm preparations during ultrastructural analyses. Hence, there are now three lines of evidence which strongly suggest that F-actin is present in the ventral hooks, although irrefutable evidence would require the biochemical analysis of the 42 kd polypeptide.

Actin has been claimed to be identified in other eutherian sperm using immunofluorescence techniques, where it has been found to be associated with either the acrosome (Talbot and Kleve, 1978; Virtanen et al., 1984), or the post-acrosomal region (Clarke and Yanagimachi, 1978; Tamblyn, 1980; Greenberg and Tamblyn, 1981; Clarke et al., 1982; Welch and O'Rand, 1985). Caution must be exercised in the interpretation of these results, however, as two studies on human sperm have reported different localizations of actin, one in the acrosomal region, the other in the PAR, which is probably due to differences in antibody specificity (Clarke et al., 1982; Virtanen et al., 1984). Nevertheless, a protein which comigrates with actin or binds to myosin ATPase has been isolated from human, hamster, bull and rabbit spermatozoa (Talbot and Kleve, 1978; Campanella et al., 1979; Greenberg and Tamblyn, 1981; Clarke et al., 1982; Ochs, 1984; Virtanen et al., 1984; Welch and O'Rand, 1985). The presence of actin in eutherian sperm is not a ubiquitous phenomenon, however, as it does not occur in rat or guinea pig spermatozoa (Campanella et al., 1979; Halenda et al., 1984). In those species in which actin has been found in the sperm head, it appears to be in the monomeric or unpolymerized form (Tamblyn, 1980; Ochs, 1984; Virtanen et al., 1984; Welch and O'Rand, 1985), and may be complexed with storage proteins (Clarke et al., 1982), although polymerization may occur during capacitation or the acrosome reaction (Petersen et al., 1978). However, the plains mouse appears to be the only eutherian species in which filamentous actin has been demonstrated in mature

epididymal sperm (Flaherty et al., 1983).

4.3.c Other Cytoskeletal or Actin-Binding Proteins

In addition to the 15 and 42 kd bands, a major band of 44 kd, as well as minor bands of 12.5, 46, 86.5 and 90 kd were detected in the sperm head extracts. These are not considered to have been contaminating tail proteins, as they did not comigrate with any bands in fractions prepared from purified tail preparations. At no stage, however, have any polypeptides been detected which had molecular weights corresponding to the heavy (200 kd) or light (17-20 kd) chains of myosin (Korn, 1978; Groschel-Stewart, 1980). Hence, it would appear that myosin is not a component of the sub-acrosomal material of cauda epididymal plains mouse sperm. This suggests that in mature sperm, the bundles of microfilaments (presumably actin) may only serve a cytoskeletal, and not contractile role. Indeed, the presence of non-muscle myosin in eutherian sperm is a controversial subject. Campanella et al. (1979) claim myosin is present in the acrosomal region of human sperm, whereas, using affinity purified antibodies, Virtanen et al. (1984) only found myosin to be present in the neck region of human sperm. Tamblyn (1981) has presented biochemical evidence for myosin in bull sperm, although its localization was not determined. Hence, the results to date do not provide any substantial evidence for the presence of non-muscle myosin in the mammalian sperm head.

The absence of non-muscle myosin from the SAM of plains mouse sperm would seem to preclude the need for other cytoplasmic contractile proteins, such as α -actinin (100 kd), tropomyosin (30-40 kd) and the troponin complex (14-20 kd, 18-27 kd, 36-38 kd) (Korn, 1978, 1982; Groschel-Stewart, 1980). Indeed, none of the polypeptides identified in this study in the sperm head extracts correspond to the above molecular

weight ranges, suggesting that these cytoskeletal proteins are probably not found in the sperm head.

It is possible, however, that one or more of the minor bands could be actin-binding proteins (ABP) (Craig and Pollard, 1982). In Section 3, it was shown that the bundles of microfilaments which form in the ventral hooks have a regular and orderly array, with a hexagonal packing. Thus, it is highly probable that such a degree of organization requires the presence of actin-binding proteins during formation of the bundles, and in maintaining the organization after initial formation. ABP have been shown to occur in a variety of cell types, and range in molecular weight from about 20-280 kd. They are usually divided into three classes, depending on whether they cross-link, cap or inhibit actin filament formation (Craig and Pollard, 1982; Korn, 1982). Hence, any of the minor bands found in the sperm head extracts could represent actin-binding proteins. If present in the ventral hooks, ABP would probably be of the cross-linking class, and may be responsible for maintaining the organization of the microfilaments, or attaching the filaments to the inner surface of the PADL. However, before any such role could definitely be assigned to any of the proteins characterized in this study, it would be necessary to assay the effect of individual proteins on actin gelation, and filament formation and cross-linking (Korn, 1978, 1982).

4.3.d Protein Composition of Other Sperm Head Structures

In addition to the SAM, the sperm head extracts contained proteins solubilized from the PADL and basal plate. Thus, it is possible that the 44 kd band, and some of the minor bands may represent either or both of these structures. The PADL represents a significant part of the material solubilized, and as it is structurally distinct from the SAM, as well as

formed at a different time during spermiogenesis, it could be expected to consist of different proteins. In this respect, the PADL may consist of several proteins, as it has two structural components, the dense lamina, and the parallel ridges (see Sections 2,3). Unfortunately, studies on other sperm are unavailable for comparison, as the PADL has not been isolated from the other components of the peri-nuclear theca. Bellve and O'Brien (1983) have suggested that the peri-nuclear theca of mouse sperm consists of a diverse population of polypeptides of 8-80 kd, with prominent bands at 13, 15, 16.5 and 25 kd. However, they did not localize specific proteins to either the SAM or PADL.

It should also be noted that there were some remnants of the acrosome and PM in the sperm head extracts, and, although the degree of contamination by these components may have been fairly insignificant, they may represent one or more of the minor bands.

4.4 Concluding Remarks

This study was carried out to ascertain the major polypeptide components present in the ventral hooks on the sperm head of the plains mouse. To this end, a procedure has been developed for the isolation of sperm heads, and solubilization of the peri-nuclear theca, which includes the pseudoperforatorium and ventral hooks. Electrophoresis of these fractions revealed major bands of 15, 42 and 44 kd, as well as minor bands of 12.5-90 kd. The predominant band is one or more polypeptides of 15 kd, which probably constitutes the sub-acrosomal material. The 42 kd band comigrates with skeletal muscle actin, thus providing further evidence for the presence of filamentous actin in the ventral hooks. However, no evidence was found for the localization of myosin or other cytoskeletal contractile proteins in these structures. The 44 kd band could represent the PADL, whilst the minor bands may be

actin-binding proteins, or proteins from the basal plate or the ridges which link the PADL and PM.

To further characterize the proteins present in the ventral hooks, it would be necessary to analyze the sperm head extracts using two-dimensional electrophoresis (O'Farrell, 1975), and isolate individual polypeptides. Monospecific or monoclonal antibodies to these proteins could be produced, which could then be used to determine the exact localization of each protein in the structural components of the sperm head.

SECTION 5 : EPIDIDYMAL MATURATION OF SPERMATOZOA

1. INTRODUCTION

As eutherian spermatozoa travel through the epididymis, they undergo a series of changes which culminate in the acquisition of fertility. These include slight morphological alterations to the sperm head, migration of the cytoplasmic droplet along the middle piece, gradual stabilization of the nucleus, peri-nuclear theca and accessory tail structures by disulphide bonds, development of motility, and changes to the properties of the plasma membrane. These changes are interrelated, and collectively enable the spermatozoon to ascend to the site of fertilization, penetrate the cumulus oophorus and zona pellucida, and fertilize the oocyte (Calvin and Bedford, 1971; Bedford and Calvin, 1974b; Bedford, 1975; Holt, 1982, 1984).

In the previous sections of this study, a detailed account has been presented of the structural organization of the hooks on the sperm head of the plains mouse. It has previously been suggested that the function of these structures may be to aid penetration of the zona pellucida (Flaherty and Breed, 1983a; Breed, 1984). Hence, it was the aim of this section to examine several aspects of sperm maturation in the epididymis which may relate to events which occur during fertilization, especially penetration of the extra-cellular coats around the oocyte. These included ultrastructural changes in the sperm head, stabilization of the nucleus, peri-nuclear theca and tail by disulphide bonds, and the acquisition of motility.

2. MATERIALS AND METHODS

2.1 Preparation of Spermatozoa

Animals were killed by cervical dislocation, and the testes and epididymides removed and cleared of fat and large blood vessels. The epididymis was divided into 7 segments based on gross anatomical features. These were proximal and distal caput, proximal, mid and distal corpus, and proximal and distal cauda (Fig. 191). Sperm suspensions were prepared from each segment by pricking the tubules with a 25G needle, and extruding sperm into warm PBS, pH 7.4. Testicular sperm suspensions were prepared as outlined in Section 3.

2.2 Light Microscopy (LM)

Unwashed sperm from each segment were fixed in 2.5% glutaraldehyde in 0.15 M cacodylate buffer (pH 7.2), and a drop of each suspension placed on a slide, a coverslip added, and the edges sealed with D.P.X. Slides were examined with phase contrast optics, and photographs taken using Ilford Pan F film.

The position of the cytoplasmic droplet was studied by examining sperm at a magnification of 400x, and recording its position in about 100 sperm. Five categories were used for the position of the droplet: neck region; proximal, middle or distal third of the middle piece; or absent. Each count was repeated three times, the results combined, and expressed as a total percentage.

The maximum head length of sperm from the proximal caput and distal cauda segments was measured at a magnification of 1000x using an ocular micrometer. Only sperm lying flat in the plane of view were used. A sperm suspension from each segment was prepared using three males, and

150 sperm were measured from each segment. The head lengths are expressed as mean $\pm$ S.E.M. ($n = 150$). Statistical analysis was carried out using a one-way analysis of variance (Sokal and Rohlf, 1973).

2.3 Transmission Electron Microscopy (TEM)

The ultrastructural changes accompanying epididymal transit were studied using sperm pellets prepared from isolated sperm suspensions as per Section 2, or epididymal tissue fixed by vascular perfusion using 5% glutaraldehyde in 0.1 M phosphate (pH 7.2), containing 2.5% PVP, 1 mM CaCl_2 and 0.1% NaN_3 , and processed using the protocol described in Section 3 (see Appendix 2).

2.4 Chemical Treatment of Sperm

The methods of Calvin and Bedford (1971) and Olson et al. (1976a) were used to examine the stabilization of structures in the sperm head and tail by disulphide bonds. Sperm suspensions were prepared from each segment and washed once by centrifugation (500 g, 10 minutes). The pellets were gently resuspended in 0.05 M borate buffer, pH 9.0, and aliquots of each suspension mixed 1:1 with treatment solutions in borate buffer to give the final concentrations shown below:

1. 0.5% sarkosyl (N-lauroyl sarcosine)
2. 0.5% sarkosyl and 1 mM DTT (Dithiothreitol)
3. 1% SDS (sodium dodecyl sulphate)
4. 1% SDS and 1 mM DTT
5. 1 mM DTT
6. 0.05 M borate buffer, pH 9.0

All samples were incubated at 21°C, and examined with phase contrast optics at regular intervals up to 120 minutes. The integrity of the nucleus, pseudoperforatorium, ventral hooks, middle piece and

principal piece was scored qualitatively for each sample. All experiments were carried out at least three times, and the results combined.

2.5 Assessment of Sperm Motility

Testicular and epididymal sperm suspensions were prepared in warm Hank's Balanced salt solution (HBSS), pH 7.4. After allowing 5 minutes for dispersal, a small aliquot of each suspension was placed inside a ring of vaseline on a pre-warmed slide, and a coverslip carefully added, leaving a gap of about 0.3-0.5 mm. After allowing 2 minutes for the subsidence of bulk fluid movements, the motility pattern of each sample was examined using phase contrast optics at a magnification of 200x. All samples were examined within 30 minutes of initial extrusion from the epididymis, and the motility patterns of epididymal sperm from four males were recorded in this study.

3. RESULTS

3.1 Light Microscopical Observations

The maximum length of the sperm head was found to decrease from $9.26 \pm 0.02 \mu\text{m}$ ($n = 150$) in proximal caput sperm, to $9.09 \pm 0.01 \mu\text{m}$ ($n = 150$) in distal cauda sperm, which was found to be statistically significant ($F_{1,198}=23.58$, $P < 0.01$). The three hooks remained close together in sperm from all regions of the epididymis, and the morphological features of the sperm head did not change during epididymal transit (Figs. 192-198).

The only detectable change was in the migration of the cytoplasmic droplet (CPD) along the middle piece (see Table 1). In about 79% of testicular sperm, the CPD was located in the neck region (Fig. 192),

SEGMENT	POSITION OF CPD (%)				
	NECK REGION	MIDDLE PIECE			ABSENT
		PROX. THIRD	MID. THIRD	DIST. THIRD	
Testis	79.2	7.3	2.4	3.8	7.3
Prox. Caput	6.9	3.6	63.6	18.1	7.8
Dist. Caput	0	0.6	39.2	51.1	9.1
Prox. Corpus	0	0.3	9.8	81.0	8.9
Mid. Corpus	0	0	2.7	90.4	6.9
Dist. Corpus	0	0	2.9	71.1	26.0
Prox. Cauda	0	0	1.4	70.9	27.7
Dist. Cauda	0	0	2.1	74.6	23.3

TABLE 1 : Position of the cytoplasmic droplet (CPD) on the tail of sperm from the testis and different segments of the epididymis. Values are expressed as a percentage of the total number of sperm examined from each region in three experiments..

whilst in about 7% of sperm, it was in the proximal third of the middle piece (Fig. 193). In proximal caput sperm, however, the CPD was usually (63%) present in the middle third of the middle piece (Fig. 194), although it was found in the distal third in 18% of sperm from this segment. In the distal caput region, most sperm possessed a bent or kinked middle piece, and the CPD was situated at this point, which was invariably in the middle or distal third (Figs. 195,196). A bent tail was not a characteristic feature of proximal corpus sperm, in 81% of which the CPD was located in the distal third of the middle piece (Fig. 197). The proportion of sperm with distal droplets increased to 90% in the mid corpus segment. In the distal corpus, however, this decreased to 71%, and there was an increase in the percentage of sperm without droplets to 26%. In cauda epididymal sperm, about 71-75% of sperm retained the CPD in the distal part of the middle piece, whilst it was absent from 23-28% of sperm (Fig. 198).

3.2 Ultrastructural Features of the Sperm Head

3.2.a Plasma Membrane

The plasma membrane over the ventral hooks did not undergo any structural changes during epididymal transit, and was closely applied to the PADL in sperm from all segments (Figs. 204,207). The peri-acrosomal plasma membrane, however, underwent a distinct conformational change. In caput sperm, the PM was closely applied to the outer acrosomal membrane (OAM), and relatively smooth in contour (Figs. 199,201). In corpus and cauda epididymal sperm, however, the PM over the equatorial segment, and sometimes also the principal segment, was more extensively ruffled or undulating, although it remained smooth on the dorsal margin of the sperm head (Figs. 205,208). This did not occur, however, on the dorsal

hook, where the PM over the equatorial segment was not ruffled (Fig. 209).

In proximal caput sperm, electron-dense material was sometimes observed in the peri-acrosomal space, between the PM and OAM (Figs. 199, 200). This was less evident in sperm from the distal caput (Fig. 202), and was not observed in sperm from the corpus or cauda epididymis (Fig. 208).

3.2.b Acrosome

The most distinctive change appeared to be a reduction in the size of the apical segment of the acrosome. In testicular and proximal caput sperm, the apical segment projected from the dorsal margin of the sperm head as a narrow ridge or plate (Figs. 137,199). In sperm from the distal caput, the extent of the apical segment was considerably reduced, and it was shorter and broader in outline (Fig. 201). In corpus and cauda sperm, the apical segment was virtually non-existent, and consisted of a slight thickening on the dorsal margin of the sperm head (Figs. 205,208).

Associated with acrosomal remodelling, there was also a change in the appearance of the acrosomal contents, especially in the principal and apical segments. In caput sperm, there existed a less electron-dense zone in association with the OAM (Figs. 199,201,202). This heterogeneous distribution of the acrosomal matrix was less noticeable in sperm from the corpus epididymis (Fig. 206), but still persisted to some degree even in mature sperm from the distal cauda (Fig. 208). The thickness of the principal segment decreased from about 75.1 ± 1.3 nm ($n = 11$) in proximal caput sperm to 62.8 ± 1.0 nm ($n = 28$) in distal cauda sperm, but the thickness of the equatorial segment was found to be 28-32 nm in sperm from all segments of the epididymis.

The acrosomal membranes in the principal segment appeared as trilaminar structures in sperm taken from all parts of the epididymis, and distinct differences were not observed (Figs. 200,206). In the equatorial segment, however, the inner and outer acrosomal membranes often exhibited a pentalaminar structure, which was due to a band of electron-dense material in close apposition to the inner leaflet. This was only observed in caput sperm (Figs. 200,203), and was not apparent in sperm from more distal segments of the epididymis (Fig. 206).

3.2.c Ventral Hooks/Sub-acrosomal Material

In sperm taken from successive regions of the epididymis, no changes were detected in the shape, orientation or structure of the two ventral hooks. The spacing between the ridges which attach the PM to the PADL was constant at 14-15 nm. However, in transverse sections of the hooks, the ridges or projections were more easily visualized in sperm from the proximal segments of the epididymis, and this seemed to have been due to a closer association of the ridges and the PM in sperm from distal segments of the epididymis (Figs. 204,207).

Similarly, there were no changes in the structure of the SAM in the dorsal hook, nor in the dorsal ridges on the dorsal margin of the sperm head (Figs. 202,206,207).

3.2.d Extra-cellular Material

The quantity and type of extra-cellular material (ECM) present in the epididymal lumen changed in different segments of the epididymis. In the proximal caput, little or no ECM was present (Fig. 199), but clumps of flocculent material appeared in the distal caput, and were found in all other segments (Figs. 201,208,209). In the mid corpus region, dense accumulations of variable glycocalyx material (VGM) surrounded the

sperm head. It consisted of granular material, and often contained areas of increased density and small granules (Fig. 209). Long strands of VGM were present in the mid corpus, with many heads lodged in these strands, although they were less evident in the cauda epididymis. The VGM bound closely to the PM on the ventral surface of the dorsal hook, and more particularly, on the elongated rather than rounded side. Although it often resided close to, or surrounded the ventral hooks, the VGM did not appear to bind to these structures (Fig. 209). Also, VGM was only occasionally observed in close proximity to the sperm head caudal to the hooks, and usually near the peri-acrosomal PM (Fig. 206).

Small, membrane-bound tubular structures were also observed near the hooks, although they did not bind to the PM. These structures were not always present, and were only observed in the corpus and cauda epididymis, where they were usually located in the concave ventral margin of the dorsal hook, or around the ventral hooks (Fig. 209). The tubular structures and VGM were only observed in the perfused tissue, and not in the washed sperm pellets.

3.3 Development of Structural Rigidity

The stabilization of various sperm structures by disulphide bonds was investigated by treating sperm from different segments of the epididymis with SDS $\pm$ DTT or sarkosyl $\pm$ DTT. The ultrastructural effects of these treatments on cauda epididymal sperm have been presented in Section 4, and in a previous communication (Flaherty and Breed, 1983a).

3.3.a Sarkosyl (Table 2)

In sperm from all segments of the epididymis, the pseudoperforatorium and ventral hooks were unaffected by this treatment. The nucleus, however, presented a different pattern. Immediately after

TIME (Mins.)	SEGMENT	NUCLEUS	PSEUDO- PERFORATORIUM	VENTRAL HOOKS	MIDDLE PIECE	PRINCIPAL PIECE
1	Testis	2+	4+	4+	2+	2+
	P.CT	3+	4+	4+	3+	2+
	D.CT	4+	4+	4+	3+	4+
	P.CP	4+	4+	4+	3+	4+
	M.CP	4+	4+	4+	3+	4+
	D.CP	4+	4+	4+	3+	4+
	CD	4+	4+	4++	3+	4+
5	Testis	0/+	4+	4+	+	+
	P.CT	+	4+	4+	3+	+
	D.CT	2+	4+	4+	3+	2+/3+
	P.CP	3+	4+	4+	3+	3+
	M.CP	4+	4+	4+	3+	3+
	D.CP	4+	4+	4+	3+	3+
	CD	4+	4+	4+	3+	4+
30	Testis	0	4+	4+	0	0
	P.CT	+	4+	4+	2+/3+	+
	D.CT	+ / 2+	4+	4+	3+	+
	P.CP	2+	4+	4+	3+	2+
	M.CP	3+	4+	4+	3+	2+
	D.CP	4+	4+	4+	3+	3+
	CD	4+	4+	4+	3+	3+
60	Testis	0	4+	4+	0	0
	P.CT	+	4+	4+	2+/3+	+
	D.CT	+	4+	4+	2+/3+	+
	P.CP	2+	4+	4+	3+	+ / 2+
	M.CP	2+ / 3+	4+	4+	3+	2+
	D.CP	4+	4+	4+	3+	3+
	CD	4+	4+	4+	3+	3+

TABLE 2 : The effect of treatment with sarkosyl on plains mouse testicular and epididymal sperm.

Abbreviations:

0 = absent
 + = extensively swollen/solubilized
 2+ = moderately swollen/solubilized
 3+ = slightly swollen/solubilized
 4+ = totally unaffected

P.CT - proximal caput
 D.CT - distal caput
 P.CP - proximal corpus
 M.CP - mid corpus
 D.CP - distal corpus
 CD - proximal/distal cauda

the start of incubation, testicular and proximal caput sperm nuclei were found to be swollen, but sperm from other regions were unaffected. As the incubation time progressed, however, caput and corpus sperm nuclei showed a variable degree of decondensation. The degree of swelling was substantially reduced in sperm from more distal segments and required longer for the initiation of decondensation. The nuclei of cauda epididymal sperm remained intact, even after 60 minutes.

The mitochondrial sheath of the middle piece appeared to be solubilized almost immediately by this treatment, but other tail structures became more resistant during epididymal transit. In testicular sperm, all remnants of the tail were solubilized after only 30 minutes. In caput and corpus sperm, the principal piece became disorganized, and the outer dense fibres splayed in various directions. The only effect on cauda sperm tails, however, was a slight curling of the distal region.

3.3.b Sarkosyl and DTT (Table 3)

The ventral hooks and pseudoperforatorium were also resistant to sarkosyl and DTT, even after incubation for 30 minutes. The nuclei of sperm from all segments became completely or partially decondensed, although the rate and degree of swelling was most pronounced in testicular and caput sperm, and the resistance to this treatment was greatest in distal corpus and cauda sperm.

This treatment also resulted in a greater disorganization and solubilization of tail structures than sarkosyl. In 30 minutes, there was complete solubilization of testicular sperm tails, whilst in sperm from more distal segments, there was a variable degree of splaying of the dense fibres.

TIME (Mins.)	SEGMENT	NUCLEUS	PSEUDO- PERFORATORIUM	VENTRAL HOOKS	MIDDLE PIECE	PRINCIPAL PIECE
1	Testis	2+	4+	4+	2+	2+
	P.CT	3+	4+	4+	3+	3+
	D.CT	3+/4+	4+	4+	3+	4+
	P.CP	4+	4+	4+	3+	4+
	M.CP	4+	4+	4+	3+	4+
	D.CP	4+	4+	4+	3+	4+
	CD	4+	4+	4+	3+	4+
2	Testis	+	4+	4+	+	+
	P.CT	2+	4+	4+	2+	2+
	D.CT	3+	4+	4+	3+	3+
	P.CP	3+	4+	4+	3+	3+
	M.CP	3+/4+	4+	4+	3+	4+
	D.CP	4+	4+	4+	3+	4+
	CD	4+	4+	4+	3+	4+
5	Testis	0/+	4+	4+	+	+
	P.CT	+	4+	4+	2+	2+
	D.CT	2+	4+	4+	2+	2+
	P.CP	2+	4+	4+	2+	2+
	M.CP	3+	4+	4+	3+	4+
	D.CP	3+	4+	4+	3+	4+
	CD	3+	4+	4+	3+	4+
15	Testis	0/+	4+	4+	+	+
	P.CT	+	4+	4+	2+	2+
	D.CT	+ / 2+	4+	4+	2+	2+
	P.CP	+ / 2+	4+	4+	2+	2+
	M.CP	+ / 2+	4+	4+	3+	3+
	D.CP	+ / 2+	4+	4+	3+	3+
	CD	2+	4+	4+	3+	3+
30	Testis	0	4+	4+	0	0
	P.CT	0/+	4+	4+	+ / 2+	+ / 2+
	D.CT	+	4+	4+	+ / 2+	+ / 2+
	P.CP	+	4+	4+	+ / 2+	2+
	M.CP	+	4+	4+	2+	2+
	D.CP	+ / 2+	4+	4+	2+	2+
	CD	+ / 2+	4+	4+	2+	2+

TABLE 3 : The effect of sarkosyl and DTT on plains mouse testicular and epididymal sperm.

TIME (Mins.)	SEGMENT	NUCLEUS	PSEUDO- PERFORATORIUM	VENTRAL HOOKS	MIDDLE PIECE	PRINCIPAL PIECE
1	Testis	2+	0	0	+	+
	P.CT	3+	+	+	2+	+
	D.CT	4+	+ / 2+	+ / 2+	2+	2+
	P.CP	4+	2+	2+	2+	2+
	M.CP	4+	2+	2+	2+	2+
	D.CP	4+	2+	2+	2+	2+
	CD	4+	2+	2+	2+	2+
15	Testis	+	0	0	0 / +	0 / +
	P.CT	3+	+	+	2+	+
	D.CT	4+	+	+	2+	+
	P.CP	4+	+	+	2+	+
	M.CP	4+	+	+	2+	+
	D.CP	4+	+ / 2+	+ / 2+	2+	+
	CD	4+	2+	2+	2+	2+
60	Testis	+	0	0	0 / +	0 / +
	P.CT	2+	0 / +	0 / +	+	0 / +
	D.CT	3+	0 / +	0 / +	+	0 / +
	P.CP	3+ / 4+	0 / +	0 / +	2+	+
	M.CP	4+	+	+	2+	+
	D.CP	4+	+ / 2+	+ / 2+	2+	+
	CD	4+	+ / 2+	+ / 2+	2+	+
120	Testis	0 / +	0	0	0	0
	P.CT	2+	0	0	+	0 / +
	D.CT	2+ / 3+	0 / +	0 / +	+	0 / +
	P.CP	3+ / 4+	0 / +	0 / +	2+	+
	M.CP	4+	0 / +	0 / +	2+	+
	D.CP	4+	+ / 2+	+ / 2+	2+	+
	CD	4+	+ / 2+	+ / 2+	2+	+

TABLE 4 : The effect of SDS on plains mouse testicular and epididymal sperm.

3.3.c SDS (Table 4)

The nuclei of testicular sperm were almost completely decondensed after incubation in SDS for 120 minutes, whilst caput sperm showed a moderate degree of nuclear swelling. The nuclei of corpus and cauda epididymal sperm were unaffected by SDS, although slight nuclear swelling was sometimes observed in proximal corpus sperm.

The ventral hooks and pseudoperforatorium of testicular sperm were completely extracted in under 1 minute, and only a few remnants remained in caput sperm, whilst these structures were elongated and swollen in corpus and cauda sperm. During the incubation period of 120 minutes, the ventral hooks of proximal caput sperm were solubilized, and only basal remnants remained in distal caput to mid corpus sperm. In distal corpus and cauda sperm, however, these structures were incompletely solubilized by SDS, as outlined in Section 4.

This treatment caused considerable damage to the tails of sperm from all segments of the epididymis within one minute of incubation. After 120 minutes, there were no remnants of testicular sperm tails, and only a few remnants of the tails from caput sperm. In corpus and cauda sperm, there was disorganization of the middle piece and loss of the mitochondrial sheath, and intense coiling of the principal piece.

3.3.d SDS and DTT (Table 5)

The nuclei of testicular and caput sperm began to decondense in 2 minutes, but 15-30 minutes was required for the initiation of nuclear swelling in corpus and cauda sperm. After 60 minutes, testicular sperm nuclei were almost fully decondensed, caput sperm nuclei were substantially swollen, and the least degree of decondensation occurred in corpus and cauda sperm nuclei. The degree of decondensation was always most pronounced in the posterior region of the nucleus.

TIME (Mins.)	SEGMENT	NUCLEUS	PSEUDO- PERFORATORIUM	VENTRAL HOOKS	MIDDLE PIECE	PRINCIPAL PIECE
1	Testis	2+	0/+	0/+	+	+
	P.CT	3+	0/+	0/+	2+	+
	D.CT	4+	+	+	2+	+
	P.CP	4+	2+	2+	2+	+
	M.CP	4+	2+	2+	2+	2+
	D.CP	4+	2+	2+	2+	2+
	CD	4+	2+/3+	2+/3+	2+	2+
5	Testis	+	0	0	0/+	0/+
	P.CT	3+	0/+	0/+	+	+
	D.CT	3+	0/+	0/+	2+	+
	P.CP	4+	0/+	0/+	2+	+
	M.CP	4+	+ / 2+	+ / 2+	2+	+
	D.CP	4+	2+	2+	2+	+
	CD	4+	2+	2+	2+	+ / 2+
15	Testis	0/+	0	0	0/+	0/+
	P.CT	2+	0	0	0/+	0/+
	D.CT	2+	0	0	+ / 2+	+
	P.CP	3+	0	0	+ / 2+	+
	M.CP	3+ / 4+	+	+	2+	+
	D.CP	4+	+ / 2+	+ / 2+	2+	+
	CD	4+	+ / 2+	+ / 2+	2+	+
30	Testis	0/+	0	0	0/+	0/+
	P.CT	+	0	0	0/+	0/+
	D.CT	2+	0	0	0/+	0/+
	P.CP	2+	0	0	+	0/+
	M.CP	2+	0	0	+	0/+
	D.CP	2+	0/+	0/+	+	0/+
	CD	2+	+	+	+	+
60	Testis	0/+	0	0	0	0
	P.CT	+	0	0	0	0
	D.CT	+	0	0	0	0
	P.CP	+ / 2+	0	0	0	0
	M.CP	+ / 2+	0	0	0/+	0/+
	D.CP	+ / 2+	0	0	0/+	0/+
	CD	+ / 2+	0	0	+	0/+

TABLE 5 : The effect of SDS and DTT on plains mouse testicular and epididymal sperm.

The pseudoperforatorium and ventral hooks of testicular, caput and proximal corpus sperm were solubilized by SDS and DTT in under 5 minutes, whereas 15-30 minutes was required to solubilize these structures in mid and distal corpus sperm, and 30-60 minutes in cauda sperm.

Within one minute of incubation, the mitochondrial sheath disappeared, the middle piece became disorganized, and the principal piece became coiled. During the 60 minute incubation period, these effects became more pronounced, especially in testicular and caput sperm. At the end of incubation, tail remnants were only observed in cauda sperm, and sometimes from mid and distal corpus sperm, although not from other regions.

3.3.e DTT/Borate

Incubation of sperm in borate buffer for up to 120 minutes resulted in no apparant changes at the light microscopical level, although sperm motility was only maintained in spermatids. Treatment with DTT did not affect the nucleus or ventral hooks, but caused a variable degree of uncoiling of the mitochondrial sheath in the middle piece of the tail.

3.4 Acquisition of Motility

The percentage of motile sperm, as well as the pattern of motility, was found to be different in the seven segments of the epididymis.

Testis -

Spermatids and spermatozoa exhibited a variable degree of flagellar activity, although forward progression was never observed. Some spermatids showed weak flagellar beats of moderate amplitude and low frequency, but the usual pattern consisted of weak vibratile motions of the principal piece. This twitching was stronger and more frequent in

spermatids than spermatozoa, and many testicular spermatozoa were completely immotile.

Proximal Caput -

In this region, some spermatozoa were incapable of motility, but many exhibited weak, vibratile motions as described above. A few sperm had a bent or kinked middle piece, and possessed a non-progressive form of forward motility, comprised of weak flagellar beats of the principal piece, and slow, stabbing head movements.

Distal Caput -

The majority of sperm from this segment possessed a bent middle piece, and were motile. The middle piece was rigid, and the cytoplasmic droplet was usually located at the point of bending in the middle or distal part of the middle piece (Figs. 194,195). The flagellar beats were of moderate amplitude, but restricted to the principal piece. Sperm traversed an erratic path, with frequent changes of direction, and forward progression was slow or non-existent. Circular motion was not observed.

Proximal Corpus -

In most sperm, the middle piece was straight, and the entire tail flexed during flagellar beating, which was of low frequency and moderate amplitude. The sperm head oscillated considerably, and spermatozoa often followed rather erratic trajectories, with only weak forward progression.

Mid Corpus -

No kinked sperm tails were observed in this region, and most spermatozoa exhibited slow forward progression. The pattern of motility was similar to the proximal corpus, although the beat frequency appeared to be greater. Some sperm showed rotation about their long axis, and the trajectories were straighter and less erratic.

Distal Corpus -

In this segment, sperm showed strong progressive motility upon dilution, with an increased beat frequency, and a decrease in the amplitude of flagellar bending. The sperm head did not execute wide sweeping movements, rather short, stabbing movements in the direction of the hooks.

Proximal and Distal Cauda -

Sperm from these two segments had an identical pattern of motility. The flagellar beat was of high frequency but low amplitude, and the middle piece was rather inflexible. The sperm head executed short, jerky movements of high frequency. There was strong forward progression, and sperm rotated frequently about their long axis, although the direction of rotation could not be ascertained.

4. DISCUSSION

In the present study, several aspects of epididymal maturation of spermatozoa in the plains mouse have been investigated, but an exhaustive study of this process has not been attempted. The epididymis has been arbitrarily divided into seven segments, based on gross anatomical features and the size of the tubules. For the purpose of this study, this was found to be sufficient to outline the broad sequence of changes which constitute epididymal maturation.

4.1 Morphological Changes Associated with Maturation

4.1.a Size of the Sperm Head

The maximum length of the sperm head was found to decrease significantly during transit from the proximal caput to distal cauda of

the epididymis. Bedford (1963a) described a similar reduction in the size of the rabbit sperm head, whilst the volume of rat sperm decreases by 10% during epididymal maturation (Brotherton, 1976). It is unlikely that the reduction in size of the apical segment of the acrosome is responsible for the decrease in the length of the sperm head, since the apical segment does not occur at either of the points between which measurements were recorded. Instead, this size reduction may be due to dehydration (Lavon et al., 1970), although such a suggestion should be viewed cautiously, as the volume of the sperm head was not determined.

4.1.b Migration of the Cytoplasmic Droplet

The cytoplasmic droplet is usually located in the neck region of immature spermatozoa when they enter the epididymis, but moves distally along the middle piece during passage of sperm through the epididymis (Bedford, 1975). In the plains mouse, migration of the cytoplasmic droplet also occurs during epididymal maturation, and seems to take place in the caput region, and is complete by the time sperm reach the mid corpus. Droplet migration has previously been shown to occur in the caput epididymis in the rat, ram, bull, stallion and elephant (Jones et al., 1974; Bedford, 1975; Suzuki and Nagano, 1980a; Amann et al., 1982).

About 70% of plains mouse sperm in the cauda epididymis retain the cytoplasmic droplets at the junction of the middle and principal pieces, although it is lost completely in the remainder. The droplet is retained in many species (Hancock, 1957; Suzuki and Nagano, 1980a; Olson et al., 1983b; Moore et al., 1984), although in others, it is lost prior to ejaculation (Bedford, 1963a; Bedford et al., 1973). In this study, loss of the cytoplasmic droplet may be an artifact of preparation due to membrane damage, and may increase in distal segments of the epididymis

due to changes in the properties of the plasma membrane which make it more susceptible to damage. Alternatively loss of the droplet may be an indication of premature cellular degeneration.

4.1.c Features of the Plasma Membrane

The sperm plasma membrane represents one of the sites of major change during epididymal maturation. Some of the changes affect the lipid content of the membrane, distribution of intra-membranous particles, integral and peripheral polypeptides, and carbohydrates and surface charges (see reviews by Holt, 1982, 1984; Bellve and O'Brien, 1983). In the present study, however, only the ultrastructural features of the PM have been examined. The structure of the membrane does not appear to change during epididymal transit, and leaflets of differing electron density were not observed, as has been reported by Bedford and Nicander (1971), although different fixation protocols may account for this difference.

In caput sperm, the plasma membrane is closely applied to the acrosome, and relatively smooth in contour. However, it becomes ruffled over the equatorial segment in corpus and cauda epididymal sperm. Electron-dense material is often observed in the peri-acrosomal space of caput sperm, but is absent in sperm from other segments, as has been reported for rat sperm (Suzuki and Nagano, 1980a). In rabbit, chinchilla and monkey sperm, the PM often becomes widely separated from the OAM and susceptible to ballooning, but only in the distal segments of the epididymis (Bedford, 1965; Fawcett and Phillips, 1969b; Bedford and Nicander, 1971). In the rat, this is associated with the disappearance of electron-dense material from the peri-acrosomal space (Suzuki and Nagano, 1980a). However, ballooning of the sperm PM was not observed in this study, and the PM always remained in close proximity to the OAM, in

both perfused tissue and sperm pellets. This could be due to the high osmolarity of the fixatives employed, which would prevent ballooning of the membrane. It is plausible that shrinkage of the sperm head due to dehydration would create an excess of membrane in sperm from the distal segments of the epididymis. In low osmolarity fixatives, the PM would balloon out away from the OAM, but would have to become pleated or ruffled in a concertina effect in high osmolarity fixatives, as observed in the present study.

4.1.d Acrosomal Remodelling

A distinct remodelling and condensation of the contents of the acrosome is a feature of epididymal maturation in many, but not all, species (Bedford, 1975). In species in which the sperm acrosome is large, especially the apical segment, such as the guinea pig, chinchilla and some primates, there is often extensive remodelling of the apical segment (Fawcett and Hollenberg, 1963; Fawcett and Phillips, 1969b; Bedford, 1974a). In most species, however, there is a slight reduction in the extent of the apical segment (Bedford, 1965; Bedford and Nicander, 1971; Jones, 1971; Suzuki and Nagano, 1980a), although there is no apparent change in the acrosome of some primates, including man (Bedford et al., 1973; Bedford, 1974a; Moore et al., 1984).

Spermatozoa of the plains mouse seem to fit into the second category, for there is a reduction in the extent of the apical segment, which occurs in the caput region in a similar manner to the rabbit (Bedford and Nicander, 1971). There is also a decrease in the thickness of the principal segment during epididymal transit, which could be due to shrinkage, or to condensation and reorganization of the acrosomal matrix. It was also noted that the contents of the principal segment become more homogeneous during transit through the corpus epididymis,

which probably accounts for loss of the pentalaminar appearance of the acrosomal membranes in the equatorial segment.

The mechanisms involved in this process are not well understood, but it has been suggested that internal factors, such as the specific organization of the acrosomal matrix, as well as external factors, may be involved (Fawcett and Hollenberg, 1963; Fawcett et al., 1971). In this study, it was noted that the apical segment of proximal caput sperm became reduced in size during the preparation of sperm pellets, which involved washing by centrifugation. This finding, combined with the narrowness of the tubules in the caput region, suggests that physical force applied to the apical segment of the acrosome during transit through this region could conceivably be involved in acrosomal remodelling. An organized array of the acrosomal matrix would predispose this segment to its final shape.

4.1.e Sub-acrosomal Material/Ventral Hooks

Several authors have reported a decrease in the size of the so-called "perforatorium", or an increase in the amount of sub-acrosomal material during epididymal maturation (Bedford and Nicander, 1971; Jones, 1971). In this study, however, no changes could be detected in the sub-acrosomal material, in either the dorsal ridges, pseudoperforatorium, or ventral hooks. The only change in the structure of the ventral hooks concerns the ridges which connect the PADL and PM. In transverse sections of the ventral hooks, these ridges were more difficult to visualize in sperm from distal segments of the epididymis. The exact nature of this change is unclear, although it is tempting to speculate that it could be due to shrinkage of the hooks as a result of dehydration (Lavon et al., 1970). However, the spacing of the ridges was found to remain constant at about 14 nm, and all the specimens used for

measurements were prepared in an identical manner. Hence, it seems unlikely that a shrinkage effect is involved, as this would be expected to decrease the spacing of the ridges. Instead, the difficulty of resolving the ridges could be the result of a closer association of the ridges and PM, which could be important for maintaining cellular integrity during zona penetration (see Section 7).

4.1.f Extra-cellular Material

In the corpus and cauda epididymis, granular material appears in the epididymal lumen, and attaches to the PM on the ventral surface of the dorsal hook. Similar material has been examined in detail in the rat epididymis, and has been called "variable glycocalyx material" or VGM (Suzuki and Nagano, 1980a). VGM has also been found to occur in association with boar epididymal sperm (Jones, 1971), and in the concave region of some hooked primate sperm (Bedford, 1974a), although it does not occur in the rabbit, guinea pig or other primate species (Fawcett and Hollenberg, 1963; Bedford, 1965; Bedford and Nicander, 1971; Bedford, 1974a).

The area of attachment of VGM to the sperm head is variable in both the rat and plains mouse, though it is usually associated with the dorsal hook. It is lost from rat sperm in the proximal cauda, but is retained by plains mouse spermatozoa in situ. In the rat, it is not removed by washing the sperm, whereas it was not present in plains mouse sperm pellets. In the rat, it is stainable with ruthenium red, and has therefore been considered as part of the glycocalyx, and its appearance coincides with changes in the intra-membranous particle distribution in the PM (Suzuki and Nagano, 1980a). The disappearance of VGM has been shown to correlate with the appearance of specific polypeptides on the peri-acrosomal plasma membrane (Rifkin and Olson, 1985). The exact

function of this material, however, remains obscure.

Another luminal structure associated with sperm consists of tubular, membrane-bound structures which are found in the vicinity of the three hooks during the later stages of maturation. These appear to correspond to the tubular structures on mouse and Chinese hamster spermatozoa (Phillips, 1975). In the Chinese hamster, these structures are much more abundant than in the plains mouse, and although their origin and function is unknown, it has been suggested that they may perform a similar function to the VGM in the rat epididymis (Yanagimachi et al., 1985).

4.2 Stabilization of Sperm Structures by Disulphide Bonds

The treatment of plains mouse sperm with sarkosyl or SDS, either with or with dithiothreitol, a disulphide reducing agent, produced different patterns of solubilization in sperm from different segments of the epididymis.

Sarkosyl and SDS alone caused nuclear swelling in testicular, caput and proximal corpus sperm, although sperm from the distal corpus and cauda regions were unaffected. However, nuclear decondensation was induced in sperm from all seven regions by these detergents in combination with dithiothreitol (DTT), suggesting that disulphide bonds are important for nuclear stability. Nevertheless, the degree of decondensation was less in sperm from the distal regions, suggesting a higher degree of nuclear stabilization by disulphide bonds. In sperm from all regions, the anterior portion of the nucleus, especially in the dorsal hook, was found to be less susceptible to solubilization.

The ventral hooks and pseudoperforatorium were found to be resistant to sarkosyl $\pm$ DTT at all levels of the epididymis, as reported previously for cauda epididymal sperm (Flaherty and Breed, 1983a).

Treatment of testicular sperm with SDS resulted in almost instantaneous solubilization of these structures, but it required about 30 minutes in caput and proximal corpus sperm, and 60-120 minutes in mid corpus sperm. The ventral hooks and pseudoperforatorium were incompletely solubilized in sperm from the distal corpus and cauda segments. However, SDS and DTT removed these structures from sperm from each segment, and the time required was greatest in cauda sperm.

Hence, it would appear that the nucleus, pseudoperforatorium and ventral hooks gradually become stabilized by disulphide bonds during transit through the epididymis. This process is complete in distal corpus or proximal cauda sperm. It has previously been shown to be a characteristic feature of sperm maturation in a wide variety of species, and is thought to be an adaptation to penetration of the extra-cellular coats around the oocyte (Calvin and Bedford, 1971; Bedford et al., 1973; Bedford, 1974a; Bedford and Calvin, 1974a). This is postulated to be a time-dependent process, in which free sulphydryl groups (-SH) are oxidized to produce disulphide (S-S) bonds (Calvin and Bedford, 1971). In the rat sperm nucleus, this cross-linking process is associated with a 20 fold decrease in the number of free -SH groups (Calvin et al., 1973), and it is believed that the disulphide bonds form between cysteine residues of adjacent protamines (Calvin, 1975).

In addition to sperm head structures, however, the resistance of sperm tails to solubilization by SDS or sarkosyl was also found to increase in sperm taken from distal segments of the epididymis. The addition of DTT resulted in a more rapid and complete solubilization, indicating that accessory tail structures also become stabilized by disulphide bonds during epididymal maturation. It has previously been shown in other species that the OMM, dense fibres, connecting piece and fibrous sheath become stabilized by disulphide bonds during epididymal

maturation (Calvin and Bedford, 1971; Bedford and Calvin, 1974b; Olson et al., 1976b). The current results, and those presented in Section 4, suggest that a similar situation exists during epididymal maturation of spermatozoa in the plains mouse.

4.3 Acquisition of Motility

A qualitative description of the acquisition of motility by epididymal plains mouse sperm has been undertaken in this study. However, due to the unavailability of micro-cinematographic facilities, it has not been possible to examine sperm velocities or the kinetics of the flagellar beat.

It was found that testicular sperm are either immotile or exhibit weak vibratile movements of the tail, but that the percentage of sperm which are capable of motility upon dilution increases from the proximal to the distal regions of the epididymis. This has been shown to be a characteristic feature of epididymal maturation in mammals (Gaddum, 1968; Hinton et al., 1979; Moore et al., 1984).

However, there is also a change in the pattern of motility (Bedford, 1975). It has been discovered in several species that a non-progressive type of motility develops in the caput region, characterized by sperm swimming in tight circles, with a rigid, curved middle piece. This pattern changes to a strong, progressive form during transit through the corpus and proximal cauda regions. This mature pattern features flagellar beats of high frequency but moderate amplitude which travel the entire length of the tail, and frequent rotation of the sperm (Blandau and Rumery, 1964; Gaddum, 1968; Fray et al., 1972; Katz et al., 1978; Hinton et al., 1979; Woolley, 1979; Mohri et al., 1983).

In the plains mouse, a similar sequence of changes occurs, but the pattern of motility exhibited by sperm in the caput region is different.

Instead of having a rigid, curved middle piece, distal caput sperm possess a rigid but bent or kinked middle piece, with the cytoplasmic droplet located at the point of bending. The principal piece exhibits weak flagellar beats, which results in slow and highly erratic trajectories, with oscillations of the sperm head in all directions. No circling patterns have been observed. This unusual feature was apparent in most distal caput sperm in four different preparations, and did not occur in sperm from the testis or distal segments of the epididymis, suggesting it was not merely an artifact of preparation. During transit through the corpus segment, it becomes replaced by the mature pattern of motility, with strong forward progression and frequent rotation. The middle piece is flexible, the frequency of beating increases, the amplitude of bending decreases, and the sperm head executes short, stabbing movements.

This changing pattern of motility may be brought about by the formation of disulphide bonds in the dense fibres and fibrous sheath of the tail (Bedford and Calvin, 1974b; Olson et al., 1976b; Chang et al., 1983), phosphorylation of axonemal microtubules (Tongkao and Chuvalatnatol, 1979), binding of forward motility protein (Hoskins et al., 1978), and changes in the responsiveness of sperm to levels of cAMP and carnitine (Hoskins et al., 1978; Hinton et al., 1981).

4.4 Concluding Remarks

It thus seems that plains mouse sperm undergo a series of changes which is characteristic of epididymal maturation in eutherian mammals. These include remodelling of the apical segment of the acrosome and migration of the cytoplasmic droplet, which occur in the proximal segments of the epididymis, as well as stabilization of sperm structures by disulphide bonds and the acquisition of motility, which are not

completed until the distal corpus or proximal cauda. However, the acquisition of fertilizing ability has not been studied in this section, for reasons which will become apparent in Section 6. Nevertheless, it seems reasonable to assume that sperm should become fertile in the distal corpus region, because the major structural changes have been completed, and the mature pattern of motility has been acquired.

The ventral hooks and pseudoperforatorium gradually become stabilized by disulphide bonds during epididymal maturation, and the PM becomes more closely adherant to the ventral hooks. These features, in conjunction with the high degree of disulphide cross-linking of the anterior portion of the nucleus, may represent adaptations to penetration of the tough extracellular coats which surround the oocytes (Bedford and Calvin, 1974a; Bedford, 1983a, 1983b). This possibility will be examined in greater detail in Section 7).

SECTION 6 : STRUCTURE OF THE OOCYTE-CUMULUS COMPLEX, AND STUDIES ON IN VITRO FERTILIZATION

1. INTRODUCTION

The structural features of the three hooks on the sperm head of the plains mouse have been extensively studied in previous sections of this study. To date, however, nothing is known about the function(s) of these unusual structures, although it has been suggested that, due to their shape and inherent rigidity, they may aid in penetration of the spermatozoon through the zona pellucida (Flaherty *et al.*, 1983; Breed, 1984). Hence, it has been the aim of this section to study the possible functions of the hooks during fertilization, and to examine the structural features of the oocyte-cumulus complex, so as to elucidate any unusual characteristics which could have necessitated the development of three hooks to aid in penetration of the egg investments. However, investigations of sperm-egg interactions in this species have been hampered by difficulties encountered in inducing superovulation, so attempts have been made to investigate the possible role of the ventral hooks in sperm-egg fusion using *in vitro* fertilization of zona-free rat and laboratory mouse oocytes by plains mouse sperm.

2. MATERIALS AND METHODS

2.1 Animals

Oocytes were obtained from mature female plains mice, 4-18 months old (body weight^{*} = 45.2 ± 0.9 g, n=35), immature female plains mice, 4-6 weeks old (body weight = 27.1 ± 0.5 g, n = 84), female BALBc laboratory

* Values are expressed as mean $\pm$ S.E.M.

mice, aged 4-6 weeks (body weight = $18.5 \pm 0.2\text{g}$, $n = 61$), and immature female white Portan rats, about 22-25 days old (body weight = $52.6 \pm 1.4\text{g}$, $n = 40$).

For in vitro fertilization studies, adult males were used which had previously been separated for at least 3 weeks. These included 5-12 month old plains mice (body weight = $58.2 \pm 2.2\text{g}$, $n = 13$), 4-11 month old BALBc laboratory mice (body weight = $41.9 \pm 1.6\text{g}$, $n = 8$), and 10-12 month old white Portan rats (body weight = $533.5 \pm 22.5\text{g}$, $n = 4$).

2.2 Induction of Ovulation

In all cases, ovulation was induced by administration of pregnant mare's serum gonadotrophin (PMSG - Folligon, Intervet Aust.), followed by human chorionic gonadotrophin (hCG - Sigma Chem. Co.). Both hormones were prepared in physiological saline, and either used immediately, or stored frozen at -20°C for not more than 2-4 weeks. The concentration was adjusted so that the required dose was given in 0.1 ml.

Adult and immature female plains mice were injected intra-peritoneally (IP) with 10-50 IU PMSG, followed 48-72 hours later by 10-75 IU hCG. As controls, female BALBc laboratory mice were given 1.5-7.5 IU PMSG, followed 48 hours later by 1.5 - 7.5 IU hCG. (Gates, 1971; Roldan et al, 1985).

For in vitro fertilization, female BALBc laboratory mice were induced to ovulate with 5 IU PMSG followed 48 hours later by 5 IU hCG (Gates, 1971). Immature female rats were given a sub-cutaneous injection of 10 IU PMSG, followed 48-56 hours later by IP administration of 10 IU hCG (Toyoda and Chang, 1974).

2.3 Light and Electron Microscopy

Plains mice were killed 15-16 hours post-hCG by cervical

dislocation or an overdose of ether, the oviducts carefully removed and rinsed free of blood. The swollen ampullae were torn with fine forceps to release the cumulus clots into warm (35°C) HT6 culture medium (see Appendix 5) or Hank's balanced salt solution (HBSS, pH 7.4). They were examined with an Olympus IMT inverted phase contrast microscope. The cumulus cells were removed by incubation for 5-10 minutes in 1 mg/ml hyaluronidase (Type III - Sigma Chem. Co.) in HT6 or HBSS. The naked oocytes were rinsed twice in culture medium, and mounted on slides inside a ring of vaseline. Some oocytes were fixed overnight in 10% buffered neutral formalin, stained for 10-40 minutes in 0.25% aceto orcein (lacmoid) in 45% acetic acid, and cleared in acetic acid prior to mounting. All preparations were examined with an Olympus Vanox microscope fitted with phase contrast optics, and photographed on Ilford Pan F.

The surface features of the zona pellucida were examined by scanning electron microscopy (SEM). Cumulus-free oocytes were fixed for 12 hours at 4°C in 2.5% glutaraldehyde/2% formaldehyde in 0.1 M cacodylate buffer (pH 7.4), then allowed to settle onto 18 mm round coverslips, which had previously been soaked overnight in 0.1-0.5% poly-L-lysine. The coverslips were then rinsed thoroughly in buffer, and dehydrated through a graded series of acetones. After critical point drying from liquid carbon dioxide, they were mounted on stubs, and coated with Au/Pd and carbon, then examined at 20 kV in a Siemens-Etec autoscan.

Intact oocyte-cumulus complexes (OCC) were prepared for transmission electron microscopy (TEM) using routine preparative procedures, and after fixation in the presence of ruthenium red (RR) to stabilize the cumulus matrix (Luft, 1971; Talbot and DiCarlantonio, 1984a; 1984b). The OCC were fixed for 1-2 hours in 2.5% glutaraldehyde

in 0.1M cacodylate (pH 7.4) $\pm$ 0.5% ruthenium red (Sigma Chem. Co.), rinsed in cacodylate buffer, then post-fixed for 2 hours in the dark in 1% OsO₄ in cacodylate buffer $\pm$ 0.5% ruthenium red. After rinsing in buffer, the RR-treated clots were dehydrated and embedded in Spurr's epoxy resin, whilst the remainder were en bloc stained in uranyl acetate prior to dehydration and embedding (see Section 2). The distribution of cumulus cells was determined from thick (0.5 μ m) sections which were stained with toluidine blue. Ultrathin sections were examined either unstained, or after double staining with uranyl acetate and lead citrate, in a Philips EM300 at 60-80 kV.

Measurements were made from electron micrographs as previously outlined (Sections 2 and 3), and a micrometer slide was used to provide calibration for light micrographs. Values are expressed as mean $\pm$ S.E.M. (n = number of observations).

2.4 In Vitro Fertilization (IVF)

The procedures used in this study were modifications of those employed by Stanger and Quinn (1982) and Toyoda and Chang (1974).

2.4.a Media Preparation

Two culture media were utilized in this section. The first was a modified BWB medium (TCM), as employed by Toyoda and Chang (1974) for rat IVF, containing 4 mg/ml Fatty acid-free Fraction V BSA (Sigma Chem. Co.). The other was a high bicarbonate Tyrode's medium (HBT6), containing 58 mM NaCl, 52 mM sodium bicarbonate, and 15 mg/ml Fraction V BSA (Sigma Chem. Co.) (Quinn et al., 1982; Quinn, personal communication). The composition of the media is given in Appendix 5. The stocks were prepared in sterile plastic tissue culture flasks and

tubes (Falcon, Lux), using AR grade chemicals (BDH, Sigma), and 4x glass distilled deionized H_2O . Both media were prepared from the stock solutions, the osmolarity adjusted to 285 (HBT6) or 308 (TCM) mOsm. by adding H_2O , and the BSA added. The solutions were then millipore filtered ($0.22 \mu m$) and stored at $4^\circ C$ for not more than 2 weeks.

Culturing was carried out using 0.5ml drops of medium in 35 mm tissue culture dishes (Falcon, Lux), overlaid with paraffin oil (BDH-liquid paraffin, Pr. 29437). The paraffin oil was autoclaved (10 minutes/2 ATM.), cooled, then equilibrated with 5% CO_2 in air before use. Dishes with culture medium drops were prepared before use, and equilibrated with an atmosphere of 5% CO_2 in air for 8-12 hours at $37.5^\circ C$. After pre-equilibration, the pH of the media was found to be 7.5-7.6 (HBT6) or 7.4 (TCM). All pasteur pipettes, instruments and pipette tips were autoclaved or sterilized by UV irradiation.

2.4.b Preparation of Sperm Suspensions

One or two males were killed by cervical dislocation, and the caudae epididymides removed, cleared of fat and major blood vessels, rinsed in culture medium (CM), and placed in a pre-equilibrated CM drop under oil at $35^\circ C$. Sperm were extruded into the CM by pricking several distal tubules, and, after allowing 10 minutes for sperm dispersal, the motility was checked and a sperm count performed (see Appendix 3). Only suspensions with >70% initial motility were used. The required sperm concentrations were obtained by serial dilution, and sperm suspensions were pre-incubated at $37.5^\circ C$ for the following times:

Plains mouse - HBT6/TCM ; $0.5-1.0 \times 10^6$ sp/ml ; 1, 2 or 4 hours

Laboratory mouse - HBT6 ; 1.0×10^6 sp/ml ; 90 minutes

Rat - TCM ; 0.5×10^6 sp/ml ; 5 hours

2.4.c Collection of Oocytes

Females were killed by cervical dislocation, 14-15 hours post-hCG, and one or two at a time. The oviducts were quickly excised, and placed in warm hepes-buffered culture medium (HT6/HTCM - see Appendix 5). The cumulus clots were carefully released from the ampullae, and transferred to 1 mg/ml hyaluronidase in HT6 or HTCM for 10 minutes to disperse the cumulus cells. The cumulus-free oocytes were washed in 3 changes of CM. When necessary, the zona pellucida was weakened by incubation for 2-5 minutes in 1 mg/ml pronase (Type XIV - Sigma Chem. Co.) in HTCM (rat), or 1 mg/ml α -chymotrypsin (Type II - Sigma Chem. Co.) in HT6 (plains mouse and laboratory mouse), and removed by pipetting repeatedly through a narrow bore pipette. The zone-free oocytes were washed 4 times in CM before insemination.

2.4.d Insemination and Examination

After pre-incubation of sperm, 8-30 oocytes, either zona-intact or zona-free, were transferred to the CM drops containing sperm, and incubated 4-8 hours at 37.5°C in an atmosphere of 5% CO₂ in air. Insemination occurred 15-16 hours post-hCG.

In experiment 1, plains mouse sperm were pre-incubated for 2 hours at 0.5×10^6 sp/ml, then zona-intact plains mouse oocytes, which were obtained from immature females given PMSG and hCG 48 hours apart, were added. In experiment 2, plains mouse sperm were pre-incubated 2 hours in either HBT6 or TCM at 1.0×10^6 sp/ml, then diluted to 0.5×10^6 sp/ml for incubation with zona-intact oocytes, or 0.1×10^6 sp/ml for zona-free oocytes. These oocytes were obtained from immature female plains mice primed with PMSG followed 72 hours later by hCG.

For the insemination of zona-free rat and laboratory mouse oocytes by plains mouse sperm, the sperm were pre-incubated for 1, 2 or 4 hours

at 1.0×10^6 sp/ml in HBT6 or TCM, then diluted to $0.01-1.0 \times 10^6$ sp/ml before addition of the zona-free oocytes. In controls, laboratory mouse sperm were pre-incubated at 1.0×10^6 sp/ml for 90 minutes, then diluted to $0.01-1.0 \times 10^6$ sp/ml, to which were added either zona-intact or zona-free mouse oocytes. Rat sperm were pre-incubated in TCM for 5 hours, then the concentration adjusted to $0.05-0.5 \times 10^6$ sp/ml at the time of insemination. These experiments were all repeated 3 times, and the results combined.

After incubation, the oocytes were removed, washed in CM, then fixed and stained with aceto orcein as outlined in 2.3. They were examined with phase contrast optics, and scored as being fertilized only if they contained either a decondensing sperm head, or two pronuclei and a sperm tail within the vitellus.

2.5 Assessment of Plains Mouse Sperm Motility and Acrosome Reactions

Plains mouse sperm suspensions were prepared in HBT6 or TCM, and incubated at 1.0×10^6 sp/ml for up to 12 hours at 37.5°C . After 0,1,2,4,6,8, and 12 hours, aliquots were removed and prepared for TEM as described in Section 2, and examined for the occurrence of acrosome reactions. Concurrently, small aliquots were removed, and a subjective assessment of the pattern of motility was performed in a similar manner to that described in Section 5. The number of motile sperm was counted in 5 fields of view at a magnification of 200x for each sample. These experiments were repeated three times, and the results combined.

3. RESULTS

3.1 Superovulation of Adult and Immature Female Plains Mice

The results of superovulation trials are outlined in Tables 6 and 7.

DOSE	TIME INTERVAL	NUMBER OF TRIALS	NUMBER OF ANIMALS	OVULATION RATE %	NUMBER OF EGGS MEAN $\pm$ S.E.M.(n)
10 IU PMSG/hCG	48	8	50	22.0 (11/50)	18.6 $\pm$ 2.4 (11)
10 IU PMSG/hCG	72	4	16	75.0 (12/16)	28.3 $\pm$ 3.6 (12)
20IU PMSG/hCG	72	2	11	91.0 (10/11)	52.1 $\pm$ 10.1(10)

TABLE 6 : Superovulation of immature (4-6 week old) female plains mice using PMSG and hCG.

DOSE	TIME INTERVAL	NUMBER OF TRIALS	NUMBER OF ANIMALS	OVULATION RATE %	NUMBER OF EGGS MEAN $\pm$ S.E.M.(n)
10 IU PMSG/hCG	48	2	12	33.3 (4/12)	12.8 $\pm$ 2.9 (4)
20 IU PMSG/hCG	48	5	31	22.5 (7/31)	6.6 $\pm$ 1.9 (7)
40 IU PMSG/hCG	48	1	7	14.3 (1/7)	3.0 $\pm$ 0.0 (1)
50 IU PMSG 75 IU hCG	48	1	6	0 (0/6)	0
10 IU PMSG/hCG	72	1	5	80.0 (4/5)	9.3 $\pm$ 2.25 (4)
20 IU PMSG/hCG	72	4	21	42.8 (9/21)	18.6 $\pm$ 4.5 (9)
50 IU PMSG 75 IU hCG	72	2	9	22.2 (2/9)	6.5 $\pm$ 1.5 (2)

TABLE 7 : Superovulation of mature (4-18 month old) female plains mice using PMSG and hCG.

In initial trials, PMSG and hCG were administered 48 hours apart, but in both mature and immature female plains mice, this resulted in low ovulation rates of up to 33%. The induction of ovulation was unpredictable using this priming protocol, as readily shown by the results of 8 trials using immature females, in which the number of animals that ovulated was 3/4, 1/8, 0/6, 0/5, 0/7, 5/9, 0/3 and 2/8. Nevertheless, about 18 eggs were collected from each female in which ovulation occurred (Table 6). With adult females, about 13 eggs were released by each female, though only 33% ovulated, and both the ovulation rate and number of eggs tended to decrease with increasing doses of gonadotrophins (Table 7).

During the latter part of this study, the time interval was increased between the injection of PMSG and hCG. The administration of hormones to immature females at an interval of 72 hours produced greater ovulation rates and numbers of oocytes (Table 6). Using 10IU PMSG/hCG, 75% of animals ovulated, and about 28 oocytes were collected from each female (range = 8-42). With 20IU PMSG/hCG, however, the ovulation rate was 91%, and each female ovulated up to 103 oocytes

DOSE	NUMBER OF TRIALS	NUMBER OF ANIMALS	OVULATION RATE %	NUMBER OF EGGS MEAN $\pm$ S.E.M.(n)
1.5 IU PMSG/hCG	3	16	100 (16/16)	8.4 $\pm$ 0.9 (16)
3.0 IU PMSG/hCG	3	15	99.3 (14/15)	13.4 $\pm$ 1.3 (14)
5.0 IU PMSG/hCG	3	15	100 (15/15)	16.7 $\pm$ 2.5 (15)
7.5 IU PMSG/hCG	3	15	100 (15/15)	32.4 $\pm$ 3.6 (15)

TABLE 8 : Induction of ovulation in 5-6 week old BALBc laboratory mice using PMSG, followed 48 hours later by hCG.

(range = 2-103) (Table 6). This protocol also resulted in a higher ovulation rate amongst adult females, although the number of oocytes obtained did not appear to be greater, except when 20IU of each hormone was given (Table 7).

As controls for these experiments, 5-6 week old BALBc laboratory mice were superovulated with PMSG and hCG. Four doses of gonadotrophins (1.5, 3.0, 5.0 and 7.5 IU) were used, and 93-100% of animals ovulated in each group (Table 8). The number of eggs ovulated was found to increase with higher doses of PMSG and hCG, but, although not shown in Table 8, this was largely associated with a higher number of immature, degenerate or fragmented oocytes, especially using 7.5 IU of PMSG/hCG.

3.2 Structure of the Oocyte-Cumulus Complex (OCC)

There were no detectable differences between oocytes obtained from mature or immature females, or from different superovulation regimes, so the results have been combined. Degenerate oocytes and their associated cumuli were not examined.

3.2.a General Organization of the OCC

Upon release from the ampullae, cumulus clots were up to 1.5 mm in length, and roughly ovoid in shape (Fig. 210), though some were more irregular (Fig. 211). Large clots tended to be compact, with many oocytes distributed randomly throughout a homogeneous array of cumulus cells (Fig. 210). Smaller clots, containing fewer than 8 oocytes, were often less regular in outline, and the cumulus cells were more diffusely arranged. The total oocyte diameter was about 100 μ m ($99.6 \pm 1.1 \mu$ m, $n = 11$), and they were found throughout the OCC, and sometimes near the edge (Fig. 211). The cumulus cells were evenly spread throughout the OCC, although regions of increased cell density were apparent, as well

as clusters of cumulus cells and red blood cells (Fig. 212). Although occasional cells were observed near the outer surface of the zona pellucida, there was no evidence to suggest the presence of a distinct corona radiata (Fig. 213).

3.2.b Structure of the Oocyte Vitellus

The vitellus was round or slightly ovoid in shape, and about $84.8 \pm 1.8 \mu\text{m}$ ($n = 9$) in diameter. The ooplasm was of moderate density, and contained a variety of cellular organelles. The second meiotic spindle, containing the arrested metaphase chromosomes, was located in the cortex, near one pole of the oocyte. It occurred in an area which was largely devoid of organelles, and was aligned tangentially to the surface (Figs. 214, 215). The chromosomes were usually aligned in a regular array on the metaphase plate (Fig. 215), although in some oocytes which otherwise appeared morphologically normal, the chromosomes were spread out and disorganized (Fig. 216).

Mitochondria were located throughout the vitellus, and often occurred in clusters, or stacked together, one on top of the other (Figs. 217, 218). They were ovoid in shape, though somewhat elongated, with a very dense matrix and predominantly transverse cristae (Fig. 217). Cisternae of smooth endoplasmic reticulum (SER) were frequently observed, usually in association with mitochondria, and contained flocculent material (Fig. 217). Polyribosomes were common, but rough endoplasmic reticulum (RER) and cytoplasmic lamellae (CPL) appeared to be absent (Fig. 218). Golgi complexes, up to $5 \mu\text{m}$ in diameter, were present in the egg cortex (Figs. 218, 219), and sometimes several could be seen in a single cross-section. Amorphous, electron-dense structures were located in the cortex of some oocytes, often closely associated with Golgi or mitochondria (Fig. 219). They were membrane-

bound, variable in shape, and often exhibited a membranous or periodic sub-structure (Fig. 220).

Cortical granules were invariably present, although the number varied greatly between oocytes, as well as in different regions of individual oocytes. The spatial density of cortical granules was about 10-130 per 100 μm , except in the zone overlying the spindle, in which they were absent. Cortical granules were usually found in close proximity to the oolemma, with only a few located in sub-cortical regions. They were either thinly spread out in a monolayer, or present in clusters of 3-10 (Figs. 219, 221, 222). They were variable in shape, though most were round or slightly ovoid. Two distinct types of granules were present, namely "dense" and "granular" forms. The dense granules consisted of an electron-dense, homogeneous core, separated from a unit membrane by a narrow space, and were 0.1-0.23 μm in diameter ($0.164 \pm 0.003 \mu\text{m}$, $n = 78$). The granular type, however, were larger in size ($0.220 \pm 0.007 \mu\text{m}$, $n = 36$), and consisted of a granular core, and a variable space to the unit membrane (Fig. 223). The dense granules were more numerous, and the granular ones were sometimes observed in the process of discharging their contents into the peri-vitelline space (Figs. 224, 225).

The surface of the vitellus was irregular in outline, with many microvilli, which were variable in size and distribution. A characteristic feature, however, was the absence of microvilli from the region overlying the meiotic spindle, where the oolemma was essentially smooth in contour (Figs. 219, 221, 222).

3.2.c Peri-vitelline Space and First Polar Body

The peri-vitelline space (PVS) was a narrow space which separated the vitellus and zona pellucida, but became obliterated in the region of

the spindle and first polar body. Apart from vitelline microvilli, the contents of the PVS were quite heterogeneous in nature. In some cases, it was virtually empty, whilst in others, it contained a variety of components, such as cortical granules (Figs. 224, 225), large double membrane septate structures, which seemed to bud off from the vitellus (Figs. 226, 227), and small granules, often enclosed in a vesicle, which were RR positive and appeared to have been released by the vitellus (Figs. 226, 228, 229).

The first polar body (PB) was frequently observed in the PVS, but was not always near the spindle (Figs. 230, 231). It was large, either round or ovoid, and often formed an indentation in the vitellus. The cytoplasm of the PB was less electron-dense than the ooplasm, and contained chromosomes, spread out and not enclosed by a nuclear envelope (Figs. 230, 231). The polar body was sometimes broken down, and appeared as a mass of membranous and vesicular elements, which were RR positive (Fig. 232).

3.2.d Zona Pellucida

The zona pellucida (ZP) was an acellular layer which totally enclosed the vitellus. In unfixed oocytes, it was about 6 μm thick ($5.9 \pm 0.1 \mu\text{m}$, $n = 29$) (Fig. 214). SEM revealed that the outer surface of the zona had a porous or spongy appearance, consisting of concentric layers of round pores of varying size (Figs. 233, 234). These pores were also evident in ultrathin sections, and were reduced in size on the inner surface of the ZP (Fig. 235).

The ZP consisted of granular or fibrillar material, and stained strongly with ruthenium red. There was no apparent difference between the inner and outer surfaces in the density or packing of the fibrous elements (Fig. 235). Oblique sections revealed an interwoven meshwork

of fine fibrils (Fig. 236), which, after staining with RR, were about 8 nm in diameter (8.1 ± 0.3 nm, $n = 15$) (Fig. 237). The orientation of the fibrils varied throughout the ZP (Fig. 235), but bands of circumferentially oriented fibrils surrounded the pores (Fig. 238). Within this fibrillar meshwork, other structures were observed, including cortical granules, remnants of corona cell processes, and the small PVS granules (Fig. 239).

3.2.e Cumulus Cells

Four types of cumulus cells were found throughout the OCC, and there did not appear to be any differential distribution of the four types. These were:

1. Small, round necrotic cells, with an electron-dense cytoplasm
2. Small cells, with extensive surface blebbing
3. Large, round cells, with a smooth surface
4. Large cells, with an irregular or microvillous surface (Figs. 240-243)

Some gap junctions were present between adjacent cumulus cells, but these were uncommon. The three non-necrotic cell types contained a similar array of organelles. The cytoplasm was moderately electron-dense, and contained large numbers of free ribosomes, distended RER filled with flocculent material, Golgi complexes, large ovoid mitochondria with transverse cristae, lipid droplets, membrane whorls, and sometimes lysosomes. The blebs sometimes had a vacuolated appearance (Figs. 241-243).

3.2.f Structure of the Cumulus Matrix

The extra-cellular matrix (ECM) of the cumulus oophorus was poorly

preserved by routine preparative procedures, but after fixation in the presence of ruthenium red, it was found to consist of electron-dense granules (24.9 ± 0.6 nm, $n = 54$) and fine filaments (4.7 ± 0.2 nm, $n = 43$). However, regional differences occurred in the distribution of these two elements throughout the OCC. In the vicinity of the oocyte, there was usually a homogeneous distribution of the two components (Figs. 244, 245), although areas containing a greater abundance or strands of granules were found in other parts of the OCC (Figs. 244, 246). There was often a slight increase in the number of granules near the zona pellucida, although strands were rarely observed, except where a cumulus cell was positioned close to the ZP (Fig. 247). Individual cumulus cells were frequently surrounded by a space which was largely devoid of granules, although others were enclosed by a dense band of granules (Figs. 247-249). Strands of aggregated granules were often present in the outer part of the OCC, and the edge consisted either of one or more narrow strands, or a broad band of granules (Fig. 250). The ECM was present in most, but not all, zona pores, and the filaments appeared to anastomose with the fibrillar elements of the ZP. The filaments were present throughout the OCC, but were most readily visualized in the clear spaces around cumulus cells, and were not prevalent in regions of large numbers of granules, such as the outer edge of the OCC.

The individual granules were round or slightly ovoid in shape, but tended to form end-end aggregates, and sometimes larger clumps. The filaments were variable in length, sometimes branched, and linked individual granules as well as aggregates, to form an interlocking meshwork of these two elements. The filaments often attached to cumulus cells, usually via the many adherant granules, and sometimes linked two processes from the same cumulus cell (Figs. 252-256).

3.3 In Vitro Fertilization

Due to initial difficulties in inducing consistent superovulation of female plains mice, only two trials were carried out using plains mouse sperm and oocytes. Instead, attempts were made to study the possible involvement of the ventral hooks in sperm-egg fusion using heterologous insemination of zona-free laboratory mouse and rat oocytes by plains mouse sperm.

3.3.a Homologous Insemination of Plains Mouse Oocytes

In experiment 1, preincubated plains mouse sperm were used to inseminate zona-intact oocytes obtained from plains mice injected with PMSG, followed 48 hours later by hCG. However, of 15 oocytes, none were fertilized, but 73% ejected a second polar body and formed a female pronucleus, and thus appeared to have been spontaneously activated (Fig. 257).

In experiment 2, zona-free or zona-intact oocytes were incubated with sperm in either HBT6 or TCM. These oocytes were obtained from animals given PMSG and hCG at an interval of 72 hours. None of 15 zona-free oocytes, and 0/37 zona-intact oocytes were fertilized in TCM. In HBT6, 0/18 zona-free and 0/46 zona-intact oocytes were penetrated. However, 6.5-11.0% of these oocytes appeared to have undergone spontaneous activation, as in experiment 1.

3.3.b Heterologous Insemination of Zona-free Mouse and Rat Oocytes

In a series of trials using zona-free laboratory mouse oocytes, none of 573 were penetrated by plains mouse sperm in HBT6, using sperm concentrations of $0.01-1.0 \times 10^6$ sp/ml, after pre-incubation for 1, 2 or 4 hours (Table 9). Sperm bound readily to the oocytes, the number increasing with the sperm concentration, but no signs of fertilization

were observed (Fig. 258; Table 9). In control experiments, laboratory mouse sperm fertilized 80% of zona-free mouse oocytes at a concentration of 0.1×10^6 sp/ml, with 22.5% polyspermy. Additional experiments using zona-intact laboratory mouse oocytes produced 79.4% fertilization at 0.2×10^6 sp/ml (Figs. 259, 260).

SPERM PRE-INCUBATION (HOURS)	SPERM CONCENTRATION ($\times 10^6$ sp/ml)	NUMBER OF TRIALS	NUMBER OF OOCYTES	FERTILIZATION RATE %	NUMBER OF BOUND SPERM
1	0.01	3	45	0	0-5
	0.05	3	40	0	2-10
	0.1	3	47	0	15-30
	0.5	3	43	0	20+
	1.0	3	41	0	20+
2	0.01	3	31	0	0-15
	0.05	3	37	0	10-30
	0.1	3	49	0	5-30
	0.5	3	33	0	20+
	1.0	3	40	0	20+
4	0.01	3	36	0	0-10
	0.05	3	38	0	3-20
	0.1	3	45	0	5-20
	0.5	3	21	0	20+
	1.0	3	27	0	20+

TABLE 9 : In vitro insemination of zona-free laboratory mouse oocytes with plains mouse sperm in HBT6.

A parallel series of experiments using zona-free rat oocytes and plains mouse sperm yielded similarly poor results. However, in some trials, up to 9% of zona-free rat oocytes were found to have been penetrated by plains mouse sperm (Table 10, 11). The sperm head and part of the middle piece were incorporated, and nuclear decondensation had taken place, though a male pronucleus did not form. The ventral hooks and pseudoperforatorium appeared to be intact. Similarly the oocytes did not eject a second polar body and form a female pronucleus (Fig. 261). In control experiments, rat sperm which were pre-incubated

SPERM PRE-INCUBATION (HOURS)	SPERM CONCENTRATION ($\times 10^6$ sp/ml)	NUMBER OF TRIALS	NUMBER OF OOCYTES	FERTILIZATION RATE %	NUMBER OF BOUND SPERM
1	0.01	3	27	0	0-6
	0.05	3	27	0	0-15
	0.1	3	33	0	10-20
	0.5	3	36	0	20+
	1.0	3	42	0	20+
2	0.01	3	36	0	0-2
	0.05	3	34	0	0-8
	0.1	3	36	0	10-20
	0.5	3	33	9.1 (3/33)	15-30
	1.0	3	29	3.4 (1/29)	10-20
4	0.01	3	52	0	0-5
	0.05	3	56	0	2-10
	0.1	3	58	0	7-20
	0.5	3	49	0	10-25
	1.0	3	55	0	5-25

TABLE 10 : In vitro insemination of zona-free rat oocytes with plains mouse sperm in TCM.

SPERM PRE-INCUBATION (HOURS)	SPERM CONCENTRATION ($\times 10^6$ sp/ml)	NUMBER OF TRIALS	NUMBER OF OOCYTES	FERTILIZATION RATE %	NUMBER OF BOUND SPERM
1	0.05	3	38	0	0-10
	0.1	3	36	0	5-20
	0.5	3	52	0	5-25
2	0.05	3	24	0	0-7
	0.1	3	25	0	0-15
	0.5	3	19	0	10-25
4	0.05	3	36	2.8 (1/36)	1-10
	0.1	3	37	2.7 (1/37)	5-20
	0.5	3	30	0	20+

TABLE 11 : In vitro insemination of zona-free rat oocytes with plains mouse sperm in HBT6.

for 5 hours, fertilized 96.6% of zona-free rat oocytes at a concentration of 0.5×10^6 sp/ml, with 90% polyspermy (Fig. 262).

3.3.c Acquisition of Hyperactivation

Upon initial release into either HBT6 or TCM, plains mouse cauda epididymal sperm showed strong forward progression with frequent rotation. They traversed straight trajectories, with a high frequency and low amplitude flagellar beat. However, after incubation for 1-2 hours, the pattern of motility changed, with an increased frequency and amplitude of flagellar bending, and greater oscillation of the sperm head. As a result of this change, sperm followed erratic paths, and often showed little or no forward progression, and sometimes swam in circles. This hyperactivation occurred in bursts, between which sperm reverted to the progressive pattern of motility.

During incubation in TCM, about 80% of sperm remained motile during the first 4 hours, after which this number decreased to about 67% during the next 4 hours, and was about 50% after 12 hours. The number of motile sperm which exhibited a hyperactivated pattern of motility remained low during the first 2 hours of incubation, then increased to a maximum of 85-88% after 4-6 hours, and declined to about 40% after 12 hours (Table 12).

A slightly different sequence of events occurred during incubation of plains mouse sperm in HBT6. During the initial 20-30 minutes required for preparation of the sperm suspensions, the motility decreased to 53% (i.e. $t = 0$), and then declined further to only about 12% after 4 hours. During the final 6 hours, of incubation, only about 4-7% of sperm remained motile in HBT6. As with TCM, however, the number of hyperactivated sperm increased to reach a maximum of 92% after 6 hours, but then declined during the final 6 hour period to 50-57% of the

TIME (HOURS)	% MOTILITY	MOTILITY PATTERN (% OF MOTILE SPERM)		
		WEAK	PROGRESSIVE	HYPERACTIVATED
0	84.0	13.8	86.2	0
1	72.6	29.8	67.2	3.0
2	80.3	12.4	58.0	29.6
4	80.1	8.9	3.2	87.9
6	66.6	14.9	0.0	85.1
8	68.1	39.8	6.4	53.8
12	49.4	16.0	45.2	38.8

TABLE 12 : Motility of plains mouse sperm during incubation in TCM for 12 hours.

motile sperm. The actual number of hyperactivated sperm was low however, due to the poor maintenance of motility (Table 13).

TIME (HOURS)	% MOTILITY	MOTILITY PATTERN (% OF MOTILE SPERM)		
		WEAK	PROGRESSIVE	HYPERACTIVATED
0	53.0	20.5	79.5	0
1	37.7	17.9	74.0	8.1
2	16.0	20.6	19.6	59.8
4	11.6	8.3	4.2	87.5
6	6.8	8.2	0.0	91.8
8	4.4	17.6	32.4	50.0
12	6.0	30.0	13.3	56.7

TABLE 13 : Motility of plains mouse sperm during incubation in HBT6 for 12 hours.

3.3.d Occurrence of Acrosome Reactions

Plains mouse sperm were incubated for various periods in HBT6 or TCM, and examined at the ultrastructural level to determine if normal acrosome reactions occurred under these culture conditions.

During the first 4 hours of incubation in TCM, there were no detectable changes in the structure of the sperm head in about 90% of sperm. In some, however, the principal segment of the acrosome, and sometimes also the equatorial segment were lost (Fig. 263). During the ensuing incubation up to 12 hours, the number of acrosome-reacted sperm increased slightly, but most sperm heads appeared to be intact.

In HBT6, however, most sperm underwent a variable degree of acrosomal loss, usually of both the principal and equatorial segments (Fig. 264). After 12 hours of incubation, the entire acrosome was absent from about 80% of sperm. A few sperm were observed which appeared to have undergone a "normal" acrosome reaction. This involved fusion of the plasma membrane and outer acrosomal membrane in the principal segment, resulting in loss of the acrosomal contents. The equatorial segment remained intact, and often exhibited periodic cross-bridges linking the inner and outer acrosomal membranes (Fig. 265).

During the course of incubation in both media, either with or without homologous or heterologous oocytes, no changes were apparent in the shape or structure of the pseudoperforatorium or ventral hooks, although the three hooks became further apart in some, but not all of the sperm (Fig. 258).

4. DISCUSSION

4.1 Induction of Superovulation in the Plains Mouse

Initial attempts to superovulate female plains mice were carried out by administration of PMSG, followed 48 hours later by hCG, as is routinely used for superovulating laboratory mice (Gates, 1971). Although good ovulatory responses were sometimes obtained with this protocol using immature female plains mice, the results were variable

and inconsistent. In 8 trials, the overall ovulation rate was only 22%, although about 18 eggs were obtained from each of the females which ovulated. Similarly, a variable response was obtained using mature females, and the best results (33% ovulation rate) were achieved by the administration of only 10 IU of PMSG and hCG. However, in some cases, only one or a few trials were performed, due to the limited number of available animals; further investigations would probably produce a clearer pattern. In control experiments with BALBc laboratory mice, 93-100% of animals ovulated when primed with gonadotrophins, suggesting that the variable response in female plains mice was not due to a lack of biological activity of the gonadotrophins employed.

In order to study fertilization, either in vivo or in vitro, it is necessary to be able to consistently obtain large numbers of oocytes. The above-mentioned protocol was therefore considered unsuitable for such studies. Hence, during the latter part of this study, attempts were made to induce more consistent superovulation by increasing the time interval between the administration of PMSG and hCG from 48 to 72 hours. Roldan et al., (1985) have used such a protocol to reliably induce superovulation in adult vesper mice (Calomys spp.). In both adult and immature female plains mice, the ovulation rate and mean number of eggs collected from each female were higher when PMSG and hCG were injected 72 hours apart. Superovulation was induced in up to 91% of immature females, yielding as many as 103 eggs per female when 20 IU of each gonadotrophin was used. Further, up to 80% of adult females ovulated using this protocol, although the ovulation rate declined when high doses of gonadotrophins were administered.

Statistical analysis of the superovulation data was not carried out because of the small sample sizes of some treatment groups, inter-litter variability in the response to PMSG and hCG (see 3.1), and the

preclusion of random sampling due to the intermittent supply of immature female plains mice.

The reasons for this improvement in the ovulation rates obtained when plains mice were injected with hormones 72 hours apart was not determined, but it may relate to the length of the oestrous cycle. In rodents such as the rat, laboratory mouse, Mongolian gerbil, golden and Chinese hamster, the oestrous cycle is usually 4-5 days in length (Ward, 1946; Bronson et al, 1966; Barfield and Beeman, 1968; Perry, 1971; Yanagimachi et al, 1983). In the hydromyine rodents, however, the length of the cycle is 5-10 days (Telfer and Breed, 1976; Watts, 1979), and more specifically, it is about 7-8 days in the plains mouse (Watts and Aslin, 1981). The induction of ovulation requires the acquisition of LH receptors by the granulosa cells during the period of follicular growth (Baird, 1984). In the plains mouse, therefore, it may take longer for the LH receptors to develop after administration of PMSG, due to the length of the oestrous cycle. Hence, when hCG is given 48 hours after PMSG, this developmental period may be incomplete in most follicles, resulting in low ovulation rates. The inconsistent response may be due to variability in the length of the oestrous cycles between individual females. Evidence for such a suggestion has been provided by the observation that, after priming both mature and immature females with PMSG, followed 48 hours later by hCG, anovulatory ovaries often contained many large, antral follicles (Flaherty, unpublished observation). This suggests that PMSG induced follicular development, but the follicles were unable to respond to administration of hCG, presumably due to insufficient development of LH receptors.

A recent study in our laboratory has examined the superovulatory response of two other hydromyine rodents, the western chestnut mouse (Pseudomys nanus) and the spinifex hopping mouse (Notomys alexis)

(McGregor, 1985). In both species, the ovulation rate tended to be higher when PMSG and hCG were given 72 hours apart, although the actual ovulation rates and number of oocytes collected were considerably lower than for the plains mouse (McGregor, 1985). This interspecific variability in the response of hydromyine rodents to exogenous gonadotrophins is difficult to explain in terms of oestrous cycle length, since it is 5-7 days in P. nanus, and 6-8 days in N. alexis (Telfer and Breed, 1976; Watts, 1979). Several other factors may be involved, however, including the age of the females, which has been shown to be important in the laboratory mouse (Gates, 1971), or the genetic background of the animals (Fraser, 1977b). The latter may be of particular importance as the colonies have been randomly inbred from only a small number of individuals. Further improvements might also be achieved by the administration of PMSG in multiple small doses over a longer period of time (McLaren, 1967).

One final point which warrants discussion is the normality of the oocytes obtained by superovulation in this study. Assuming a cycle length of 7-8 days (Watts and Aslin, 1981), some of the eggs obtained by the 72 hour protocol are likely to have been quite precocious in comparison with spontaneously ovulated oocytes. However, this may not be of great concern, as it has been shown that, in the golden hamster at least, precociously ovulated oocytes are capable of normal fertilization and embryonic development (Yanagimachi, 1984). Another unknown factor is the exact post-ovulatory age of the oocytes obtained in this study. It has been assumed that ovulation takes place about 11-14 hours post-hCG, as in the laboratory mouse (Gates, 1971). Hence, at the time of oocyte collection (15 hours post-hCG), they should have only been in the oviduct for 1-4 hours. However, due to a limited supply of animals, this was not verified, and it is thus possible that the oocytes may have

been ovulated prior to this time as a result of an endogenous LH surge, which would mean they were aged at the time of collection. Hence, future studies on superovulation in the plains mouse should aim to determine the exact time of ovulation in relation to the administration of gonadotrophins.

4.2 Structure of the Oocyte-Cumulus Complex

The structure of the plains mouse OCC has been studied at the light and electron microscopical levels, and no major differences were apparent between oocytes from animals given 10 or 20 IU of PMSG and hCG at 48 or 72 hours intervals. The OCC were assessed as being mature on the basis of having a first polar body, arrested metaphase chromosomes, no corona cells penetrating the zona pellucida, and an expanded and mucified cumulus oophorus (Zamboni, 1970; 1972). At this stage, however, it is not known if the structure of the OCC obtained by superovulation is truly indicative of the OCC after spontaneous ovulation.

4.2.a Comparison With Other Eutherian Species

In the plains mouse, the organization of the vitellus and distribution of organelles appears to be similar to the laboratory mouse oocyte (Weakley, 1968; Zamboni, 1970; Phillips and Shalgi, 1980a), and thus only three aspects will be discussed in this section.

Firstly, cytoplasmic lamellae (CPL) were absent from the vitellus of the plains mouse. In rat, mouse and hamster oocytes, CPL are found throughout the vitellus, especially in the rat and hamster, and consist of stacks of thin sheets, up to 7 μ m in length (Weakley, 1968; Green, 1985). It has been suggested that CPL may assume the function of yolk (Szollosi, 1971).

The second meiotic metaphase spindle lies close to the surface at one pole of the plains mouse oocyte, and the oolemma in this region is relatively smooth and devoid of microvilli, as reported for hamster and laboratory mouse oocytes (Longo, 1974a; Nicosia et al., 1977). In some oocytes, the organization of the spindle appeared to have been disrupted, and the chromosomes were spread out in disarray, although these oocytes otherwise appeared morphologically normal. Migration and disorganization of the spindle occurs in mouse, rabbit and hamster oocytes which have been aged in situ in the oviducts (Szollosi, 1971; Longo, 1974a; 1974b; 1980). However, it seems unlikely that this would have caused the spindle disorganization of the plains mouse oocytes, since only a small number displayed this feature, and the morphological characteristics of the majority of oocytes were entirely normal. It seems more likely, therefore, that such oocytes may have been undergoing the initial stages of atresia when induced to ovulate by the administration of hCG.

Cortical granules, which are small, membrane-bound structures which reside adjacent to the oolemma, are a characteristic feature of all mammalian oocytes (Gulyas, 1980). In plains mouse oocytes, the cortical granules range in diameter from about 0.1-0.3 μm , which is at the lower end of the mammalian size range (Gulyas, 1980). Two types of granules are present, the "dense" and "granular" forms, which differ in size and the appearance of their core. Similarly, "light" and "dark" cortical granules occur in the oocytes of the laboratory mouse (Nicosia et al., 1977), pig (Szollosi and Gerard, 1983), and golden hamster (Szollosi, 1967). In human and rabbit oocytes, however, the cortical granules tend to be uniform in appearance (Zamboni and Mastroianni, 1966; Sathananthan and Trounson, 1982). Although the functional significance of different types of granules is unknown, they may reflect differences in maturity

or function (Gulyas, 1980).

Unlike most species, the plains mouse cortical granules were not separated from the oolemma by a space of a few hundred angstroms; rather, they were found at small but varying distances from it. It is not known if this merely reflects inter-specific variation, or if plains mouse oocytes undergo "cortical maturation" after ovulation, as postulated in the mouse (Zamboni, 1970; 1972). Some granules were also observed in the act of releasing their contents into the PVS, and these were usually the "granular" type. Nicosia et al. (1977) reported the release of "light" granules from laboratory mouse oocytes incubated in vitro in the presence of sperm, although such a premature cortical discharge did not appear to affect fertilization (Wolf et al., 1979). Hence, in plains mouse oocytes, the "granular" cortical granules may serve a similar function to the "light" granules found in laboratory mouse oocytes.

The zona pellucida is about 5-15 μm thick in most eutherian oocytes (Wolf, 1983). Hence, at only 6 μm thick, the plains mouse zona is comparatively thin. Its sub-structure appears to consist of an interlocking fibrillar meshwork, as described in zonae from other species (Zamboni and Mastroianni, 1966; Baranska et al., 1975; Dudkiewicz et al., 1976; Talbot, 1984). However, in contrast to reports for human, porcine and hamster zonae (Stegner and Wartenberg, 1961; Dudkiewicz et al., 1976; Dietl and Czuppon, 1984), no gradient seems to exist in the density of zona fibrils from the inner to outer aspect of the plains mouse zona. The ZP is more homogeneous on its inner aspect, though this is probably due to the porous nature of the outer aspect. SEM revealed similar surface features to those reported for the mouse zona pellucida (Phillips and Shalgi, 1980a), but the pores are not as large as in the cat or rabbit zonae (Dudkiewicz and Williams, 1977).

The homogeneity of the inner aspect of the zona seems to indicate that the number and size of the pores decreases from the outer to inner surfaces, as reported for the hamster zona by Phillips and Shalgi (1980b).

Plains mouse oocytes were found to be surrounded by fairly evenly spaced cumulus cells and ECM, and a distinct corona radiata was not evident. Hence, it is unlike the situation in the laboratory mouse or hamster OCC, in which there is a decreased cell spacing near the oocyte, which has been postulated to represent an increased barrier to sperm penetration (Talbot and DiCarlantonio, 1984a; 1984b). Though the cumulus cell spacing throughout the plains mouse OCC was not measured, it appeared that there is not an increased barrier to penetration near the oocyte.

The structure of the cumulus ECM was studied after fixation in the presence of ruthenium red. For comparison, similar studies have only been reported for the mouse and hamster OCC (Talbot and DiCarlantonio, 1984a; 1984b), and the results of the present study indicate a number of differences in the structure of the plains mouse ECM. Firstly, although the ECM consisted of granules and filaments distributed throughout the OCC as in these other two species, the granules were found to be considerably smaller in the plains mouse OCC (25 nm) in comparison to the hamster or mouse OCC (37-43 nm) (Talbot and DiCarlantonio, 1984b). This is probably a species-specific difference, and not the result of differences in staining technique, as the thickness of the filaments was similar (4.6-5.1 nm) in all three species. Secondly, dense aggregations of ECM were not found on the surface of the plains mouse zona. Talbot and DiCarlantonio (1984b) have postulated that this occurs in hamster and mouse OCC due to changes occurring in the oviduct, as it does not appear in follicular OCCs.

It was noted in this study that there seems to be a differential distribution of the ECM components in the OCC. Granules are scarce in the vicinity of cumulus cells, although filaments are found in such regions, and in areas of high granule density, the filaments are difficult to localize. And finally, dense strands of aggregated granules occur in the outer part of the OCC, as described for laboratory mouse and hamster OCC (Talbot and DiCarlantonio, 1984b). Although the functional significance of this differential distribution is unknown, it may be important for maintaining the structural integrity of the OCC, or alternatively, it may represent an artifact of fixation and dehydration.

4.2.b OCC Structure in the Hydromyinae

The structure of the OCC has recently been examined in two other hydromyine rodents, the western chesnut mouse and spinifex hopping mouse (McGregor, 1985). A number of substantial differences were found in the structure of the OCC between these two species, and with respect to the plains mouse. Firstly, the diameter of the vitellus is less than in the plains mouse, and although the distribution of organelles is similar, there were found to be two major differences. Two types of cortical granules were not observed in oocytes of these other two species, and granules were less abundant than in the plains mouse oocyte, although they were present in higher numbers in the sub-cortical cytoplasm. At this stage it is not known if the number of cortical granules relates to the efficiency of fertilization in these species (Wabik-Sliz, 1979). CPL were found in both these species, though they are absent from plains mouse oocytes. Secondly, the structure of the zona pellucida is similar in all three species, but at 10-12 μm in thickness, it is considerably thicker in chesnut and hopping mouse oocytes (McGregor, 1985).

In the OCC of the chesnut and plains mouse, the oocytes were found to be surrounded by evenly spaced cumulus cells, and no corona radiata is present. The ECM is distributed homogeneously throughout the OCC. In the hopping mouse OCC, however, the cumulus cells are larger, a distinct 3-5 cell thick corona radiata is present, and the ECM occurs in strands between the cumulus cells. A dense band of granules was found on both chesnut and hopping mouse zonae. The other major difference between the ECM of these hydromyine species is in the dimensions of the granules and filaments, which are almost twice as large as in the plains mouse ECM (McGregor, 1985).

Hence, within the Hydromyinae, there appears to be considerable variation between species in the structure of the OCC. It is doubtful that these are due to technical differences, as preparations were carried out in the same manner, and often at the same time in all three species. The functional significance of this variation will be discussed in Section 7.

4.3 Fertilization Studies

In previous sections of this study, the structural properties of the sperm head of the plains mouse have been examined in detail. Thus far, however, nothing is known about the possible function of the three hooks, which are a unique and characteristic feature of the Hydromyinae (Breed and Sarafis, 1979; Breed, 1980). Hence, the primary aim of this study was to investigate the possible role of the three hooks in the process of fertilization.

4.3.a Fertilization in the Plains Mouse

A detailed study of fertilization in vivo has not been undertaken for several reasons. Firstly, a limited supply of animals was

available, as well as limited facilities for mating large numbers of animals. Secondly, previous work in our laboratory has shown that it is difficult to obtain vaginal smears by lavage in this species without inducing long periods of anoestrus (Breed, personal communication). Thirdly, data obtained from the breeding colony records showed that, when plains mice were mated randomly, only 30% mated successfully within three cycle lengths of pairing, and only 44% mated within three months of pairing (Flaherty and Peirce, unpublished observations). Hence, this species doesn't appear to breed well in the laboratory. The low mating rates may be due to acyclic females or incompatibility of animals. Fourthly, superovulation of adult female plains mice produced variable results, and the improvements associated with the 72 hour protocol came too late during the course of this study. Lastly, no data is available on the timing of ovulation and fertilization in vivo in this species.

As an alternative, it was intended to study gamete interactions under controlled in vitro conditions. However, such studies were hampered by initial difficulties encountered in consistently superovulating female plains mice, and only two experiments have been conducted. In these trials, none of the zona-intact or zona-free oocytes were fertilized by plains mouse sperm pre-incubated for 2 hours in either HBT6 or TCM. In HBT6, however, some of the oocytes underwent spontaneous activation, ejecting the second polar body and forming a single pronucleus. It seems unlikely that activation was induced by leaving the oocytes in the oviducts for too long after death, as occurs in the rat (Keefer and Schuetz, 1982), since they were removed from the oviducts and placed in culture medium within 2 minutes of cervical dislocation. Activation only occurred in HBT6, and not in TCM, suggesting that it may have been the result of inadequate culture conditions. An alternative explanation is that the release of acrosomal

contents from immotile sperm may have triggered this activation, since sperm became immotile and lost the acrosome within a few hours of incubation in HBT6.

4.3.b Heterologous IVF

Several studies have implicated the post-acrosomal region (PAR) as the initial site of sperm-egg fusion (Piko, 1969; Yanagimachi and Noda, 1970b; 1970c; Thompson et al, 1974). It has been postulated that capacitation induces a change in the physiological properties of the plasma membrane in this region, such that it becomes able to fuse with the oolemma (Yanagimachi and Noda, 1970d). In Sections 2 and 3, it was shown that the peripheral layer of the two ventral hooks consists of an extension of the PAR. Hence, attempts were made to study the possible role of the ventral hooks in sperm-egg fusion, using in vitro fertilization of zona-free laboratory mouse and rat oocytes by plains mouse sperm. The zona-free hamster egg would have been the most suitable candidate for such a study, since it has been shown to fuse with sperm from at least eleven species (Yanagimachi, 1981), but unfortunately, these were unavailable for study.

Plains mouse sperm bound readily to zona-free laboratory mouse oocytes, but none were penetrated, in spite of the use of various pre-incubation times and sperm concentrations. A few zona-free rat oocytes were fertilized in some trials, though the fertilization rate did not exceed 9%. Fertilization was always monospermic, the sperm head and middle piece were incorporated and nuclear decondensation occurred, but a male pronucleus did not form, and the oocyte was not activated to complete the second meiotic division. Hanada and Chang (1972) observed a similar failure of foreign sperm to activate oocytes during in vitro cross-fertilization of zona-free oocytes. These results were considered

inadequate to justify an ultrastructural analysis of sperm-egg fusion.

The process of sperm-egg fusion has been shown to be less species-specific than penetration of the zona pellucida, but there still exists a variable degree of specificity. Unlike the hamster oocyte, the oolemma of most species presents some barrier to cross-fertilization (Yanagimachi, 1977; 1981). Several studies have shown that zona-free laboratory mouse oocytes are rarely penetrated by human, rat, hamster, guinea pig, deer mouse or gerbil sperm (Hanada and Chang, 1972; 1978; Quinn, 1979). Hence, the failure of plains mouse sperm to fuse with zona-free laboratory mouse oocytes is not entirely unexpected, and may be due to the relatively high degree of specificity shown by the mouse oolemma. The zona-free rat oocyte, however, is readily fertilized by laboratory mouse sperm (Hanada and Chang, 1972; Quinn, 1979), although in the present study, only a few were fertilized by plains mouse sperm.

Although the specificity of the oolemma could account for the inability of plains mouse sperm to penetrate zona-free mouse and rat oocytes, it is also possible that the culture media employed were unsuitable for capacitation and induction of the acrosome reaction in plains mouse sperm. The acrosome reaction is a necessary prerequisite for sperm-egg fusion, as well as penetration of the zona pellucida (Yanagimachi and Noda, 1970d; Wolf et al, 1976; Hanada and Chang, 1978; Yanagimachi, 1981). It is characterized by point fusions between the plasma membrane and outer acrosomal membrane in the apical and principal segments, producing a vesiculated acrosomal ghost and thereby releasing the contents of the acrosome (Barros et al, 1967; Yanagimachi and Usui, 1974; Russell et al, 1979).

Ultrastructural analysis of cultured sperm revealed that during incubation in TCM, only a few sperm underwent a "normal" acrosome reaction, and in the majority of sperm, the sperm head remained intact

after incubation for up to 12 hours. Sperm motility was well maintained in TCM, and after 4-6 hours, most sperm exhibited an altered pattern of motility, characterized by an increased amplitude of flagellar bending, and greater oscillations of the sperm head. This probably represents a form of hyperactivation, as originally described in hamster sperm by Yanagimachi (1970), and subsequently shown to be a feature of capacitation in many species (Yanagimachi, 1972; Fraser, 1977; Cooper et al, 1979; Cummins, 1982; Mortimer et al, 1984). It would appear, therefore, that although plains mouse sperm become hyperactivated during incubation in TCM, few undergo the acrosome reaction, which would in turn explain the low rates of fertilization of zona-free rat oocytes.

Similarly, incubation of sperm of HBT6 was found to be insufficient for induction of the acrosome reaction. During the course of ultrastructural analyses of cultured sperm, only a few were encountered which appeared to have undergone a "normal" acrosome reaction. In most sperm, the entire acrosome was lost, including the equatorial segment. Intermediate forms, displaying swelling or vesiculation of the principal segment, were rarely observed, suggesting that most sperm were undergoing "false" or degenerative acrosome reactions. This correlates well with the poor maintenance of motility in HBT6, although many of the remaining motile sperm showed a hyperactivated pattern of motility. Fleming et al (1982) have shown that acrosome-reacted guinea pig sperm only remain fertile for a short time. Hence, the low number of motile sperm which had undergone the acrosome reaction would probably account for the failure of plains mouse sperm to penetrate zona-free laboratory mouse or rat oocytes in this medium.

It would seem that, under the culture conditions employed in this study, plains mouse sperm can undergo the initial phase of capacitation, which is sufficient to initiate hyperactivation, but inadequate for

induction of the acrosome reaction (Barros and Berrios, 1977).

4.4 Concluding Remarks

This study, though unsuccessful in some aspects, has provided a method for consistently inducing superovulation in both mature and immature female plains mice. Further investigation, however, will be required to determine the time of ovulation and the fertility of the resulting oocytes. The ultrastructure of the oocyte-cumulus complex has been examined in detail, but the cumulus oophorus and zona pellucida do not appear to represent sufficiently formidable barriers to necessitate the development of three hooks to aid in penetration of these investments (see Section 7). However, the biochemical and biophysical properties of these structures have yet to be ascertained.

Although preliminary attempts at in vitro fertilization of plains mouse or zona-free laboratory mouse and rat oocytes have been largely unsuccessful, it has been shown that the culture conditions will have to be extensively modified to provide a suitable environment for capacitation and induction of the acrosome reaction. Alternatively, ionophore-induced acrosome reactions may overcome this obstacle (Shams-Borhan and Harrison, 1981). Hence, with these improvements, it should be possible to study gamete interactions in the plains mouse either in vivo or in vitro.

SECTION 7 : **FUNCTIONAL ASPECTS OF SPERM HEAD MORPHOLOGY IN THE PLAINS MOUSE**

The principal aim of this study was to investigate the structural and functional aspects of the three hooks on the sperm head of the plains mouse, Pseudomys australis. The structural features of the sperm head have been discussed in detail in Sections 2-5, but before presenting a discussion of the possible functional significance of this unusual sperm head morphology, a cumulative description will be given.

1. **STRUCTURE OF THE PLAINS MOUSE SPERM HEAD**

The present study has provided a comprehensive ultrastructural analysis of many aspects of the sperm head morphology in this species. Thus, in combination with previous studies carried out in our laboratory (Flaherty and Breed, 1983; Flaherty et al., 1983), it is now possible to present a unified description of sperm head morphology in the plains mouse.

The sperm head is falciform in shape, with three curved hooks projecting from its apical margin. The single dorsal hook changes shape from asymmetrical at its base to becoming dorso-ventrally flattened and crescentic near its tip. It is structurally similar to the rat sperm head, and contains nuclear, sub-acrosomal and acrosomal material. The sub-acrosomal material (SAM) continues beyond the nucleus as the pseudoperforatorium, although this structure does not contain filamentous actin.

The two ventral hooks have a unique shape and structure, thus far only described in the Hydromyinae (Flaherty and Breed, 1983a; Breed, 1984). There is a single hook basally, which bifurcates to produce two

curved hooks. These are initially round, but become bilaterally flattened apically, with constricted dorsal and ventral margins. The hooks consist of a core of SAM, which contains one or more 15 kd polypeptides, and becomes cross-linked by disulphide bonds during epididymal maturation. Bundles of 6 nm microfilaments, presumably actin based on NBD-phalloidin staining and electrophoretic analysis of hook extracts, occur in the hooks, and appear to be responsible for the acquisition and maintenance of the shape of the apical regions of the hooks. The peripheral layer of the ventral hooks consists of an extension of the post-acrosomal dense lamina, with parallel ridges spaced 14-15 nm apart firmly attaching the plasma membrane to the hooks.

The structure of the sperm head posterior to the hooks seems to follow the general eutherian pattern as outlined by Fawcett (1970; 1975). The main features include tapering of the head from caudal to apical, as well as ventral to dorsal aspects; presence of the principal segment of the acrosome on the dorsal margin, and the equatorial segment on the lateral surfaces of the sperm head; accumulation of SAM on the dorsal margin, forming two ridges; and location of the post-acrosomal region on the caudal and ventral margins of the sperm head.

2. FUNCTIONAL DESIGN OF THE SPERM HEAD IN RELATION TO PENETRATION OF THE OOCYTE-CUMULUS COMPLEX

2.1 Mechanical versus Enzymatic Modes of Penetration

The zona pellucida, and cumulus oophorus in some species, probably represent significant barriers to sperm penetration and subsequent fertilization (Austin, 1961; Bedford, 1983a, 1983b).

Hyaluronidase has been postulated to assist sperm penetration of the cumulus oophorus by dissolving the hyaluronic acid matrix, thereby

allowing sperm to glide between the cumulus cells (Austin, 1948; Cummins, 1982). Evidence for such a role comes from the ability of this enzyme to disperse the cumulus cells (McLean and Rowlands, 1942); localization of hyaluronidase in the principal segment of the acrosome, the contents of which are dispersed during the acrosome reaction (Barros et al., 1967; Morton, 1977); release of hyaluronidase during the acrosome reaction in the guinea pig (Rogers and Yanagimachi, 1975a), ram (Shams-Borhan and Harrison, 1981) and hamster (Zao et al., 1985); initiation of the acrosome reaction in vivo during or before penetration of the cumulus (Austin and Bishop, 1958b; Bedford, 1972; Mahi and Yanagimachi, 1976; Cummins and Yanagimachi, 1982); and finally, inhibition of cumulus dispersion and fertilization by anti-hyaluronidase antibodies (Metz et al., 1972). However, hyaluronidase does not disperse the bovine cumulus oophorus, suggesting the need for other enzymes (Lorton and First, 1979), such as the corona-penetrating esterase (Zaneveld and Williams, 1970; Bradford et al., 1976) or arylsulphatase and β -N-acetylhexosaminidase (Rodger and Young, 1981).

It has been suggested that acrosomal enzymes, particularly a trypsin-like protease called acrosin, may effect or aid sperm penetration through the tough zona pellucida (McRorie and Williams, 1974). The evidence for and against such a role for acrosin has been reviewed in detail in Section 1. To date, however, the evidence has been somewhat circumstantial and direct evidence has not been provided (Moore and Bedford, 1983). Other enzymes may also be involved in penetration of the zona pellucida, either alone or acting synergistically with acrosin or each other (Morton, 1977; Srivastava et al., 1979; Farooqui and Srivastava, 1980; Ninjoor and Srivastava, 1985).

However, there is general consensus that sperm motility and some

form of mechanical force is involved in sperm penetration of the zona pellucida, although whether penetration is effected by purely mechanical means, or in combination with enzymic activity, is the subject of considerable debate (Yanagimachi, 1981; Bedford, 1983b; Moore and Bedford, 1983). As a component of capacitation, sperm exhibit a different pattern of motility, termed hyperactivation (Yanagimachi, 1981), which may or may not involve an increase in the power output (Katz et al., 1978; Johnson et al., 1981), but has been postulated to be well suited for maximizing the thrust applied by the sperm to the zona (Katz and Yanagimachi, 1981). Direct observations of sperm penetrating the zona pellucida have shown that they do not glide smoothly through it, but move forward a short distance at a time with constant and active flagellar beating (Yanagimachi, 1966; Sato and Blandau, 1979a; Gaddum-Rosse et al., 1984). Indeed, based on calculations of the forward thrust developed by sperm motility and the viscoelastic nature of the zona pellucida, Green and Purves (1984) calculated that eutherian sperm are capable of penetrating the zona by mechanical means alone.

Bedford (1983b) has postulated that the sperm head has undergone a number of structural changes or adaptations to assist penetration of the tough extra-cellular coats around the oocyte. Thus, it is bilaterally flattened, and tapers anteriorly to a point, which may concentrate the thrust or stress on the zona pellucida (Bedford, 1983b; Green and Purves, 1984). There is also an apical accumulation of sub-acrosomal material in both spatulate and hooked sperm heads, which has been suggested to act as a mechanical tool to aid penetration of the zona (Austin and Bishop, 1958a; Nicander and Bane, 1966; Yanagimachi and Noda, 1970b; Bedford, 1983b). During epididymal maturation of spermatozoa, the nucleus and sub-acrosomal material are stabilized by extensive disulphide bonding, which is viewed as necessary for

withstanding the rigors associated with penetration of the resilient zona (Calvin and Bedford, 1971; Bedford and Calvin, 1974a).

2.2 Functional Design of the Plains Mouse Sperm Head

The above discussion suggests that a considerable degree of mechanical force is probably involved in sperm penetration of the zona pellucida. It has previously been postulated that the ventral hooks on the sperm head of hydromyine rodents may function in assisting penetration of this tough extra-cellular coat (Flaherty et al., 1983; Breed, 1984). In this study, it was originally hoped to test this hypothesis by examining gamete interactions in the plains mouse, either in vivo or in vitro. However, for reasons outlined in Section 6, this aim has not been fulfilled. Nevertheless, the following discussion represents an analysis of the functional design of the plains mouse sperm head in relation to the possible role the ventral hooks may assume in mechanical penetration of the zona pellucida.

2.2.a Shape of the Sperm Head and Hooks

The sperm head of the plains mouse is bilaterally flattened, and is narrower on its apical and dorsal margins. These are features of most eutherian sperm (Nicander and Bane, 1966; Fawcett, 1970; 1975), and apart from reducing the resistance of the sperm head to traversal of the zona pellucida, would also concentrate the thrust generated by flagellar activity on a small area of the zona (Bedford, 1983b; Green and Purves, 1984).

The shape of the dorsal hook would be of considerable importance, since it probably represents the leading edge, and therefore the portion of the sperm head through which much of the force would be exerted on the zona pellucida. It is rounded on its dorsal surface, with plate-

like lateral extensions, especially in the apical half of the hook. This could provide the hook with two narrow edges for cleaving a path through the zona. However, the basal asymmetry of the hook would seem to provide a more effective cleavage surface on one side of the hook. This may be responsible for determining the angle of the penetration curve, although such asymmetry does not occur in rat (Lalli and Clermont, 1981), golden hamster (Yanagimachi and Noda, 1970a) or laboratory mouse (Flaherty, unpublished observation) sperm. It is also noteworthy that the shape of the hook is different from rat and laboratory mouse sperm, in which the apical hook is roughly triangular in shape (Lalli and Clermont, 1981; Flaherty, unpublished observation). It is tempting to speculate that this may relate to the additional presence of the two ventral hooks.

The two ventral hooks are rounded near their base, but are bilaterally flattened and taper to a fine point in their apical half, with constricted dorsal and ventral margins. The shape of the apex of the hooks is not unlike that of an arrowhead, and would appear to be well adapted for cleaving a path through the zona pellucida. However, the possible involvement of the ventral hooks in this process would also depend on two other factors:

1. During zona penetration, the resilience of this structure would probably tend to maintain the close-knit orientation of the three hooks, which would limit their effectiveness as mechanical cleavage devices. During in vitro incubation, some sperm retained the close orientation of the hooks as observed in unwashed epididymal sperm, whilst in others, the hooks became further spread apart. Though the orientation of the hooks during fertilization in vivo has not been ascertained, it seems unlikely that they would be spread apart, as this would undoubtedly enhance the possibility of damage, and movement of the sperm head

against the zona would probably force the hooks together.

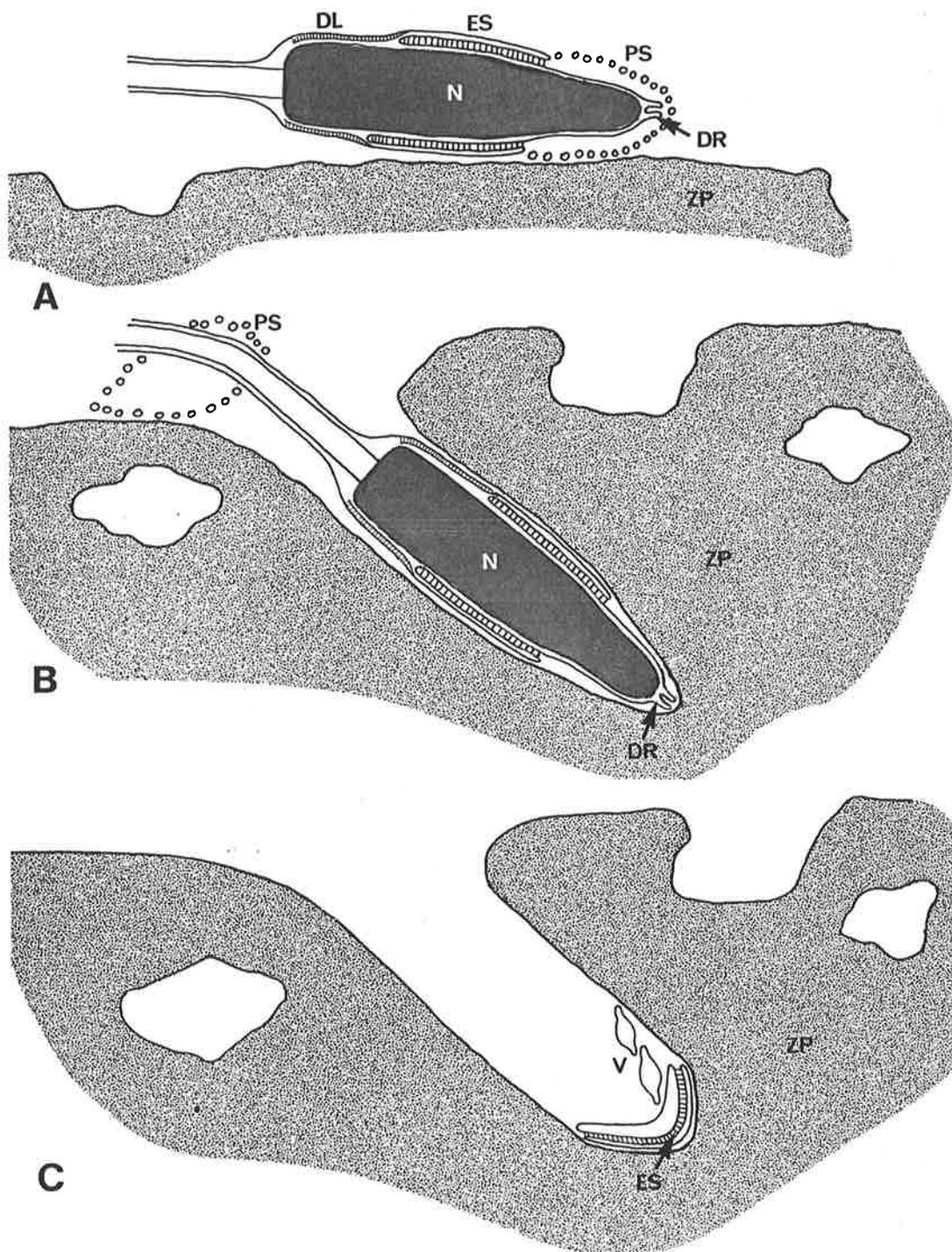
2. The assistance to zona penetration rendered by the ventral hooks would also depend to some extent on the orientation of the sperm head in the zona pellucida. Studies of fertilization in a variety of species have shown that sperm lie flat on the surface of the zona, then penetrate it at an oblique angle to its outer surface (Dickmann and Dziuk, 1964; Yanagimachi, 1966; Bedford, 1972; Yanagimachi and Phillips, 1984). The presence of cumulus cells around the oocyte might direct sperm towards the zona at such an angle (Shalgi and Phillips, 1983), since sperm penetrate the ZP of cumulus-free oocytes in vitro at an angle of 90° (Sato and Blandau, 1979a; Gaddum-Rosse et al., 1984). Hence, although zona penetration was not studied in the plains mouse, it is probable that the sperm head would similarly lie flat on the surface of the zona. During penetration of the ZP, therefore, the anterior margin of the sperm head, namely the dorsal surface of the dorsal hook, would form the leading edge (see Text Fig. 3). The two ventral hooks would probably be situated behind the dorsal hook, and would not be in direct contact with the zona material in the direction of sperm penetration. It is noteworthy that the dorsal hook contains highly cross-linked nuclear material, whereas the ventral hooks do not. This may impart sufficient rigidity to the dorsal hook to enable it to withstand the rigors of zona penetration, whereas this is unnecessary in the ventral hooks. Hence, the ventral hooks could not assume a principal role in penetration of the ZP, although sideways movements of the sperm head would enable them to assist in this process, but only on one side of the penetration slit, and not at its leading edge. However, in the constricted confines of the ZP penetration slit, it is unlikely that large amplitude oscillations of the sperm head would be possible, (Yanagimachi, 1966; Sato and Blandau, 1979a), thereby further

TEXT FIGURE 3

Hypothetical orientation of the plains mouse sperm head during binding to, and penetration of, the zona pellucida (ZP). The nucleus (N), post-acrosomal dense lamina (DL), equatorial segment (ES), ventral hooks (V), sub-acrosomal dorsal ridges (DR), and vesiculated acrosomal ghost in the principal segment (PS) are shown.

- A. The sperm head lies flat on the surface of the zona pellucida, and binding occurs over the acrosomal ghost, or plasma membrane in the equatorial segment.

- B,C. During penetration of the zona pellucida, the dorsal surface of the sperm head would be the first point of contact (arrows). This region contains either the dorsal ridges (DR), or the equatorial segment (ES) on the anterior part of the dorsal hook. The two ventral hooks (V) would probably be situated in the ventral concavity of the dorsal hook.



restricting the effectiveness of the two ventral hooks.

2.2.b Orientation of the Three Hooks

The orientation of the three hooks is symmetrical near their base, but towards the tip of the hooks, the dorsal hook becomes tilted to one side, and the two ventral hooks move off line in a stacked arrangement (see Section 2, Fig. 5). At this point, it is not known if this orientation is maintained during penetration of the ZP. However, assuming that it is, such an arrangement could conceivably protect the hooks from damage. It only occurs near the tip of the hooks, where all three are thin. The slight concavity in the dorsal hook could provide space for the close apposition of the middle hook, and the constricted dorsal and ventral margins of the ventral hooks might facilitate the close apposition of these two structures. Such an arrangement would produce a compact and resistant tip, with the close proximity of the three hooks to each other providing enhanced rigidity and protection. The overall shape of the sperm head, therefore, would not be unlike other murid rodents (Friend, 1936). The hook on the sperm head of the rat and laboratory mouse is triangular in shape, and remains so near the tip of the hook (Lalli and Clermont, 1981; Flaherty, unpublished observation). This is unlike the tip of the dorsal hook on the plains mouse sperm head, which is very thin and dorso-ventrally flattened, and possibly also less rigid, requiring the closeness of the ventral hooks for structural support.

2.2.c Possible Structural Adaptations to Zona Penetration

Bedford and Calvin (1974a) demonstrated that the nucleus and perinuclear material are stabilized by disulphide bonds in many, if not all, eutherian sperm, and the degree of cross-linking is higher in the

anterior regions of the sperm head. This has been suggested to be an adaptation to penetration of the resilient zona pellucida (ZP) (Bedford and Calvin, 1974a; Bedford, 1983b). Similarly, in plains mouse sperm, the nuclear material and pseudoperforatorium in the dorsal hook have been shown to become stabilized by disulphide bonds during transit through the epididymis. The nucleus posterior to the hooks has a lower degree of cross-linking, presumably since it would not be the major region of the sperm head pushing against the ZP. Contrary to a previous report (Flaherty and Breed, 1983a), the sub-acrosomal material in the core of the ventral hooks is also cross-linked by disulphide bonds. The caudal half of the hooks was found to be most resistant to treatment with SDS and DTT, suggesting that a higher degree of disulphide bonding may be necessary to stabilize the base of the hooks, due to the relative paucity of actin filaments in this region. In Section 3, it was proposed that the main function of actin filaments was to shape the ventral hooks during step 13 of spermiogenesis. However, in mature sperm, the actin filaments may also help to retain the shape and rigidity of the hooks, as proposed by Flaherty et al. (1983). Incubation of sperm in SDS results in elongation and swelling of the ventral hooks, as well as loss of their curvature and distinctive shape. The disulphide cross-linked core of SAM persists after such treatment, although the actin filaments, dense lamina and other SAM are solubilized (see Section 4). Thus, it would seem that disulphide bonding does not maintain the shape or curvature of the hooks, such a function probably being performed by the actin filaments. At this stage, it seems unlikely that the actin filaments would have a contractile role in cauda epididymal sperm, since electrophoretic analysis of sperm extracts failed to reveal any polypeptides corresponding to either the heavy or light chains of myosin. Hence, the actin filaments probably assume a

skeletal role in maintaining the shape, curvature and rigidity of the ventral hooks.

There is an accumulation of SAM on the dorsal margin of the sperm head, including the dorsal hook, and two disulphide bonded ridges indent the principal segment of the acrosome. However, the ridges are only present from about two thirds of the way along the dorsal hook, to the posterior border of the acrosome, which is the area enclosed by the principal segment. During the acrosome reaction, the contents of this segment would be released, exposing the two sub-acrosomal ridges, covered by the resilient inner acrosomal membrane (Huang and Yanagimachi, 1985). The position of the ridges suggests that they may serve to concentrate force on the zona, although the significance of two ridges, rather than one, is unknown at present.

The PAR is located on the caudal and ventral margins of the sperm head, along the ventral margin of the dorsal hook as in rat and laboratory mouse sperm (Bishop and Walton, 1960), and also constitutes the peripheral layer of the ventral hooks. This region has been implicated as the initial site of sperm-egg fusion (Yanagimachi and Noda, 1970c; 1970d), although more recent studies suggest that the equatorial segment represents a specialized area for fusion (Bedford et al., 1979; Moore and Bedford, 1983). Ultrastructural analysis of the PAR in a variety of species has revealed that the plasma membrane is firmly attached to the underlying dense lamina, and this tight association is mediated by parallel rows of ridges or projections (Fawcett and Ito, 1965; Gordon, 1972; Pedersen, 1972; Olson et al., 1983a). A similar structural arrangement occurs in plains mouse sperm, and the plasma membrane was difficult to remove completely with Triton X-100, especially on the ventral hooks. This suggests that one of the major functions of the post-acrosomal dense lamina (PADL) may be to

firmly attach the plasma membrane to the sperm head. Thus, after the acrosome reaction, the entire exposed surface of the sperm head would be stabilized and protected against damage, since the inner acrosomal membrane is firmly attached to the underlying SAM, and the equatorial segment is stabilized by periodic bridges linking the inner and outer acrosomal membranes (Bedford et al., 1979). Unsuccessful attempts were made to study the possible involvement of the PADL in the ventral hooks during sperm-egg fusion. However, it is difficult to envisage why ventral hooks should have developed for such a function, since the PADL and equatorial segment, which are regarded as the regions involved in fusion (Yanagimachi, 1981), cover much of the remainder of the sperm head. Regardless of the exact function of the hooks, however, the presence of the PADL on the ventral hooks could be viewed as a structural adaptation to prevent damage or loss of the plasma membrane on the hooks during penetration of the ZP.

In the plains mouse, the equatorial segment occupies much of the lateral surface of the sperm head, as in the rat and laboratory mouse (Phillips, 1977a; 1977b). It is also found on the lateral and ventral surfaces of the dorsal hook, as well as on its dorsal surface near the tip. A similar distribution occurs on the apical hook of the rat sperm head (Lalli and Clermont, 1981), although the functional significance of such an arrangement is obscure. In spatulate sperm, the equatorial segment is present in the mid-posterior region as a band or crescentic area (Nicander and Bane, 1966). In the plains mouse sperm head, not only does the equatorial segment occur as a band on the lateral surfaces, but also along the dorsal hook. This would seem to be unnecessary for sperm-egg fusion, unless a slightly different mechanism of fusion or incorporation occurs. In addition, it would expose an important region of the sperm head to damage during zona penetration.

It has been postulated that several structural features have arisen to protect the equatorial segment of eutherian sperm. This segment is either situated in a slight recess, or has a ridge of SAM on its anterior border (Bedford et al., 1979), and fine striations or bridges link the inner and outer acrosomal membranes (Wooding, 1973; Bedford et al., 1979; Russell et al., 1980). In the present study, three features were noted which would tend to protect the equatorial segment of plains mouse sperm during zona penetration. Firstly, cross-bridges link the acrosomal membranes in this region. A pattern of regularly arranged particles was also found on the outer acrosomal membrane, which in bull sperm, correlates with these cross-bridges (Olson and Winfrey, 1985). Secondly, the ventral and caudal borders of the equatorial segment are protected by a distinct ridge or outpushing of the PADL, and an accumulation of SAM. This has also been studied in other eutherian sperm (Courstens et al., 1976). And thirdly, a deep depression occurs at the base of the hooks, which also represents the anterior border of the equatorial segment. This extends caudally as an indentation of the sperm head at the junction of the principal and equatorial segments. Hence, the equatorial segment is situated in a slight hollow, and its ventral and caudal borders are protected by an overlying ridge. The equatorial segment on the dorsal hook, however, seems to be unprotected, although it may be stabilized to a greater degree than on the lateral surfaces of the sperm head, obviating the need for further structural adaptations to protect it during zona penetration. This is supported by observations that, after treatment of sperms heads with TX100 or SB₁₆, acrosomal remnants were found on the dorsal hook, but rarely in other regions of the sperm head (see Section 4).

The function of the two finger-like acrosomal projections at the base of the ventral hooks is unknown. These are a constant feature of

the hook structure, and are formed in an orderly manner during spermiogenesis. The contents of these projections are released during the acrosome reaction in vitro, but a function for the enzymes seems doubtful, since the projections are only open at the base of the hooks, which would not be in direct contact with the zona material at the leading edge of the penetration slit.

Hence, it appears that the plains mouse sperm head is suitably designed to concentrate significant shearing force on the zona pellucida, especially along the dorsal margin of the sperm head and dorsal hook. The shape of the sperm head, the shape and orientation of the hooks, the presence of dorsal sub-acrosomal ridges, and the rigidity of various sperm head structures can be viewed as structural adaptations to assist mechanical penetration of the zona pellucida. However, it seems unlikely that the two ventral hooks would assume a significant role in sperm penetration, due mainly to their position ventral to the dorsal hook, although this obviously requires experimental verification.

2.3 Relationship of Sperm Head Morphology to OCC Structure

Bedford (1983b) has postulated that the eutherian sperm head has undergone structural modifications to enable it to withstand penetration of the toughened oocyte investments. Though it may be doubtful that the ventral hooks on the sperm head of the plains mouse play a major role in penetration of the zona pellucida, they may contribute to this process. Hence, the structure of the oocyte-cumulus complex (OCC) was investigated to determine if the ventral hooks developed in response to an increase in the barriers to sperm penetration. However, it was found that in superovulated OCC, the cumulus cells are loosely arranged, a distinct corona radiata is absent, and the zona pellucida is quite thin. Thus, the structural features of the plains mouse OCC do not appear to

represent significantly greater barriers to sperm penetration. A study of the OCC structure in two other hydromyine rodents has revealed considerable interspecific variation (McGregor, 1985). In the western chestnut mouse, Pseudomys nanus, the organization of the cumulus oophorus is similar to the plains mouse, but the zona is considerably thicker (10 versus 6 μm). This is unusual, since the sperm head morphology is similar in both species, though the ventral hooks are actually less well developed in the chestnut mouse (Breed, 1983). In the hopping mouse, Notomys alexis, the sperm head has a short, truncated dorsal hook, but ventral hooks are usually absent (Breed and Sarafis, 1983). However, in the OCC of this species, the cumulus cells are large and tightly packed, a corona radiata is present, and the zona pellucida is 11-12 μm in thickness (McGregor, 1985). Hence, from these studies it would appear that there is little or no correlation between the presence of three hooks on the sperm head, and increased barriers to sperm penetration afforded by the zona pellucida and cumulus oophorus. However, such a statement assumes that the observed structural features are truly indicative of tubal OCC after spontaneous ovulation, though to date, these have not been studied. It has also been shown that the biochemical properties of the zona pellucida vary between eutherian species (Dunbar, 1983), suggesting that although the plains mouse zona is thin, its structural and biochemical properties may render it more difficult to penetrate, thereby necessitating the three hooks. However, it is difficult to reconcile such significant biochemical and morphological differences between species, especially in an isolated, monophyletic group such as the Hydromyinae (Watts and Aslin, 1981).

2.4 Significance of Variation in Sperm Head Morphology in the Hydromyinae

The Hydromyinae comprises 12 genera, which are subdivided into 3

tribes, the Uromyini, Conilurini and Hydromyini. Karyotypic and electrophoretic studies have revealed that this sub-family is probably monophyletic, and diverged from the ancestral stock about 15-20 million years ago (mya) (Baverstock et al., 1977, 1983; Watts and Aslin, 1981). Studies of sperm morphology in the Hydromyinae have shown that in 25 out of 30 species, including representatives from all 12 genera, three hooks occur on the apical margin of the sperm head (Breed and Sarafis, 1979; Breed, 1980). It has thus been suggested that a three-hooked sperm head probably represents the ancestral form. In those species in which the hooks are reduced or absent, this would be a secondarily derived condition (Breed and Sarafis, 1979; Breed, 1983; 1984).

To date, however, it has not been possible to explain why such an unusual sperm head morphology appeared, or if it has any functional significance. Further studies are required to determine if the ventral hooks are involved in penetration of the zona pellucida. An alternative hypothesis, therefore, is that the ancestral hydromyine sperm head morphology may have arisen as a post-mating reproductive isolation mechanism (Dobzhansky et al., 1977; Nei et al., 1983), resulting in isolation of the Hydromyinae from the ancestral murid stock. Phillips et al (1985) have also suggested that variations in sperm morphology may have evolved as reproductive isolation mechanisms. This probably would have taken place about 15-20 mya. The appearance of three hooks could be an example of a punctuated speciation event, arising as a pleiotropic effect due to mutation of a single regulatory gene (Gould, S.J., 1980). Alternatively, the ancestral sperm head morphology may have arisen by allopatric speciation (Dobzhansky et al., 1977). It is difficult to envisage how such an isolation mechanism would have functioned, but it may have involved a differential distribution of zona receptor sites on the sperm head (O'Rand et al, 1985; Sullivan and Bleau, 1985), or the

ventral hooks may have carried a factor necessary for activation of the oocyte.

In those species which possess three hooks on the sperm head, there is considerable inter-specific variation in the absolute and relative lengths of the hooks, their orientation, and the size and shape of the sperm head posterior to the hooks (Breed and Sarafis, 1979; Breed, 1980; 1983; 1984). This variation probably arose as a result of speciation and radiation of the Hydromyinae, either before or after the invasion of the Australian continent. The time at which this occurred is unknown, but it is thought that the Uromyini and Hydromyini entered Australia less than 2 mya, whereas the Conilurini, of which the plains mouse is a member, radiated into Australia about 5-10 mya (Watts and Aslin, 1981; Hand, 1984). The conservation of the three-hooked sperm head morphology in 25 out of 30 species (Breed and Sarafis, 1979; Breed, 1980), may also reflect the high degree of conservation of karyotypes in this group (Baverstock et al., 1983).

In some species of Pseudomys and Notomys, however, the hooks have become reduced or lost altogether, though this is probably a secondarily derived character (Breed, 1983; 1984). It is possible that pre-mating isolation mechanisms developed during the radiation of the Hydromyinae, thus obviating the need for the ventral hooks to function as a post-mating isolation mechanism, and might therefore explain why some species reverted to the ancestral murid sperm head morphology or to other forms. One in particular, P. shortridgei, which is genetically distinct from the other members of this genus (Baverstock et al., 1981), has diverged significantly and has an ovoid sperm head, with a rather bulbous acrosome (Breed, 1983). It also suggests that, if the ventral hooks do have some functional significance in sperm penetration, then it has been lost during this divergence. However, this must remain speculative

until fertilization has been studied in these species.

Another possible explanation for the presence of the three hooks is that they arose through a punctuated evolutionary event, but are of no adaptive value and merely represent an example of non-adaptive variation (Ayala, 1978). This probably would have occurred at the time of divergence of the group (Watts and Aslin, 1981). Whilst the orientation of the hooks may preclude any function in the events of fertilization, as discussed in 2.2, it would not be detrimental to sperm penetration of the OCC. Hence, the reduction in size or loss of the hooks in the sperm of some species may simply represent the loss of a non-adaptive characteristic. Indeed, Cummins (1980) has suggested that variations in mammalian sperm morphology may be due to genetic drift rather than selective pressures.

Another possible explanation for the occurrence of the ventral hooks is that they developed in response to markedly increased barriers to sperm penetration afforded by the egg investments. However, as a result of speciation and radiation within the Hydromyinae during the last 20 mya, these increased barriers may have become unnecessary and therefore reduced, although the ventral hooks still remain in many species as an evolutionary "appendix" of neutral selective value.

3. CONCLUSIONS

The present study has investigated the structural and functional aspects of sperm head morphology in the plains mouse, Pseudomys australis, with special emphasis on the two ventral hooks, which are a characteristic feature of the hydromyine rodents.

Using a variety of ultrastructural and electrophoretic techniques, it has been shown for the first time that the ventral hooks consist of a core of sub-acrosomal material, enclosed within an extension of the

post-acrosomal dense lamina. Bundles of actin filaments maintain the curvature of the hooks, and the shape of their apical regions. The dorsal hook has many structural features in common with other hooked murid sperm, whilst the sperm head posterior to the hooks follows the basic mammalian pattern. During epididymal maturation, the nucleus and sub-acrosomal material become stabilized by disulphide cross-linking, especially in the dorsal and ventral hooks.

Preliminary attempts to study gamete interactions in vitro have been largely unsuccessful, due to difficulties in superovulating female plains mice, and unsuitable culture conditions. Nevertheless, the possible functional significance of the hooks has been discussed. After an analysis of the structural design of the sperm head, the features of the oocyte-cumulus complex, and the variability in sperm head morphology within the Hydromyinae, it is concluded that the ventral hooks have probably not arisen to aid penetration of the spermatozoon through the oocyte investments. Instead, it is suggested that they may have arisen as a post-mating reproductive isolation mechanism during the divergence of the Hydromyinae from the ancestral murid stock about 20 mya, or they may represent an example of non-adaptive variation. However, further studies are required to clarify these issues, and a clear picture of the significance of these unique structures will not be obtained until more is known about the biophysical properties of the zona pellucida, and the process of fertilization in the plains mouse.

APPENDIX 1

Conditions of Housing of Animals

All the animals used in this study were housed in the Medical School animal house at the University of Adelaide. They were maintained on either a 12L:12D or 14L:10D lighting regime, at a regulated temperature of 20-25°C. Males and females were caged separately in groups of 3-6 per cage. For breeding, one male and one or two females were housed together, and young were weaned at about 28 days of age. The animals were fed on mouse pellets, given water al libitum, and mixed seed and fresh fruit was supplied once a week. For in vitro fertilization experiments, males were housed individually for at least 3 weeks before use.

APPENDIX 2

Perfusion Fixation Solutions

1. Rinse Solution : 9.0 g NaCl
 25.0 g polvinylpyrrolidone (PVP, 40 kd)
 0.25 g heparin
 5.0 g procain-hydrochloride

Dissolve in 1 litre of dist. H₂O, adjust to pH 7.4, and millipore filter (0.45 µm).

2. Fixative I : 45 ml 0.2M NaH₂PO₄
 405 ml 0.2M Na₂HPO₄
 60 ml 25% glutaraldehyde
 60 ml 25% formaldehyde
 25 g PVP

Stir till dissolved, adjust to pH 7.4, make up to 1 litre with dist. H₂O, and filter.

3. Fixative 2 : 45 ml 0.2M NaH₂PO₄
 405 ml 0.2M Na₂HPO₄
 120 ml 25% glutaraldehyde
 120 ml 25% formaldehyde
 25 g PVP

Stir till dissolved, adjust to pH 7.4, make up to 1 litre with dist. H₂O, and filter.

(Forssmann et al., 1977)

4. Azide-containing fixative : 45 ml 0.2M NaH₂PO₄
 405 ml 0.2M Na₂HPO₄
 200 ml 25% glutaraldehyde
 1.0 g sodium azide (NaN₃)

Dissolve NaN₃, adjust to pH 7.4, make up to 1 litre with dist. H₂O, and filter.

(Based on Minassian and Huang, 1979).

APPENDIX 3

Sperm Counting Procedure

- Prepare a 1:10 or 1:20 dilution of the sperm suspension with 10% buffered neutral formalin.
- Apply to haemocytometer, and count number of sperm in N squares (area of squares = 0.04 mm^2).

$$x = \frac{n \times D}{N \times V} \quad \text{sp/mm}^3$$

$$= \frac{n \times D \times 10^3}{N \times V} \quad \text{sp/ml}$$

where n = total number of sperm counted
 D = dilution
 N = number of squares counted
 V = volume of each square

- Haemocytometer depth = $\frac{1}{3}$ 0.1 mm. Hence volume (V) represented by each square is 0.004 mm^3 .

$$x = \frac{n \times D \times 10^3}{N \times 0.004} \quad \text{sp/ml}$$

$$= \frac{n \times D \times 10^6}{N \times 4} \quad \text{sp/ml}$$

if D = 10, N = 5

$$x = \frac{n}{2} \times 10^6 \text{ sp/ml}$$

APPENDIX 4

SDS-PAGE Components

Stacking Gel : 4.2% acrylamide (20:1 acryl./bis-acryl.)
0.125M tris-HCL, pH 6.8
0.1% SDS (sodium dodecyl sulphate)
0.6 μ l TEMED (Tetra methylethylene diamine)
125 μ l riboflavin (4 mg/100 ml)

Separating Gel : 5-15% acrylamide (36.5 : 1 acryl./bis-acryl.)
0.125M tris-HCL, pH 8.9
0.1% SDS
18.25% sucrose (15% gel only)
0.25 μ l/ml TEMED
3 μ l/ml 10% ammonium persulphate

Electrode Buffer : 0.025M tris-glycine, pH 8.3
0.1% SDS

Loading Buffer : 62.5 mM tris-HCL, pH 6.8
10% glycerol
2% SDS
50 μ l/ml B-mercapto-ethanol

APPENDIX 5

Chemical Composition of Culture Media

COMPONENT	FINAL CONCENTRATION IN CM (mM)			
	HBT6	HT6	TCM	HTCM
NaCl	58.0	99.4	94.6	94.6
KCL	1.42	1.42	4.78	4.78
MgSO ₄ .7 H ₂ O	0.71	0.71	1.19	1.19
Na ₂ HPO ₄	0.36	0.36	-	-
KH ₂ PO ₄	-	-	1.19	1.19
NaHCO ₃	52.0	4.0	25.07	4.0
CaCl ₂ .2H ₂ O	1.78	1.78	1.71	1.71
Na. Hepes	-	21.0	-	21.0
Glucose	5.56	5.56	5.56	5.56
Na. lactate	24.9	24.9	21.58	21.58
Na. pyruvate	0.47	0.47	0.50	0.50
Phenol red	0.001%	0.001%	0.001%	0.001%
Penicillin G (µg/ml)	60	60	60	60
Strep. sulphate (µg/ml)	50	50	50	50
* BSA (mg/ml)	15	5	4	4
osmolality (mOsm)	285	285	308	308
** pH	7.5-7.6	7.5	7.4	7.5

* HBT6/HT6 - Fraction V BSA
 TCM/HTCM - Fatty acid-free Fraction V BSA

** HBT6/TCM - after 8 hour equilibration with 5% CO₂ in air
 HT6/HTCM - in air

NOTE - 10x stock solutions were prepared individually for pyruvate, CaCl₂, hepes and NaHCO₃, whilst the other components were all present in another stock.

BIBLIOGRAPHY

- ACOTT, T.S. and HOSKINS, D.D. (1978).
Bovine sperm forward motility protein. Partial purification and characterization.
J. Biol. Chem. 253 : 6744-6750
- ACOTT, T.S. and HOSKINS, D.D. (1981).
Bovine sperm forward motility protein : binding to epididymal spermatozoa.
Biol. Reprod. 24 : 234-240
- ACOTT, T.S. and CARR, D.W. (1984).
Inhibition of bovine spermatozoa by caudal epididymal fluid: II. Interaction of pH and a quiescence factor.
Biol. Reprod. 30 : 926-935
- ACOTT, T.S., JOHNSON, D.J., BRANDT, H. and HOSKINS, D.D. (1979).
Sperm forward motility protein : tissue distribution and species cross reactivity.
Biol. Reprod. 20 : 247-252
- ACOTT, T.S., KATZ, D.F. and HOSKINS, D.D. (1983).
Movement characteristics of bovine epididymal spermatozoa : effects of forward motility protein and epididymal maturation.
Biol. Reprod. 29 : 389-399
- AFZELIUS, B. (1959).
Electron microscopy of the sperm tail. Results obtained with a new fixative.
J. Biophys. Biochem. Cytol. 5 : 269-278
- AHUJA, K.K. (1982).
Fertilization studies in the hamster : The role of cell-surface carbohydrates.
Exp. Cell Res. 140 : 353-362
- AHUJA, K.K. (1984).
Lectin-coated agarose beads in the investigation of sperm capacitation in the hamster.
Dev. Biol. 104 : 131-142
- AHUJA, K.K. and TZARTOS, S.J. (1981).
Investigation of sperm receptors in the hamster zona pellucida by using univalent (Fab) antibodies to hamster ovary.
J. Reprod. Fert. 61 : 257-264
- AHUJA, K.K. and BOLWELL, G.P. (1983).
Probable asymmetry in the organization of components of the hamster zona pellucida.
J. Reprod. Fert. 69 : 49-55

- AITKEN, R.J., HOLME, E., RICHARDSON, D.W. and HULME, M. (1982).
Properties of intact and univalent (Fab) antibodies raised against
isolated, solubilized, mouse zonae pellucidae.
J. Reprod. Fert. 66 : 327-334
- ALLISON, A.C. and HARTREE, E.F. (1970).
Lysosomal enzymes in the acrosome and their possible role in
fertilization.
J. Reprod. Fert. 21 : 501-515
- AMANN, R.P., HAY, S.R. and HAMMERSTEDT, R.H. (1982).
Yield, characteristics, motility and cAMP content of sperm isolated
from seven regions of the ram epididymis.
Biol. Reprod. 27 : 723-733
- ANDERSON, E. and ALBERTINI, D.F. (1976).
Gap junctions between the oocyte and companion follicle cells in
the mammalian ovary.
J. Cell Biol. 71 : 680-686
- AUSTIN, C.R. (1948).
Function of hyaluronidase in fertilization.
Nature 162 : 63-64
- AUSTIN, C.R. (1951a).
Observations on the penetration of the sperm into the mammalian
egg.
Aust. J. Sci. Res. B. 4 : 581-596
- AUSTIN, C.R. (1951b).
The formation, growth and conjugation of the pronuclei in the rat
egg.
JJ. Roy. Microsc. Soc. 71 : 295-306
- AUSTIN, C.R. (1952).
The "capacitation" of the mammalian sperm.
Nature 170 : 326
- AUSTIN, C.R. (1956).
Cortical granules in hamster eggs.
Exp. Cell Res. 10 : 533-540
- AUSTIN, C.R. (1957).
Fertilization, early cleavage and associated phenomena in the field
vole (Microtus agrestis).
J. Anat. 91 : 1-11
- AUSTIN, C.R. (1961).
The Mammalian Egg.
C.C. Thomas Publ., Springfield, Ill.
- AUSTIN, C.R. (1967).
Capacitation of spermatozoa.
Int. J. Fert. 12 : 25-31

- AUSTIN, C.R. and BISHOP, M.W.H. (1958a).
Some features of the acrosome and perforatorium in mammalian spermatozoa.
Proc. Roy. Soc. B. 149 : 234-240
- AUSTIN, C.R. and BISHOP, M.W.H. (1958b).
Role of the rodent acrosome and perforatorium in fertilization.
Proc. Roy. Soc. B. 149 : 241-248
- AUSTIN, C.R. and BRADEN, A.W.H. (1954).
Time relations and their significance in the ovulation and penetration of eggs in rats and rabbits.
Aust. J. Biol. Sci. 7 : 179-194
- AUSTIN, C.R. and BRADEN, A.W.H. (1956).
Early reactions of the rodent egg to spermatozoon penetration.
J. Exp. Biol. 33 : 358-365
- AUSTIN, C.R. and SHORT, R.V. (1982).
Reproduction in Mammals. Book 1. Germ cells and fertilization.
2nd Ed. Cambridge Univ. Press, Cambridge.
- AVRAMOVA, Z., DESSEV, G. and TSANEV, R. (1980).
DNA-associated proteins of ram sperm nuclei.
FEBS letts. 118 : 58-62
- AYALA, F.J. (1978).
The mechanisms of evolution.
Sci. Am. 239 : 48-61
- BABCOCK, D.F., STAMERJOHN, D.M. and HUTCHINSON, T. (1978).
Calcium redistribution in individual cells correlated with ionophore action on motility.
J. Exp. Zool. 204 : 391-400
- BACA, M. and ZAMBONI, L. (1967).
The fine structure of human follicular oocytes.
J. Ultrastruct. Res. 19 : 354-381
- BACCETTI, B. (1979).
The evolution of the acrosomal complex. In : The Spermatozoon. Maturation, motility, surface properties and comparative aspects. Fawcett, D.W. and Bedford, J.M. (eds.) pp.305-329
Urban and Schwarzenberg, Baltimore.
- BACCETTI, B., PALLINI, V. and BURRINI, A.G. (1976).
The accessory fibers of the sperm tail. III. High-sulfur and low-sulfur components in mammals and cephalopods.
J. Ultrastruct. Res. 57 : 289-308
- BAIRD, D.T. (1984).
The ovary. In : Reproduction in Mammals. Book 3. Hormonal Control of Reproduction. Austin, C.R. and Short, R.V. (eds.) 2nd Ed. pp.91-114. Cambridge Univ. Press, Cambridge.

- BALHORN, R. (1982).
A model for the structure of chromatin in mammalian sperm.
J. Cell Biol. 93 : 298-305
- BALHORN, R., GLEDHILL, B.L. and WYROBEK, A.J. (1977).
Mouse sperm chromatin proteins : quantitative isolation and partial characterization.
Biochemistry 16 : 4074-4080
- BALL, G.D., BELLIN, M.E., AX, R.L. and FIRST, N.L. (1982).
Glycosaminoglycans in bovine cumulus-occyte complexes : morphology and chemistry.
Mol. Cell. Endocr. 28 : 113-122
- BANE, A. and NICANDER, L. (1963).
The structure and formation of the perforatorium in mammalian sperm.
Int. J. Fert. 8 : 865-866
- BANGHAM, A.D. (1961).
Electrophoretic characteristics of ram and rabbit spermatozoa.
Proc. Roy. Soc. B. 155 : 292-305
- BARAK, L.S., YOCUM, R.R., NOTHNAGEL, E.A. and WEBB, W.W. (1980).
Fluorescence staining of the actin cytoskeleton in living cells with 7-nitrobenz-2-oxa-1, 3-diazole-phalloidin.
Proc. Natl. Acad. Sci. USA 77 : 980-984
- BARANSKA, W., KONWINSKI, M. and KUJAWA, M. (1975).
Fine structure of the zona pellucida of unfertilized egg cells and embryos.
J. Exp. Zool. 192 : 193-202
- BARFIELD, M.A. and BEEMAN, E.A. (1968).
The oestrus cycle in the Mongolian gerbil, Meriones unguiculatus.
J. Reprod. Fert. 17 : 247-251
- BARKER, L.D.S. and AMANN, R.P. (1971).
Epididymal physiology. II. Immunofluorescent analyses of epithelial secretion and absorption, and of bovine sperm maturation.
J. Reprod. Fert. 26 : 319-332
- BARON, C. and THOMPSON, T.E. (1975).
Solubilization of bacterial membrane proteins using alkyl glucosides and dioctanoyl phosphatidylcholine.
Biochim. Biophys. Acta. 382 : 276-285
- BARROS, C. and FRANKLIN, L.E. (1968).
Behaviour of the gamete membranes during sperm entry into the mammalian egg.
J. Cell. Biol. 37 : C13-C18
- BARROS, C. and YANAGIMACHI, R. (1971).
Induction of zona reaction in golden hamster eggs by cortical granule material.
Nature 233 : 268-269

- BARROS, C. and YANAGIMACHI, R. (1972).
Polyspermy-preventing mechanisms in the golden hamster egg.
J. Exp. Zool. 180 : 251-266
- BARROS, C. and BERRIOS, M. (1977).
Is the activated spermatozoon really capacitated?
J. Exp. Zool. 201 : 65-72
- BARROS, C. and HERRERA, E. (1977).
Ultrastructural observations on the incorporation of guinea-pig spermatozoa into zona-free hamster oocytes.
J. Reprod. Fert. 49 : 47-50
- BARROS, C., BEDFORD, J.M., FRANKLIN, L.E. and AUSTIN, C.R. (1967).
Membrane vesiculation as a feature of the mammalian acrosome reaction.
J. Cell. Biol. 34 : C1-C5
- BATTAGLIA, D.E. and YANAGIMACHI, R. (1979).
Enhanced and co-ordinated movement of the hamster oviduct during the periovulatory period.
J. Reprod. Fert. 56 : 515-520
- BATTAGLIA, D.E. and GADDUM-ROSSE, P. (1984).
Rat eggs normally exhibit a variety of surface phenomena during fertilization.
Gamete Res. 10 : 107-118
- BAVERSTOCK, P.R., WATTS, C.H.S. and HOGARTH, J.T. (1977).
Chromosome evolution in Australian rodents. I. The Pseudomyinae, the Hydromyinae and the Uromys/Melomys group.
Chromosoma 61 : 95-125
- BAVERSTOCK, P.R., WATTS, C.H.S., ADAMS, M. and COLE, S.R. (1981).
Genetical relationships among Australian rodents (Muridae).
Aust. J. Zool. 29 : 289-303
- BAVERSTOCK, P.R., WATTS, C.H.S., GELDER, M. and JAHNKE, A. (1983).
G-banding homologies of some Australian rodents.
Genetica 60 : 105-117
- BEARER, E.L. and FRIEND, D.S. (1980).
Anionic lipid domains : correlation with functional topography in a mammalian cell membrane.
Proc. Natl. Acad. Sci. USA 77 : 6601-6605
- BEARER, E.L. and FRIEND, D.S. (1982).
Modifications of anionic-lipid domains preceding membrane fusion in guinea pig sperm.
J. Cell. Biol. 92 : 604-615
- BEATTY, R.A. and SHARMA, K.N. (1960).
Genetics of gametes. III. Strain differences in spermatozoa from eight inbred strains of mice.
Proc. Roy. Soc. Edin. B. 68 : 25-53

- BEDFORD, J.M. (1963a).
Morphological changes in rabbit spermatozoa during passage through the epididymis.
J. Reprod. Fert. 5 : 169-177
- BEDFORD, J.M. (1963b).
Changes in the electrophoretic properties of rabbit spermatozoa during passage through the epididymis.
Nature 200 : 1178-1180
- BEDFORD, J.M. (1964).
Fine Structure of the sperm head in ejaculate and uterine spermatozoa of the rabbit.
J. Reprod. Fert. 7 : 221-228
- BEDFORD, J.M. (1965).
Changes in the fine structure of the rabbit sperm head during passage through the epididymis.
J. Anat. 99 : 891-906
- BEDFORD, J.M. (1966).
Development of the fertilizing ability of spermatozoa in the epididymis of the rabbit.
J. Exp. Zool. 163 : 319-330
- BEDFORD, J.M. (1967).
Observations on the fine structure of spermatozoa of the bush baby (Galago senegalensis), the African green monkey (Cercopithecus aethiops) and man.
Am. J. Anat. 121 : 443-460
- BEDFORD, J.M. (1968).
Ultrastructural changes in the sperm head during fertilization in the rabbit.
Am. J. Anat. 123 : 329-358
- BEDFORD, J.M. (1969).
Limitations of the uterus in the development of the fertilizing ability (capacitation) of spermatozoa.
J. Reprod. Fert. Suppl. 8 : 19-26
- BEDFORD, J.M. (1970).
Sperm capacitation and fertilization in mammals.
Biol. Reprod. Suppl. 2 : 128-158
- BEDFORD, J.M. (1972).
An electron microscopic study of sperm penetration into the rabbit egg after natural mating.
Am. J. Anat. 133 : 213-254
- BEDFORD, J.M. (1974a).
Biology of primate spermatozoa.
Contrib. Primatol. 3 : 97-139

- BEDFORD, J.M. (1974b).
Mechanisms involved in penetration of spermatozoa through the investments of the mammalian egg. In : Physiology and Genetics of Reproduction. Coutinho, E.M. and Fuchs, F. (Eds). Pt. B. pp.55-68. Plenum Press, New York.
- BEDFORD, J.M. (1975).
Maturation, transport, and fate of spermatozoa in the epididymis. In : Handbook of Physiology. Section 7, Vol. V, pp.303-317. Hamilton, D.W. and Greep, R.O. (Eds.) Amer. Physiol. Soc., Washington.
- BEDFORD, J.M. (1977).
Sperm/egg interaction : the specificity of human spermatozoa. Anat. Rec. 188 : 477-488
- BEDFORD, J.M. (1983a).
Significance of the need for sperm capacitation before fertilization in eutherian mammals. Biol. Reprod. 28 : 108-120
- BEDFORD, J.M. (1983b).
Form and function of eutherian spermatozoa in relation to the nature of egg vestments. In : Fertilization of the Human Egg In Vitro. Beier, H.M. and Lindner, N.R. (Eds.) pp.133-196. Springer-Verlag, Berlin.
- BEDFORD, J.M. and CHANG, M.C. (1962).
Removal of decapacitation factor from seminal plasma by high-speed centrifugation. Am. J. Physiol. 202 : 179-181
- BEDFORD, J.M. and NICANDER, L. (1971).
Ultrastructural changes in the acrosome and sperm membranes during maturation of spermatozoa in the testis and epididymis of the rabbit and monkey. J. Anat. 108 : 527-543
- BEDFORD, J.M. and CALVIN, H.I. (1974a).
The occurrence and possible functional significance of -S-S- crosslinks in sperm heads, with particular reference to eutherian mammals. J. Exp. Zool. 188 : 137-156
- BEDFORD, J.M. and CALVIN, H.I. (1974b).
Changes in -s-s- linked structures in the sperm tail during epididymal maturation, with comparative observations in sub-mammalian species. J. Exp. Zool. 187 : 181-204
- BEDFORD, J.M. and CROSS, N.L. (1978).
Normal penetration of rabbit spermatozoa through a trypsin- and acrosin- resistant zona pellucida. J. Reprod. Fert. 54 : 385-392

- BEDFORD, J.M. and WITKIN, S.S. (1983).
Influence of complement depletion on sperm function in the female rabbit.
J. Reprod. Fert. 69 : 523-528
- BEDFORD, J.M., CALVIN, H. and COOPER, G.W. (1973).
The maturation of spermatozoa in the human epididymis.
J. Reprod. Fert. Suppl. 18 : 199-213
- BEDFORD, J.M., MOORE, H.D.M. and FRANKLIN, L.E. (1979).
Significance of the equatorial segment of the acrosome of the spermatozoon in eutherian mammals.
Exp. Cell. Res. 119 : 119-126
- BELLVE, A.R. and CARRAWAY, R. (1979).
Characterization of two basic chromosomal proteins isolated from mouse spermatozoa.
J. Cell. Biol. 79 : 177a
- BELLVE, A.A.R. and O'BRIEN, D.A. (1983).
The mammalian spermatozoon : structure and temporal assembly. In : Mechanism and Control of Animal Fertilization. Hartmann, J.F. (Ed.) pp.55-137.
Academic Press, New York.
- BELLVE, A.R., ANDERSON, E. and HANLEY-BOWDOIN, L. (1975).
Synthesis and amino acid composition of basic proteins in mammalian sperm nuclei.
Dev. Biol. 47 : 349-365
- BERNSTEIN, M.H. and TEICHMAN, R.J. (1972).
Regional differentiation in the heads of spermatozoa of rabbit, man and bull.
Am. J. Anat. 133 : 165-178
- BERRIOS, M., FLECHON, J.E. and BARROS, C. (1978).
Ultrastructure of Octodon degus spermatozoon with special reference to the acrosome.
Am. J. Anat. 151 : 39-54
- BERRUTI, G. and MARTEGANI, E. (1984).
Dansylalanylsylchloromethyl ketone as a fluorescent probe for localization of acrosin activity in boar and human spermatozoa.
J. Histochem. Cytochem. 32 : 526-530
- BHATTACHARYYA, A.K., GOODPASTURE, J.D. and ZANEVELD, L.J.D. (1979).
Acrosin of mouse spermatozoa.
Am. J. Physiol. 237 : E40-E44
- BHATTACHARYYA, A.K. and ZANEVELD, L.J.D. (1983).
The sperm head. In : Biochemistry of Mammalian Reproduction. Zaneveld, L.J.D. and Chatterton, S.K. (Eds.) Pt. 1, pp.119-151.
Wiley, New York.
- BISHOP, M.W.H. and AUSTIN, C.R. (1957).
Mammalian spermatozoa.
Endeavour 16 : 137-150

- BISHOP, M.W.H. and WALTON, A. (1960).
Spermatogenesis and the structure of mammalian spermatozoa. In :
Marshall's Physiology of Reproduction. Parkes, A.S. (Ed.). Vol. 1,
pp.1-
Longmans, London.
- BLANDAU, R.J. and ODOR, D.L. (1949).
The total number of spermatozoa reaching various segments of the
reproductive tract in the female albino rat at intervals after
insemination.
Anat. Rec. 103 : 93-109
- BLANDAU, R.J. and ODOR, D.L. (1952).
Observations on sperm penetration into the ooplasm and changes in
the cytoplasmic components of the fertilizing spermatozoon in rat
ova.
Fertil. Steril. 3 : 13-26
- BLANDAU, R.J. and RUMERY, R.E. (1964).
The relationship of swimming movements of epididymal spermatozoa to
their fertilizing capacity.
Fertil. Steril. 15 : 571-579
- BLAQUIER, J.A., CAMEO, M.S. and BURGOS, M.H. (1972).
The role of androgens in the maturation of epididymal spermatozoa
in the guinea pig.
Endocrinology 90 : 839-842
- BLEIL, J.D. and WASSARMAN, P.M. (1980a).
Structure and function of the zona pellucida : Identification and
characterization of the proteins of the mouse oocyte's zona
pellucida.
Dev. Biol. 76 : 185-202
- BLEIL, J.D. and WASSARMAN, P.M. (1980b).
Synthesis of zona pellucida proteins by denuded and follicle-
enclosed mouse oocytes during culture in vitro.
Proc. Natl. Acad. Sci. USA 77 : 1029-1033
- BLEIL, J.D. and WASSARMAN, P.M. (1980c).
Mammalian sperm-egg interaction : identification of a glycoprotein
in mouse egg zonae pellucidae possessing receptor activity for
sperm.
Cell. 20 : 873-882
- BLEIL, J.D. and WASSARMAN, P.M. (1983).
Sperm-egg interactions in the mouse : sequence of events and
induction of the acrosome reaction by a zona pellucida
glycoprotein.
Dev. Biol. 95 : 317-324
- BLEIL, J.D., BEALL, C.F. and WASSARMAN, P.M. (1981).
Mammalian sperm-egg interaction : fertilization of mouse eggs
triggers modification of the major zona pellucida glycoprotein,
ZP2.
Dev. Biol. 86 : 189-197

- BLOOM, G. and NICANDER, L. (1961).
On the ultrastructure and development of the protoplasmic droplet of spermatozoa.
Z. Zellforsch Mikrosk. Anat. 55 : 833-834
- BOUSQUET, D., LEVEILLE, M.C., ROBERTS, K.D., CHAPDELAINE, A. and BLEAU, G. (1981).
The cellular origin of the zona pellucida antigen in the human and hamster.
J. Exp. Zool. 215 : 215-218
- BRADEN, A.W.H. (1952).
Properties of the membranes of rat and rabbit eggs.
Aust. J. Sci. Res. Ser. B. 5 : 460-471
- BRADEN, A.W.H. (1953).
Distribution of sperms in the genital tract of the female rabbit after coitus.
Aust. J. Biol. Sci. 6 : 693-705
- BRADEN, A.W.H. and AUSTIN, C.R. (1954).
The number of sperms about the eggs in mammals and its significance for normal fertilisation.
Aust. J. Biol. Sci. 7 : 543-551
- BRADEN, A.W.H., AUSTIN, C.R. and DAVID, H.A. (1954).
The reaction of the zona pellucida to sperm penetration.
Aust. J. Biol. Sci. 7 : 391-409
- BRADFORD, M.M., McRORIE, R.A. and WILLIAMS, W.L. (1976).
Involvement of esterases in sperm penetration of the corona radiata of the ovum.
Biol. Reprod. 15 : 102-106
- BRADFORD, M.M., DUDKIEWICZ, A.B., PENNY, G.S. DYCKES, D.F., BURLEIGH, B.D., WOOLEY, R.E. and McRORIE, R.A. (1981).
Localization of proacrosin on the inner acrosomal membrane of spermatozoa in rabbits and hamsters.
Am. J. Vet. Res. 42 : 1082-1083
- BRADLEY, M.P., RAYNS, D.G. and FORRESTER, I.T. (1980).
Effects of filipin, digitonin, and polymyxin B on plasma membrane of ram spermatozoa - an EM study.
Arch. Androl. 4 : 195-204
- BREED, W.G. (1980).
Further observations on spermatozoal morphology and male reproductive tract anatomy of Pseudomys and Notomys species (Mammalia : Rodentia).
Trans. Roy. Soc. S. Aust. 104 : 51-55
- BREED, W.G. (1983).
Variation in sperm morphology in the Australian rodent genus, Pseudomys (Muridae).
Cell Tiss. Res. 229 : 611-625

- BREED, W.G. (1984).
Sperm head structure in the Hydromyinae (Rodentia : muridae) : A further evolutionary development of the subacrosomal space in mammals.
Gamete Res. 10 : 31-44
- BREED, W.G. and SARAFIS, V. (1979).
On the phylogenetic significance of spermatozoal morphology and male reproductive tract anatomy in Australian rodents.
Trans. Roy. Soc. S. Aust. 103 : 127-135
- BREED, W.G. AND SARAFIS, V. (1983).
Variation in sperm head morphology in the Australian rodent Notomys alexis.
Aust. J. Zool. 31 : 313-316
- BRISCOE, D.A., FOX, B.J. and INGLEBY, S. (1981).
Genetic differentiation between Pseudomys pilligaensis and related Pseudomys (Rodentia : Muridae).
Aust. Mammal. 4 : 89-92
- BROGGARD-HANSEN, K. and HJORT, T. (1971).
Immunofluorescent studies on human spermatozoa. II. Characterization of spermatozoal antigens and their occurrence in spermatozoa from the male partners of infertile couples.
Clin. Exp. Immunol. 9 : 21-31
- BRONSON, F.H., DAGG, C.P. and SNELL, G.D. (1966).
Reproduction. In : Biology of the Laboratory Mouse. Green, E.L. (Ed.) pp.187-204. 2nd ed.
McGraw-Hill, New York.
- BROOKS, D.E. (1981).
Secretion of proteins and glycoproteins by the rat epididymis : regional differences, androgen-dependence, and effects of protease inhibitors, procaine and tunicamycin.
Biol. Reprod. 25 : 1099-1117
- BROOKS, D.E. (1983a).
Effect of androgens on protein synthesis and secretion in various regions of the rat epididymis, as analyzed by two-dimensional gel electrophoresis.
Mol. Cell. Endocrin. 29 : 255-270
- BROOKS, D.E. (1983b).
Selective binding of specific rat epididymal secretory proteins to spermatozoa and erythrocytes.
Gamete Res. 7 : 367-376
- BROOKS, D.E. and HIGGINS, S.J. (1980).
Characterization and androgen-dependence of proteins associated with luminal fluid and spermatozoa in the rat epididymis.
J. Reprod. Fert. 59 : 363-375
- BROOKS, D.E. and TIVER, K. (1983).
Localization of epididymal secretory proteins on rat spermatozoa.
J. Reprod. Fert. 69 : 651-657

- BROTHERTON, J. (1976).
Difference in size between spermatozoa from the cauda epididymis and the caput epididymis of the rat.
J. Reprod. Fert. 48 : 365-366
- BROWN, C.R. (1982).
Effects of ram sperm acrosin on the investments of sheep, pig, mouse and gerbil eggs.
J. Reprod. Fert. 64 : 457-462
- BROWN, C.R. (1983).
Purification of mouse sperm acrosin, its activation from proacrosin and effect on homologous egg investments.
J. Reprod. Fert. 69 : 289-295
- BROWN, C.R. and HARRISON, R.A.P. (1978).
The activation of proacrosin in spermatozoa from ram, bull and boar.
Biochim. Biophys. Acta. 526 : 202-217
- BROWN, C.R. and CHENG, W.T.K. (1985).
Limited proteolysis of the porcine zona pellucida by homologous sperm acrosin.
J. Reprod. Fert. 74 : 257-260
- BROWN, C.R., ANDANI, Z. and HARTREE, E.F. (1975).
Studies on ram acrosin. Isolation from spermatozoa, activation by cations and organic solvents, and influence of cations on its reaction with inhibitors.
Biochem. J. 149 : 133-146
- BROWN, C.R., von GLOS, K.I. and JONES, R. (1983).
Changes in plasma membrane glycoproteins of rat spermatozoa during maturation in the epididymis.
J. Cell Biol. 96 : 256-264
- BRYAN, J.H.D. (1974)
Capacitation in the mouse : The response of murine acrosomes to the environment of the female reproductive tract.
Biol. Reprod. 10 : 414-421
- BUSTOS-OBREGON, E. and FLECHON, J.E. (1975).
Comparative scanning electron microscope study of boar, bull and ram spermatozoa.
Cell Tiss. Res. 161 : 329-341
- CABOT, C.L. and OLIPHANT, G. (1978).
The possible role of immunological complement in induction of rabbit sperm acrosome reaction.
Biol. Reprod. 19 : 666-672
- CALVIN, H.I. (1975).
Keratinoid proteins in the heads and tails of mammalian spermatozoa. In : Biology of the Male Gamete. Duckett, J.G. and Racey, P.A. (Eds.) pp.257-273.
Academic Press, London.

- CALVIN, H.I. (1976a).
Comparative analysis of the nuclear basic proteins in rat, human, guinea pig, mouse and rabbit spermatozoa.
Biochim. Biophys. Acta. 434 : 377-389
- CALVIN, H.I. (1976b).
Isolation and subfractionation of mammalian sperm heads and tails.
In : Methods in Cell Biology. Prescott, D.M. (Ed.) Vol. 13, pp.85-104.
Academic Press, New York.
- CALVIN, H.I. and BEDFORD, J.M. (1971).
Formation of disulphide bonds in the nucleus and accessory structures of mammalian spermatozoa during maturation in the epididymis.
J. Reprod. Fert. Suppl. 13 : 65-75
- CALVIN, H.I., YU, C.C. and BEDFORD, J.M. (1973).
Effects of epididymal maturation, zinc (II), and copper (II) on the reactive sulphydryl content of structural elements in rat spermatozoa.
Exp. Cell Res. 81 : 333-341
- CALVIN, H.I., HWANG, F.H.F. and WOHLRAB, H. (1975).
Localization of zinc in a dense fiber-connecting piece fraction of rat sperm tails analogous chemically to hair keratin.
Biol. Reprod. 13 : 228-239
- CAMPANELLA, C., GABBIANI, G., BACCETTI, B., BURRINI, A.G. and PALLINI, V. (1979).
Actin and Myosin in the vertebrate acrosomal region.
J. Submicr. Cytol. 11 : 53-71
- CARRANCO, A., REYES, R., MAGDELANO, V.M., HUACUJA, L., HERNANDEZ, O., ROSADO, A., MERCHANT, H. and DELGADO, N.M. (1983).
Heparin-induced nuclei decondensation of mammalian epididymal spermatozoa.
Arch. Androl. 10 : 213-218
- CASTELLANI-CERESA, L., COTELLI, F., LORA-LAMIA, C. and BERRUTI, G. (1980).
The spermatozoon of Talpa europaea with particular reference to the acrosome region.
J. Ultrastruct. Res. 72 : 112-118
- CHANG, M.C. (1951).
Fertilizing capacity of spermatozoa deposited into the fallopian tubes.
Nature 168 : 697-698
- CHANG, M.C. (1957).
A detrimental effect of seminal plasma on the fertilizing capacity of sperm.
Nature 179 : 258-259

- CHANG, M.C. (1984).
The meaning of sperm capacitation. A historical perspective.
J. Androl. 5 : 45-50
- CHANG, M.C. and HUNT, D.M. (1956).
Effects of proteolytic enzymes on the zona pellucida of fertilized and unfertilized mammalian eggs.
Exp. Cell Res. 11 : 497-499
- CHANG, M.C. and HUNT, D.M. (1962).
Morphological changes of sperm head in the ooplasm of mouse, rat, hamster and rabbit.
Anat. Rec. 142 : 417-425
- CHANG, T.S.K., SMYTH, T., VINDIVICH, D. and ALBERTSEN, P.C. (1983).
Induction of immature and mature motility patterns in hamster caput epididymal spermatozoa.
Biol. Reprod. 28 : Suppl. 1 : 134
- CHAYKIN, S. and WATSON, J.G. (1983).
Reproduction in mice : spermatozoa as factors in the development and implantation of embryos.
Gamete Res. 7 : 63-73
- CHIQUELINE, A.D. (1960).
The development of the zona pellucida of the mammalian ova.
Am. J. Anat. 106 : 149-155
- CHOLEWA-STEWART, J. and MASSARD, E.J. (1972).
Thermally induced dissolution of the murine zona pellucida.
Biol. Reprod. 7 : 166-169
- CLARKE, G.N. and YANAGIMACHI, R. (1978).
Actin in mammalian sperm heads.
J. Exp. Zool. 205 : 125-132
- CLARKE, G.N., CLARKE, F.M. and WILSON, S. (1982).
Actin in human spermatozoa.
Biol. Reprod. 26 : 319-327
- CLEGG, E.D., MORRE, D.J. and LUNSTRA, D.D. (1975).
Porcine sperm membranes : in vivo phospholipid changes, isolation and electron microscopy. In : The Biology of the Male Gamete. Duckett, J.G. and Racey, P.A. (Eds.) pp.321-335.
Academic Press, London.
- CLERMONT, Y., EINBERG, E., LE BLOND, C.P. and WAGNER, S. (1955).
The perforatorium - an extension of the nuclear membrane of the rat spermatozoon.
Anat. Rec. 121 : 1-12
- COELINGH, J.P. and ROZIJN, T.H. (1975).
Comparative studies on the basic nuclear proteins of mammalian and other spermatozoa. In : The Biology of the male Gamete. Duckett, J.G. and Racey, P.A. (Eds.) pp.245-256.
Academic Press, London.

- COELINGH, J.P., ROZIJN, T.H. and MONFOORT, C.H. (1969).
Isolation and partial characterization of a basic protein from bovine sperm heads.
Biochim. Biophys. Acta. 188 : 353-356
- COHEN, J. (1983).
Selection among spermatozoa. In : The Sperm Cell. Andre, J. (Ed.) pp.33-37.
Martinus-Nijhoff, The Hague.
- COHEN, J. and McNAUGHTON, D.C. (1974).
Spermatozoa : the probable selection of a small population by the genital tract of the female rabbit.
J. Reprod. Fert. 39 : 297-310
- COOPER, G.W. and BEDFORD, J.M. (1971a).
Acquisition of surface charge by the plasma membrane of mammalian spermatozoa during epididymal maturation.
Anat. Rec. 169 : 300-301
- COOPER, G.W. and BEDFORD, J.M. (1971b).
Charge density change in the vitelline surface following fertilization of the rabbit egg.
J. Reprod. Fert. 25 : 431-436
- COOPER, G.W. and BEDFORD, J.M. (1976).
Asymmetry of spermiation and sperm surface charge patterns over the giant acrosome in the musk shrew Suncus murinus.
J. Cell Biol. 69 : 415-428
- COOPER, G.W., OVERSTREET, J.W. and KATZ, D.F. (1979).
The motility of rabbit spermatozoa recovered from the female reproductive tract.
Gamete Res. 2 : 35-42
- COOPER, T.G. (1984).
The onset and maintenance of hyperactivated motility of spermatozoa from the mouse.
Gamete Res. 9 : 55-74
- CORNETT, L.E. and MEIZEL, S. (1978).
Stimulation of in vitro activation and the acrosome reaction of hamster spermatozoa by catecholamines.
Proc. Natl. Acad. Sci. USA 75 : 4954-4958
- COURTENS, J.L. and FOURNIER-DELPECH, S. (1979).
Modifications in the plasma membranes of epididymal ram spermatozoa during maturation and incubation in utero.
J. Ultrastruct. Res. 68 : 136-148
- COURTENS, J.L., COUROT, M. and FLECHON, J.E. (1976).
The perinuclear substance of boar, bull, ram and rabbit spermatozoa.
J. Ultrastruct. Res. 57 : 54-64

- COURTENS, J.L., ROZINEK, J. and FOURNIER-DELPECH, S. (1982).
Binding of epididymal proteins to the spermatozoa of ram.
Andrologia 14 : 509-514
- COURTENS, J.L., DELALEU, B. and LOIR, M. (1983).
Microtubules and spermiogenesis. In : The Sperm Cell. Andre, J.
(Ed.) pp.236-244.
Martinus Nijhoff, The Hague.
- CRAIG, S.W. and POLLARD, T.D. (1982).
Actin-binding proteins.
Trends Biochem. Sci. 7 : 88-92
- CRAN, D.G., HAY, M.F. and MOOR, R.M. (1979).
The fine structure of the cumulus oophorus during follicular
development in the sheep.
Cell Tiss. Res. 202 : 439-451.
- CRAN, D.G., MOOR, R.M. and HAY, M.F. (1980).
Fine structure of the sheep oocyte during antral follicle
development.
J. Reprod. Fert. 59 : 125-132
- CROZET, N. (1984).
Ultrastructural aspects of in vivo fertilization in the cow.
Gamete Res. 10 : 241-251
- CROZET, N. and DUMONT, M. (1984).
The site of the acrosome reaction during in vivo penetration of the
sheep oocyte.
Gamete Res. 10 : 97-105
- CUMMINS, J.M. (1976).
Effects of epididymal occlusion on sperm maturation in the hamster.
J. Exp. Zool. 197 : 187-190
- CUMMINS, J.M. (1980).
Decondensation of sperm nuclei of Australian marsupials : Effects
of air drying and of calcium and magnesium.
Gamete Res. 3 : 351-367
- CUMMINS, J.M. (1982).
Hyperactivated motility patterns of ram spermatozoa recovered from
the oviducts of mated ewes.
Gamete Res. 6 : 53-63
- CUMMINS, J.M. and YANAGIMACHI, R. (1982).
Sperm-egg ratios and the site of the acrosome reaction during
in vivo fertilization in the hamster.
Gamete Res. 5 : 239-256
- CUMMINS, J.M. and WOODALL, P.F. (1985).
On mammalian sperm dimensions.
J. Reprod. Fert. 75 : 153-175

- CUTHBERTSON, K.S.R., WHITTINGHAM, D.G. and COBBOLD, P.H. (1981).
Free Ca^{2+} increases in exponential phases during mouse oocyte activation.
Nature 294 : 754-757
- CZAKER, R. (1985).
Morphogenesis and cytochemistry of the postacrosomal dense lamina during mouse spermiogenesis.
J. Ultrastruct. Res. 90 : 26-39
- DACHEUX, J.L. and VOGLMAYR, J.K. (1983).
Sequence of sperm cell surface differentiation and its relationship to exogenous fluid proteins in the ram epididymis.
Biol. Reprod. 29 : 1033-1046
- DA SILVA SASSO, W. (1959).
Existence of hyaluronic acid at the zona pellucida of the rabbit ovum.
Acta Anat. 36 : 352-357
- DAVIS, B.K. (1981).
Timing of fertilization in mammals : sperm cholesterol/phospholipid ratio as a determinant of the capacitation interval.
Proc. Natl. Acad. Sci. USA 78 : 7560-7564
- DAVIS, B.K., BYRNE, R. and HUNGUND, B. (1979).
Studies on the mechanism of capacitation. II : evidence for lipid transfer between plasma membrane of rat sperm and serum albumin during capacitation in vitro.
Biochim. Biophys. Acta. 558 : 257-266
- DAVIS, B.K., BYRNE, R. and BEDIGIAN, K. (1980).
Studies on the mechanism of capacitation : albumin-mediated changes in plasma membrane lipids during in vitro incubation of rat sperm cells.
Proc. Natl. Acad. Sci. USA 77 : 1546-1550
- DAWSON, R.M.C. and SCOTT, T.W. (1964).
Phospholipid composition of epididymal spermatozoa prepared by density gradient centrifugation.
Nature 202 : 292-293
- DE VRIES, J.W.A., WILLEMSEN, R. and GEUZE, H.J. (1985).
Immunocytochemical localization of acrosin and hyaluronidase in epididymal and ejaculated porcine spermatozoa.
Eur. J. Cell Biol. 37 : 81-88
- DICKMANN, Z. (1964).
The passage of spermatozoa through and into the zona pellucida of the rabbit egg.
J. Exp. Biol. 41 : 177-182
- DICKMANN, Z. and DZIUK, P.J. (1964).
Sperm penetration of the zona pellucida of the pig egg.
J. Exp. Biol. 41 : 603-608

- DIETL, J. and CZUPPON, A.B. (1984).
Ultrastructural studies of the porcine zona pellucida during the solubilization process by Li-3, 5-diiodosalicylate.
Gamete Res. 9 : 45-54
- DOBZHANSKY, T., AYALA, F.J., STEBBINS, G.L. and VALENTINE, J.W. (1977).
Evolution.
Freeman, San Francisco.
- DOOHER, G.B. (1981).
Differences in the rate of redistribution of receptors for concanavalin A in vivo and in vitro on spermatozoa from normal mice and from sterile mice carrying different T/t locus haplotypes.
Gamete Res. 4 : 105-111
- DOOHER, G.B. and BENNETT, D. (1973).
Fine structural observations on the development of the sperm head in the mouse.
Am. J. Anat. 136 : 339-362
- DOTT, H.M. (1975).
Morphology of stallion spermatozoa.
J. Reprod. Fert. Suppl. 23 : 41-46.
- DOTT, H.M. and DINGLE, J.T. (1968).
Distribution of lysosomal enzymes in the spermatozoa and cytoplasmic droplets of bull and ram.
Exp. Cell Res. 52 : 523-540
- DRELL, D.W. and DUNBAR, B.S. (1984).
Monoclonal antibodies to rabbit and pig zonae pellucidae distinguish species-specific and shared antigenic determinants.
Biol. Reprod. 30 : 445-457
- DRESDNER, R.D. and KATZ, D.F. (1981).
Relationships of mammalian sperm motility and morphology to hydrodynamic aspects of cell function.
Biol. Reprod. 25 : 920-930
- DUDKIEWICZ, A.B. (1984).
Purification of boar acrosomal arylsulfatase A and possible role in the penetration of cumulus cells.
Biol. Reprod. 30 : 1005-1014
- DUDKIEWICZ, A.B. and WILLIAMS, W.L. (1977).
Fine structural observations of the mammalian zona pellucida by scanning electron microscopy.
S.E.M. 1977/II : 317-324
- DUDKIEWICZ, A.B., SHIVERS, C.A. and WILLIAMS, W.L. (1976).
Ultrastructure of the hamster zona pellucida treated with zona precipitating antibody.
Biol. Reprod. 14 : 175-185
- DUKELOW, W.R., CHERNOFF, H.N. and WILLIAMS, W.L. (1966).
Enzymatic characterization of decapacitation factor.
Proc. Soc. Exp. Biol. Med. 121 : 396-398

- DUKELOW, W.R., CHERNOFF, H.N. and WILLIAMS, W.L. (1967).
Properties of decapacitation factor and presence in various species.
J. Reprod. Fert. 14 : 393-399
- DUNBAR, B.S. (1983).
Morphological, biochemical, and immunochemical characterization of the mammalian zona pellucida. In : Mechanism and Control of Animal Fertilization. Hartmann, J.F. (Ed.) pp.139-175.
Academic Press, New York.
- DUNBAR, B.S., WARDRIP, N.J. and HEDRICK, J.L. (1980).
Isolation, physicochemical properties, and macromolecular composition of zona pellucida from porcine oocytes.
Biochemistry 19 : 356-365
- DUNBAR, B.S., LIU, C. and SAMMONS, D.W. (1981).
Identification of the three major proteins of porcine and rabbit zonae pellucidae by high resolution two-dimensional gel electrophoresis : comparison with serum, follicular fluid, and ovarian cell proteins.
Biol. Reprod. 24 : 1111-1124
- DUNBAR, B.S., DUDKIEWICZ, A.B. and BUNDMAN, D.S. (1985).
Proteolysis of specific porcine zona pellucida glycoproteins by boar acrosin.
Biol. Reprod. 32 : 619-630
- DYSON, A.L.M.B. and ORGEBIN-CRIST, M.C. (1973).
Effect of hypophysectomy, castration and androgen replacement upon the fertilizing ability of rat epididymal spermatozoa.
Endocrinology 93 : 391-402
- DZIUK, P.J. and DICKMANN, Z. (1965).
Sperm penetration through the zona pellucida of the sheep egg.
J. Exp. Zool. 158 : 237-240
- EAST, I.J. and DEAN, J. (1984).
Monoclonal antibodies as probes of the distribution of ZP-2, the major sulfated glycoprotein of the murine zona pellucida.
J. Cell Biol. 98 : 795-800
- EBENSPERGER, C. and BARROS, C. (1984).
Changes at the hamster oocyte surface from the germinal vesicle stage to ovulation.
Gamete Res. 9 : 387-397
- EDOY, E.M. and KOEHLER, J.K. (1982).
Restricted domains of the sperm surface.
S.E.M. 1982/III : 1313-1323
- EDELMAN, G.M. and MILLETTE, C.F. (1971).
Molecular probes of spermatozoan structures.
Proc. Natl. Acad. Sci. USA 68 : 2436-2440

- EDELMAN, G.M. and MILLETTE, C.F. (1975).
Chemical dissection and surface mapping of spermatozoa. In :
Functional Anatomy of the Spermatozoon. Afzelius, B.A. (Ed.).
pp.349-357.
Pergamon Press, New York.
- ELDER, F.F.B. and HSU, T.C. (1981).
Silver-staining patterns of mammalian epididymal spermatozoa.
Cytogen. Cell Genet. 30 : 157-167
- ELFVIN, L.G. (1968).
An ultrastructural difference between the outer and inner membrane
of the middle piece mitochondria in rat spermatozoa.
J. Ultrastruct. Res. 24 : 259-268
- ELIAS, P.M., FRIEND, D.S. and GOERKE, J. (1979).
Membrane sterol heterogeneity. Freeze-fracture detection with
saponins and filipin.
J. Histochem. Cytochem. 27 : 1247-1260
- ELLIOTT, M. and HIGGINS, J.A. (1983).
Capacitation and the acrosome reaction in guinea pig spermatozoa
increase the availability of surface aminophospholipids for
labelling by trinitrobenzene sulphonate.
Cell Biol. Int. Rep. 7 : 1091-1096
- ENG, L.A. and OLIPHANT, G. (1978).
Rabbit sperm reversible decapacitation by membrane stabilization
with a highly purified glycoprotein from seminal plasma.
Biol. Reprod. 19 : 1083-1094
- ESBENSHADE, K.L. and CLEGG, E.D. (1976).
Electrophoretic characterization of proteins in the plasma membrane
of porcine spermatozoa.
J. Reprod. Fert. 47 : 333-337
- ESBENSHADE, K.L. and CLEGG, E.D. (1980).
Surface proteins of ejaculated porcine sperm and sperm incubated in
the uterus.
Biol. Reprod. 23 : 530-537
- EVENSON, D.P., WITKEN, S.S., de HARVEN, E. and BENDICH, A. (1978).
Ultrastructure of partially decondensed human spermatozoal
chromatin.
J. Ultrastruct. Res. 63 : 178-187
- FAIN-MAUREL, M.A., DADOUNE, J.P. and REGER, J.F. (1984).
A cytochemical study on surface charges and lectin-binding sites in
epididymal and ejaculated spermatozoa of Macaca fascicularis.
Anat. Rec. 208 : 375-382
- FAROOQUI, A.A. and SRIVASTAVA, P.N. (1979).
Isolation, characterization and the role of rabbit testicular
arylsulphatase A in fertilization.
Biochem. J. 181 : 331-337

- FAROOQUI, A.A. and SRIVASTAVA, P.N. (1980).
Isolation of b-N-acetylhexosaminidase from rabbit semen and its role in fertilization.
Biochem. J. 191 : 827-834
- FAWCETT, D.W. (1965).
The anatomy of the mammalian spermatozoon with particular reference to the guinea pig.
Z. Zellforsch. Mikrosk. Anat. 67 : 279-296
- FAWCETT, D.W. (1970).
A comparative view of sperm ultrastructure.
Biol. Reprod. Suppl. 2 : 90-127
- FAWCETT, D.W. (1975).
The mammalian spermatozoon.
Dev. Biol. 44 : 394-436
- FAWCETT, D.W. and HOLLENBERG, R.D. (1963).
Changes in the acrosome of guinea pig spermatozoa during passage through the epididymis.
Z. Zellforsch. Mikrosk. Anat. 60 : 276-292
- FAWCETT, D.W. and ITO, S. (1965).
The fine structure of bat spermatozoa.
Am. J. Anat. 116 : 567-610
- FAWCETT, D.W. and PHILLIPS, D.M. (1969a).
The fine structure and development of the neck region of the mammalian spermatozoon.
Anat. Rec. 165 : 153-184
- FAWCETT, D.W. and PHILLIPS, D.M. (1969b).
Observations on the release of spermatozoa and on changes in the head during passage through the epididymis.
J. Reprod. Fert. Suppl. 6 : 405-418
- FAWCETT, D.W., ANDERSON, W.A. and PHILLIPS, D.M. (1971).
Morphogenetic factors influencing the shape of the sperm head.
Dev. Biol. 26 : 220-251
- FEUCHTER, F.A., VERNON, R.B. and EDDY, E.M. (1981).
Analysis of the sperm surface with monoclonal antibodies : topographically restricted antigens appearing in the epididymis.
Biol. Reprod. 24 : 1099-1110
- FLAHERTY, S.P. (1981).
The structure and chemical composition of the hooks on the sperm head of the plains mouse, Pseudomys australis.
Honours Thesis, University of Adelaide.
- FLAHERTY, S.P. and BREED, W.G. (1983a).
The sperm head of the plains mouse, Pseudomys australis : ultrastructure and effects of chemical treatments.
Gamete Res. 8 : 231-244

- FLAHERTY, S.P. and BREED, W.G. (1983b).
Formation of the ventral hooks on the sperm head of the plains mouse, Pseudomys australis.
Proc. 15th Conf. Aust. Soc. Reprod. Biol. Canberra. Abstr. 36
- FLAHERTY, S.P., BREED, W.G. and SARAFIS, V. (1983).
Localization of actin in the sperm head of the plains mouse, Pseudomys australis.
J. Exp. Zool. 225 : 497-500
- FLECHON, J.E. (1979a).
Sperm glycoproteins of the boar, bull, rabbit and ram.
I. Acrosomal glycoproteins.
Gamete Res. 2 : 43-51
- FLECHON, J.E. (1979b).
Sperm glycoproteins of the boar, bull, rabbit and ram. II. Surface glycoproteins and free acidic groups.
Gamete Res. 2 : 53-64
- FLECHON, J.E. and MORSTIN, J. (1975).
Localisation des glycoprotéines et des charges négatives et positives dans le revêtement de surface des spermatozoïdes éjaculés de lapin et de taureau.
Ann. Histochim. 20 : 291-300
- FLECHON, J.E. and HUNTER, R.H.F. (1981).
Distribution of spermatozoa in the utero-tubal junction and isthmus of pigs, and their relationship with the luminal epithelium after mating : a scanning electron microscope study.
Tissue Cell. 13 : 127-139
- FLEMING, A.D. and YANAGIMACHI, R. (1981).
Effects of various lipids on the acrosome reaction and fertilizing capacity of guinea pig spermatozoa with special reference to the possible involvement of lysophospholipids in the acrosome reaction.
Gamete Res. 4 : 253-273
- FLEMING, A.D. and YANAGIMACHI, R. (1982).
Fertile life of acrosome-reacted guinea pig spermatozoa.
J. Exp. Zool. 220 : 109-115
- FLEMING, A.D., YANAGIMACHI, R. and YANAGIMACHI, H. (1981).
Spermatozoa of the Atlantic bottlenosed dolphin, Tursiops truncatus.
J. Reprod. Fert. 63 : 509-514
- FLEMING, W.N. and SAACKE, R.G. (1972).
Fine structure of the bovine oocyte from the mature Graafian follicle.
J. Reprod. Fert. 29 : 203-213
- FLICKINGER, C. and FAWCETT, D.W. (1967).
The junctional specializations of Sertoli cells in the seminiferous epithelium.
Anat. Rec. 158 : 207-222

- FLORMAN, H.M. and STOREY, B.T. (1982).
Mouse gamete interactions : the zona pellucida is the site of the acrosome reaction leading to fertilization in vitro.
Dev. Biol. 91 : 121-130
- FLORMAN, H.M., BECHTOL, K.B. and WASSARMAN, P.M. (1984).
Enzymatic dissection of the functions of the mouse egg's receptor for sperm.
Dev. Biol. 106 : 243-255
- FORSSMANN, W.G., ITO, S., WEIHE, E., AOKI, A., DYM, M. and FAWCETT, D.W. (1977).
An improved perfusion fixation method for the testis.
Anat. Rec. 188 : 307-314
- FOURNIER-DELPECH, S., COLAS, G., COUROT, M. ORTAVANT, R. and BRICE, G. (1979).
Epididymal sperm maturation in the ram : motility, fertilizing ability and embryonic survival after uterine artificial insemination in the ewe.
Ann. Biol. Anim. Biochem. Biophys. 19 : 597-605
- FOURNIER-DELPECH, S., COURTENS, J.L., PISSELET, C.L., DELALEU, B. and COUROT, M. (1982).
Acquisition of zona binding by ram spermatozoa during epididymal passage, as revealed by interaction with rat oocytes.
Gamete Res. 5 : 403-408
- FOURNIER-DELPECH, S., HAMAMAH, S., TANANIS-ANTHONY, C., COUROT, M. and ORGBIN-CRIST, M.C. (1984).
Hormonal regulation of zona-binding ability and fertilizing ability of rat epididymal spermatozoa.
Gamete Res. 9 : 21-30
- FRANKE, W.W., GRUND, C., FINK, A., WEBER, K., JOCKUSCH, B.M., ZENTGRAF, H. and OSBORN, M. (1978).
Location of actin in the microfilament bundles associated with the junctional specializations between Sertoli cells and spermatids.
Biol. Cell. 31 : 7-14
- FRANKLIN, L.E. and FUSSELL, E.N. (1972).
Evolution of the apical body in golden hamster spermatids with some reference to primates.
Biol. Reprod. 7 : 194-206
- FRANKLIN, L.E., BARROS, C. and FUSSELL, E.N. (1970).
The acrosomal region and the acrosome reaction in sperm of the golden hamster.
Biol. Reprod. 3 : 180-200
- FRASER, L.R. (1977a).
Motility patterns in mouse spermatozoa before and after capacitation.
J. Exp. Zool. 202 : 439-444

- FRASER, L.R. (1977b).
Fertilization and pre-implantation development in vitro of mouse eggs obtained following stimulation with different doses of pregnant mare serum. A comparison of the responses in two strains. Differentiation 9 : 29-35
- FRASER, L.R. (1981).
Di butyryl cyclic AMP decreases capacitation time in vitro in mouse spermatozoa.
J. Reprod. Fert. 62 : 63-72
- FRASER, L.R. (1982).
p-aminobenzamidine, an acrosin inhibitor, inhibits mouse sperm penetration of the zona pellucida, but not the acrosome reaction.
J. Reprod. Fert. 65 : 185-194
- FRASER, L.R. (1983).
Potassium ions modulate expression of mouse sperm fertilizing ability, acrosome reaction and hyperactivated motility in vitro.
J. Reprod. Fert. 69 : 539-553
- FRASER, L.R. and DANDEKAR, P.V. (1975).
The relationship between zona digestion and cortical granule disappearance in rabbit eggs inseminated in vitro.
Biol. Reprod. 13 : 123-125
- FRASER, L.R. and QUINN, P.J. (1981).
A glycolytic product is obligatory for initiation of the sperm acrosome reaction and whiplash motility required for fertilization in the mouse.
J. Reprod. Fert. 61 : 25-35
- FRAY, C.S., HOFFER, A.P. and FAWCETT, D.W. (1972).
A reexamination of motility patterns of rat epididymal spermatozoa.
Anat. Rec. 173 : 301-308
- FRIEND, D.S. (1977).
The organization of the spermatozoal membrane. In : Immunobiology of Gametes. Edidin, M. and Johnson, M.H. (Eds.) Vol. 4, pp.5-30.
Alden Press, Oxford.
- FRIEND, D.S. (1980).
Freeze-fracture alterations in guinea-pig sperm membranes preceding gamete fusion. In : Membrane-Membrane Interactions. Gilula, N.B. (Ed). pp.153-165.
Raven Press, New York.
- FRIEND, D.S. (1982).
Plasma-membrane diversity in a highly polarized cell.
J. Cell Biol. 93 : 243-249
- FRIEND, D.S. and FAWCETT, D.W. (1974).
Membrane differentiations in freeze-fractured mammalian sperm.
J. Cell Biol. 63 : 641-664

- FRIEND, D.S. and BEARER, E.L. (1981).
b-hydroxysterol distribution as determined by freeze-fracture cytochemistry.
Histochem. J. 13 : 535-546
- FRIEND, D.S. and HEUSER, J.E. (1981).
Orderly particle arrays on the mitochondrial outer membrane of rapidly-frozen sperm.
Anat. Rec. 199 : 159-175
- FRIEND, D.S., ORCI, L., PERRELET, A. and YANAGIMACHI, R. (1977).
Membrane particle changes attending the acrosome reaction in guinea pig spermatozoa.
J. Cell Biol. 74 : 561-577
- FRIEND, G.F. (1936).
The sperms of the British muridae.
Quart. J. Microsc. Soc. 78 : 419-443
- FRIESS, A.E. and SINOWATZ, F. (1984).
Con A- and WGA-binding sites on bovine epididymal spermatozoa : TEM of specimens in toto.
Biol. Cell. 50 : 279-284
- FUKUDA, Y., MADDOCK, M.B. and CHANG, M.C. (1979).
In vitro fertilization of two species of deermouse eggs by homologous or heterologous sperm and penetration of laboratory mouse eggs by deermouse sperm.
J. Exp. Zool. 207 : 481-490
- GAASTRA, W., LUKKES-HOFSTRA, J. and KOLK, A.H.J. (1978).
Partial covalent structure of two basic chromosomal proteins from human spermatozoa.
Biochem. Genet. 16 : 525-529
- GADDUM, P. (1968).
Sperm maturation in the male reproductive tract : development of motility.
Anat. Rec. 161 : 471-479
- GADDUM-ROSSE, P. (1981).
Some observations on sperm transport through the uterotubal junction of the rat.
J. Anat. 160 : 333-341
- GADDUM-ROSSE, P. and BLANDAU, R.J. (1973a).
Ciliary activity in the human and Macaca nemestrina oviduct.
Am. J. Anat. 138 : 269-275
- GADDUM-ROSSE, P. and BLANDAU, R.J. (1973b).
In vitro studies on ciliary activity within the oviducts of the rabbit and pig.
Am. J. Anat. 136 : 91-104
- GADDUM-ROSSE, P. and BLANDAU, R.J. (1976).
Comparative observations on ciliary currents in mammalian oviducts.
Biol. Reprod. 14 : 605-609

- GADDUM-ROSSE, P., BLANDAU, R.J., LANGLEY, L.B. and SATO, K. (1982).
Sperm tail entry into the mouse egg in vitro.
Gamete Res. 6 : 215-223
- GADDUM-ROSSE, P., BLANDAU, R.J., LANGLEY, L.B. and BATTAGLIA, D.E. (1984).
In vitro fertilization in the rat : observations on living eggs.
Fertil. Steril. 42 : 285-292
- GARBERS, D.L., WAKABAYASHI, T. and REED, P.W. (1970).
Enzyme profile of the cytoplasmic droplet from bovine epididymal spermatozoa.
Biol. Reprod. 3 : 327-337
- GARNER, D.L., SALISBURY, G.W. and GRAVES, C.N. (1971).
Electrophoretic fractionation of bovine acrosomal proteins and proteinase.
Biol. Reprod. 4 : 93-100
- GARNER, D.L., REAMER, S.A., JOHNSON, L.A. and LESSLEY, B.A. (1977).
Failure of immunoperoxidase staining to detect acrosin in the equatorial segment of spermatozoa.
J. Exp. Zool. 201 : 309-315
- GATES, A.H. (1971).
Maximizing yield and developmental uniformity of eggs. In : Methods in Mammalian Embryology. Daniel, J.C. (Ed.) pp.64-75
Freeman, San Francisco.
- GAUNT, S.J. (1983).
Spreading of a sperm surface antigen within the plasma membrane of the egg after fertilization in the rat.
J. Embryol. Exp. Morph. 75 : 259-270
- GAUNT, S.J., BROWN, C.R. and JONES, R. (1983).
Identification of mobile and fixed antigens on the plasma membrane of rat spermatozoa using monoclonal antibodies.
Exp. Cell Res. 144 : 275-284
- GILULA, N.B., EPSTEIN, M.L. and BEERS, W.H. (1978).
Cell-to-cell communication and ovulation. A study of the cumulus-oocyte complex.
J. Cell Biol. 78 : 58-75
- GLOVER, T.D. and NICANDER, L. (1971).
Some aspects of structure and function in the mammalian epididymis.
J. Reprod. Fert. Suppl. 13 : 39-50
- GONENNE, A. and ERNST, R. (1978).
Solubilization of membrane proteins by sulfobetaines, novel zwitterionic surfactants.
Anal. Biochem. 87 : 28-38
- GOODPASTURE, J.C., REDDY, J.M. and ZANEVELD, L.J.D. (1981).
Acrosin, proacrosin, and acrosin inhibitor of guinea pig spermatozoa capacitated and acrosome-reacted in vitro.
Biol. Reprod. 25 : 44-55

- GORDON, M. (1972).
Observations on the post-acrosomal region and the neck membranes of rabbit spermatozoa stained en bloc with uranyl acetate.
Z. Zellforsch Mikrosk. Anat. 131 : 15-25
- GORDON, M. (1973).
Localization of phosphatase activity on the membranes of the mammalian sperm head.
J. Exp. Zool. 185 : 111-120
- GORDON, M. and DANDEKAR, P.V. (1976).
Electron microscope assessment of fertilization of rabbit ova treated with concanavalin A and wheat germ agglutinin.
J. Exp. Zool. 198 : 437-442
- GORDON, M., DANDEKAR, P.V. and BARTOSZEWICZ, W. (1975).
The surface coat of epididymal, ejaculated and capacitated sperm.
J. Ultrastruct. Res. 50 : 199-207
- GORDON, M., DANDEKAR, P.V. and EAGER, P.R. (1978).
Identification of phosphatases on the membranes of guinea pig sperm.
Anat. Rec. 191 : 123-134
- GOULD, K.G. (1980).
Scanning electron microscopy of the primate sperm.
Int. Rev. Cytol. 63 : 323-355
- GOULD, S.J. (1980).
Is a new and general theory of evolution emerging?
Paleobiol. 6 : 119-130
- GREEN, D.P.L. (1978a).
The mechanism of the acrosome reaction. In : Development in Mammals. Johnson, M.H. (Ed.) Vol. 3, pp.65-81.
North-Holland, Amsterdam.
- GREEN, D.P.L. (1978b).
The osmotic properties of the acrosome of guinea-pig sperm.
J. Cell Sci. 32 : 165-176
- GREEN, D.P.L. (1978c).
The activation of proteolysis in the acrosome reaction of guinea-pig sperm.
J. Cell Sci. 32 : 153-164
- GREEN, D.P.L. (1985).
An analysis of the structure of the bilaminar lamellae of the hamster egg.
J. Ultrastruct. Res. 91 : 30-37
- GREEN, D.P.L. and HOCKADAY, A.R. (1978).
The histochemical localization of acrosin in guinea-pig sperm after the acrosome reaction.
J. Cell Sci. 32 : 177-184

- GREEN, D.P.L. and PURVES, R.D. (1984).
Mechanical hypothesis of sperm penetration.
Biophys. J. 45 : 659-662
- GREEN, J.A. and DRYDEN, G.L. (1976).
Ultrastructure of epididymal spermatozoa of the Asiatic musk shrew,
Suncus Murinus.
Biol. Reprod. 14 : 327-331
- GREENBERG, B.J. and TAMBLYN, T.M. (1981).
Actin from mature bovine spermatozoa.
J. Cell Biol. 91 : 191a
- GREVE, J.M., SALZMANN, G.S., ROLLER, R.J. and WASSARMAN, P.M. (1982).
Biosynthesis of the major zona pellucida glycoprotein secreted by
oocytes during mammalian oogenesis.
Cell 31 : 749-759
- GROSCHER-STEWART, U. (1980).
Immunochemistry of cytoplasmic contractile proteins.
Int. Rev. Cytol. 65 : 193-254
- GULYAS, B.J. (1980).
Cortical granules of mammalian eggs.
Int. Rev. Cytol. 63 : 357-392
- GULYAS, B.J. and SCHMELL, E.D. (1980).
Ovoperoxidase activity in ionophore treated mouse eggs. I. Electron
microscopic localization.
Gamete Rec. 3 : 267-277
- GURAYA, S.S. (1969).
Histochemical nature of cortical granules in the human egg.
Z. Zellforsch. Mikrosk. Anat. 94 : 32-35
- GUSSE, M. and CHEVALIER, P. (1980).
Electron microscopic evidence for the presence of globular
structures in different sperm chromatins.
J. Cell Biol. 87 : 280-284
- GWATKIN, R.B.L. (1964).
Effects of enzymes and acidity on the zona pellucida of the mouse
egg before and after fertilization.
J. Reprod. Fert. 7 : 990-1005
- GWATKIN, R.B.L. (1982).
Receptor for sperm on the mammalian ovum. In : In Vitro
Fertilization and Embryo Transfer. Hafez, E.S.E. and Semm, K.
(Eds.) pp.3-11
MTP Press, Lancaster.
- GWATKIN, R.B.L. and WILLIAMS, D.T. (1977).
Receptor activity of the hamster and mouse zona pellucida before
and after the zona reaction.
J. Reprod. Fert. 49 : 55-59

- GWATKIN, R.B.L., WILLIAMS, D.T., HARTMANN, J.F. and KNIAZUK, M. (1973).
The zona reaction of hamster and mouse eggs : production in vitro
by a trypsin-like protease from cortical granules.
J. Reprod. Fert. 32 : 259-265
- GWATKIN, R.B.L., ANDERSEN, O.F. and WILLIAMS, D.T. (1980).
Large scale isolation of bovine and pig zonae pellucidae :
chemical, immunological, and receptor properties.
Gamete Res. 3 : 217-231
- HADDAD, A. and NAGAI, M.E.T. (1977).
Radioautographic study of glycoprotein biosynthesis and renewal in
the ovarian follicles of mice and the origin of the zona pellucida.
Cell Tiss. Res. 177 : 347-369
- HALEND, R.M., PRIMAKOFF, P. and MYLES, D.G. (1984).
Evidence for the presence of actin in guinea pig spermatogenic
cells and its loss prior to formation of mature sperm.
J. Cell Biol. 99 : 394A
- HAMILTON, D.W. (1975).
Structure and function of the epithelium lining the ductuli
efferentes, ductus epididymidis, and ductus deferens in the rat.
In : Handbook of Physiology. Greep, R.O. and Astwood, E.D. (Eds.)
Section 7, Vol. 5, pp.259-301.
Amer. Physiol. Soc., Washington.
- HAMILTON, D.W. (1980).
UDP-galactose : N-acetylglucosamine galactosyltransferase in fluids
from rat rete testis and epididymis.
Biol. Reprod. 23 : 377-385
- HAMILTON, D.W. and GOULD, R.P. (1982).
Preliminary observations on enzymatic galactosylation of
glycoproteins on the surface of rat caput epididymal sperm.
Int. J. Androl. Suppl. 5 : 73-80
- HAMMOND, J. and WALTON, A. (1934).
Notes on ovulation and fertilisation in the ferret.
J. Exp. Biol. 11 : 307-319
- HANADA, A. and CHANG, M.C. (1972).
Penetration of zona-free eggs by spermatozoa of different species.
Biol. Reprod. 6 : 300-309
- HANADA, A. and CHANG, M.C. (1978).
Penetration of the zona-free or intact eggs by foreign spermatozoa
and the fertilization of deer mouse eggs in vitro.
J. Exp. Zool. 203 : 27-286
- HANCOCK, J.L. (1957).
The morphology of boar spermatozoa.
J. Roy. Microsc. Soc. 76 : 84-97
- HANCOCK, J.L. and TREVAN, D.J. (1957).
The acrosome and post-nuclear cap of bull spermatozoa.
J. Roy. Microsc. Soc. 76 : 77-83

- HAND, S. (1984).
Australia's oldest rodents : master mariners from Malaysia. In : Vertebrate Zoogeography and Evolution in Australasia. Archer, M. and Clayton, G. (Eds.) pp.905-912.
Hesperian Press, Carlisle.
- HARRISON, R.A.P. (1983).
The acrosome, its hydrolases, and egg penetration. In : The Sperm Cell. Andre, J. (Ed.). pp.259-273.
Martinus-Nijhoff, The Hague.
- HARRISON, R.A.P., FLECHON, J.E. and BROWN, C.R. (1982).
The location of acrosin and proacrosin in ram spermatozoa.
J. Reprod. Fertil. 66 : 349-358
- HARTMANN, J.F. (1983).
Mammalian fertilization : gamete surface interactions in vitro.
In : Mechanism and Control of Animal Fertilization. Hartmann, J.F. (Ed.) pp.325-364.
Academic Press, New York.
- HARTREE, E.F. (1977).
Spermatozoa, eggs and proteinases.
Biochem. Soc. Trans. 5 : 375-394
- HARTREE, E.F. and SRIVASTAVA, P.N. (1965).
The chemical composition of the acrosomes of ram spermatozoa.
J. Reprod. Fert. 9 : 47-60
- HASTINGS, R.A., ENDERS, A.C. and SCHLAFKE, S. (1972).
Permeability of the zona pellucida to protein tracers.
Biol. Reprod. 7 : 288-296
- HERR, J.C. and EDDY, E.M. (1980).
Detection of mouse sperm antigens by a surface labelling and immunoprecipitation approach.
Biol. Reprod. 22 : 1263-1274
- HINTON, B.T., DOTT, H.M. and SETCHELL, B.P. (1979).
Measurement of the motility of rat spermatozoa collected by micropuncture from the testis and from different regions along the epididymis.
J. Reprod. Fert. 55 : 167-172
- HINTON, B.T., BROOKS, D.E., DOTT, H.M. and SETCHELL, B.P. (1981).
Effects of carnitine and some related compounds on the motility of rat spermatozoa from the caput epididymidis.
J. Reprod. Fert. 61 : 59-64
- HIRAO, Y. and YANAGIMACHI, R. (1978a).
Effects of various enzymes on the ability of hamster egg plasma membranes to fuse with spermatozoa.
Gamete Res. 1 : 3-12

- HIRAO, Y. and YANAGIMACHI, R. (1978b).
Temperature dependence of sperm-egg fusion and post-fusion events
in hamster fertilization.
J. Exp. Zool. 205 : 433-438
- HOLT, W.V. (1979).
Development and maturation of the mammalian acrosome.
J. Ultrastruct. Res. 68 : 58-71
- HOLT, W.V. (1980).
Surface-bound sialic acid on ram and bull spermatozoa : deposition
during epididymal transit and stability during washing.
Biol. Reprod. 23 : 847-857
- HOLT, W.V. (1982).
Functional development of the mammalian sperm plasma membrane.
Oxford Rev. Reprod. Biol. 4 : 195-240
- HOLT, W.V. (1984).
Membrane heterogeneity in the mammalian spermatozoon.
Int. Rev. Cytol. 87 : 159-194
- HOLTZ, W. and SMIDT, D. (1976).
The fertilizing capacity of epididymal spermatozoa in the pig.
J. Reprod. Fert. 46 : 227-229
- HORAN, A.H. and BEDFORD, J.M. (1972).
Development of the fertilizing ability of spermatozoa in the
epididymis of the Syrian hamster.
J. Reprod. Fert. 30 : 417-423
- HOSKINS, D.D., BRANDT, H. and ACOTT, T.S. (1978).
Initiation of sperm motility in the mammalian epididymis.
Fed. Proc. 37 : 2534-2542
- HUACUJA, L., DELGADO, N.M., MERCHANT, H., PANCARDO, R. and ROSADO, A.
(1977).
Cyclic AMP induced incorporation of ^{33}Pi into human spermatozoa
membrane components.
Biol. Reprod. 17 : 89-96
- HUANG, T.T.F. and YANAGIMACHI, R. (1984).
Fucoidin inhibits attachment of guinea pig spermatozoa to the zona
pellucida through binding to the inner acrosomal membrane and
equatorial domains.
Exp. Cell Res. 153 : 363-373
- HUANG, T.T.F. and YANAGIMACHI, R. (1985).
Inner acrosomal membrane of mammalian spermatozoa : its properties
and possible functions in fertilization.
Am. J. Anat. 174 : 249-268
- HUANG, T.T.F., FLEMING, A.D. and YANAGIMACHI, R. (1981).
Only acrosome-reacted spermatozoa can bind to and penetrate zona
pellucida : a study using the guinea pig.
J. Exp. Zool. 217 : 287-290

- HUANG, T.T.F., OHZU, E. and YANAGIMACHI, R. (1982).
Evidence suggesting that L-fucose is part of a recognition signal for sperm-zona pellucida attachment in mammals.
Gamete Res. 5 : 355-361
- HUANG, T.T.F., HARDY, D., YANAGIMACHI, H., TEUSCHER, C., TUNG, K., WILD, G. and YANAGIMACHI, R. (1985).
pH and protease control of acrosomal content stasis and release during the guinea pig sperm acrosome reaction.
Biol. Reprod. 32 : 451-462
- HUANG-YANG, Y.H.J. and MEIZEL, S. (1975).
Purification of rabbit testis proacrosin and studies of its active form.
Biol. Reprod. 12 : 232-238
- HUNEAU, D., HARRISON, R.A.P. and FLECHON, J.E. (1984).
Ultrastructural localization of proacrosin and acrosin in ram spermatozoa.
Gamete Res. 9 : 425-440
- HUNTER, A.G. and NORNES, H.O. (1969).
Characterization and isolation of a sperm-coating antigen from rabbit seminal plasma with capacity to block fertilization.
J. Reprod. Fert. 20 : 419-427
- HUNTER, R.H.F. (1976).
Sperm-egg interactions in the pig : monospermy, extensive polyspermy, and the formation of chromatin aggregates.
J. Anat. 122 : 43-59
- HUNTER, R.H.F. (1981).
Sperm transport and reservoirs in the pig oviduct in relation to the time of ovulation.
J. Reprod. Fert. 63 : 109-117
- HUNTER, R.H.F. (1984).
Pre-ovulatory arrest and peri-ovulatory redistribution of competent spermatozoa in the isthmus of the pig oviduct.
J. Reprod. Fert. 72 : 203-211
- HUNTER, R.H.F. and HALL, J.P. (1974).
Capacitation of boar spermatozoa : synergism between uterine and tubal environments.
J. Exp. Zool. 188 : 203-214
- HUNTER, R.H.F. and NICHOL, R. (1983).
Transport of spermatozoa in the sheep oviduct : preovulatory sequestering of cells in the caudal isthmus.
J. Exp. Zool. 228 : 121-128
- ILLISON, L. (1966).
The fine structure of the mature spermatozoan head and neck of the mouse.
J. Anat. 100 : 949-950

- INOUE, M. and WOLF, D.P. (1974).
Comparative solubility properties of the zonae pellucidae of unfertilized and fertilized mouse ova.
Biol. Reprod. 11 : 558-565
- INOUE, M. and WOLF, D.P. (1975a).
Comparative solubility properties of rat and hamster zonae pellucidae.
Biol. Reprod. 12 : 535-540
- INOUE, M. and WOLF, D.P. (1975b).
Fertilization-associated changes in the murine zona pellucida : a time sequence study.
Biol. Reprod. 13 : 546-551
- ISAHAKIA, M. and ALEXANDER, N.J. (1984).
Interspecies cross-reactivity of monoclonal antibodies directed against human sperm antigens.
Biol. Reprod. 30 : 1015-1026
- JEDLICKI, A. and BARROS, C. (1985).
Scanning electron microscope study of in vitro prepenetration gamete interactions.
Gamete Res. 11 : 121-131
- JI, I., YOO, B.Y. and JI, T.H. (1981a).
Surface proteins and glycoproteins of ejaculated bovine sperm. I. Iodination and proteolytic digestion.
Biol. Reprod. 24 : 617-626
- JI, I., YOO, B.Y. and JI, T.H. (1981b).
Surface proteins and glycoproteins of ejaculated bovine sperm. II. Molecular composition of the midpiece and mainpiece.
Biol. Reprod. 24 : 627-636
- JOHNSON, L.A., GARNER, D.L., TRUITT-GILBERT, A.J. and LESSLEY, B.A. (1983).
Immunocytochemical localization of acrosin on both acrosomal membranes and in the acrosomal matrix of porcine spermatozoa.
J. Androl. 4 : 222-229
- JOHNSON, L.L., KATZ, D.F. and OVERSTREET, J.W. (1981).
The movement characteristics of rabbit spermatozoa before and after activation.
Gamete Res. 4 : 275-282
- JOHNSON, M. and EDIDIN, M. (1978).
Lateral diffusion in plasma membrane of mouse egg is restricted after fertilisation.
Nature 272 : 448-450
- JOHNSON, M.H., EAGER, D., MUGGLETON-HARRIS, A. and GRAVE, H.M. (1975).
Mosaicism in organisation of concanavalin A receptors on surface membrane of mouse egg.
Nature 257 : 321-322

- JONES, H.P., LENZ, R.W., PALEVITZ, B.A. and CORMIER, M.J. (1980).
Calmodulin localization in mammalian spermatozoa.
Proc. Natl. Acad. Sci. USA 77 : 2772-2776
- JONES, R., BROWN, C.R., VON GLOS, K.I. and PARKER, M.G. (1980).
Hormonal regulation of protein synthesis in the rat epididymis.
Characterization of androgen-dependent and testicular fluid-
dependent proteins.
Biochem. J. 188 : 667-676
- JONES, R., GAUNT, S.J., BROWN, C.R. and SETCHELL, B.P. (1983).
Sperm maturation antigens. In : The Sperm Cell. Andre, J. (Ed.)
pp.131-134.
Martinus-Nijhoff, The Hague.
- JONES, R.C. (1970).
Studies of the ultrastructure of the head of some mammalian
spermatozoa with particular reference to the perforatorium.
7th Int. Congr. Electr. Micr. Grenoble 3 : 641-642
- JONES, R.C. (1971).
Studies on the structure of the head of boar spermatozoa from the
epididymis.
J. Reprod. Fert. Suppl. 13 : 51-64
- JONES, R.C. (1973).
Changes occurring in the head of boar spermatozoa : vesiculation or
vacuolation of the acrosome?
J. Reprod. Fert. 33 : 113-118
- JONES, R.C. (1975).
The ultrastructure of spermatozoa from some hystricomorph rodents.
In : The Functional Anatomy of the Spermatozoon. Afzelius, B.A.
(Ed.) pp.251-258.
Pergamon Press, Oxford.
- JONES, R.C., ROWLANDS, I.W. and SKINNER, J.D. (1974).
Spermatozoa in the genital ducts of the african elephant, Loxodonta
africana.
J. Reprod. Fert. 41 : 189-192
- KANG, Y.H. (1974).
Development of the zona pellucida in the rat oocyte.
Am. J. Anat. 139 : 535-566
- KARNOVSKY, M.J. (1967).
The ultrastructural basis of capillary permeability studied with
peroxidase as a tracer.
J. Cell Biol. 35 : 213-236
- KASHIWABARA, T., TANAKA, R. and MATSUMOTO, T. (1965).
Tail-to-tail agglomeration of bull spermatozoa by phytoagglutinins
present in soybeans.
Nature 207 : 831-832

- KATZ, D.F. and YANAGIMACHI, R. (1980).
Movement characteristics of hamster spermatozoa within the oviduct.
Biol. Reprod. 22 : 759-764
- KATZ, D.F. and YANAGIMACHI, R. (1981).
Movement characteristics of hamster and guinea pig spermatozoa upon attachment to the zona pellucida.
Biol. Reprod. 25 : 785-791
- KATZ, D.F., YANAGIMACHI, R. and DRESDNER, R.D. (1978).
Movement characteristics and power output of guinea-pig and hamster spermatozoa in relation to activation.
J. Reprod. Fert. 52 : 167-172
- KEEFER, C.L. and SCHUETZ, A.W. (1982).
Spontaneous activation of ovulated rat oocytes during in vitro culture.
J. Exp. Zool. 224 : 371-377
- KESSEL, R.G. and SPAZIANI, E. (1969).
Nuclear morphogenesis in rat spermatids.
J. Cell Biol. 43 : 67a-68a
- KILLIAN, G.J. and AMANN, R.P. (1973).
Immunoelectrophoretic characterization of fluid and sperm entering and leaving the bovine epididymis.
Biol. Reprod. 9 : 489-499
- KINSEY, W.H. and KOEHLER, J.K. (1976).
Fine structural localization of concanavalin A binding sites on hamster spermatozoa.
J. Supramol. Struct. 5 : 185-198
- KINSEY, W.H. and KOEHLER, J.K. (1978).
Cell surface changes associated with in vitro capacitation of hamster sperm.
J. Ultrastruct. Res. 64 : 1-13
- KIRTON, K.T. and HAFS, H.D. (1965).
Sperm capacitation by uterine fluid or Beta-amylase in vitro.
Science 150 : 618-619
- KOEHLER, J.K. (1966).
Fine structure observations in frozen-etched bovine spermatozoa.
J. Ultrastruct. Res. 16 : 359-375
- KOEHLER, J.K. (1970).
A freeze-etching study of rabbit spermatozoa with particular reference to head structures.
J. Ultrastruct. Res. 33 : 598-614
- KOEHLER, J.K. (1972).
Human sperm head ultrastructure : a freeze-etching study.
J. Ultrastruct. Res. 39 : 520-539

- KOEHLER, J.K. (1975).
Studies on the distribution of antigenic sites on the surface of rabbit spermatozoa.
J. Cell Biol. 67 : 647-659
- KOEHLER, J.K. (1976).
Changes in antigenic site distribution on rabbit spermatozoa after incubation in "capacitating" media.
Biol. Reprod. 15 : 444-456
- KOEHLER, J.K. (1978a).
Observations on the fine structure of vole spermatozoa with particular reference to cytoskeletal elements in the mature sperm head.
Gamete Res. 1 : 247-257
- KOEHLER, J.K. (1978b).
The mammalian sperm surface : studies with specific labelling techniques.
Int. Rev. Cytol. 54 : 73-107
- KOEHLER, J.K. (1981).
Lectins as probes of the spermatozoon surface.
Arch. Androl. 6 : 197-217
- KOEHLER, J.K. and PERKINS, W.D. (1974).
Fine structure observations on the distribution of antigenic sites on guinea pig spermatozoa.
J. Cell Biol. 60 : 789-795
- KOEHLER, J.K. and GADDUM-ROSSE, P. (1975).
Media induced alterations of the membrane associated particles of the guinea pig sperm tail.
J. Ultrastruct. Res. 51 : 106-118
- KOEHLER, J.K., DE CURTIS, I., STENCHEVER, M.A. and SMITH, D. (1982).
Interaction of human sperm with zona-free hamster eggs : a freeze-fracture study.
Gamete Res. 6 : 371-386
- KOEHLER, J.K., WURSCHMIDT, U. and LARSEN, M.P. (1983).
Nuclear and chromatin structure in rat spermatozoa.
Gamete Res. 8 : 357-370
- KOEHLER, J.K., SMITH, D. and KARP, L.E. (1984).
The attachment of acrosome-intact sperm to the surface of zona-free hamster oocytes.
Gamete Res. 9 : 197-205
- KOHANE, A.C., GONZALEZ-ECHEVERRIA, F.M.C., PINEIRO, L. and BLAQUIER, J.A. (1980a).
Interaction of proteins of epididymal origin with spermatozoa.
Biol. Reprod. 23 : 737-742

- KOHANE, A.C., CAMEO, M.S., PINEIRO, L., GARBERI, J.C. and BLAQUIER, J.A. (1980b).
Distribution and site of production of specific proteins in the rat epididymis.
Biol. Reprod. 23 : 181-187
- KOPECNY, V. and PAVLOK, A. (1975a).
Autoradiographic study of mouse spermatozoan arginine-rich nuclear proteins in fertilization.
J. Exp. Zool. 191 : 85-96
- KOPECNY, V. and PAVLOK, A. (1975b).
Incorporation of arginine-³H into chromatin of mouse eggs shortly after sperm penetration.
Histochemistry 45 : 341-345
- KOPECNY, V. and PAVLOK, A. (1984).
Association of newly synthesized ³H-arginine-labeled proteins with chromatin structures in fertilization.
Gamete Res. 9 : 399-408
- KORN, E.D. (1978).
Biochemistry of actomyosin-dependant cell motility (A Review).
Proc. Natl. Acad. Sci. USA 75 : 588-599
- KORN, E.D. (1982).
Actin polymerization and its regulation by proteins from non muscle cells.
Physiol. Rev. 62 : 672-737
- KRUIP, T.A.M., CRAN, D.G., VAN BENEDEN, T.H. and DIELEMAN, S.J. (1983).
Structural changes in bovine oocytes during final maturation in vivo.
Gamete Res. 8 : 29-47
- KRZANOWSKA, H. and LORENC, E. (1983).
Influence of egg investments on in vitro penetration of mouse eggs by misshapen spermatozoa.
J. Reprod. Fert. 68 : 57-62
- KUZAN, F.B., FLEMING, A.D. and SEIDEL, G.E. (1984).
Successful fertilization in vitro of fresh intact oocytes by perivitelline (acrosome-reacted) spermatozoa of the rabbit.
Fertil. Steril. 41 : 766-770
- LAEMMLI, U.K. (1970).
Cleavage of structural proteins during the assembly of the head of bacteriophage T4.
Nature 227 : 680-685
- LALLI, M. and CLERMONT, Y. (1981).
Structural changes of the head components of the rat spermatid during late spermiogenesis.
Am. J. Anat. 160 : 419-434

- LAM, D.M.K., FURRER, R. and BRUCE, W.R. (1970).
The separation, physical characterization, and differentiation kinetics of spermatogonial cells of the mouse.
Proc. Natl. Acad. Sci. USA 65 : 192-199
- LAMBAISE, J.T. and AMANN, R.P. (1973).
Infertility of rabbit testicular spermatozoa collected in their native fluid environment.
Fertil. Steril. 24 : 65-67
- LAMBERT, H. (1981).
Temperature dependence of capacitation in bat sperm monitored by zona-free hamster ova.
Gamete Res. 4 : 525-533
- LAMBERT, H. (1984).
Role of sperm-surface glycoproteins in gamete recognition in two mouse species.
J. Reprod. Fert. 70 : 281-284
- LAMBERT, H. and VAN LE, A. (1984).
Possible involvement of a sialylated component of the sperm plasma membrane in sperm-zona interaction in the mouse.
Gamete Res. 10 : 153-163
- LAMBERT, R.W. (1979).
Spermiogenesis and spermatozoal morphology in the plains mouse, Pseudomys australis.
Honours Thesis, University of Adelaide.
- LANGLAIS, J., ZOLLINGER, M., PLANTE, L., CHAPDELAIN, A., BLEAU, G. and ROBERTS, K.D. (1981).
Localization of cholesteryl sulfate in human spermatozoa in support of a hypothesis for the mechanism of capacitation.
Proc. Natl. Acad. Sci. USA 78 : 7266-7270
- LAVON, U., VOLCANI, R. and DANON, D. (1970).
The lipid content of bovine spermatozoa during maturation and aging.
J. Reprod. Fert. 23 : 215-222
- LE BLOND, C.P. and CLERMONT, Y. (1952a).
Definition of the stages of the cycle of the seminiferous epithelium in the rat.
Ann. N.Y. Acad. Sci. 55 : 548-573
- LE BLOND, C.P. and CLERMONT, Y. (1952b).
Spermiogenesis of rat, mouse, hamster and guinea pig as revealed by the "periodic acid-fuchsin sulfurous acid" technique.
Am. J. Anat. 90 : 167-215
- LEE, M.C. and DAMJANOV, I. (1985).
Lectin binding sites on human sperm and spermatogenic cells.
Anat. Rec. 212 : 282-287

- LEGAULT, Y., BLEAU, G., CHAPDELAINE, A. and ROBERTS, K.D. (1979a).
The binding of sterol sulfates to hamster spermatozoa.
Steroids 34 : 89-99
- LEGAULT, Y., BOUTHILLIER, M., BLEAU, G., CHAPDELAINE, A. and ROBERTS, K.D. (1979b).
The sterol and sterol sulfate content of the male hamster reproductive tract.
Biol. Reprod. 20 : 1213-1219
- LENZ, R.W., AX, R.L., GRIMER, H.J. and FIRST, N.L. (1982).
Proteoglycan from bovine follicular fluid enhances an acrosome reaction in bovine spermatozoa.
Biochem. Biophys. Res. Commun. 106 : 1092-1098
- LENZ, R.W., BELLIN, M.E. and AX, R.L. (1983a).
Rabbit spermatozoa undergo an acrosome reaction in the presence of glycosaminoglycans.
Gamete Res. 8 : 11-19
- LENZ, R.W., BALL, G.D., LOHSE, J.K., FIRST, N.L. and AX, R.L. (1983b).
Chondroitin sulfate facilitates an acrosome reaction in bovine spermatozoa as evidenced by light microscopy, electron microscopy and in vitro fertilization.
Biol. Reprod. 28 : 683-690
- LEVINSKY, H., SINGER, R., MALIK, Z., SAGIV, M., COHEN, A.M., SERVADIO, C. and ALLALOUF, D. (1983).
Distribution of sialic acid in human sperm membranes.
Arch. Androl. 10 : 209-212
- LEWIN, L.M., WEISSENBERG, R., SOBEL, J.S., MARCUS, Z. and NEBEL, L. (1979).
Differences in concanavalin A-FITC binding to rat spermatozoa during epididymal maturation and capacitation.
Arch. Androl. 2 : 279-281
- LEWIN, L.M., NEVO, Z., GABSU, A. and WEISSENBERG, R. (1982).
The role of sperm-bound hyaluronidase in the dispersal of the cumulus oophorus surrounding rat ova.
Int. J. Androl. 5 : 37-44
- LIVOLANT, F. (1984).
Cholesteric organization of DNA in the stallion sperm head.
Tissue Cell 16 : 535-555
- LLANOS, M.N. and MEIZEL, S. (1983).
Phospholipid methylation increases during capacitation of golden hamster sperm in vitro.
Biol. Reprod. 28 : 1043-1051
- LONGO, F.J. (1974a).
An ultrastructural analysis of spontaneous activation of hamster eggs aged in vivo.
Anat. Rec. 179 : 27-56

- LONGO, F.J. (1974b).
Ultrastructural changes in rabbit eggs aged in vivo.
Biol. Reprod. 11 : 22-39
- LONGO, F.J. (1978).
Effects of cytochalasin B on sperm-egg interactions.
Dev. Biol. 67 : 249-265
- LONGO, F.J. (1980).
Aging of mouse eggs in vivo and in vitro.
Gamete Res. 3 : 379-393
- LONGO, F.J. and CHEN, D.Y. (1985).
Development of cortical polarity in mouse eggs: involvement of the meiotic apparatus.
Dev. Biol. 107 : 382-394
- LORTON, S.P. and FIRST, N.L. (1979).
Hyaluronidase does not disperse the cumulus oophorus surrounding bovine ova.
Biol. Reprod. 21 : 301-308
- LUFT, J.H. (1971).
Ruthenium red and violet. I. Chemistry, purification, methods of use for electron microscopy and mechanism of action.
Anat. Rec. 171 : 347-368
- LUI, C.W., CORNETT, L.E. and MEIZEL, S. (1977).
Identification of the bovine follicular fluid protein involved in the in vitro induction of the hamster sperm acrosome reaction.
Biol. Reprod. 17 : 34-41
- MAHI, C.A. and YANAGIMACHI, R. (1975).
Induction of nuclear decondensation of mammalian spermatozoa in vitro.
J. Reprod. Fert. 44 : 293-296
- MAHI, C.A. and YANAGIMACHI, R. (1976).
Maturation and sperm penetration of canine ovarian oocytes in vitro.
J. Exp. Zool. 196 : 189-196
- MAHI-BROWN, C.A. and YANAGIMACHI, R. (1983).
Parameters influencing ovum pickup by oviductal fimbria in the golden hamster.
Gamete Res. 8 : 1-10
- MARSTON, J.H. and CHANG, M.C. (1966).
The morphology and timing of fertilization and early cleavage in the Mongolian gerbil and deer mouse.
J. Embryol. Exp. Morphol. 15 : 169-191
- MATTHEWS, M. and ADLER, N.T. (1977).
Facilitative and inhibitory influences of reproductive behaviour on sperm transport in rats.
J. Comp. Physiol. Psychol. 91 : 727-741

- MAXWELL, W.L. (1982).
The acrosomal zonule.
Tissue Cell 14 : 283-288
- McCLEAN, D. and ROWLANDS, I.W. (1942).
Role of hyaluronidase in fertilisation.
Nature 150 : 627-628
- McCULLOH, D.H., REXROAD, C.A. and LEVITAN, H. (1981).
Rabbit egg membrane potential's response at fertilization.
J. Cell Biol. 91 : 176a
- McGREGOR, L.K. (1985).
Does the structure of the oocyte and its surrounding investments relate to the morphology of the spermatozoa in Australian rodents.
Honours Thesis, University of Adelaide.
- McLAREN, A. (1967).
Factors affecting the variation in response of mice to gonadotropic hormones.
J. Endocrin. 37 : 147-154
- McRORIE, R.A. and WILLIAMS, W.L. (1974).
Biochemistry of mammalian fertilization.
Ann. Rev. Biochem. 43 : 77-803
- McRORIE, R.A., TURNER, R.B., BRADFORD, M.M. and WILLIAMS, W.L. (1976).
Acrolysin, the aminoproteinase catalyzing the initial conversion of proacrosin to acrosin in mammalian fertilization.
Biochem. Biophys. Res. Commun. 71 : 492-498
- MEISTRICH, M.L., REID, B.O. and BARCELLONA, W.J. (1976).
Changes in sperm nuclei during spermiogenesis and epididymal maturation.
Exp. Cell Res. 99 : 72-78
- MEIZEL, S. (1978).
The mammalian sperm acrosome reaction : a biochemical approach.
In : Development in Mammals. Johnson, M.H. (Ed.) Vol. 3, pp.1-64.
North-Holland, Amsterdam.
- MEIZEL, S. (1984).
The importance of hydrolytic enzymes to an exocytotic event, the mammalian sperm acrosome reaction.
Biol. Rev. 59 : 125-157
- MEIZEL, S. and HUANG-YANG, Y.H.J. (1973).
Further studies of an inactive form of a trypsin-like enzyme in rabbit testes.
Biochem. Biophys. Res. Commun. 53 : 1145-1150
- MEIZEL, S. and MUKERJI, S.K. (1975).
Proacrosin from rabbit spermatozoa : partial purification and initial biochemical characterization.
Biol. Reprod. 13 : 83-93

- MEIZEL, S. and MUKERJI, S.K. (1976).
Biochemical studies of proacrosin and acrosin from hamster cauda epididymal spermatozoa.
Biol. Reprod. 14 : 444-450
- MEIZEL, S. and WORKING, P.K. (1980).
Further evidence suggesting the hormonal stimulation of hamster sperm acrosome reactions by catecholamines in vitro.
Biol. Reprod. 22 : 211-216
- MEIZEL, S., LUI, C.W., WORKING, P.K. and MRSNY, R.J. (1980).
Taurine and hypotaurine : their effects on motility, capacitation and the acrosome reaction of hamster sperm in vitro and their presence in sperm and reproductive tract fluids of several mammals.
Dev. Growth Diff. 22 : 483-494
- MERCHANT, H. and CHANG, M.C. (1971).
An electron microscopic study of mouse eggs matured in vivo and in vitro.
Anat. Rec. 171 : 21-38
- METZ, C.B., SEIGUER, A.C. and CASTRO, A.E. (1972).
Inhibition of the cumulus dispersing and hyaluronidase activities of sperm by heterologous and isolagous antisperm antibodies.
Proc. Soc. Exp. Biol. Med. 140 : 776-781
- MEYENHOFER, M., ANDERSON, O.F., MARX, B.S. and GWATKIN, R.B.L. (1977).
The zona reaction : an SEM and light microscope study employing hamster gametes.
S.E.M. 1977/II : 343-347
- MILLETTE, C.F., SPEAR, P.G., GALL, W.E. and EDELMAN, G.M. (1973).
Chemical dissection of mammalian spermatozoa.
J. Cell Biol. 58 : 662-675
- MINASSIAN, H. and HUANG, S.N. (1979).
Effect of sodium azide on the ultrastructural preservation of tissues.
J. Microsc. 117 : 243-253
- MIYAMOTO, H. and CHANG, M.C. (1972).
Fertilization in vitro of mouse and hamster eggs after the removal of follicular cells.
J. Reprod. Fert. 30 : 309-312
- MOHRI, H. and YANAGIMACHI, R. (1980).
Characteristics of motor apparatus in testicular, epididymal and ejaculated spermatozoa. A study using demembrated sperm models.
Exp. Cell Res. 127 : 191-196
- MOHRI, H., ISHIJIMA, S. and HIRAMOTO, Y. (1983).
Movement characteristics of hamster spermatozoa. In : The Sperm Cell. Andre, J. (Ed.) pp.345-348.
Martinus-Nijhoff, The Hague.

MONFOORT, C.H., SCHIPHOF, R., ROZIJN, T.H. and STEYN-PARVE, E.P. (1973).

Amino acid composition and carboxyl-terminal structure of some basic chromosomal proteins of mammalian spermatozoa.

Biochim. Biophys. Acta. 322 : 173-177

MOOR, R.M., SMITH, M.W. and DAWSON, R.M.C. (1980).

Measurement of intercellular coupling between oocytes and cumulus cells using intracellular markers.

Exp. Cell Res. 126 : 15-29

MOORE, H.D.M. (1979).

The net surface charge of mammalian spermatozoa as determined by isoelectric focusing. Changes following sperm maturation, ejaculation, incubation in the female tract, and after enzyme treatment.

Int. J. Androl. 2 : 449-462

MOORE, H.D.M. (1980).

Localization of specific glycoproteins secreted by the rabbit and hamster epididymis.

Biol. Reprod. 22 : 705-718

MOORE, H.D.M. (1981a).

Glycoprotein secretions of the epididymis in the rabbit and hamster : localization on epididymal spermatozoa and the effect of specific antibodies on fertilization in vivo.

J. Exp. Zool. 215 : 77-85

MOORE, H.D.M. (1981b).

An assessment of the fertilizing ability of spermatozoa in the epididymis of the marmoset monkey (Callithrix jacchus).

Int. J. Androl. 4 : 321-330

MOORE, H.D.M. and BEDFORD, J.M. (1978a).

Ultrastructure of the equatorial segment of hamster spermatozoa during penetration of oocytes.

J. Ultrastruct. Res. 62 : 110-117

MOORE, H.D.M. and BEDFORD, J.M. (1978b).

An in vivo analysis of factors influencing the fertilization of hamster eggs.

Biol. Reprod. 19 : 879-885

MOORE, H.D.M. and BEDFORD, J.M. (1983).

The interaction of mammalian gametes in the female. In : Mechanism and Control of Animal Fertilization. Hartmann, J.F. (Ed.). pp.453-497.

Academic Press, New York.

MOORE, H.D.M. and HARTMAN, T.D. (1984).

Localization by monoclonal antibodies of various surface antigens of hamster spermatozoa and the effect of antibody on fertilization in vitro.

J. Reprod. Fert. 70 : 175-183

- MOORE, H.D.M., HARTMAN, T.D. and PRYOR, J.P. (1983).
Development of the oocyte-penetrating capacity of spermatozoa in the human epididymis.
Int. J. Androl. 6 : 310-318
- MOORE, H.D.M., HARTMAN, T.D. and HOLT, W.V. (1984).
The structure and epididymal maturation of the spermatozoon of the common marmoset (Callithrix jacchus).
J. Anat. 138 : 227-235
- MORTIMER, D. and THOMPSON, T.E. (1976).
Nuclear pores in the spermatozoon of the rat.
Experientia 32 : 104-106
- MORTIMER, D., COUROT, A.M., GIOVANGRANDI, Y., JEULIN, C. and DAVID, G. (1984).
Human sperm motility after migration into, and incubation in, synthetic media.
Gamete Res. 9 : 131-144
- MORTON, B.E., SAGADRACA, R. and FRASER, C. (1978).
Sperm motility within the mammalian epididymis : species variation and correlation with free calcium levels in epididymal plasma.
Fertil. Steril. 29 : 695-698
- MORTON, D.B. (1975).
Acrosomal enzymes : immunochemical localization of acrosin and hyaluronidase in ram spermatozoa.
J. Reprod. Fert. 45 : 375-378
- MORTON, D.B. (1976).
Lysosomal enzymes in mammalian spermatozoa. In : Lysosomes in Biology and Pathology. Dingle, J.T. and Dean, R.T. (Eds.) Vol. 5, pp.203-255.
North-Holland, Amsterdam.
- MORTON, D.B. (1977).
Immunoenzymic studies on acrosin and hyaluronidase in ram spermatozoa. In : Immunobiology of Gametes. Edidin, M. and Johnson, M.H. (Eds.) pp.115-155.
Cambridge Univ. Press, London.
- MYLES, D.G. and PRIMAKOFF, P. (1983).
Establishment of sperm surface topography during development analyzed with monoclonal antibodies. In : The Sperm Cell. Andre, J. (Ed.) pp.127-130.
Martinus-Nijhoff, The Hague.
- MYLES, D.G. and PRIMAKOFF, P.M. (1984).
Localized surface antigens of guinea pig sperm migrate to new regions prior to fertilization.
J. Cell Biol. 99 : 1634-1641
- NAGY, F. and EDMONDS, R.H. (1973).
Some observations on the fine structure of armidillo spermatozoa.
J. Reprod. Fert. 34 : 551-553

- NEI, M., MARUYAMA, T. and WU, C.I. (1983).
Models of evolution and reproductive isolation.
Genetics 103 : 557-579
- NEVO, A.C., MICHAELE, I. and SCHINDLER, H. (1961).
Electrophoretic properties of bull and of rabbit spermatozoa.
Exp. Cell Res. 23 : 69-83
- NICANDER, L. and BANE, A. (1962).
Fine structure of boar spermatozoa.
Z. Zellforsch Mikrosk. Anat. 57 : 390-405
- NICANDER, L. and BANE, A. (1966).
Fine structure of the sperm head in some mammals, with particular reference to the acrosome and the subacrosomal substance.
Z. Zellforsch Mikrosk. Anat. 72 : 496-515
- NICOLSON, G.L. and YANAGIMACHI, R. (1972).
Terminal saccharides on sperm plasma membranes : identification by specific agglutinins.
Science 177 : 276-279
- NICOLSON, G.L., LACORBIERE, M. and YANAGIMACHI, R. (1972).
Quantitative determination of plant agglutinin membrane sites on mammalian spermatozoa.
Proc. Soc. Exp. Biol. Med. 141 : 661-663
- NICOLSON, G.L., YANAGIMACHI, R. and YANAGIMACHI, H. (1975).
Ultrastructural localization of lectin-binding sites on the zonae pellucidae and plasma membranes of mammalian eggs.
J. Cell Biol. 66 : 263-274
- NICOLSON, G.L., USUI, N., YANAGIMACHI, R., YANAGIMACHI, H. and SMITH, J.R. (1977).
Lectin-binding sites on the plasma membranes of rabbit spermatozoa. Changes in surface receptors during epididymal maturation and after ejaculation.
J. Cell Biol. 74 : 950-962
- NICOLSON, G.L., BRODGINSKI, A.B., BEATTIE, G. and YANAGIMACHI, R. (1979).
Cell surface changes in the proteins of rabbit spermatozoa during epididymal passage.
Gamete Res. 2 : 153-162
- NICOSIA, S.V., WOLF, D.P. and INOUE, M. (1977).
Cortical granule distribution and cell surface characteristics in mouse eggs.
Dev. Biol. 57 : 56-74
- NICOSIA, S.V., WOLF, D.P. and MASTROIANNI, L. (1978).
Surface topography of mouse eggs before and after insemination.
Gamete Res., 1 : 145-155

- NIKOLOPOULOU, M., SOUCEK, D.A. and VARY, J.C. (1985).
Changes in the lipid content of boar sperm plasma membranes during epididymal maturation.
Biochim. Biophys. Acta 815 : 486-498
- NINJOOR, V. and SRIVASTAVA, P.N. (1985).
Partial purification and characterization of an alkaline proteinase from the rabbit testis.
Gamete Res. 11 : 69-82
- NODA, Y.D. and YANAGIMACHI, R. (1976).
Electron microscopic observations of guinea pig spermatozoa penetrating eggs in vitro.
Dev. Growth Diff. 18 : 15-23
- NOLAND, T.D., OLSON, G.E. and GARBERS, D.L. (1983).
Purification and partial characterization of plasma membranes from bovine spermatozoa.
Biol. Reprod. 29 : 987-998
- OAKBERG, E.F. (1956).
A description of spermiogenesis in the mouse and its use in analysis of the cycle of the seminiferous epithelium and germ cell renewal.
Am. J. Anat. 99 : 391-413
- O'BRIEN, D.A. and BELLVE, A.R. (1980a).
Protein constituents of the mouse spermatozoon. I. An electrophoretic characterization.
Dev. Biol. 75 : 386-404
- O'BRIEN, D.A. and BELLVE, A.R. (1980b).
Protein constituents of the mouse spermatozoon. II. Temporal synthesis during spermatogenesis.
Dev. Biol. 75 : 405-418
- OCHS, D. (1984).
Actin in post-ejaculatory human sperm.
Biol. Reprod. 30 Suppl. 1 : 78
- ODOR, D.L. (1960).
Electron microscopic studies on ovarian oocytes and unfertilized tubal ova in the rat.
J. Biophys. Biochem. Cytol. 7 : 567-574
- O'FARRELL, P.H. (1975).
High resolution two-dimensional electrophoresis of proteins.
J. Biol. Chem. 250 : 4007-4021
- OH, Y.K. and BRACKETT, B.G. (1976).
Ultrastructure of rabbit ova recovered from ovarian follicles and inseminated in vitro.
Fertil. Steril. 26 : 665-685

- OIKAWA, T., YANAGIMACHI, R. and NICOLSON, G.L. (1975).
Species differences in lectin-binding sites of the zona pellucida
of rodent eggs.
J. Reprod. Fert. 43 : 137-140
- OLIPHANT, G. and BRACKETT, B.G. (1973).
Immunological assessment of surface changes of rabbit sperm
undergoing capacitation.
Biol. Reprod. 9 : 404-414
- OLSON, G.E. and LINCK, R.W. (1977).
Observations of the structural components of flagellar axonemes and
central pair microtubules from rat sperm.
J. Ultrastruct. Res. 61 : 21-43
- OLSON, G.E. and SAMMONS, D.W. (1980).
Structural chemistry of outer dense fibres of rat sperm.
Biol. Reprod. 22 : 319-332
- OLSON, G.E. and DANZO, B.J. (1981).
Surface changes in rat spermatozoa during epididymal transit.
Biol. Reprod. 24 : 431-443
- OLSON, G.E. and GOULD, K.G. (1981).
Characterization of sperm surface and seminal plasma glycoproteins
of the chimpanzee.
J. Reprod. Fert. 62 : 185-191
- OLSON, G.E. and ORGEBIN-CRIST, M.C. (1982).
Sperm surface changes during epididymal maturation.
Ann. N.Y. Acad. Sci. 383 : 372-392
- OLSON, G.E. and WINFREY, V.P. (1985).
Structure of membrane domains and matrix components of the bovine
acrosome.
J. Ultrastruct. Res. 90 : 9-25
- OLSON, G.E., HAMILTON, D.W. and FAWCETT, D.W. (1976a).
Isolation and characterization of the perforatorium of rat
spermatozoa.
J. Reprod. Fert. 47 : 293-297
- OLSON, G.E., HAMILTON, D.W. and FAWCETT, D.W. (1976b).
Isolation and characterization of the fibrous sheath of rat
epididymal spermatozoa.
Biol. Reprod. 14 : 517-530
- OLSON, G.E., NOLAND, T.D., WINFREY, V.P. and GARBERS, D.L. (1983a).
Substructure of the post-acrosomal sheath of bovine spermatozoa.
J. Ultrastruct. Res. 85 : 204-218
- OLSON, G.E., WINFREY, V.P., NOLAND, T.D. and GARBERS, D.L. (1983b).
Ultrastructure of a crystalline inclusion in the cytoplasmic
droplet of bovine spermatozoa.
Gamete Res. 8 : 325-333

- O'RAND, M.G. (1978).
Capping of sperm-specific autoantigens on spermatids, residual bodies and ejaculate spermatozoa.
Anat. Rec. 190 : 497
- O'RAND, M.G., MATTHEWS, J.E., WELCH, J.E. and FISHER, S.J. (1985).
Identification of zona binding proteins of rabbit, pig, human, and mouse spermatozoa on nitrocellulose blots.
J. Exp. Zool. 235 : 423-428
- ORGBIN-CRIST, M.C. (1967a).
Sperm maturation in the rabbit epididymis.
Nature 216 : 816-818
- ORGBIN-CRIST, M.C. (1967b).
Maturation of spermatozoa in the rabbit epididymis : Fertilizing ability and embryonic mortality in does inseminated with epididymal spermatozoa.
Ann. Biol. Anim. Biochem. Biophys. 7 : 373-389
- ORGBIN-CRIST, M.C. (1973).
Maturation of spermatozoa in the rabbit epididymis : effect of castration and testosterone replacement.
J. Exp. Zool. 185 : 301-310
- ORGBIN-CRIST, M.C., DANZO, B.J. and DAVIES, J. (1975).
Endocrine control of the development and maintenance of sperm fertilizing ability in the epididymis. In : Handbook of Physiology. Hamilton, D.W. and Greep, R.D. (Eds.). Section 7, Vol. 5, pp.319-333.
Amer. Physiol. Soc., Washington.
- OVERSTREET, J.W., (1983).
Transport of gametes in the reproductive tract of the female mammal. In : Mechanism and Control of Animal Fertilization. Hartmann, J.F. (Ed.). pp.499-543.
Academic Press, New York.
- OVERSTREET, J.W. and BEDFORD, J.M. (1974).
Transport, capacitation and fertilizing ability of epididymal spermatozoa.
J. Exp. Zool. 189 : 203-214
- OVERSTREET, J.W. and COOPER, G.W. (1975).
Reduced sperm motility in the isthmus of the rabbit oviduct.
Nature 258 : 718-719
- OVERSTREET, J.W., and BEDFORD, J.M. (1976).
Embryonic mortality in the rabbit is not increased after fertilization by young epididymal spermatozoa.
Biol. Reprod. 15 : 54-57
- OVERSTREET, J.W. and KATZ, D.F. (1977).
Sperm transport and selection in the female genital tract. In : Development in Mammals. Johnson, M.H. (Ed.). Vol. 2, pp.31-65.
North-Holland, Amsterdam.

- OVERSTREET, J.W. and COOPER, G.W. (1979).
Effect of ovulation and sperm motility on the migration of rabbit spermatozoa to the site of fertilization.
J. Reprod. Fert. 55 : 53-59
- PALLINI, V. (1979).
Isolation of mitochondria from bull epididymal spermatozoa. In : The Spermatozoon. Maturation, motility, surface properties and comparative aspects. Fawcett, D.W. and Bedford, J.M. (Eds.). pp.401-410.
Urban and Schwarzenberg, Baltimore.
- PALLINI, V., BACCETTI, B. and BURRINI, A.G. (1979).
A peculiar cysteine-rich polypeptide related to some unusual properties of mammalian sperm mitochondria. In : The Spermatozoon. Maturation, motility, surface properties and comparative aspects. Fawcett, D.W. and Bedford, J.M. (Eds.). pp.141-151.
Urban and Schwarzenberg, Baltimore.
- PARKS, J.E. and HAMMERSTEDT, R.H. (1985).
Development changes occurring in the lipids of ram epididymal spermatozoa plasma membrane.
Biol. Reprod. 32 : 658-668
- PAUFLER, S.K. and FOOTE, R.H. (1968).
Morphology, motility and fertility of spermatozoa recovered from different areas of ligated rabbit epididymides.
J. Reprod. Fert. 17 : 125-137
- PAVLOK, A. (1974).
Development of the penetration activity of mouse epididymal spermatozoa in vivo and in vitro.
J. Reprod. Fert. 36 : 203-205
- PEDERSEN, H. (1972).
The post-acrosomal region of the spermatozoa of man and Macaca arctoides.
J. Ultrastruct. Res. 40 : 366-377
- PEDERSEN, H. (1974).
The human spermatozoon.
Dan. Med. Bull. 21 Suppl. 1 : 1-36
- PERREAULT, S.D. and ZIRKIN, B.R. (1982).
Sperm nuclear decondensation in mammals : role of sperm-associated proteinase in vivo.
J. Exp. Zool. 224 : 253-257
- PERREAULT, S., ZANEVELD, L.J.D. and ROGERS, B.J. (1980).
Inhibition of fertilization in the hamster by sodium aurothiomalate, a hyaluronidase inhibitor.
J. Reprod. Fert. 60 : 461-467
- PERREAULT, S.D., ZIRKIN, B.R. and ROGERS, B.J. (1982).
Effect of trypsin inhibitors on acrosome reaction of guinea pig spermatozoa.
Biol. Reprod. 26 : 343-351

- PERRY, J.S. (1971).
The Ovarian Cycle of Mammals.
 Oliver and Boyd, Edinburgh.
- PETERSON, R., RUSSELL, L., BUNDMAN, D. and FREUND, M. (1978).
 Presence of microfilaments and tubular structures in boar spermatozoa after chemically inducing the acrosome reaction.
Biol. Reprod. 19 : 459-466
- PETERSON, R.N., ASHRAF, M. and RUSSELL, L.D. (1983).
 Effect of calmodulin antagonists on calcium uptake by boar spermatozoa.
Biochem. Biophys. Res. Commun. 114 : 28-33
- PETERSON, R.N., HENRY, L., HUNT, W., SAXENA, N. and RUSSELL, L.D. (1985).
 Further characterization of boar sperm plasma membrane proteins with affinity for the porcine zona pellucida.
Gamete Res. 12 : 91-100
- PFALLER, W., ROVAN, E. and MAIRBAURL, H. (1976).
 A comparison of the ultrastructure of spray-frozen and freeze-etched or freeze-dried bull and boar spermatozoa with that after chemical fixation.
J. Reprod. Fert. 48 : 285-290
- PHILLIPS, D.M. (1972).
 Substructure of the mammalian acrosome.
J. Ultrastruct. Res. 38 : 591-604
- PHILLIPS, D.M. (1975).
 Cell surface structure of rodent sperm heads.
J. Exp. Zool. 191 : 1-8
- PHILLIPS, D.M. (1977a).
 Surface of the equatorial segment of the mammalian acrosome.
Biol. Reprod. 16 : 128-137
- PHILLIPS, D.M. (1977b).
 Surfaces of mammalian spermatozoa as revealed in platinum replicas.
 In : Male Reproductive System. Yates, R.D. and Gordon, M. (Eds.).
 pp.1-13.
 Masson, New York.
- PHILLIPS, D.M. (1977c).
 Mitochondrial disposition in mammalian spermatozoa.
J. Ultrastruct. Res. 58 : 144-154
- PHILLIPS, D.M. and OLSON, G.E. (1975).
 Mammalian sperm motility - structure in relation to function. In :
The Functional Anatomy of the Spermatozoon. Afzeluis, B.A. (Ed.).
 pp.117-126.
 Pergamon Press, Oxford.

- PHILLIPS, D.M. and SHALGI, R. (1980a).
Surface architecture of the mouse and hamster zona pellucida and oocyte.
J. Ultrastruct. Res. 72 : 1-12
- PHILLIPS, D.M. and SHALGI, R. (1980b).
Surface properties of the zona pellucida.
J. Exp. Zool. 213 : 1-8
- PHILLIPS, D.M. and SHALGI, R. (1982).
Sperm penetration into rat ova fertilized in vivo.
J. Exp. Zool. 221 : 373-378
- PHILLIPS, D.M. and YANAGIMACHI, R. (1982).
Difference in the manner of association of acrosome-intact and acrosome-reacted hamster spermatozoa with egg microvilli as revealed by scanning electron microscopy.
Dev. Growth Diff. 24 : 543-551
- PHILLIPS, D.M., SHALGI, R., KRAICER, P. and SEGAL, S.J. (1978).
The rat oocyte-cumulus complex during ovulation and fertilization as seen with the SEM.
S.E.M. 1978/II : 1113-1122
- PHILLIPS, D.M., SHALGI, R. and DEKEL, N. (1985).
Mammalian fertilization as seen with the scanning electron microscope.
Am. J. Anat. 174 : 357-372
- PICKWORTH, S., YERGANIAN, G. and CHANG, M.C. (1968).
Fertilization and early development in the Chinese hamster, Cricetulus griseus.
Anat. Rec. 162 : 197-207
- PIKO, L. (1969).
Gamete structure and sperm entry in mammals. In : Fertilization. Metz, C.B. and Monroy, A. (Eds.). Vol. 2, pp.325-403. Academic Press, New York.
- PLATTNER, H. (1971).
Bull spermatozoa : a re-investigation by freeze-etching using widely different cryofixation procedures.
J. Submicr. Cytol. 3 : 19-32
- PLOEN, L., EKWALL, H. and AFZELIUS, B.A. (1979).
Spermiogenesis and the spermatozoa of the European common shrew (Sorex araneus L.)
J. Ultrastruct. Res. 68 : 149-159
- POGANY, G.C., CORZETT, M., WESTON, S. and BALHORN, R. (1981).
DNA and protein content of mouse sperm. Implications regarding sperm chromatin structure.
Exp. Cell Res. 136 : 127-136

- POLAKOSKI, K.L. and McRORIE, R.A. (1973).
Boar acrosin. II. Classification, inhibition and specificity studies of a proteinase from sperm acrosomes.
J. Biol. Chem. 248 : 8183-8188
- POLAKOSKI, K.L. and PARRISH, R.F. (1977).
Boar proacrosin. Purification and preliminary activation studies of proacrosin isolated from ejaculated boar sperm.
J. Biol. Chem. 252 : 1888-1894
- POLAKOSKI, K.L., WILLIAMS, W.L. and McRORIE, R.A. (1973).
Partial purification and characterization of an acidic proteinase in sperm acrosomes.
Fed. Proc. 32 : 310
- PRIMAKOFF, P.M. and MYLES, D.G. (1983).
A map of the guinea pig sperm surface constructed with monoclonal antibodies.
Dev. Biol. 98 : 417-428
- PRIMAKOFF, P.M., HEATON, M.R. and MYLES, D.G. (1983).
Species specificity of hybridoma antibodies to surface antigens of guinea pig sperm.
Gamete Res. 8 : 385-394
- PRUSLIN, F.H. and RODMAN, T.C. (1983).
Proteins of de-membrated protamine-depleted mouse sperm.
Exp. Cell Res. 144 : 115-126
- PRUSLIN, F.H. and RODMAN, T.C. (1985).
Proteins of demembrated mouse sperm heads. Characterization of a major sperm-unique component.
J. Biol. Chem. 260 : 5654-5659
- QUINN, P. (1979).
Failure of human spermatozoa to penetrate zona-free mouse and rat ova in vitro.
J. Exp. Zool. 210 : 497-506
- QUINN, P.J. and WHITE, I.G. (1967).
Phospholipid and cholesterol content of epididymal and ejaculated ram spermatozoa and seminal plasma in relation to cold shock.
Aust. J. Biol. Sci. 20 : 1205-1215
- QUINN, P., BARROS, C. and WHITTINGHAM, D.G. (1982).
Preservation of hamster oocytes to assay the fertilizing capacity of human spermatozoa.
J. Reprod. Fert. 66 : 161-168
- RATTNER, J.B. and BRINKLEY, B.R. (1972).
Ultrastructure of mammalian spermiogenesis. III. The organization and morphogenesis of the manchette during rodent spermiogenesis.
J. Ultrastruct. Res. 41 : 209-218

- REPIN, V.S. and AKIMOVA, I.M. (1976).
The microelectrophoretic analysis of protein patterns of mammalian oocyte and zygote zonae pellucidae.
Biokhimiya (USSR) 41 : 39-44
- REYNOLD, E.S. (1963).
The use of lead citrate at high pH as an electron-opaque stain in electron microscopy.
J. Cell Biol. 17 : 208-212
- RIFKIN, J.M. and OLSON, G.E. (1985).
Characterization of maturation-dependent extrinsic proteins of the rat sperm surface.
J. Cell Biol. 100 : 1582-1591
- ROBERTS, M.L., SCOUTEN, W.H. and NYQUIST, S.E. (1976).
Isolation and characterization of the cytoplasmic droplet in the rat.
Biol. Reprod. 14 : 421-424
- RODGER, J.C. and YOUNG, R.J. (1981).
Glycosidase and cumulus dispersal activities of acrosomal extracts from opossum (marsupial) and rabbit (eutherian) spermatozoa.
Gamete Res. 4 : 507-514
- RODMAN, T.C., PRUSLIN, F.H., HOFFMANN, H.P. and ALLFREY, V.C. (1981).
Turnover of basic chromosomal proteins in fertilized eggs : a cytoimmunochemical study of events in vivo.
J. Cell Biol. 90 : 351-361
- RODMAN, T.C., PRUSLIN, F.H. and ALLFREY, V.G. (1982).
Mechanisms of displacement of sperm basic nuclear proteins in mammals. An in vitro simulation of post-fertilization results.
J. Cell Sci. 53 : 227-244
- RODMAN, T.C., PRUSLIN, F.H., CHIORAZZI, N., MICHELIS, M.A. and WINSTON, R. (1983).
p15, a nuclear-associated protein of human sperm : Identification of four variants and their occurrence in normal and abnormal seminal cells.
Gamete Res. 8 : 129-147
- RODMAN, T.C., PRUSLIN, F.H. and ALLFREY, V.C. (1984).
Protamine-DNA association in mammalian spermatozoa.
Exp. Cell Res. 150 : 269-281
- ROGERS, B.J. (1978).
Mammalian sperm capacitation and fertilization in vitro : a critique of methodology.
Gamete Res. 1 : 165-223
- ROGERS, B.J. and YANAGIMACHI, R. (1975a).
Release of hyaluronidase from guinea-pig spermatozoa through an acrosome reaction initiated by calcium.
J. Reprod. Fert. 44 : 135-138

- ROGERS, B.J. and YANAGIMACHI, R. (1975b).
Retardation of guinea pig sperm acrosome reaction by glucose : the possible importance of pyruvate and lactate metabolism in capacitation and the acrosome reaction.
Biol. Reprod. 13 : 568-575
- ROGERS, B.J., UENO, M. and YANAGIMACHI, R. (1981).
Fertilization by guinea pig spermatozoa requires potassium ions.
Biol. Reprod. 25 : 639-648
- ROLDAN, E.R.S., VITULLO, A.D., MERANI, M.S. and VON LAWZEWITSCH, I. (1985).
Cross fertilization in vivo and in vitro between three species of vesper mice, Calomys (Rodentia, Cricetidae).
J. Exp. Zool. 233 : 433-442
- ROMRELL, L.J. and ROSS, M.H. (1979).
Characterization of Sertoli cell-germ cell junctional specializations in dissociated testicular cells.
Anat. Rec. 193 : 23-42
- ROOMANS, G.M. (1975).
Tannic acid fixation of human spermatozoa.
J. Reprod. Fert. 43 : 197-200
- ROSS, M.H. (1976).
The Sertoli cell junctional specialization during spermiogenesis and at spermiation.
Anat. Rec. 186 : 79-104
- ROWLEY, M.J., TESHIMA, F. and HELLER, C.G. (1970).
Duration of transit of spermatozoa through the human male ductular system.
Fert. Steril. 21 : 390-396
- RUSSELL, L.D. (1977).
Observations on rat Sertoli ectoplasmic ("junctional") specializations in their association with germ cells of the rat testis.
Tissue Cell 9 : 475-498
- RUSSELL, L.D. (1979a).
Further observations on tubulobulbar complexes formed by late spermatids and Sertoli cells in the rat testis.
Anat. Rec. 194 : 213-232
- RUSSELL, L.D. (1979b).
Spermatid-Sertoli tubulobulbar complexes as devices for elimination of cytoplasm from the head region of late spermatids of the rat.
Anat. Rec. 194 : 233-246
- RUSSELL, L.D. (1980).
Sertoli-germ cell interrelations : a review.
Gamete Res. 3 : 179-202

- RUSSELL, L. and CLERMONT, Y. (1976).
Anchoring device between Sertoli cells and late spermatids in rat seminiferous tubules.
Anat. Rec. 185 : 259-278
- RUSSELL, L.D. and MALONE, J.P. (1980).
A study of Sertoli-spermatid tubulobulbar complexes in selected mammals.
Tissue Cell 12 : 263-285
- RUSSELL, L., PETERSON, R. and FREUND, M. (1979).
Direct evidence for formation of hybrid vesicles by fusion of plasma and outer acrosomal membranes during the acrosome reaction in boar spermatozoa.
J. Exp. Zool. 208 : 41-56
- RUSSELL, L., PETERSON, R.N. and FREUND, M. (1980).
On the presence of bridges linking the inner and outer acrosomal membranes of boar spermatozoa.
Anat. Rec. 198 : 449-459
- RUSSELL, L.D., LEE, I.P., ETTLIN, R. and PETERSON, R.N. (1983a).
Development of the acrosome and alignment, elongation and entrenchment of spermatids in procarbazine-treated rats.
Tissue Cell 15 : 615-626
- RUSSELL, L.D., PETERSON, R.N., RUSSELL, T.A. and HUNT, W. (1983b).
Electrophoretic map of boar sperm plasma membrane polypeptides and localization and fractionation of specific polypeptide subclasses.
Biol. Reprod. 28 : 393-413
- RUSSELL, L.D., PETERSON, R.N., HUNT, W. and STRACK, L.E. (1984).
Posttesticular surface modifications and contributions of reproductive tract fluids to the surface polypeptide composition of boar spermatozoa.
Biol. Reprod. 30 : 959-978
- SAACKE, R.G. and ALMQUIST, J.O. (1964a).
The ultrastructure of bovine spermatozoa. I. The head of normal ejaculated sperm.
Am. J. Anat. 115 : 143-162
- SAACKE, R.G. and ALMQUIST, J.O. (1964b).
The ultrastructure of bovine spermatozoa. II. The neck and tail of normal ejaculated sperm.
Am. J. Anat. 115 : 163-184
- SACCO, A.G., YUREWICZ, E.C., SUBRAMANIAN, M.G., and DE MAYO, F.J. (1981).
Zona pellucida composition : species cross-reactivity and contraceptive potential of antiserum to a purified pig zona antigen (PPZA).
Biol. Reprod. 25 : 997-1008
- SACCO, A.G., YUREWICZ, E.C. and ZHANG, S. (1983).
Immunoelectrophoretic analysis of the porcine zona pellucida.
J. Reprod. Fert. 68 : 21-31

- SALING, P.M. (1981).
Involvement of trypsin-like activity in binding of mouse spermatozoa to zonae pellucidae.
Proc. Natl. Acad. Sci. USA 78 : 6231-6235
- SALING, P.M. (1982).
Development of the ability to bind to zonae pellucidae during epididymal maturation : reversible immobilization of mouse spermatozoa by lanthanum.
Biol. Reprod. 26 : 429-436
- SALING, P.M. and STOREY, B.T. (1979).
Mouse gamete interactions during fertilization in vitro.
Chlortetracycline as a fluorescent probe for the mouse sperm acrosome reaction.
J. Cell Biol. 83 : 544-555
- SALING, P.M. and LAKOSKI, K.A. (1985).
Mouse sperm antigens that participate in fertilization. II. Inhibition of sperm penetration through the zona pellucida using monoclonal antibodies.
Biol. Reprod. 33 : 527-536
- SALING, P.M., STOREY, B.T. and WOLF, D.P. (1978).
Calcium-dependent binding of mouse epididymal spermatozoa to the zona pellucida.
Dev. Biol. 65 : 515-525
- SALING, P.M., SOWINSKI, J. and STOREY, B.T. (1979).
An ultrastructural study of epididymal mouse spermatozoa binding to zonae pellucidae in vitro : sequential relationship to the acrosome reaction.
J. Exp. Zool. 209 : 229-238
- SALING, P.M., IRONS, G. and WAIBEL, R. (1985).
Mouse sperm antigens that participate in fertilization. I. Inhibition of sperm fusion with the egg plasma membrane using monoclonal antibodies.
Biol. Reprod. 33 : 515-526
- SARAFIS, V., LAMBERT, R.W. and BREED, W.G. (1981).
Sperm head morphology of the plains mouse Pseudomys australis.
J. Reprod. Fert. 61 : 399-401
- SATHANANTHAN, A.H. and TROUNSON, A.O. (1982).
Ultrastructural observations on cortical granules in human follicular oocytes cultured in vitro.
Gamete Res. 5 : 191-198
- SATO, K. (1979).
Polyspermy - preventing mechanisms in mouse eggs fertilized in vitro.
J. Exp. Zool. 210 : 353-359

- SATO, K. and BLANDAU, R.J. (1979a).
Time and process of sperm penetration into cumulus-free mouse eggs fertilized in vitro.
Gamete Res. 2 : 295-304
- SATO, K. and BLANDAU, R.J. (1979b).
Second meiotic division and polar body formation in mouse eggs fertilized in vitro.
Gamete Res. 2 : 283-293
- SAXENA, N.K., RUSSELL, L.D., SAXENA, N. and PETERSON, R.N. (1984).
Monoclonal antibodies to boar sperm reveal apparent new surface domains.
J. Cell Biol. 99 : 259A
- SCHMELL, E.D. and GULYAS, B.J. (1980a).
Mammalian sperm-egg recognition and binding in vitro. I. Specificity of sperm interactions with live and fixed eggs in homologous and heterologous inseminations of hamster, mouse and guinea pig oocytes.
Biol. Reprod. 23 : 1075-1085
- SCHMELL, E.D. and GULYAS, B.J. (1980b).
Ovoperoxidase activity in ionophore treated mouse eggs. II. Evidence for the enzyme's role in hardening the zona pellucida.
Gamete Res. 3 : 279-290
- SCHMELL, E.D., YUAN, L.C., GULYAS, B.J. and AUGUST, J.T. (1982).
Identification of mammalian sperm surface antigens. I. Production of monoclonal anti-mouse sperm antibodies.
Fertil. Steril. 37 : 249-257
- SCHWARZ, M.A. and KOEHLER, J.K. (1979).
Alterations in lectin binding to guinea pig spermatozoa accompanying in vitro capacitation and the acrosome reaction.
Biol. Reprod. 21 : 1295-1307
- SCOTT, T.W., VOGLMAYER, J.K. and SETCHELL, B.P. (1967).
Lipid composition and metabolism in testicular and ejaculated ram spermatozoa.
Biochem. J. 102 : 456-461
- SELLENS, M.H. and JENKINSON, E.J. (1975).
Permeability of the mouse zona pellucida to immunoglobulin.
J. Reprod. Fert. 42 : 153-157
- SENGER, P.L. and SAACKE, R.G. (1970).
Unusual mitochondria of the bovine oocyte.
J. Cell Biol. 46 : 405-408
- SHALGI, R. and KRAICER, P.F. (1978).
Timing of sperm transport, sperm penetration and cleavage in the rat.
J. Exp. Zool. 204 : 353-360

- SHALGI, R. and PHILLIPS, D.M. (1980a).
Mechanics of sperm entry in cycling hamsters.
J. Ultrastruct. Res. 71 : 154-161
- SHALGI, R. and PHILLIPS, D.M. (1980b).
Mechanics of in vitro fertilization in the hamster.
Biol. Reprod. 23 : 433-444
- SHALGI, R. and PHILLIPS, D.M. (1982).
Sperm penetration into rat ova fertilized in vitro.
J. Androl. 3 : 382-387
- SHALGI, R. and PHILLIPS, D.M. (1983).
Role of the cumulus in zona penetration. In : The Sperm Cell.
Andre, J. (Ed.). pp.90-93.
Martinus-Nijhoff, The Hague.
- SHALGI, R., PHILLIPS, D.M. and KRAICER, P.F. (1978).
Observation on the incorporation cone in the rat.
Gamete Res. 1 : 27-35
- SHAMS-BORHAN, G. and HARRISON, R.A.P. (1981).
Production, characterization, and use of ionophore-induced,
calcium-dependant acrosome reaction in ram spermatozoa.
Gamete Res. 4 : 407-432
- SHIMIZU, S., ITO, M. and DEAN, J. (1982).
Glycoproteins of mouse zona pellucida : analysis of their
reactivity to lectins.
Biochem. Biophys. Res. Commun. 109 : 449-454
- SHUR, B.D. and HALL, N.G. (1982).
A role for mouse sperm surface galactosyltransferase in sperm
binding to the egg zona pellucida.
J. Cell Biol. 95 : 574-579
- SIMIONESCU, N. and SIMIONESCU, M. (1976).
Galloylglucoses of low molecular weight as mordant in electron
microscopy. I. Procedure, and evidence for mordanting effect.
J. Cell Biol. 70 : 608-621
- SINOWATZ, F. and FRIESS, A.E. (1983).
Localization of lectin receptors on bovine epididymal spermatozoa
using a colloidal gold technique.
Histochemistry 79 : 335-344
- SIPSKI, M.L. and WAGNER, T.E. (1977).
The total structure and organization of chromosomal fibers in
eutherian sperm nuclei.
Biol. Reprod. 16 : 428-440
- SOKAL, R.R. and ROHLF, F.J. (1973).
Introduction to biostatistics.
Freeman, San Francisco.

- SOUPART, P. and NOYES, R.W. (1964).
Sialic acid as component of zona pellucida of mammalian ovum.
J. Reprod. Fert. 8 : 251-253
- SRIVASTAVA, P.N. and NINJOOR, V. (1982).
Isolation of rabbit testicular cathepsin D and its role in the activation of proacrosin.
Biochem. Biophys. Res. Commun. 109 : 63-69
- SRIVASTAVA, P.N., ADAMS, C.E. and HARTREE, E.F. (1965).
Enzymic action of acrosomal preparations on the rabbit ovum in vitro.
J. Reprod. Fert. 10 : 61-67
- SRIVASTAVA, P.N., ZANEVELD, L.J.D. and WILLIAMS, W.L. (1970).
Mammalian sperm acrosomal neuraminidases.
Biochem. Biophys. Res. Commun. 39 : 575-582
- SRIVASTAVA, P.N., AKRUK, S.R. and WILLIAMS, W.L. (1979).
Dissolution of rabbit zona by sperm acrosomal extract : effect of calcium.
J. Exp. Zool. 207 : 521-529
- SRIVASTAVA, P.N., BREWER, J.M. and WHITE, R.A. (1982).
Hydrolysis of p-nitrophenylphosphorylcholine by alkaline phosphatase and phospholipase C from rabbit sperm-acrosome.
Biochem. Biophys. Res. Commun. 108 : 1120-1125
- STACKPOLE, C.W. and DEVORKIN, D. (1974).
Membrane organization in mouse spermatozoa revealed by freeze-etching.
J. Ultrastruct. Res. 49 : 167-187
- STAMBAUGH, R. and BUCKLEY, J. (1968).
Zona pellucida dissolution enzymes of the rabbit sperm head.
Science 161 : 585-586
- STAMBAUGH, R. and BUCKLEY, J. (1969).
Identification and subcellular localization of the enzymes affecting penetration of the zona pellucida by rabbit spermatozoa.
J. Reprod. Fert. 19 : 423-432
- STAMBAUGH, R. and SMITH, M. (1978).
Tubulin and microtubule-like structures in mammalian acrosomes.
J. Exp. Zool. 203 : 135-141
- STAMBAUGH, R., BRACKETT, B.G. and MASTROIANNI, L. (1969).
Inhibition of in vitro fertilization of rabbit ova by trypsin inhibitors.
Biol. Reprod. 1 : 223-227
- STANGER, J.D. and QUINN, P. (1982).
Fertilization of cumulus-free, zona-intact mouse ova in vitro at high and low sperm concentrations.
Gamete Res. 5 : 61-70

- STEFANINI, M., OURA, C. and ZAMBONI, L. (1969).
Ultrastructure of fertilization in the mouse. 2. Penetration of sperm into the ovum.
J. Submicr. Cytol. 1 : 1-23
- STEGNER, H.E. and WARTENBERG, H. (1961).
Elektronenmikroskopische und histotopochemische untersuchungen uber struktur und bildung der zona pellucida menschlicher eizellen.
Z. Zellforsch. Mikrosk. Anat. 53 : 702-713
- STOREY, B.T., LEE, M.A., MULLER, C., WARD, C.R. and WIRTSHAFTER, D.G. (1984).
Binding of mouse spermatozoa to the zonae pellucidae of mouse eggs in cumulus : evidence that the acrosomes remain substantially intact.
Biol. Reprod. 31 : 1119-1128
- SUAREZ, S.S., KATZ, D.F. and MEIZEL, S. (1984).
Changes in motility that accompany the acrosome reaction in hyperactivated hamster spermatozoa.
Gamete Res. 10 : 253-265
- SULLIVAN, R. and BLEAU, G. (1985).
Interaction of isolated components from mammalian sperm and egg.
Gamete Res. 12 : 101-116
- SUZUKI, F. (1981).
Changes in intramembranous particle distribution in epididymal spermatozoa of the boar.
Anat. Rec. 199 : 361-376
- SUZUKI, F. and NAGANO, T. (1980a).
Epididymal maturation of rat spermatozoa studied by thin sectioning and freeze-fracture.
Biol. Reprod. 22 : 1219-1231
- SUZUKI, F. and NAGANO, T. (1980b).
Morphological relationship between the plasma membrane and the microtubules in the endpiece of the boar spermatozoon.
J. Electron Micr. 29 : 190-192
- SUZUKI, F. and YANAGIMACHI, R. (1983).
Freeze-fracture observations of ovulated hamster oocytes with their cumulus cells.
Cell Tiss. Res. 231 : 265-274
- SWENSON, C.E. and DUNBAR, B.S. (1982).
Specificity of sperm-zona interaction.
J. Exp. Zool. 219 : 97-104
- SZOLLOSI, D. (1962).
Cortical granules : a general feature of mammalian eggs.
J. Reprod. Fert. 4 : 223-224

- SZOLLOSI, D. (1967).
Development of cortical granules and the cortical reaction in rat and hamster eggs.
Anat. Rec. 159 : 431-446
- SZOLLOSI, D. (1971).
Morphological changes in mouse eggs due to aging in the fallopian tube.
Am. J. Anat. 130 : 209-226
- SZOLLOSI, D. (1972).
Changes in some cell organelles during oogenesis in mammals. In : Oogenesis. Biggers, J.D. and Schuetz, A.W. (Eds.). pp.47-64.
Univ. Park Press, Baltimore.
- SZOLLOSI, D. and HUNTER, R.H.F. (1978).
The nature and occurrence of the acrosome reaction in spermatozoa of the domestic pig, Sus scrofa.
J. Anat. 127 : 33-41
- SZOLLOSI, D. and GERARD, M. (1983).
Cytoplasmic changes in the mammalian oocytes during the preovulatory period. In : Fertilization of the Human Egg In Vitro. Beier, H.M. and Lindner, H.R. (Eds.). pp.35-55.
Springer-Verlag, Berlin.
- TADANO, Y. and YAMADA, K. (1978).
The histochemistry of complex carbohydrates in the ovarian follicles of adult mice.
Histochemistry 57 : 203-215
- TALBOT, P. (1984).
Hyaluronidase dissolves a component in the hamster zona pellucida.
J. Exp. Zool. 229 : 309-316
- TALBOT, P. (1985).
Sperm penetration through oocyte investments in mammals.
Am. J. Anat. 174 : 331-346
- TALBOT, P. and FRANKLIN, L.E. (1974).
Hamster sperm hyaluronidase. II. Its release from sperm in vitro in relation to the degenerative and normal acrosome reaction.
J. Exp. Zool. 189 : 321-332
- TALBOT, P. and FRANKLIN, L.E. (1976).
Morphology and kinetics of the hamster sperm acrosome reaction.
J. Exp. Zool. 198 : 163-176
- TALBOT, P. and FRANKLIN, L.E. (1978).
Surface modification of guinea pig sperm during in vitro capacitation : an assessment using lectin-induced agglutination of living sperm.
J. Exp. Zool. 203 : 1-14
- TALBOT, P. and KLEVE, M.G. (1978).
Hamster sperm cross react with anti-actin.
J. Exp. Zool. 204 : 131-136

- TALBOT, P. and CHACON, R. (1982).
Ultrastructural observations on binding and membrane fusion between human sperm and zona pellucida-free hamster oocytes.
Fertil. Steril. 37 : 240-248
- TALBOT, P. and DI CARLANTONIO, G. (1984a).
Architecture of the hamster oocyte-cumulus complex.
Gamete Res. 9 : 261-272
- TALBOT, P. and DI CARLANTONIO, G. (1984b).
The oocyte-cumulus complex : ultrastructure of the extracellular components in hamsters and mice.
Gamete Res. 10 : 127-142
- TALBOT, P., SUMMERS, R.G., HYLANDER, B.L., KEOUGH, E.M. and FRANKLIN, L.E. (1976).
The role of calcium in the acrosome reaction : an analysis using inophore A23187.
J. Exp. Zool. 198 : 383-392
- TALBOT, P., DI CARLANTONIO, G., ZAO, P., PENKALA, J. and HAIMO, L.T. (1985).
Motile cells lacking hyaluronidase can penetrate the hamster oocyte cumulus complex.
Dev. Biol. 108 : 387-398
- TAMBLYN, T.M. (1980).
Identification of actin in boar epididymal spermatozoa.
Biol. Reprod. 22 : 727-734
- TAMBLYN, T.M. (1981).
Evidence for non-muscle myosin in bovine ejaculated spermatozoa.
Gamete Res. 4 : 499-506
- TASH, J.S. and MEANS, A.R. (1983).
Cyclic adenosine 3',5' monophosphate, calcium and protein phosphorylation in flagellar motility.
Biol. Reprod. 28 : 75-104
- TEICHMAN, R.J. and BERNSTEIN, M.H. (1971).
Fine structure localizations of acid phosphatase in rabbit and bull sperm heads.
J. Reprod. Fert. 27 : 243-248
- TEICHMAN, R.J., FUJIMOTO, M. and YANAGIMACHI, R. (1972).
A previously unrecognized material in mammalian spermatozoa as revealed by malachite green and pyronine.
Biol. Reprod. 7 : 73-81
- TEICHMAN, R.J., CUMMINS, J.M. and TAKEI, G.H. (1974).
The characterization of a malachite green stainable, glutaraldehyde extractable phospholipid in rabbit spermatozoa.
Biol. Reprod. 10 : 565-577

- TELFER, S. and BREED, W.G. (1976).
The effect of age on the female reproductive tract of the hopping mouse Notomys alexis.
Aust. J. Zool. 24 : 533-540
- TELKKA, A., FAWCETT, D.W. and CHRISTENSEN, A. (1961).
Further observations on the structure of mammalian sperm tail.
Anat. Rec. 141 : 231-245
- TESARIK, J. and DVORAK, M. (1982).
Human cumulus oophorus preovulatory development.
J. Ultrastruct. Res. 78 : 60-72
- TESORIERO, J.V. (1984).
Comparative cytochemistry of the developing ovarian follicles of the dog, rabbit and mouse : origin of the zona pellucida.
Gamete Res. 10 : 301-318
- THOMPSON, R.S., MOORE-SMITH, D. and ZAMBONI, L. (1974).
Fertilization of mouse ova in vitro : An electron microscopic study.
Fertil. Steril. 25 : 222-249
- TILNEY, L.G., BONDER, E.M. and DE ROSIER, D.J. (1981).
Actin filaments elongate from their membrane-associated ends.
J. Cell Biol. 90 : 485-494
- TONGKAO, D. and CHULAVATNATOL, M. (1979).
Phosphorylation of microtubules of rat spermatozoa during epididymal maturation. In : The Spermatozoon. Maturation, motility, surface properties and comparative aspects. Fawcett, D.W. and Bedford, J.M. (Eds.). pp.129-133.
Urban and Schwarzenberg, Baltimore.
- TOOWICHARANONT, P. and CHULAVATNATOL, M. (1983a).
Measurement of anionic sites of rat epididymal spermatozoa using tritiated polycationized ferritin.
J. Reprod. Fert. 69 : 303-306
- TOOWICHARANONT, P. and CHULAVATNATOL, M. (1983b).
Direct assay of bound sialic acids on rat spermatozoa from caput and cauda epididymis.
J. Reprod. Fert. 67 : 275-280
- TOSHIMORI, K. (1982).
Penetration of the mouse sperm head through the zona pellucida in vivo : an electron microscope study at 200 kV.
Biol. Reprod. 26 : 475-481
- TOYAMA, Y. (1976).
Actin-like filaments in the Sertoli cell junctional specializations in the swine and mouse testis.
Anat. Rec. 186 : 477-492
- TOYAMA, Y. and NAGANO, T. (1983).
Boar spermatozoa observed by rapid-freeze and deep-etch method.
Anat. Rec. 206 : 171-179

- TOYODA, Y. and CHANG, M.C. (1974).
Fertilization of rat eggs in vitro by epididymal spermatozoa and the development of eggs following transfer.
J. Reprod. Fert. 36 : 9-22
- TRIANA, L.R., BABCOCK, D.F., LORTON, S.P., FIRST, N.L. and LARDY, H.A. (1980).
Release of acrosomal hyaluronidase follows increased membrane permeability to calcium in the presumptive capacitation sequence for spermatozoa of the bovine and other mammalian species.
Biol. Reprod. 23 : 47-59
- TSANEV, R. and AVRAMOVA, Z. (1981).
Nonprotamine nucleoprotein ultrastructures in mature ram sperm nuclei.
Eur. J. Cell Biol. 24 : 139-145
- TUNG, K.S.K. (1975).
Human sperm antigens and antisperm antibodies. I. Studies on vasectomy patients.
Clin. Exp. Immunol. 20 : 93-104
- TUNG, K.S.K. (1976).
Human sperm antigens and antisperm antibodies. II. Studies on acrosomal antigens.
Clin. Exp. Immunol. 24 : 292-299
- TUNG, K.S.K., YANAGIMACHI, H. and YANAGIMACHI, R. (1982).
Sperm autoantigens and fertilization. III. Ultrastructural localization of guinea pig autoantigens.
Anat. Rec. 202 : 241-253
- TURNER, T.T. (1984).
Resorption versus secretion in the rat epididymis.
J. Reprod. Fert. 72 : 509-514
- TURNER, T.T. and HOWARDS, S.S. (1978).
Factors involved in the initiation of sperm motility.
Biol. Reprod. 18 : 571-578
- TURNER, T.T. and REICH, G.W. (1985).
Cauda epididymal sperm motility : a comparison among five species.
Biol. Reprod. 32 : 120-128
- URCH, U.A., WARDRIP, N.J. and HEDRICK, J.L. (1985a).
Limited and specific proteolysis of the zona pellucida by acrosin.
J. Exp. Zool. 233 : 479-483
- URCH, U.A., WARDRIP, N.J. and HEDRICK, J.L. (1985b).
Proteolysis of the zona pellucida by acrosin : the nature of the hydrolysis products.
J. Exp. Zool. 236 : 239-243
- USSELMAN, M.C., CONE, R.A., CARR, D.W. and ACOTT, T.S. (1984).
Species diversity of sperm motility regulation in the caudal epididymis.
Biol. Reprod. 30 Suppl. 1 : 82

- USUI, N. and YANAGIMACHI, R. (1976).
Behaviour of hamster sperm nuclei incorporated into eggs at various stages of maturation, fertilization and early development.
J. Ultrastruct. Res. 57 : 276-288
- UYEMURA, D.G. and SPUDICH, J.A. (1980).
Biochemistry and regulation of non-muscle actins. In : Molecular Organization and Cell Function. Goldberger, R.F. (Ed.). Vol. 2, pp.317-338.
Plenum Press, New York.
- VAIDYA, R.A., GLASS, R.H., DANDEKAR, P. and JOHNSON, K. (1971).
Decrease in the electrophoretic mobility of rabbit spermatozoa following intra-uterine incubation.
J. Reprod. Fert. 24 : 299-301
- VIERULA, M. and RAJANIEMI, H. (1980).
Radioiodination of surface proteins of bull spermatozoa and their characterization by sodium dodecyl sulphate-polyacrylamide gel electrophoresis.
J. Reprod. Fert. 58 : 483-489
- VIJAYASARATHY, S., SHIVAJI, S. and BALARAM, P. (1982).
Bull sperm plasma and acrosomal membranes : fluorescence studies of lipid phase fluidity.
Biochem. Biophys. Res. Commun. 108 : 585-591
- VIRTANEN, I., BADLEY, R.A., PAASIVUO, R. and LEHTO, V.P. (1984).
Distinct cytoskeletal domains revealed in sperm cells.
J. Cell Biol. 99 : 1083-1091
- VOGL, A.W. and SOUCY, L.J. (1985).
Arrangement and possible function of actin filament bundles in ectoplasmic specializations of ground squirrel Sertoli cells.
J. Cell Biol. 100 : 814-825
- VOGL, A.W., LIN, Y.C., DYM, M. and FAWCETT, D.W. (1983a).
Sertoli cells of the golden-mantled ground squirrel (Spermophilus lateralis) : A model system for the study of shape change.
Am. J. Anat. 168 : 83-98
- VOGL, A.W., LINCK, R.W. and DYM, M. (1983b).
Colchicine-induced changes in the cytoskeleton of the golden-mantled ground squirrel (Spermophilus lateralis) Sertoli cells.
Am. J. Anat. 168 : 99-108
- VOGL, A.W., SOUCY, L.T. and LEW, G.T. (1985).
Distribution of actin in isolated seminiferous epithelia and denuded tubule walls of the rat.
Anat. Res. 213 : 63-71
- VOGLMAYER, J.K. (1975).
Metabolic changes in spermatozoa during epididymal transit. In : Handbook of Physiology. Hamilton, D.W. and Greep, R.O. (Eds.). Sect. 7, Vol. 5, pp.437-452.
Amer. Physiol. Soc., Washington.

- VOGLMAYR, J.K. and SAWYER, R.F. (1984).
Interactions of the ram sperm cell surface with luminal fluids of the uterus, oviduct and cauda epididymidis.
Biol. Reprod. 30 Suppl. 1 : 114
- VOGLMAYR, J.K., FAIRBANKS, G., JACKOWITZ, M.A. and COLELLA, J.R. (1980).
Post-testicular developmental changes in the ram sperm cell surface and their relationship to luminal fluid proteins of the reproductive tract.
Biol. Reprod. 22 : 655-667
- VOGLMAYR, J.K., FAIRBANKS, G., VESPA, D.B. and COLELLA, J.R. (1982).
Studies on mechanisms of surface modifications in ram spermatozoa during the final stages of differentiation.
Biol. Reprod. 26 : 483-500
- VOGLMAYR, J.K., FAIRBANKS, G. and LEWIS, R.G. (1983).
Surface glycoprotein changes in ram spermatozoa during epididymal maturation.
Biol. Reprod. 29 : 767-775
- WABIK-SLIZ, B. (1979).
Number of cortical granules in mouse oocytes from inbred strains differing in efficiency of fertilization.
Biol. Reprod. 21 : 89-97
- WALT, H. and HEDINGER, C. (1983).
Loss of activity of motile components in rat spermatids during differentiation to virtually immotile testicular spermatozoa. In : The Sperm Cell. Andre, J. (Ed.). pp.376-379.
Martinus-Nijhoff, The Hague.
- WARD, M.C. (1946).
A study of the estrous cycle and the breeding of the golden hamster (Cricetus auratus).
Anat. Rec. 94 : 139-161
- WASSARMAN, P.M. (1983).
Oogenesis : synthetic events in the developing mammalian egg. In : Mechanism and Control of Animal Fertilization. Hartmann, J.F. (Ed.). pp.1-54.
Academic Press, New York.
- WATANABE, M., MURAMATSU, T., SHIRANE, H. and UGAI, K. (1981).
Discrete distribution of binding sites for Dolichos biflorus agglutinin (DBA) and for peanut agglutinin (PNA) in mouse organ tissues.
J. Histochem. Cytochem. 29 : 779-790
- WATTS, C.H.S. (1979).
Reproductive parameters of some Australian rodents.
Aust. Zool. 20 : 305-311
- WATTS, C.H.S. and ASLIN, H.J. (1981).
The Rodents of Australia.
Angus and Robertson, Sydney.

- WEAKLEY, B.S. (1966).
Electron microscopy of the oocyte and granulosa cells in the developing ovarian follicles of the golden hamster (Mesocricetus auratus).
J. Anat. 100 : 503-534
- WEAKLEY, B.S. (1968).
Comparison of cytoplasmic lamellae and membranous elements in the oocytes of five mammalian species.
Z. Zellforsch. Mikrosk. Anat. 85 : 109-123
- WEINMAN, D.E. and WILLIAMS, W.L. (1964).
Mechanism of capacitation of rabbit spermatozoa.
Nature 203 : 423-424
- WELCH, J.E. and O'RAND, M.G. (1985).
Identification and distribution of actin in spermatogenic cells and spermatozoa of the rabbit.
Dev. Biol. 109 : 411-417
- WILLSON, J.T. and KATSH, S. (1965).
Cytoimmunological studies of guinea pig sperm antigens. I. Testicular versus epididymal spermatozoa.
Z. Zellforsch. Mikrosk. Anat. 65 : 16-26
- WINCEK, T.J., PARRISH, R.F. and POLAKOSKI, K.L. (1979).
Fertilization : a uterine glycosaminoglycan stimulates the conversion of sperm proacrosin to acrosin.
Science 203 : 553-554
- WOLF, D.E. and ZIOMEK, C.A. (1983).
Regionalization and lateral diffusion of membrane proteins in unfertilized and fertilized mouse eggs.
J. Cell Biol. 96 : 1786-1790
- WOLF, D.E. and VOGLMAYR, J.K. (1984).
Diffusion and regionalization in membranes of maturing ram spermatozoa.
J. Cell Biol. 98 : 1678-1684
- WOLF, D.P. (1981).
The mammalian egg's block to polyspermy. In : Fertilization and Embryonic Development In Vitro. Mastroianni, L. and Biggers, J.D. (Eds.). pp.183-197.
Plenum Press, New York.
- WOLF, D.P. (1983).
The ovum before and after fertilization. In : Biochemistry of Mammalian Reproduction. Zaneveld, L.J.D. and Chatterton, R.T. (Eds.). Pt.1. pp.231-259.
Wiley, New York.
- WOLF, D.P. and HAMADA, M. (1977).
Induction of zonal and egg plasma membrane blocks to sperm penetration in mouse eggs with cortical granule exudate.
Biol. Reprod. 17 : 350-354

- WOLF, D.P. and ARMSTRONG, P.B. (1978).
Penetration of the zona-free mouse egg by capacitated epididymal sperm : cinemicrographic observations.
Gamete Res. 1 : 39-46
- WOLF, D.P. and SOPER, K. (1978).
The block to sperm penetration in zona-free mouse eggs.
Dev. Biol. 64 : 1-10
- WOLF, D.P., INOUE, M. and STARK, R.A. (1976).
Penetration of zona-free mouse ova.
Biol. Reprod. 15 : 213-221
- WOLF, D.P., HAMADA, M. and INOUE, M. (1977).
Kinetics of sperm penetration into and the zona reaction of mouse ova inseminated in vitro.
J. Exp. Zool. 201 : 29-36
- WOLF, D.P., NICOSIA, S.V. and HAMADA, M. (1979).
Premature cortical granule loss does not prevent sperm penetration of mouse eggs.
Dev. Biol. 71 : 22-32
- WOLGEMUTH, D.J., CELENZA, J., BUNDMAN, D.S. and DUNBAR, B.S. (1984).
Formation of the rabbit zona pellucida and its relationship to ovarian follicular development.
Devel. Biol. 106 : 1-14
- WOOD, D.M. and DUNBAR, B.S. (1981).
Direct detection of two cross-reactive antigens between porcine and rabbit zonae pellucidae by radioimmunoassay and immuno-electrophoresis.
J. Exp. Zool. 217 : 423-433
- WOODING, F.B.P. (1973).
The effect of triton X-100 on the ultrastructure of ejaculated bovine sperm.
J. Ultrastruct. Res. 42 : 502-516
- WOODING, F.B.P. and O'DONNELL, J.M. (1971).
A detailed ultrastructural study of the head membranes of ejaculated bovine sperm.
J. Ultrastruct. Res. 35 : 71-85.
- WOOLLEY, D.M. (1970a).
A "posterior ring" in the spermatozoa of species of Muridae.
J. Reprod. Fert. 23 : 361-363
- WOOLLEY, D.M. (1970b).
The midpiece of the mouse spermatozoon : its form and development as seen by surface replication.
J. Cell Sci. 6 : 865-879
- WOOLLEY, D.M. (1971).
Striations in the peripheral fibers of rat and mouse spermatozoa.
J. Cell Biol. 49 : 936-939

- WOOLLEY, D.M. (1977).
Evidence for "twisted plane" undulations in golden hamster sperm tails.
J. Cell Biol. 75 : 851-865
- WOOLLEY, D.M. (1979).
Interpretation of the pattern of sperm tail movements. In : The Spermatozoon. Maturation, motility, surface properties and comparative aspects. Fawcett, D.W. and Bedford, J.M. (Eds.). pp.69-79.
Urban and Schwarzenberg, Baltimore.
- WOOLLEY, D.M. and NICKELS, S.N. (1985).
Microtubule termination patterns in mammalian sperm flagella.
J. Ultrastruct. Res. 90 : 221-234
- WU, T.C., LEE, M.C., WAN, Y.J. and DAMJANOV, I. (1984).
Lectin binding sites of the mouse ovary, intraovarian and ovulated ova.
Histochemistry 80 : 527-533
- WYKER, R. and HOWARDS, S.S. (1977).
Micropuncture studies of the motility of rete testis and epididymal spermatozoa.
Fertil. Steril. 28 : 108-112
- YANAGIMACHI, R. (1966).
Time and process of sperm penetration into hamster ova in vivo and in vitro.
J. Reprod. Fert. 11 : 359-370
- YANAGIMACHI, R. (1970).
The movement of golden hamster spermatozoa before and after capacitation.
J. Reprod. Fert. 28 : 193-196
- YANAGIMACHI, R. (1972).
Fertilization of guinea pig eggs in vitro.
Anat. Rec. 174 : 9-20
- YANAGIMACHI, R. (1975).
Acceleration of the acrosome reaction and activation of guinea pig spermatozoa by detergents and other reagents.
Biol. Reprod. 13 : 519-526
- YANAGIMACHI, R. (1977).
Specificity of sperm-egg interactions. In : Immunobiology of Gametes. Edidin, M. and Johnson, M.H. (Eds.). pp.255-295.
Cambridge Univ. Press, Cambridge.
- YANAGIMACHI, R. (1978).
Calcium requirement for sperm-egg fusion in mammals.
Biol. Reprod. 19 : 949-958

- YANAGIMACHI, R. (1981).
Mechanisms of fertilization in mammals. In : Fertilization and Embryonic Development In Vitro. Mastroianni, L. and Biggers, J.D. (Eds.). pp.81-180.
Plenum Press, New York.
- YANAGIMACHI, R. (1982).
Requirement of extracellular calcium ions for various stages of fertilization and fertilization-related phenomena in the hamster.
Gamete Res. 5 : 323-344
- YANAGIMACHI, R. (1984).
Developmental ability of precociously superovulated golden hamster eggs.
Gamete Res. 9 : 231-237
- YANAGIMACHI, R. and NODA, Y.D. (1970a).
Fine structure of the hamster sperm head.
Am. J. Anat. 128 : 367-388
- YANAGIMACHI, R. and NODA, Y.D. (1970b).
Ultrastructural changes in the hamster sperm head during fertilization.
J. Ultrastruct. Res. 31 : 465-485
- YANAGIMACHI, R. and NODA, Y.D. (1970c).
Electron microscopic studies of sperm incorporation into the golden hamster egg.
Am. J. Anat. 128 : 429-462
- YANAGIMACHI, R. and NODA, Y.D. (1970d).
Physiological changes in the postnuclear cap region of mammalian spermatozoa : A necessary preliminary to the membrane fusion between sperm and egg cells.
J. Ultrastruct. Res. 31 : 486-493
- YANAGIMACHI, R. and USUI, N. (1974).
Calcium dependence of the acrosome reaction and activation of guinea pig spermatozoa.
Exp. Cell Res. 89 : 161-174
- YANAGIMACHI, R. and MAHI, C.A. (1976).
The sperm acrosome reaction and fertilization in the guinea-pig : a study in vivo.
J. Reprod. Fert. 46 : 49-54
- YANAGIMACHI, R. and NICOLSON, G.L. (1976).
Lectin-binding properties of hamster egg zona pellucida and plasma membrane during maturation and preimplantation development.
Exp. Cell Res. 100 : 249-257
- YANAGIMACHI, R. and PHILLIPS, D.M. (1984).
The status of acrosomal caps of hamster spermatozoa immediately before fertilization in vivo.
Gamete Res. 9 : 1-19

- YANAGIMACHI, R. and SUZUKI, F. (1985).
A further study of lysolecithin-mediated acrosome reaction of guinea pig spermatozoa.
Gamete Res. 11 : 29-40
- YANAGIMACHI, R., NODA, Y.D., FUGIMOTO, M. and NICOLSON, G.L. (1972).
The distribution of negative surface charges on mammalian spermatozoa.
Am. J. Anat. 135 : 497-520
- YANAGIMACHI, R., NICOLSON, G.L., NODA, Y.D. and FUJIMOTO, M. (1973).
Electron microscopic observations of the distribution of acidic anionic residues on hamster spermatozoa and eggs before and during fertilization.
J. Ultrastruct. Res. 43 : 344-353
- YANAGIMACHI, R., YANAGIMACHI, H. and ROGERS, B.J. (1976).
The use of zona-free animal ova as a test-system for the assessment of the fertilizing capacity of human spermatozoa.
Biol. Reprod. 15 : 471-476
- YANAGIMACHI, R., KAMIGUCHI, Y., SUGAWARA, S. and MIKAMO, K. (1983).
Gametes and fertilization in the Chinese hamster.
Gamete Res. 8 : 97-117
- YANAGIMACHI, R., KAMIGUCHI, Y., MIKAMO, K., SUZUKI, F., and YANAGIMACHI, H. (1985).
Maturation of spermatozoa in the epididymis of the Chinese hamster.
Am. J. Anat. 172 : 317-330
- YANG, C.H. and SRIVASTAVA, P.N. (1974a).
Separation and properties of hyaluronidase from ram sperm acrosomes.
J. Reprod. Fert. 37 : 17-25
- YANG, C.H. and SRIVASTAVA, P.N. (1974b).
Purification and properties of arylsulfatases from rabbit sperm acrosomes.
Proc. Soc. Exp. Biol. Med. 145 : 721-725
- YANG, W.H., LIN, L.L., WANG, J.R. and CHANG, M.C. (1972).
Sperm penetration through zona pellucida and perivitelline space in the hamster.
J. Exp. Zool. 179 : 191-206
- YOUNG, L.G. and GOODMAN, S.A. (1980).
Characterization of human sperm cell surface components.
Biol. Reprod. 23 : 826-835
- YOUNG, L.G. and GOODMAN, S.A. (1982).
Analysis of lipid and protein components of ejaculated bull sperm surface and seminal plasma.
Gamete Res. 6 : 281-291
- YOUNG, R.J. (1979).
Rabbit sperm chromatin is decondensed by a thiol-induced proteolytic activity not endogenous to its nucleus.

Biol. Reprod. 20 : 1001-1004

- YOUNG, R.J. and SWEENEY, K. (1979).
The structural organization of sperm chromatin.
Gamete Res. 2 : 265-282
- YOUNG, R.J. and COOPER, G.W. (1983a).
Dissociation of intermolecular linkages of the sperm head and tail by primary amines, aldehydes, sulphydryl reagents and detergents.
J. Reprod. Fert. 69 : 1-10
- YOUNG, R.J. and COOPER, G.W. (1983b).
Stabilization in vivo and in vitro of the spermatozoon head and tail to detachment induced by primary amine, thiol and detergent.
Gamete Res. 7 : 277-28
- YOUNG, W.C. (1931).
A study of the function of the epididymis. III. Functional changes undergone by spermatozoa during their passage through the epididymis and vas deferens in the guinea-pig.
J. Exp. Biol. 8 : 151-175
- YUREWICZ, E.G., SUBRAMANIAN, M.G. and SACCO, A.G. (1984).
Purification of a 55K porcine zona macromolecule and its evaluation as a contraceptive target antigen.
Biol. Reprod. 30 Suppl. 1. pp.74.
- ZAMBONI, L. (1970).
Ultrastructure of mammalian oocytes and ova.
Biol. Reprod. Suppl. 2 : 44-65
- ZAMBONI, L. (1972).
Comparative studies on the ultrastructure of mammalian oocytes. In : Oogenesis. Biggers, J.D. and Schuetz, A.W. (Eds.). pp.5-45. Univ. Park Press, Baltimore.
- ZAMBONI, L. and MASTROIANNI, L. (1966).
Electron microscopic studies on rabbit ova. I. The follicular oocyte.
J. Ultrastruct. Res. 14 : 95-117
- ZAMBONI, L. and STEFANINI, M. (1971).
The fine structure of the neck of mammalian spermatozoa.
Anat. Rec. 169 : 155-172
- ZAMBONI, L., ZEMJANIS, R. and STEFANINI, M. (1971).
The fine structure of monkey and human spermatozoa.
Anat. Rec. 169 : 129-154
- ZANEVELD, L.J.D. and WILLIAMS, W.L. (1970).
A sperm enzyme that disperses the corona radiata and its inhibition by decapacitation factor.
Biol. Reprod. 2 : 363-368
- ZANEVELD, L.J.D., ROBERTSON, R.T., KESSLER, M., SRIVASTAVA, P.N. and WILLIAMS, W.L. (1970).
Inhibition of fertilization in vivo by mammalian trypsin

inhibitors.
Fed. Proc. 29 : 644

ZANEVELD, L.J.D., ROBERTSON, R.T., KESSLER, M. and WILLIAMS, W.L. (1971).

Inhibition of fertilization in vivo by pancreatic and seminal plasma trypsin inhibitors.
J. Reprod. Fert. 25 : 387-392

ZAO, P.Z.R., MEIZEL, S. and TALBOT, P. (1985).

Release of hyaluronidase and b-N-acetylhexosaminidase during in vitro incubation of hamster sperm.
J. Exp. Zool. 234 : 63-74

ZIRKIN, B.R., SOUCEK, D.A., CHANG, T.S.K. and PERREAULT, S.D. (1985).

In vitro and in vivo studies of mammalian sperm nuclear decondensation.
Gamete Res. 11 : 349-365