

·Review·

Role of the epididymis in sperm competition

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Abstract

Although it is generally understood that the testes recruited kidney ducts for reproductive function during the evolution of vertebrates, little is understood of the biological significance of the adaptation. In the context of the evolution of the mammalian epididymis, this report provides evidence that a major role of the epididymis is to enhance a male's chance of achieving paternity in a competitive mating system. A unique example of sperm cooperation in monotremes is used as evidence that the epididymis produces sperm competition proteins to form groups of 100 sperm into bundles that have a forward motility nearly thrice that of individual spermatozoa. As it required 3-h incubation *in vitro* under capacitation conditions to release motile sperm from the bundles, it is suggested that the monotremes provide an example of capacitation that is quite different from capacitation in higher mammals. It is suggested that variation between species in the intensity of sperm competition could explain the variation that occurs between species in the amount of post-testicular sperm maturation and storage in the epididymis, an explanation of why the human epididymis does not play as important a role in reproduction as the epididymis of most mammals. (*Asian J Androl* 2007 July; 9: 493–499)

Keywords: epididymis; sperm maturation; sperm storage; capacitation; sperm competition; sperm cooperation

1 Introduction

It is generally understood that during evolution the testes have taken over kidney ducts for reproductive function [1]. However, the biological significance of the adaptation has not been satisfactorily resolved. The development of extragonadal duct systems in the female has the obvious advantage that fertilization and embryonic development can proceed in a protected environment. However, the value of developing the complex, testicular excurrent duct system of mammals is not so obvious. It is well established that mammalian spermatozoa are not capable of fertilizing an ovum when they leave the testis and only acquire this capacity during passage through the epididymis, by a process that involves struc-

tural and molecular changes. This epididymal development is correlated with a subsequent need for spermatozoa to undergo a period of capacitation before they can immediately fertilize an ovum [2–5]. These developments are in distinct contrast to the extragonadal duct system of other endotherms, such as non-passerine birds in which sperm are capable of fertilizing an ovum when they leave the testis (although the capacity is enhanced during epididymal transit) and capacitation is not required [6, 7].

It has been suggested that a major role of the epididymis is to enhance a male's chance of achieving paternity in a competitive mating system [8]. That is, it is an adaptation involved in what is referred to as sperm competition, the processes involved in the inter-male competition to achieve paternity and which play an important role in evolution [9–11]. Sperm competition is significant in driving reproductive adaptations in males as there are many more mitotic divisions in the male than female germ line [12, 13]. It has been estimated that, depending upon the species and genes under consideration,

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embryos might have up to six times more mutations originating in the male than female germ line, and this has led to the hypothesis that evolution is male driven [14, 15].

2 Sperm cooperation

2.1 Sperm bundles in monotremes

Our work on the monotremes, the platypus and Australian echidna, provide some of the most convincing evidence that the mammalian epididymis is adapted for sperm competition. From an evolutionary perspective the monotremes are the earliest offshoot of the mammalian line and an enigmatic mixture of mammalian, reptilian and specialized characteristics that make them uniquely suitable for research into the evolution of mammals. The reproductive tracts of the platypus and echidna are similar and indicate that both species are involved in a high level of sperm competition. They have large testes relative to body mass (e.g. 0.6%–1.3% of body mass in the echidna) and a long, highly specialized penis. Also, their courtship behavior involves considerable inter-male rivalry to mate involving venomous spurs in platypus [16], and the formation of ‘trains’ in echidna, in which a number of males follow in line behind an oestrous female [17]. A unique characteristic of monotremes is that their sperm form into bundles (Figure 1) as they transit the epididymis [18]. The bundles form as spermatozoa pass through the isthmus joining the initial and terminal segments of the epididymis in a



Figure 1. Scanning electron micrograph of a bundle of echidna spermatozoa in the terminal segment of the ductus epididymidis showing how approximately 100 spermatozoa are wrapped around one another in a helical manner and appear to be bound together along most of the head. $\times 2900$. (Replicated with permission from Djakiew *et al.* [19].)

manner that suggests that at least two proteins are involved in a specific sequence of processes (Figure 2). The spermatozoa initially group into a sphere with the rostral ends of their heads facing the centre, presumably held in place by protein. Then, groups (of approximately 100 spermatozoa in the echidna) re-orientate, lying roughly side-by-side forming a V-shaped bundle with the tips of the rostral ends of their heads embedded in material that is electron dense, and most of the rest of the heads are supported by a more flocculent electron dense material (see Figures 2 and 3 in [19]).

1-D gels of micropuncture samples of luminal fluid from along the epididymis of the echidna [19] show a pattern of proteins similar to samples of luminal fluid from eutherian [20] or marsupial [21] mammals. The concentration of protein increases along most of the caput epididymidis, but the pattern does not change significantly. The pattern is also much the same in samples from the cauda epididymidis, and our earlier work found no convincing evidence that new proteins are secreted coincident with the formation of sperm bundles [19]. However, when the synthesis and secretion of proteins along the epididymis was examined (Figure 3) it was found that at least two new proteins, with molecular weights of approximately 60 000 Da and 76 000 Da, were secreted into the region of the duct where spermatozoa formed into bundles. As the secretion of these proteins coincides with sperm bundle formation, they are good candidates to be sperm competition proteins in the echidna. Our findings on the platypus are consistent with the echidna except that the molecular weights are lower for the proteins associated with sperm bundle formation.

When epididymal luminal fluid and ejaculated semen

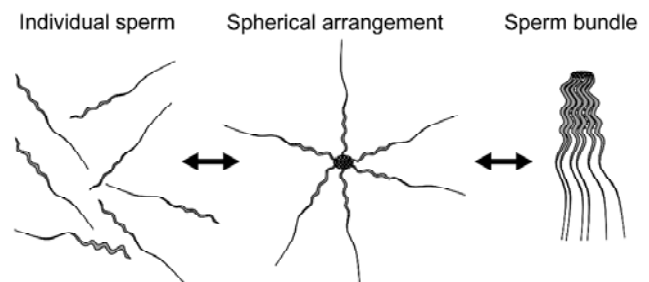


Figure 2. Proposed mechanism of formation of bundles during passage of sperm through the isthmus of the monotreme epididymis. Initially, sperm group with the rostral ends of their heads facing the centre, held in place by a dense mass of protein. Sperm then re-orientate facing the same direction to form a V-shaped bundle with the rostral ends of their heads embedded in the dense mass of protein, and most of the rest of their heads supported by a less-dense mass of protein. (See Figures 15–19 in Djakiew *et al.* [18] for light micrographs of bundle formation.)

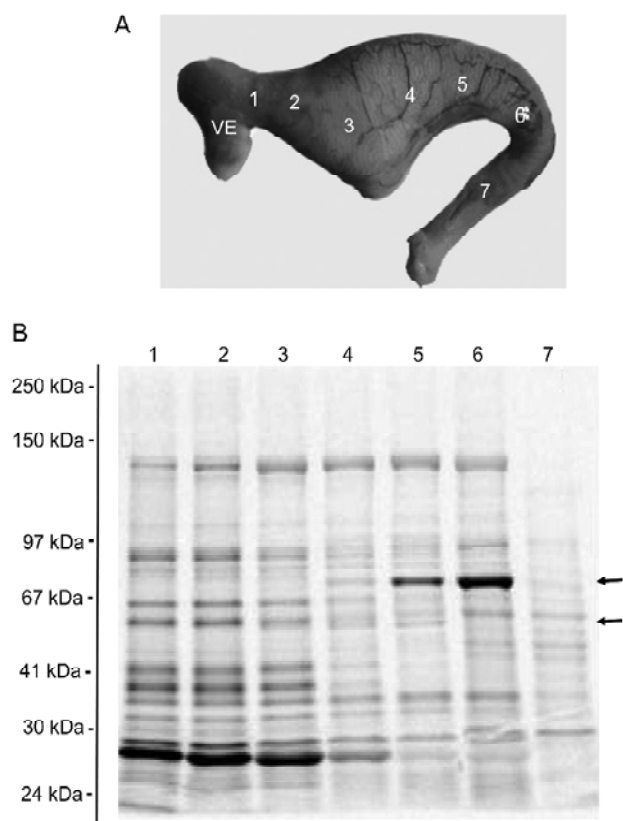


Figure 3. Secretome of the echidna epididymis. (A): Epididymis of the echidna showing the location where samples were taken for the preparation of 1-D gel. VE, ductuli efferentes. 1–4, caput epididymidis. 5, isthmus joining caput and cauda epididymidis. 6, cauda epididymidis. 7, ductus deferens. (B): 1-D fluorograph of samples of luminal fluid collected after ^{35}S -methionine was incorporated into proteins during *in vitro* incubation of lengths of duct [81]. The arrows show the occurrence of the major bands that appear coincident with the formation of sperm into bundles.

from the echidna were diluted and incubated *in vitro*, the sperm bundles moved forward at a velocity of approximately $140\ \mu\text{m}/\text{sec}$, and three times faster than individual sperm. The bundles are also faster than the sauropsid sperm from the Japanese quail ($50\ \mu\text{m}/\text{s}$) and mammalian sperm, such as that of humans ($40\text{--}50\ \mu\text{m}/\text{s}$ [22]) and rams ($110\ \mu\text{m}/\text{s}$ [23]). The bundles of echidna sperm persist during incubation *in vitro* for at least 2.5 h and seem to require a signal to disperse motile sperm. In capacitation medium the bundles only dispersed motile sperm after incubation for 3 h in the presence of a cAMP analogue and a phosphodiesterase inhibitor, implying a role for a cAMP signaling pathway as in higher mammals [5, 24].

2.2 Sperm cooperation in marsupials and eutherians

The form and method of formation of sperm bundles in monotremes is unique among the vertebrates. The method of formation clearly involves a sequence of processes and proteins that can reversibly bind to the sperm membrane and to themselves. Although the process occurs in the epididymis and involves epididymal proteins, it is necessary to resolve how the epididymal proteins interact with components of sperm which are present when sperm leave the testis.

Other forms of sperm cooperation have been recorded among mammals. Guinea pig sperm form rouleaux during passage through the epididymis, but the formation does not persist *in vitro* or in the female reproductive tract [10]. Sperm from most American marsupials [25, 26] form pairs during epididymal passage. Compared to individual spermatozoa *in vitro*, the pairs have slightly higher velocity in normal medium and considerably greater velocity when the viscosity of the medium is increased [27]. The common wood mouse, *Apodemus sylvaticus* [28], displays unique aggregations *in vitro* resulting in “trains” of hundreds or thousands of cells, which significantly increase sperm progressive motility and their ability to penetrate viscous media. Dispersal is associated with most of the sperm undergoing a premature acrosome reaction. Although it is likely that the capacity to form sperm trains develops during epididymal transit, this has not been determined.

3 Sperm maturation and storage in the epididymis

Sperm competition has produced the male reproductive equivalent of an arms race resulting in the evolution of numerous adaptations to enhance males' capacity to achieve paternity in competitive mating systems [10, 29, 30]. Natural selection can ensure that when a male develops a characteristic to enhance his success in sperm competition, it can eventually become a characteristic of the species, or even an Order or Class. Presumably, this is how the mammalian epididymis has become specialized for sperm maturation and storage, adaptations to increase the quality and quantity of sperm in an ejaculate. Indeed, work on heterospermic inseminations shows that both these characteristics are very important in sperm competition [31–35]. Furthermore, even when a characteristic becomes universal among a group its importance between subgroups might depend on the intensity of the selective pressure for the characteristic. Consequently, as there is variation in the intensity of sperm competition among mammalian species, there should also be variation in the amount of post-testicular sperm maturation and sperm storage in the epididymis.

3.1 Sperm maturation in the epididymis

There has been little discussion relating post-testicular sperm maturation to sperm competition. This lack of discussion is surprising considering the amount of published work on the role of epididymal proteins in post-testicular sperm maturation and the convincing work on *Drosophila*, which shows that their accessory glands of reproduction produce at least four sperm competition proteins, and that the genes for these proteins are under strong, positive Darwinian selection [36, 37]. In this respect, the demonstration that epididymal proteins are implicated in developing and improving the capacity of sperm to fertilize ova [38, 39] could be interpreted as evidence that the epididymis secretes sperm competition proteins.

Some variation among scrotal mammals has been identified in the importance of post-testicular sperm maturation among mammals. For example, vaso-epididymovasostomy of humans and laboratory species has shown that post-testicular sperm maturation is not as important in humans as in the rat and the rabbit [40–42]. Furthermore, there is considerable variation between species in the extent that the acrosome is modified in the epididymis. It varies from not at all in monotremes [43–45] and some primates, including man [46–48], modest modification in lagomorphs, ungulates and elephants [49–51], and spectacular changes in some rodents [52].

3.2 Sperm storage in the epididymis

There is some acceptance that the cauda epididymidis of scrotal mammals plays a role in sperm competition because it is adapted to store sperm [53]. This ensures that, during several days of intense mating, daily sperm output by a male can greatly exceed daily sperm production by the testes [1, 8]. Although there is little understanding of the mechanism of sperm storage there is some knowledge of how the specialization is achieved. The cauda epididymidis provides an environment lower than testicular temperature [54] and a unique milieu [55] that keeps the sperm at a metabolic rate one-third to one-fifth that of diluted epididymal spermatozoa [56]. There is also significant circumstantial evidence showing the evolution of the cauda epididymidis for sperm storage [53, 57]. Even amongst the Aves a sperm storage region that looks like the mammalian cauda epididymidis has developed among the passerines [58], presumably an adaptation associated with the greater demands of flight in passerines (than non-passerines) limiting the mass of the testes.

Furthermore, there is considerable variation between species of scrotal mammals in the proportion of extragonadal sperm that are stored in the cauda epididymidis: from, for example, 26% in the spinifex hopping mouse, *Notomys alexis* [59], 29% in *Smithopsis macrura* [60], 44% in the

Rhesus monkey [61], 53% in humans [62], 74% in the Plains rat, *Pseudomys australis* [59], 79% in the ram [63] and 84% in the hamster [61]. There are also reports that cats, gorillas, marmosets and talapoin monkeys store relatively few sperm in the cauda epididymidis [64]. Curiously, some species store a significant proportion of extragonadal sperm in the ductus deferens. For example, the spinifex hopping mouse, *Notomys alexis*, has a similar number of spermatozoa in the ductus deferens (mainly to the middle and urethral regions) and cauda epididymidis [65]. Also, the common shrew, *Sorex araneus*, has a distal distended region of the ductus deferens which appears to function as a sperm reservoir [66].

3.3 Epididymal function and sperm competition in humans

The discussion above indicates that in humans the epididymis is less developed for sperm maturation and storage than in most other mammals. It is suggested that this is because sperm competition is less important in driving human evolution than it is in most other mammals, a consequence of the protection and help that a male can contribute towards rearing his children. Smith [67] reviews the evidence for sperm competition in humans and concludes that it occurs particularly “in the contexts of communal sex, rape, prostitution, courtship, and (most commonly) facultative polyandry”. However, most estimates of the occurrence of cuckoldry in humans over the past tens of thousands of years, are small. Even hunter-gatherer societies, such as the Australian aborigines, recognized a relationship between coitus and pregnancy, had very strict mores determining relationships and have been shown to be accurate in knowing paternity [68]. Some of the estimate of 2% cuckoldry in the !Kung has been attributed to errors in labeling of samples [11]. Evidence from Y chromosome studies indicate that cuckoldry has averaged only approximately 1.3% per generation in England since the late Middle Ages [69]. It is suggested that the higher average cuckoldry rates that have been estimated for recent decades [70, 71] might be related to increased affluence and social security, and there has been insufficient time for them to influence the current status of the human epididymis.

4 Sperm capacitation in mammals

Monotremes have sauropsid-like sperm that have some mammalian characteristics, such as the way the acrosome lies over the head and the presence of a cytoplasmic droplet that migrates along the midpiece [43–45]. However, no structural changes (other than the droplet migration) have been recognized after monotreme

sperm leave the testis and it is not known whether monotreme spermatozoa require post-testicular sperm maturation and capacitation like higher mammals, or whether they are like the avian model and are involved in little post-testicular maturation and do not require capacitation before they are capable of fertilizing an ovum.

The formation of sperm bundles in monotremes, their persistence during incubation *in vitro*, and subsequent dissociation in capacitation medium raises a question about the nature of sperm capacitation. There is no doubt that the spermatozoa are firmly held within the bundles so it is unlikely that they could fertilize an ovum until after they disperse from the bundles. Consequently, it could be argued that the need for a period of incubation before the bundles disperse is consistent with the definition of capacitation. Also, the dispersion of monotreme's spermatozoa from bundles involves the cAMP signaling pathway, like capacitation in higher mammals (see above). However, the development of the need for capacitation and the capacitation process itself are quite different in the monotremes from the process in higher mammals. Little is known of the mechanism in marsupials [5, 72]. In eutherians, it has been shown that the concentration of cholesterol in the sperm membrane increases in the epididymis [73, 74] and capacitation involves the loss of cholesterol from the sperm membrane [4]. Protein has been implicated as an acceptor of cholesterol during capacitation and there are several lipid carrying proteins secreted by the epididymis that could act as donors [75, 76]. However, no more direct involvement of protein has been identified. The development of the need for capacitation in monotremes directly involves protein in the formation of sperm bundles. The protein probably binds to the rostral tip of the sperm membrane and to itself to form spheres of sperm all with their heads facing the centre, and then protein probably binds to the lateral membrane of the sperm head and to itself to form the V-shaped bundles. Questions to be resolved are: do the receptors for the protein(s) develop on the sperm membrane in the testis or epididymis; and what is the nature of the bonds between the epididymal protein and the sperm membrane, and between epididymal proteins to hold the sperm together in a bundle?

It is perhaps noteworthy that the difference in forms of capacitation and post-testicular sperm maturation between the monotremes and the higher mammals is correlated with an increase in the structural differentiation of the ductus epididymidis. The monotreme epididymis is definitely mammalian [18, 19, 77, 78] and the ductus epididymidis of the echidna (and the platypus epididymis is as big) is as long relative to testis mass as the ductus in eutherian [79] and marsupial [80] mammals. Most of

the epididymis of monotremes (96% in the echidna) is similar in structure and dependence on luminal fluid from the testis to the initial segment of the rat and other laboratory and domestic animals [18, 77, 78]. However, there is only one other segment: a short, terminal segment. Marsupials and eutherians are differentiated into at least six structurally distinct segments, the initial segments being much shorter than in the monotremes (37% and 15% respectively of the total length of the ductus epididymidis).

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