



THE GREAT
SPERM RACE



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THE GREAT SPERM RACE



The documentary *The Great Sperm Race* describes the tortuous journey of the sperm in their quest to fertilise the egg. From storage in the male testes to fusion with the egg, this accompanying paper summarises the latest scientific thinking about human sperm delivery and transport, which formed the basis of the programme.

Of the approximately 250 million human sperm cells that enter the vagina just a few thousand are able to enter the fallopian tubes and just one will fertilise the egg. There are many obstacles that sperm must overcome to reach their destination. These include: the acidic atmosphere of the vagina, the mucus of the cervix, the narrowness of the uterotubal junction (the entrance to the cervix), the white blood cells of the immune system, which see the sperm as a pathogenic 'foreign invader' to destroy, and what scientists think is a molecular screening process at the final checkpoint that allows only some sperm through.



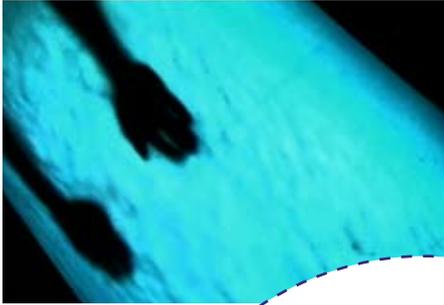
However, a number of mechanisms aid the process. The sperm are guided along their journey by chemical and temperature signals. There is also evidence of a 'sperm reservoir' in the fallopian tubes, where sperm bind to the epithelial lining of the tubes, reducing the chance of fertilisation by multiple sperm. During ovulation, the sperm are hyperactivated to help them penetrate the mucus in the fallopian tubes and the outer coating of the egg. And scientists have also proposed that the sperm outer membrane fuses with the egg outer membrane to facilitate fertilisation.



Sperm can vary greatly in their features and the paper discusses several theories on why this is. One hypothesises that two distinct populations of sperm exist: those that are fit enough to reach the oviduct and those that have errors, which are inevitably killed along the journey to the egg. Another, controversial, theory, suggests the existence of 'kamikaze' sperm and a division of labour of sorts between different sperm. There are also various theories about sperm competition and what happens when sperm from different males overlap. Finally, the paper discusses evidence that sperm counts have decreased in the last 50 years, possibly due to environmental effects.

This paper, written by the programme's Science Producer, Jennifer Beamish, is in the style of a review article, a type of scientific paper where the author summarises current thinking on the field. Like all scientific papers it has undergone 'peer review', where an anonymous panel of experts checks the paper for accuracy before it is published. Each of the reviewer comments has been left in to ensure balance and illustrate the peer review process.

ABSTRACT



Reviewer:

I believe the paper is scientifically accurate. I am familiar with many of the papers cited and have checked a selection of the others on PubMed, confirming that they are correctly cited.

Reviewer:

The report is basically well written and well-referenced, a comprehensive summary of sperm transport that covers most areas adequately.

In *The Great Sperm Race* we propose to incorporate the latest scientific thinking on human sperm delivery and transport through the female reproductive tract that occurs at coitus, from storage in the male epididymis to fusion with the oocyte plasma membrane in the oviduct and formation of a fertilised ovum.

Spermatozoa undergo physical stresses during ejaculation and throughout the female tract. Of an average 2.5×10^8 human sperm cells introduced into the anterior vagina during coitus only a few thousand reach the uterotubal junction and gain entry to the fallopian tubes and only one will fertilise an oocyte. Various impediments exist that sperm must overcome as they travel through the female reproductive tract: the acidic pH of the vagina; the mucus micro-architecture of the cervix; the narrow uterotubal junction; the presence of phagocytosing leukocytes in the vagina, cervix and uterus and finally we discuss the possibility of discrimination of spermatozoa by the oocyte.

Sperm may be guided to the oocyte by a combination of thermotaxis and chemotaxis. Formation of a functional reservoir through epithelial binding of sperm to the oviductal isthmus may reduce the likelihood of polyspermic fertilisation. Motility hyperactivation assists sperm in penetrating mucus in the fallopian tubes and the cumulus oophorus and acrosome exocytosis may assist penetration of the oocyte's zona pellucida that precedes fusion with the oocyte plasma membrane.

We also discuss the various theories of sperm heterogeneity posited to account for the remarkable pleiomorphism in sperm morphology. Cohen proposed two distinct populations of sperm, those that can reach the oviduct and those that due to meiotic errors during spermatogenesis cannot and instead succumb to leukocytic phagocytosis en route. Baker and Bellis proposed the contentious 'kamikaze' sperm hypothesis that posits a functionally adaptive 'division of labour' between sperm. We also discuss sperm competition theory and posit the likely in vivo scenario if more than one sperm population overlap.

We conclude with a discussion of male fertility in which we examine evidence that sperm counts have decreased in the last 50 years possibly due to in utero and other environmental effects.

It is worth noting that in vitro fertilisation involves few if any of the in vivo selection processes described in this document and consequently success rates are much higher. If for instance 100,000 or more sperm are introduced to an egg in vitro then fertilisation is likely and up to 90% of sperm can be successful if inserted by injection directly into the egg (Barratt, telephone conversation 1/2008).

Reviewer:

Overall the scientific accuracy of the document is reasonable. But there are some important, and rather fundamental, omissions that largely debunk the unfounded concept of kamikaze sperm etc. And, as often happens, people don't realise that the process is different in humans from most animals for very simple logistic reasons.

INTRODUCTION

Passage of sperm through the female reproductive tract is regulated to maximise the chances that high-fertility sperm with normal morphology and vigorous motility reach the oocyte and once there have the best chance of success. In humans there is evidence that successful fertilisation can take place if ejaculation occurs 5 days before ovulation (Wilcox et al, 1995).

Sperm must survive transit through a series of tough obstacles and of the millions introduced only thousands will reach the fallopian tubes, possibly only tens will encounter the oocyte and only one will ordinarily fertilise it. In discussing sperm delivery and transport this document will draw heavily from the structure of Suarez and Pacey (2006).



PRODUCTION AND MATURATION OF SPERMATOZOA IN THE MALE REPRODUCTIVE TRACT

Spermatozoa are produced and matured in the male reproductive tract over a 90 day period. They are created through a process of meiotic cell division in the testes and are further matured in the epididymis where they undergo dynamic changes and acquire key proteins. They are then stored in the ampulla of the vas deferens prior to ejaculation.

Because sperm are terminally differentiated cells and deprived of an active transcription and translation apparatus they must survive in the female without benefit of reparative mechanisms available to many other cells. As such they have finite resources available to them on their journey through the female reproductive system (Suarez and Pacey, 2006).

Spermatozoa comprise a head, midpiece and tail. The head contains the haploid genome; the midpiece houses mitochondria spirally arranged around the first portion of the tail and responsible for oxidative phosphorylation and provide much of the energy needed for sperm motility. Coating the head is a membrane called the acrosome rich in enzymes including hyaluronidase which may be necessary to penetrate the oocyte's cumulus and zona pellucida.

SITE OF SEMEN DEPOSITION

Spermatozoa are delivered into the anterior vagina during coitus. Semen has been observed pooled near the cervical os forming a loose coagulated gel. Within minutes sperm begin to leave this coagulum to swim into the cervical canal (Sobrero and MacLeod, 1962). It has been proposed that semen serves to hold the sperm at the cervical os (Harper 1994) and to protect sperm from the harsh acidic environment of the vagina.

VAGINAL DEFENCES AGAINST INFECTIOUS ORGANISMS MAY AFFECT SPERM

The vagina is an open tract and as such is vulnerable to infection from outside, especially at the time of coitus. As a consequence it is well equipped with antimicrobial defences including an acidic pH of 5 or under which is microbicidal to many sexually transmitted disease pathogens. The pH of semen plasma is around 7 (Roberts, 1986) and has the potential to neutralise vaginal acid. Vaginal pH was measured by radio-telemetry in a fertile human couple during coitus. The pH rose from

4.3 to 7.2 within 8s of the arrival of semen and no change was detected when the partner used a condom (Fox et al 1973). Vaginal washing of women with high levels of detectable seminal antigens had a median pH of 6.1 whereas the median pH of washings lacking detectable antigens was 3.7 (Bouvet et al, 1997). Contraceptive gel designed to maintain a low vaginal pH after coitus has been shown to immobilise human sperm in vitro and in vivo (Amaral et al 2004).

In addition to pH buffers, seminal plasma contains inhibitors of immune responses, including protective components that coat sperm (Suarez and Oliphant 1982; Dostal et al 1997).

Reviewer:

This completely overlooks all of the evidence that the ejaculate and the sperm themselves have various means of suppressing or disarming the female immune system (e.g. the high levels of prostaglandins in the seminal plasma). Otherwise, it would be very common place for women to become sensitised to sperm and raise antibodies against them, preventing fertilisation.

SPERM PLEIOMORPHISM: DIFFERENT SPERM MORPHOLOGIES

Sperm are present in huge quantities in any one ejaculate: up to half a billion. Why so many are produced is thought to be because of the intense selection that occurs in the female tract, with only a relatively few spermatozoa being accepted, resulting in an unusually rapid evolutionary rate among the proteins involved in achieving fertilisation. Because so many steps are required, variability and meiotic assortment among relevant reproductive genes provide limitless ways to affect sperm function. The huge numbers of spermatozoa produced by spermatogenesis represent the results of such recombinations, as though males must strive to cover every likely eventuality of the selective process (Holt and Van Look, 2004).

A common conclusion that emerges from the literature is that most spermatozoa are not capable of fertilisation. However there is no a priori equivalence between sperm morphological normality and fertilising ability. There is intense discussion about what constitutes a 'normal' spermatozoon and indeed about whether there is such a thing as 'normal' owing to the fact that certain so-called 'abnormal' sperm can achieve fertilisation (Mortimer and Menkveld, 2001; Holt, 2005).

Reviewer:
One point missed is that the poor morphology of most 'normal' human sperm is probably related to the different organisation of spermatogenesis in the human compared with most other mammals. There is also the lack of competition: humans are monogamous (that is, most humans only have one partner at a time). If a woman only mates with one person at a time, how can there be sperm competition? So there is little evolutionary pressure on men to have high counts of normal sperm.

High fertilisation rates are obtained provided more than 14% of spermatozoa are classified as 'normal' (Kruger et al 1986; Mortimer and Menkveld, 2001) and in IVF the figure can be as low as 4%. According to Mortimer and Menkveld the 'normal' sperm must have a head with a smooth oval configuration with a well-defined acrosome constituting 40%-70% of the anterior sperm head. The normal head length is between 4 and 5 μm and width between 2.5 and 3.5 μm . The head width must be between three-fifths and two-thirds the head length. But there is no absolute correlation between 'normal' head shape and genetic quality. Direct comparisons of sperm phenotypes and chromosomal complements showed that morphologically 'normal' spermatozoa from infertile men contained a significantly higher incidence of genetic abnormalities than normal sperm from a cohort of fertile controls (Ryu et al 2001).

Classifying spermatozoa as 'normal' seems to be more of a problem in humans than for other species like bull in which pleiomorphism is uncommon. Almost all bull spermatozoa are 'normal' sperm. Unusual sperm head shapes in species like the bull are indeed indicative of defective spermatogenesis (Burgoyne, 1975; Russell et al 1991). Furthermore in many experiments where equal numbers of 'normal' sperm from two or more animals have been mixed and inseminated together (Dziuk, 1996) the conception rates are usually skewed towards one of the males (Steward et al 1974; Beatty et al 1976; Robl and Dziuk, 1988; Berger, 1995; Stahlberg et al, 2000). Thus even though the number of sperm inseminated were controlled there were still functional differences between ejaculates implying that the female reproductive tract itself is capable of making the distinction between sperm from different males. How this happens is as yet unknown but Holt posits that sperm heterogeneity within each ejaculate holds the key, (Holt, 2005). Sperm DNA must be good enough to support embryonic development; its DNA delivery mechanism must be fully functional (head, midpiece and tail); its surface characteristics must not attract the attention of phagocytes within the female and sperm are so well endowed with molecular receptors (Meizel, 2004) to which they must respond appropriately that the integrity of biochemical signalling pathways within each sperm is also likely to be critical to their fertility (Holt, 2005). Many of these abilities are invisible unless sperm are analysed physiologically rather than merely morphologically.

In humans it may also be likely that some so-called 'abnormal' sperm have the fertilising ability of 'normal' sperm despite their appearance.

THEORIES THAT ACCOUNT FOR SPERM HETEROGENEITY IN HUMANS:

A precise understanding of why and how some spermatozoa are better able to reach and fertilise an oocyte under natural conditions remains an unknown. But here are a few theories that have been posited to explain why sperm heterogeneity might exist which though unproven are nevertheless worth mentioning.

COHEN'S TWO-POPULATION THEORY

Smallcombe and Tyler (1980) and Pandya and Cohen (1985) showed in human and animal studies that the immunoglobulin factor IgG and massive leukocytosis was elicited by spermatozoa, not by mere copulation. Cohen postulated that spermatozoa emerge from the seminal coagulate up to 15 minutes after pooling to coincide with the female immune response. Spermatozoa comprise of two distinct groups: one that results from errors in the meiotic process and is coated with IgG, and a tiny minority that remain uncoated. The coated majority exit the coagulate first and are immediately targeted and phagocytosed by the circulating leukocytes; the second uncoated minority travel unchallenged (Cohen, 1990; 1998).

Advances in DNA assessment have not yet provided direct support for Cohen's theory though it is still described as an attractive and logical hypothesis (Holt and Van Look, 2004).

EISENBACH'S THEORY OF RIPE SPERM

Eisenbach proposed a population of 'ripe' sperm to correspond with the tiny proportion of sperm that are both chemotactic and capacitating (Cohen-Dayag et al, 1995); as the ripe sperm age they are replaced and the remaining great majority of non-fertile sperm are either aged or juvenile.

This theory also remains unproven. Perhaps changes in membrane lipids (Haidl and Cooper 1997) could be involved; membrane-damaged sperm can be involved in fertilisation if assisted (Ahmadi and Ng, 1997) but they may normally be discriminated against in vivo.

THE 'KAMIKAZE' THEORY OF SPERM

The most notorious theory of sperm heterogeneity is the 'kamikaze' sperm hypothesis of Baker and Bellis (Baker and Bellis, 1998 and 1989) that proposed a functionally adaptive division of labour between sperm: 'egg-getters', 'blockers' and 'killers'. Given that sperm consist of a diminutive single-cell structure, the 'kamikaze' theory's requirement that sperm carry a self-recognition system that must differentiate between not just different genes but different sets of genes from a rival male may be unlikely.

The 'kamikaze' theory found favour with the media and audiences and has been rapidly disseminated through Robin Baker's popular science book *Sperm Wars, Infidelity, Sexual Conflict and other Bedroom Battles*. A mathematical model used to examine the evolutionary conditions that might lead to the evolution and maintenance of a 'soldier' class of sperm concluded that these conditions are not stringent. 'Soldier' sperm may be particularly likely to evolve in contexts where there is substantial variance in the number of sperm from different males available to compete for fertilisations, due to factors such as mating order effects, between male differences in ejaculate size and copulation timing in relation to ovulation (Kura and Nakashima, 2000). Otherwise the theory remains unproven and is largely discredited.

SPERM COMPETITION THEORY

The Baker and Bellis 'kamikaze' theory was hypothesised within the context of sperm competition theory. Sperm competition occurs when spermatozoa from more than one male have the opportunity to fertilise a single female during the same fertile period (Parker, 1970; 1998). The morphological pleiomorphism of human sperm is thought by evolutionary biologists to be indicative of relatively low levels of sperm

competition in our species' history and prehistory. Evolutionary biologists argue that because human social systems do not generally involve the intensive male–male competitive matings as seen in other primate societies, there is less pressure to drive up the rate and quality of sperm production. In species with high levels of multiple matings like bulls and chimpanzees, sperm morphology tends to be relatively homogenous, more plentiful and of higher quality (Dixson, 2001).

Several studies have demonstrated that large testes size and hence a greater sperm production capacity is a feature of species which exhibit multi-male mating systems (Harcourt et al 1981). Comparative analyses of testis size in humans and great apes have shown that humans tend to group with the polygynous gorilla (one male–multifemale) and have relatively small testis/bodyweight ratios relative to the multimale–multifemale chimpanzees.

Likewise gorillas and humans have similar levels of pleiomorphism compared to chimpanzees (Seuanez, 1981). Chimpanzees also have roughly twice the number of mitochondria in their sperm midpiece than humans (Bedford, 1974) which correlates with the finding that chimpanzee sperm midpiece volume is almost twice that of humans (Anderson and Dixson, 2002). Sperm competition in the chimpanzee may have resulted in the evolution of bioenergetically superior sperm in comparison to humans.

However human sperm showed significantly higher mitochondrial loading than gorilla sperm which may indicate occasional sperm competition in our species. (Anderson et al, 2007).

It looks unlikely that human males have experienced such high levels of sperm competition as chimps but it is unlikely that sperm competition has been completely absent over human evolutionary history (Pound et al, 2006). In modern society paternal discrepancy rates vary between 0.8 and 30% (median 3.7%) (Bellis et al, 2005) and infidelity rates vary between 30 and 50% (the Myth of Monogamy, Barash and Lipton, 2001) making it unlikely that some form of sperm competition is absent in present day society.

SPERM FROM 'RIVAL' HUMAN MALES – HOW MIGHT THEY BEHAVE IN UTERO?

A review of the literature on andrology and sperm competition theory suggests that rival male sperm samples might behave neutrally with respect to each other in vivo. Our own conclusion is that sperm 'compete' against any sperm, be they from their own 'group' or another. Their ability to reach and fertilise the oocyte is a factor of their own design and build – their ability to do everything correctly that is required en route through the female tract – and critically their timing at every stage. So if all else is equal in design and build terms, the successful spermatozoon is the one that is in exactly the right place at the right time, at each stage on this extraordinary journey.

VAGINAL FLOWBACK

After semen deposition in the anterior vagina there is substantial sperm loss almost immediately though how much is still not precisely known. In a 5 year study of 11 female volunteer Baker and Bellis (1993) examined the characteristics of sperm loss from the vagina following coitus ('flowback'). They found that flowback occurred in 94% of copulations with the median time to the emergence of flowback of 30 min.

Reviewer:

It is worth noting that sperm are not stored to any extent in humans, which is completely different to many animals that are promiscuous seasonal breeders (i.e. they mate with any female 'on heat'). Thus they maintain a high sperm count despite multiple matings every day over a period of weeks. This demands a sperm storage capacity otherwise sperm counts would drop with each ejaculation. Also, the higher the sperm count, the greater the chance of fertilising the eggs in a female who is mating with several males successively.

Baker and Bellis estimated a median of about 65% of sperm was retained. In 12% of copulations about 100% of the sperm were eliminated which supports the notion that only a minority of sperm travel beyond the vagina. Another research group deposited 5–40 µm albumen microspheres radioactively tagged with technetium into the cranial vagina of women to determine how sperm might be transported (Kunz et al, 1996). They found that spheres were only rapidly and maximally transported into the uterine cavity and beyond during peak fertility. At all other times the spheres didn't advance beyond the vagina.

X AND Y SPERM – DIFFERENCES IN MOTILITY

In any given ejaculate there are equal amounts of X and Y chromosome-bearing sperm (X and Y sperm). Motility differences between X and Y sperm have been reported in bull sperm (Penfold et al, 1998). When the following motion parameters were measured: curvilinear, straight-line, and average path velocity; mean angular displacement (MAD); beat cross-frequency; amplitude of lateral head displacement; linearity (LIN); and straightness of path (STR) significant differences were seen between X and Y sperm for MAD, LIN, and STR. No difference was observed for the other parameters. The results indicate that in a simple salts solution, Y bull sperm do not swim faster than X sperm but may be distinguished from X sperm on the basis of LIN and STR. Holt at the London Zoological Society hopes to replicate this work in 2008 (telephone conversation, 11/07).

SPERM TRANSPORT THROUGH THE CERVIX

THE CERVIX AS A PHYSICAL OBSTACLE

Sperm enter the cervical canal rapidly where they encounter cervical mucus. Under the influence of oestrogen during ovulation the cervix secretes highly hydrated mucus, often exceeding 96% water in women (Katz et al, 1997). The extent of hydration is correlated with penetrability of sperm (Morales et al, 1993). Coitus on the day of maximal mucus hydration in women is more closely correlated with incidence of pregnancy than when timed with respect to basal body temperature indicators of ovulation (Bigelow et al, 2004).

Cervical mucus presents more of a barrier to sperm of abnormal morphology and poor motility and is considered to be a means of sperm selection, supporting the passage of normal motile sperm while discouraging passage of microbes and sperm with abnormal motility (Hanson and Overstreet, 1981; Barros et al, 1984; Katz et al, 1990; 1997). Cervical mucus has also been shown in vitro to be a selective barrier against spermatozoa that though morphologically normal are carrying genetic structural abnormalities (Bianchi et al, 2004).

The greatest barrier to sperm penetration of cervical mucus is at its border, because here the mucus microarchitecture is more compact (Yudin et al, 1989). Components of seminal plasma may assist sperm in penetrating the mucus border. More human sperm were found to enter cervical mucus in vitro when an inseminate was diluted 1:1 with whole seminal plasma than when it was diluted with Tyrode's medium even though the sperm swam faster in the medium (Overstreet et al, 1980).



CERVICAL DEFENCES AGAINST INFECTIOUS ORGANISMS MAY AFFECT SPERM

Vaginal insemination stimulates the migration of leukocytes, particularly neutrophils and macrophages into the cervix as well as into the vagina presenting another obstacle to spermatozoa (Tyler, 1977; Pandya and Cohen, 1985; Barrett and Pockley, 1998). There is evidence from animal work (Taylor, 1982) that the immune response may be a selective one. Cohen in turn postulates that it may be a discriminatory response (Cohen, 1998). In his two-population theory those sperm without IgG coating – the so-called 'rapid transport' spermatozoa – are able to avoid phagocytosis in the cervix and can get to the oviduct rapidly. Animal experiments have shown that the high-fertility sperm that make it through the cervix and uterus to the oviduct are uncoated with IgG (Cohen and Tyler, 1980) and that these high-fertility sperm are not attacked by leukocytes (Taylor, 1982). It has also been demonstrated that neutrophils will bind to human sperm and ingest them only if serum that contains both serological complement and complement-fixing anti-sperm antibodies is present (D'Cruz et al, 1992). Complement proteins are also present in cervical mucus (Matthur et al, 1988) along with regulators of complement activity (Jensen et al, 1995). Thus there is a potential for antibody-mediated destruction of sperm in the cervical mucus as well as leukocytic capture of sperm.

ARE SPERM STORED IN THE CERVIX?

Cervical crypts are thought to entrap and store sperm (Fawcett and Raviola, 1994; Harper, 1994) and scanning electron microscopy of the human cervix indicates that mucosal grooves forming a preferential pathway for sperm could be present though a comprehensive study of the human cervix is needed to determine whether sperm follow these grooves to traverse the cervical canal.

Vigorously motile sperm have been recovered from the cervix up to 5 days after insemination (Gould et al, 1984). It is not known if these sperm had re-entered the cervix from the uterus though it may be unlikely as very few sperm have been recovered from human uteri 24 h after coitus (Rubbenstein et al, 1951; Moyer et al, 1970) and those sperm were greatly outnumbered by leukocytes (Thompson et al, 1992). If some sperm are protected from phagocytosis as some of the evidence suggests they may be able to travel from a cervical reservoir to the oviduct post coitus.

SPERM TRANSPORT THROUGH THE UTERUS

The human uterine cavity is a few cms in length and could be traversed in less than 10 mins by sperm swimming at about 5 mm/min, which is the swimming rate of sperm in aqueous medium (Mortimer and Swan, 1995). Experimental limitations make calculating the actual rate of passage of sperm difficult to ascertain but it is likely that transport of sperm is aided by proovarian contractions of the myometrium. Ultrasonography of the human uterus has revealed cranially directed waves of uterine smooth muscle contractions that increase in intensity during the late follicular phase (Lyons et al 1991; Kunz et al, 1996). The uterine contractions in women during the periovulatory period are limited to the layer of myometrium directly beneath the endometrium (Lyons et al, 1991; de Ziegler et al, 2001).

Contractile activity of uterine muscle may draw sperm and watery midcycle mucus from the cervix into the uterus and may assist sperm movement through the uterine cavity (Fukuda and Fukuda, 1994). The volume of uterine fluid in midcycle women is only about 100 µl (Casslen, 1986) and cervical mucus is plentiful enough to fill the lumen.



There is evidence that there is preferential transport to the isthmus ipsilateral to the dominant follicle than to the contralateral isthmus. Kunz et al (1996) deposited 5–40 μm albumen microspheres radioactively tagged with technetium into the cranial vagina of women to determine how sperm might be transported. They found that spheres were only rapidly and maximally transported into the uterine cavity and beyond during the midcycle late follicular phase.

Rapid transport of sperm through the uterus by myometrial contractions may enhance sperm survival by propelling them past immunological defences of the female. As is the case in the vagina and cervix, coitus induces a leukocytic infiltration of the uterine cavity, which reaches a peak several hours after insemination. Phagocytosis was observed several hours after insemination and therefore might be directed primarily against damaged sperm. However normal sperm may also be attacked because they have lost much of the immune protection afforded by seminal plasma constituents (Suarez and Oliphant, 1982; Dostal et al, 1997). However animal experiments have shown that leukocytes have little if any impact on those sperm that succeed in reaching the oviduct if reinseminated back into the female: they are more likely to fertilise eggs than competing ejaculated sperm (Cohen and MacNaughton, 1974; Overstreet and Katz, 1977).

TRANSPORT THROUGH THE UTEROTUBAL JUNCTION (UTJ)

The uterotubal junction (UTJ) presents anatomical, physiological and mucous barriers to sperm passage in humans. The entrance traverses a thick muscular layer of uterine wall (Hafez and Black, 1969) and the narrow lumen may be mucus-filled (Jansen, 1980), which impedes the progress of sperm. It has been observed that capacitated sperm are unable to swim through the narrow junction (Nakanishi et al, 2004) and in rodents that sperm with linear, progressive motility are more successful at passing through (Gaddum-Rosse, 1981; Shalgi et al 1992).

There is likely to be some form of molecular selection at work also. Male mice that are null mutants for genes encoding different sperm surface proteins, including calmegin and ACE, are infertile because their sperm fail to get through the UTJ, despite having normal sperm morphology/motility. Thus it's likely that certain epitopes are available and exposed on the surface of sperm to interact with the UTJ and somehow promote sperm passage (Krege et al, 1995; Ikawa et al, 1997; Cho et al, 1998; Hagaman et al, 1998; Yamagata et al, 2002).

A SPERM RESERVOIR IN THE FALLOPIAN TUBE – EPITHELIAL BINDING

The Fallopian tube provides a haven for sperm. Unlike the vagina, cervix and uterus, the tube does not respond to insemination with an influx of leukocytes (Rodriguez-Martinez et al, 1990). Overall data of human sperm distribution in the fallopian tubes of women have not provided a clear picture of the events of sperm transport. Sperm recovered at various times in different regions of the fallopian tube have varied so much in numbers that the data do not permit the construction of a model for the pattern of tubal transport (Williams et al, 1993).

Since pregnancy has been shown to result from intercourse as long as five days before ovulation (Wilcox et al, 1995) human sperm must be stored somewhere in the female tract. A visibly distinct sperm reservoir has been seen in the fallopian tubes of animals (Yanagimachi and Chang, 1963; Harper, 1973; Overstreet et al, 1978; Suarez, 1987) but not in humans. However it's likely that a functional reservoir does exist, created by detaining human sperm in the tubal isthmus (Pacey et al, 1995).

There is strong evidence that such a reservoir is created when sperm intermittently bind to the epithelium lining the tube. In humans motile sperm have been observed to bind their heads to the apical surface of endosalpingeal epithelium in vitro (Pacey et al, 1995a; Baillie et al, 1997; Reeve et al, 2003). In addition in vitro, sperm fertility and

motility are maintained longer when sperm are incubated with endosalpingeal epithelium (Kervancioglu et al, 1994).

It may be that the intermittent binding of sperm in the tubal reservoir serves to prevent polyspermic fertilisation by allowing only a few sperm at a time to reach the oocyte at the other end of the tube (the ampulla). Artificially increased sperm numbers at the site of fertilisation in animals increases the incidence of polyspermy (Polge et al, 1970; Hunter, 1973). While intermittent sticking would not hold sperm in a distinct reservoir indefinitely it would slow their progress towards the ampulla. Sperm progress would also be slowed by the mucus in the lumen. Finally the architecture of the mucosal lining of the human fallopian tube must act to slow sperm progress. The mucosal folds increase in height and complexity towards the ovary, thus offering increasingly greater obstacles to the advancement of sperm into and through the ampulla. Slowing the advancement of spermatozoa like this could serve the function of a reservoir, that is, prolonging the availability of sperm in the fallopian tube.

The amino acid sequence Arg-Gly-Asp (RGD) has been implicated in sperm binding to isthmic endosalpingeal epithelium but not to the ampullary endosalpingeal epithelium that lies at the other end (ampulla) of the tube (Reeve et al, 2003). Human sperm–endosalpingeal interaction in vitro appears to be disrupted in tissue donated from women who have had a previous diagnosis of endometriosis (Reeve et al, 2005) suggesting for the first time that poor sperm interaction with the endosalpingeal epithelium might be associated with reduced fertility, such as that often observed in women with endometriosis.

ADVANCEMENT OF SPERM BEYOND THE TUBAL RESERVOIR

Theoretically sperm could be released from the reservoir either through loss of binding sites on the epithelium or by alterations in the sperm themselves. In women relatively equal numbers of sperm bind to endosalpingeal explants recovered at different times of the ovarian cycle (Baillie et al, 1997) so it appears that the epithelium does not release sperm by reducing binding sites. Instead current evidence indicates that changes in sperm bring about their own release.

CAPACITATION AND HYPERACTIVATION TO MOVE SPERM FROM ISTHMUS TO AMPULLA

Sperm undergo two changes in preparation for fertilisation: capacitation and hyperactivation that together may serve to firstly detach spermatozoa from their isthmic ‘reservoir’ and then speed sperm movement to the ampulla as the time of ovulation approaches.

Reviewer:
My understanding is that hyperactivation is part of the capacitation process? But I am not an expert in this area.

Capacitation involves changes in the plasma membrane, including shedding of proteins and cholesterol, that prepare sperm to undergo the acrosome reaction and fertilise oocytes (De Jonge, 2005) and this loss or modification of proteins on the surface of the plasma could reduce affinity for endosalpingeal epithelium.

Hyperactivation is a change in flagellar beating that typically involves an increase in the flagellar bend amplitude. This can provide the force necessary for overcoming the attraction between sperm and epithelium (Ho and Suarez, 2001).

Animal experiments have shown that only hyperactivated spermatozoa detach from endosalpingeal epithelium (DeMott and Suarez, 1992) and that capacitated sperm bind less well (Lefebvre and Suarez, 1996) indicating that capacitation-induced changes in the sperm head surface are responsible for reducing binding affinity, though the pull produced by hyperactivation can almost certainly hasten detachment of bound sperm from the epithelium. The epithelium in turn may play a role in sperm release by secreting factors that alter sperm. For example hormonal signals that



induce ovulation or signals from the pre-ovulatory follicle could stimulate the epithelium to secrete factors that trigger capacitation and hyperactivation, thereby bringing about sperm release. Tubal fluid and medium conditioned by cultured endosalpingeal cells have been demonstrated to enhance capacitation in bull sperm in vitro (Chian et al, 1995; Mahmoud and Parrish, 1996)

HYPERACTIVATION AND THE FINAL STAGES OF SPERM TRANSPORT

In aqueous solution hyperactivated sperm swim vigorously in circular or erratic patterns but in vivo the physical environment encountered by sperm is quite different and evidence indicates that hyperactivation is required by sperm to progress towards the oocyte and penetrate its vestments. Hyperactivation enhances the ability of sperm to swim through viscoelastic substances such as mucus in the tubal lumen and the extracellular matrix of the cumulus oophorus (Jansen, 1980). Hyperactivated sperm penetrate artificial mucus such as viscoelastic solutions of long-chain polyacrylamide or methylcellulose, far more effectively than non-hyperactivated sperm (Suarez et al, 1991b; Suarez and Dai, 1992; Quill et al, 2003).

Hyperactivation also endows sperm with greater flexibility for turning around in pockets of mucosa (Suarez et al, 1983; Suarez and Osman, 1987) and may thus assist sperm in navigating the increasingly complex tubal maze. In animals that can't hyperactivate but are otherwise capable of vigorous progressive motility experiments show that they do not penetrate artificial mucus nor can they penetrate the oocyte's zona pellucida (Quill et al, 2001, 2003; Ren et al, 2001; Carlson et al, 2003).

TAXIS OF SPERM TOWARDS OOCYTES

There is good evidence from animal studies of a guidance system that helps sperm reach the oocyte. Long-range thermotaxis guides capacitated sperm released from intimate contact with the endosalpinx towards the fertilisation zone. Animal work shows that sperm tend to swim towards warmer temperatures and the ampulla is two degrees warmer than the isthmus (Bahat et al, 2003).

Shorter range chemotaxis may also guide the spermatozoa towards the oocyte (Eisenbach, 1999; Babcock, 2003). Sperm are equipped with a mechanism for turning towards the oocyte in response to chemotactic factors: they can switch back and forth between symmetrical flagellar beating and the asymmetrical flagellar beating of hyperactivation (Suarez et al, 1987) and so they can swim straight ahead and turn around. Controversially, sperm have also been reported to turn towards, or accumulate in a gradient of follicular fluid (Ralt et al, 1991, 1994; Cohen-Dayag et al, 1994, 1995; Fabro et al, 2002) which could accompany the oocyte into the fallopian tube.

One interesting consequence of capacitation is the apparent activation or unmasking of receptors that respond to chemical stimuli from oocytes or thermal gradients within the oviduct (Fabro et al, 2002; Bahat et al, 2003). Although the details of this process are unknown it is clear that this provides yet another sperm selection point.

Odorant receptors have been localised to a spot on the base of the flagellum of human sperm (Spehr et al, 2003) and placing sperm in a gradient of the odorant bourgeonal (lily of the valley) caused them to orient into the gradient and triggered a calcium and cAMP-mediated signalling cascade (Spehr et al, 2004). Nevertheless a chemotactic odorant has yet to be identified. If one were found it could have vast implications for the development of contraceptives, as well as assessment and treatment of infertility.

THE NATURE OF OOCYTE CHEMOTAXIS

It is unlikely that follicular fluid is the single source of sperm chemotaxis in vivo for two main reasons. Firstly, at ovulation only a very small fraction of follicular fluid is transported into the oviduct along with the oocyte (Hansen et al, 1991; Brussow et al, 1998; Hunter et al, 1999) and secondly, if sperm chemotaxis is essential for fertilisation the chemoattractant gradient should be maintained for as long as the oocyte survives and can be fertilised i.e. ca 24 hours post-ovulation (Harper, 1982). This requires a continuous supply of chemoattractant; sperm chemotaxis has been observed to both the oocyte's surrounding cumulus cells and more potently to the oocyte itself, concluding that both are necessary chemotactic sources (Sun et al, 2005).

OOCYTE DISCRIMINATION OF ABNORMAL SPERM

There is also evidence of a process whereby morphologically abnormal spermatozoa or non-capacitated or non-acrosome-reacted sperm are deselected by the oocyte and its surrounding cumulus cells. In vivo interaction between the oocyte and the few remaining sperm is likely to involve spermatozoa that are morphologically normal due to the selecting nature of the female reproductive system. Nevertheless evidence from in vitro work shows that those sperm able to traverse the cumulus mass were more likely to have normal morphology, be capacitated and acrosome-reacted with a distinct motility pattern and better zona-binding capacity suggesting that sperm selection continues to the end (Hong et al, 2004).

As mentioned already there is some evidence that chemotaxis excludes those sperm that are non-capacitated; only those sperm that capacitate appear to be chemotactically responsive (Cohen-Dayag et al, 1994, 1995; Oliveira et al, 1999; Fabro et al, 2002).

THE CUMULUS–OOCYTE COMPLEX (COC)

Sperm and oocyte have very different functional roles and contributions. The sperm merely delivers paternal chromosomes to the site of fertilisation while the oocyte will provide all the nourishment and genetic programming necessary to sustain embryonic development for almost a week after conception. The volume of the oocyte is therefore much greater than that of the spermatozoon.

A woman usually ovulates one oocyte each month, about 400 in her reproductive lifetime. Ovulation occurs before completion of oocyte maturation and the oocyte that leaves the follicle is arrested in metaphase of the second meiotic division during which time metabolism has been discontinued. To develop further, this secondary oocyte must await stimulation by fertilisation.

The oocyte is surrounded by intercellular materials, including a thin transparent gelatinous layer of protein and polysaccharides called the zona pellucida. As the oocyte is ovulated a surrounding mass of follicular cells called the cumulus oophorus accompanies it resulting in the cumulus–oocyte complex (COC).

The COC serves as the final sperm filter and probably only 10 or 20 spermatozoa make it through the cumulus to reach the zona pellucida. The race to the finish will be governed by effective ligand-receptor interactions, functionally active signal transduction cascade pathways and a bit of luck (De Jonge, 2005).



THE CUMULUS

At this stage in the fertilisation process there are probably no more than tens of sperm that have reached and begun to penetrate through the cumulus.

The cumulus is an extracellular matrix of ca 5,000 cells held together primarily by hyaluronate (hyaluronic acid) (De Jonge, 2005). Hyaluronidase activity by the sperm plasma membrane protein PH-20 acting on its hyaluronate substrate facilitates sperm penetration of the cumulus matrix (Lin et al, 1994).

As already described it's possible that the intercellular matrix of the cumulus acts similarly as cervical mucus to filter sperm with more normal morphology selectively (Sun et al, 2004). In addition it has been shown in vitro that hyaluronate selectively binds spermatogenically and genomically mature, viable and acrosome-intact human sperm (Huszar et al, 2003).



THE ZONA PELLUCIDA

The zona is composed of a small number of glycoproteins; in addition to mediating the interaction with sperm, it is involved in the prevention of polyspermy and the protection of the developing embryo prior to implantation (Conner et al, 2005).

It is likely that the sperm population that has successfully traversed the cumulus is heterogeneous relative to expression and receptivity for zona glycoprotein and functionality of signal transduction mechanisms that will ultimately participate in the zona-induced acrosome reaction, zona penetration and fertilisation. Upon contact with the zona there may only be slightly more than a handful of sperm that completely fulfil the preceding elements; perhaps chance is the final determinant for which of these is the fertilising sperm (De Jonge, 2005).

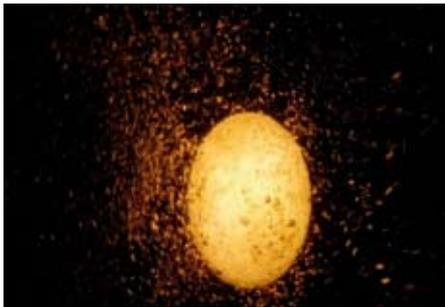
ZONA BINDING AND THE ACROSOME REACTION

The very few spermatozoa that have reached and now bind to the zona pellucida receive a signal to acrosome react i.e. release by Ca^{2+} stimulated exocytosis of the contents of their large secretory granule, the acrosome. The membranes in the head of the spermatozoon are reorganised; multiple fusions occur between the outer region of the acrosomal membrane and the plasma membrane that overlies the acrosome. The hybrid vesicles that result are released into the surrounding environment along with fluid contents of the acrosome. Loss of these anterior portions of membrane reveals the inner acrosomal membrane which together with the original posterior head plasma membrane form the new head cell membrane of the acrosome-reacted sperm.

The acrosomal contents are a rich source of enzymes, including hyaluronidase and the protease, acrosin, that may function in penetration of the sperm through the zona. The hybrid vesicles released during the acrosome reaction could also carry such enzymes on their surfaces. A combination of motility, proteases and glycosidases are likely involved in penetrating the zona (Yanagimachi, 1994; Ohmura et al, 1999; Zhu et al, 2001; Primakoff, 2002).

THE FATE OF THE FERTILISING SPERMATOZOON

During or after the acrosome reaction, the fertilising sperm detaches from the zona cutting a penetration slit that is just as wide as the sperm head. Regardless of how many sperm that manage to penetrate the zona successfully only one will fertilise and activate the oocyte. As the first sperm enters the narrow perivitelline space it binds to the oocyte's plasma membrane by the lateral face of the head, with the firm point of attachment between the sperm and egg plasma membranes occurring at the equatorial segment. Sperm surface protein candidates include sperm members of the



ADAM family (Primakoff et al, 2000). Egg surface proteins are likely to include an active site in CD9 that associates with and regulates the egg fusion machinery (Zhu et al, 2002).

Sperm-egg fusion stimulates the first signalling pathway in development.

FERTILISATION

When the cell membranes of the sperm and oocyte merge, the sperm enters the ooplasm of the oocyte, triggering oocyte activation, a series of changes in metabolic activity including a rise in metabolic rate. The cell membrane of the oocyte undergoes immediate electrical changes that block the entry of other sperm and enzymes produced by the fertilized ovum alter receptor sites so that sperm already bound are detached and others are prevented from binding.

Fertilisation stimulates the ovum to complete its second meiotic division and form a diploid zygote, producing a large mature ovum. After oocyte activation and meiosis the nuclear material remaining within the ovum reorganises as the female pronucleus. The successful spermatozoon enters the cytoplasm, its tail is shed and the nucleus in the head develops into a structure called the male pronucleus which in turn migrates to the centre of the cell and fuses with the female pronucleus. Within 12 hours genetic fusion has completed with the formation of a diploid zygote, a fertilised egg, which will in turn undergo cleavage and produce billions of specialised cells.

THE FATE OF NON-FERTILISING SPERM

After fertilisation, any sperm remaining in the female reproductive tract may be phagocytosed by isthmic epithelial cells (Chakraborty and Nelson, 1975; Rasweiler, 1987) or may be eliminated into the peritoneal cavity (Mortimer and Templeton, 1982) where they in turn may be phagocytosed. There have been cases where sperm have not been rendered non-functional as is evidenced by the numerous case reports of human tubal pregnancies that arose in spite of lack of access of sperm from the uterus into the oviduct on the side of ovulation (Metz and Mastroianni, 1979; Brown et al, 1987; Ansari and Miller, 1994). In these cases the only route available to the sperm was through the peritoneal cavity.

Reviewer:

The authors say nothing about polyspermia – the excessive secretion of semen – which I think is an interesting phenomenon worth mentioning.

MALE INFERTILITY

Failure or severe difficulty in conceiving a child is a surprisingly common worldwide problem. The incidence of 'sub-fertility' seems to be increasing, with defects in sperm (1 in 15 men) being the single most common cause. On the basis of semen assessment up to one-fifth of 18-year-old boys can be classed as sub-fertile. (Hull et al, 1985; Sharpe and Irvine, 2004; Anderson, 2005).

SPERM QUALITY – DECLINING MALE FERTILITY

A large body of data suggests that sperm counts have been declining in Europe and the US though interpretation of these trends remains controversial and the role of the environment uncertain.

Reviewer:

Despite stressing the importance of sperm counts to male fertility, there is no mention of the two key factors that determine a man's sperm count: 1) the number of Sertoli cells per testis (varies more than 100-fold between individuals, though it is unknown why), which is determined largely by events in fetal and early postnatal life; 2) the frequency of ejaculation.

A meta-analysis of 61 studies of sperm density revealed a decline in sperm concentrations of healthy men from 113 million/ml in 1938 to 66 million/ml in 1990 (Carlsen et al, 1992). The analysis was repeated and the main conclusion remained the same: in the 1950s sperm concentrations were higher than in the 1970s (Olsen et al, 1995; Swan et al, 1997). The meta-analysis was extended to include studies until 1996 and it confirmed the declining trend in the US and Europe (Swan et al, 2000).

A large study of Denmark, Finland, France and Scotland showed that Finnish men were found to have higher sperm counts than the others (Jorgensen et al, 2001). A common feature in the decline of sperm concentrations is its association with year of birth: younger cohorts have lower sperm concentrations than the older generations (Auger et al, 1995; Irvine et al, 1996; Licht, 1998; Thierfelder et al, 1999). The lowest sperm counts were found in 18–19 year old Danish men in 1997–8 (Anderson et al, 2000). The median sperm concentration was 41 million/ml and 18% had concentrations below 20 million/ml which is considered a threshold for sub-fertility by the World Health Organisation.

The reasons for declining semen quality is largely unknown though there is good evidence that environmental agents can alter semen quality (Swan, 2003).

A study of maternal smoking in relation to semen quality reported lower sperm density. Inhibin-B and elevated follicular stimulating hormone associated with high in utero exposure (over 10 cigarettes per day) (Storgaard et al, 2003).

In other studies exposure to one class of ubiquitous chemical/pesticide, the phthalates, was examined in relation to sperm concentration, motility and morphology using urinary metabolites as biomarkers (Duty et al, 2003; Swan, 2006). Higher levels of two of these metabolites were associated with reduced sperm concentration and one with reduced sperm motility. 'Higher levels' were not particularly high (less than half the levels found in a representative US population (CDC, National Report on Human exposure to environmental chemicals, 2001)) suggesting that phthalates may adversely affect male reproductive function even at the low ambient levels measured in the study.

Taken together these two studies provide good (though not conclusive) evidence that environmental agents, even at low levels, can alter semen quality.

Several other plausible agents are being investigated for their ability to alter male reproduction (organochlorine pesticides, bisphenol-A, triazines, etc) and the number is likely to increase (Swan, 2003). Because these exposures are fairly ubiquitous, it is probable that subjects in both the above studies have been exposed to low levels of multiple agents. Teasing out the separate and combined effects of this 'chemical soup' must be met before concluding that any specific exposure has altered semen quality let alone caused a worldwide decline in semen quality (Swan, 2003).

FEMALE FERTILITY – OOCYTE QUALITY

Female sub-fertility is to a large part dependent on the 'maternal age effect', characterized by a negative relationship between maternal age and reproductive efficiency, and remains a poorly understood phenomenon. Current data suggest that oocyte physiology determines this relationship and one theory proposes that free radical attack on the mitochondria of primordial oocytes residing in the ovary and subsequent deficiency in oxidative phosphorylation may be the primary cause of oocyte degradation (Wilding et al, 2005).

Reviewer:

This section is poor. The main reason women's fertility decreases with age is errors in meiosis, which lead to non-disjunction of the chromosomes and therefore chromosomally unbalanced embryos.

CONCLUSION

The union between the sperm and oocyte is arguably the most important interaction in biology and possibly the least well understood. As more and more is discovered about the processes and mechanisms involved in achieving in vivo and in vitro fertilisation the better placed scientists will be to make important progress in the development of improvements in assisted conception technologies; contraceptives and human oocyte storage enabling women to delay successful pregnancy to later in life.

REFERENCES

- Ahmadi A and Ng S-C (1997) Fertilization and development of mouse eggs injected with membrane-damaged spermatozoa. *Hum Reprod* 12, 2797-2801
- Amaral E, Perdigao A, Souza MH, Mauck C, Waller D, Zaneveld L and Faundes A (2004) Postcoital testing after the use of a bio-adhesive acid buffering gel (ACIDFORM) and a 2% nonoxynol-9 product. *Contraception* 70, 492-497.
- Andersen AG et al (2000) High frequency of sub-optimal semen quality in an unselected population of young men. *Human Reprod*, 15, 366-372
- Anderson AN et al (2005) High frequency of sub-optimal semen quality in an unselected population of young men. *Hum. Reprod.* 15, 366-372
- Anderson MJ, Chapman SJ, Videan EN, Evans E, Fritz J, Stoinski T, Dixon AF and Gagneux P (2007) Functional evidence for differences in sperm competition in humans and chimpanzees. *A J Phys Anthropol* 134, 274-280
- Anderson MJ and Dixon AF (2002) Sperm competition: motility and the midpiece in primates. *Nature* 416: 496
- Ansari AH and Miller ES (1994) Sperm transmigration as a cause of ectopic pregnancy. *Arch Androl* 32,1-4.
- Auger J et al (1995) Decline in semen quality among fertile men in Paris during the past 20 years. *New England Journal Med*, 332, 281-285
- Babcock DF (2003) Development: smelling the roses? *Science* 299,1993-1994.
- Bahat A, Tur-Kaspa I, Gakamsky A, Giojalas LC, Breitbart H and Eisenbach M (2003) Thermotaxis of mammalian sperm cells: a potential navigation mechanism in the female genital tract. *Nat Med* 9,149-150.
- Baillie HS, Pacey AA, Warren MA, Scudamore IW and Barratt CL (1997) Greater numbers of human spermatozoa associate with endosalpingeal cells derived from the isthmus compared with those from the ampulla. *Hum Reprod* 12,1985-1992.
- Baker RR and Bellis MA (1988) 'Kamikaze' sperm in mammals?'. *Animal Behaviour* 36, 3, 936-939
- Baker RR and Bellis MA (1989) Elaboration of the kamikaze sperm hypothesis: a reply to Harcourt. *Animal Behaviour* 37, 5, 865-867
- Baker RR and Bellis MA (1993) Human sperm competition: ejaculate adjustment by females and a function for the female orgasm. *Anim Behav* 6, 887-909
- Barash and Lipton (2001) The myth of monogamy, published by Barratt CLR and Pockley AG (1998) Sperm survival in the female reproductive tract: presence of immunosuppression or absence of recognition? in Debate: new perspectives on immunorecognition of gametes and embryos. *Mol Hum Reprod* 4, 4, 309-317
- Barros C, Vigil P, Herrera E, Arguello B and Walker R (1984) Selection of morphologically abnormal sperm by human cervical mucus. *Arch Adrol* 12 (Suppl.), 95-107
- Beatty RA, Stewart DL, Spooner RL and Hancock JL (1976) Evaluation by the heterospermic insemination technique of the differential effect of freezing at -196°C on the fertility of individual bull semen. *J Reprod Fertil* 47, 377-379
- Bedford JM (1974) Biology of primate spermatozoa. *Contrib Primatol*, 3, 97-139
- Bellis MA, Hughes K, Hughes S and Ashton JR (2005) Measuring paternal discrepancy and its public health consequences. *J Epidemiol Community Health* 59, 749-754
- Berger T (1995) Proportion of males with lower fertility spermatozoa estimated from heterospermic insemination. *Theriogenology* 43, 769-775
- Bianchi PG, De Agostini A, Fournier J, Guidetti C, Tarozzi N, Bizzaro D, Manicardi GC. Human cervical mucus can act in vitro as a selective barrier against spermatozoa carrying fragmented DNA and chromatin structural abnormalities. *J Assist Reprod Genet.* 21, 4, 97-102.
- Bigelow JL, Dunson DB, Stanford JB, Ecochard R, Gnath C and Colombo B (2004) Mucus observations in the fertile window: a better predictor of conception than timing of intercourse. *Hum Reprod* 19, 889-892
- Bouvet JP, Gresenguet G and Belec L (1997) Vaginal pH neutralization by semen as a cofactor of HIV transmission. *Clin Microbiol Infect* 3, 19-23.
- Brown C, La Vigne WE and Padilla SL (1987) Unruptured pregnancy in a heterotopic Fallopian tube: evidence for transperitoneal sperm migration. *Am J Obstet Gynecol* 156,88-90.
- Brussow K-P, Ratky J, Schneider F, Torner H, Kanitz W and Solti L (1998) Does follicular fluid have any importance in the transfer of porcine oocytes into the oviduct? *Theriogenology* 49, 340
- Burgoyne PS (1975) sperm phenotype and its relationship to somatic and germ line genotype – study using mouse aggregation chimeras. *Dev Biol* 44, 63-76
- Carlsen E, Giwercman A, Keiding N, Shakkebaek NE (1992) Evidence for decreasing quality of semen during past 50 years. *BMJ*, 305, 609-613
- Casslen B (1986) Uterine fluid volume: cyclic variations and possible extra uterine contributions. *J Reprod Med* 31, 506-510.
- Chakraborty J and Nelson L (1975) Fate of surplus sperm in the Fallopian tube of the white mouse. *Biol Reprod* 12, 455-463.
- Chian R-C, LaPointe S and Sirard M-A (1995) Capacitation in vitro of bovine spermatozoa by oDev 42, 318-324.
- Cho C, Bunch DO, Faure JE, Goulding EH, Eddy EM, Primakoff P and Myles DG (1998) Fertilization defects in sperm from mice lacking fertilin beta. *Science* 281,1857-1859.
- Cohen, J (1990) The function of human semen coagulation and liquefaction in vivo. In Mashiach S, Ben-Rafael Z, Laufer N and Schenker JG (eds) *Advances in in vitro fertilization and assisted reproduction technologies*. Plenum Press, New York, USA, 443-452
- Cohen, J (1998) Spermatozoa and antibodies in Debate: new perspectives on immunorecognition of gametes and embryos. *Mol Hum Reprod* 4, 4, 309-317
- Cohen J and McNaughton D (1974) Spermatozoa: the probable selection of a small population by the genital tract of the female rabbit. *J Reprod Fert*, 39, 297-310
- Cohen-Dayag A, Ralt D, Tur-Kaspa I, Manor M, Makler A, Dor J, Mashiach S and Eisenbach M (1994) Sequential acquisition of chemotactic responsiveness by human spermatozoa. *Biol Reprod* 50,786-790.
- Cohen-Dayag A, Tur-Kaspa I, Dor J et al (1995) Sperm capacitation in humans is transient and correlates with chemotactic responsiveness to follicular factors. *Proc Natl Acad Sci USA* 92, 11039-11043
- Cohen-Dayag A, Tur-Kaspa I, Dor J, Mashiach S and Eisenbach M (1995) Sperm capacitation in humans is transient and correlates with chemotactic responsiveness to follicular factors. *Proc Natl Acad Sci USA* 92, 11039-11043.
- Connor SJ, Lefievre L, Hughes DC and Barratt CLR (2005) Cracking the egg: increased complexity in the zona pellucida. *Hum Reprod*, 20, 5, 1148-1152
- D'Cruz OJ, Wang B-L and Haas GG Jr (1992) Phagocytosis of immunoglobulin G and C3-bound human sperm by human polymorphonuclear leucocytes is not associated with the release of oxidative radicals. *Biol Reprod* 46, 721-732
- De Jonge C (2005) Biological basis for human capacitation. *Hum Reprod Update* 11,205-214.
- DeMott RP and Suarez SS (1992) Hyperactivated sperm progress in the mouse oviduct. *Biol Reprod* 46,779-785.
- de Ziegler D, Bulletti C, Fanchin R, Epiney M and Brioschi PA (2001) Contractility of the nonpregnant uterus: the follicular phase. *Ann N Y Acad Sci* 943,172-184.
- Dixon AF (1998) *Primate sexuality*. Oxford University Press.
- Dostal J, Veselsky L, Marounek M, Zelezna B and Jonakova V (1997) Inhibition of bacterial and boar epididymal sperm immunogenicity by boar seminal immunosuppressive component in mice. *J Reprod Fertil* 111, 135-141
- Duty SM et al (2003) Phthalate exposure and human semen parameters. *Epidemiol*, 14, 269-277
- Dziuk PJ (1996) Factors that influence the proportion of offspring sired by a male following heterospermic insemination. *Anim Reprod Sci* 43, 65-88.
- Eisenbach M (1999) Mammalian sperm chemotaxis and its association with capacitation. *Dev Genet* 25,87-94.
- Fabro G, Rovasio RA, Civalero S, Frenkel A, Caplan SR, Eisenbach M and Giojalas LC (2002) Chemotaxis of capacitated rabbit spermatozoa to follicular fluid revealed by a novel directionality-based assay. *Biol Reprod* 67,1565-1571.
- Fawcett DF and Raviola E (1994) Bloom and Fawcett, *A Textbook of Histology*, 12th edn. Chapman & Hall, New York, 946 pp.
- Fox CA, Meldrum SJ and Watson BW (1973) Continuous measurement by radio-telemetry of vaginal pH during human coitus. *J Reprod Fertil* 33, 69-75
- Fukuda M and Fukuda K (1994) Uterine endometrial cavity movement and cervical mucus. *Hum Reprod* 9,1013-1016.
- Gaddum-Rosse P (1981) Some observations on sperm transport through the uterotubal junction of the rat. *Am J Anat* 160,333-341.
- Gould JE, Overstreet JW and Hanson FW (1984) Assessment of human sperm function after recovery from the female reproductive tract. *Biol Reprod* 31,888-894.
- Hagaman JR, Moyer JS, Bachman ES, Sibony M, Magyar PL, Welch JE, Smithies O, Krege JH and O'Brien DA (1998) Angiotensin-converting enzyme and male fertility. *Proc Natl Acad Sci USA* 95,2552-2557.
- Haidl G and Opper C (1997) Changes in lipids and membrane anisotropy in human spermatozoa during epididymal maturation. *Hum Reprod* 12, 2720-2723
- Hafez ESE and Black DL (1969) The mammalian uterotubal junction. In Hafez ESE and Blandau RJ (eds), *The Mammalian Oviduct: Comparative Biology and Methodology*. The University of Chicago Press, Chicago, IL, pp. 85-128.
- Hanson FW and Overstreet JW (1981) The interaction of human spermatozoa with cervical mucus in vivo. *Am J Obstet Gynecol* 140, 173-178
- Harcourt AH, Harvey PH, Larson SG and Short RV (1981) Testis weight, body weight and breeding system in primates. *Nature*, 293, 55-57
- Harper MJK (1973) Relationship between sperm transport and penetration of eggs in the rabbit oviduct. *Biol Reprod* 8,441-450.
- Harper MJK (1982) Sperm and egg transport. In Austin CR and Short RV (eds) *Germ Cells and Fertilization*. Cambridge University Press, Cambridge, UK, 1, 102-127

- Harper MJK (1994) Gamete and zygote transport. In Nobil I and Neill JD (eds), *The Physiology of Reproduction*, 2nd edn. Raven Press Ltd., New York, NY, pp. 123–187.
- Ho HC and Suarez SS (2001) Hyperactivation of mammalian spermatozoa: function and regulation. *Reproduction* 122,519–526.
- Hong SJ, Chiu PC, Lee KF, Tse JM, Ho PC, Yeung WS (2004) Establishment of a capillary-cumulus model to study the selection of sperm for fertilization by the cumulus oophorus. *Hum Reprod*. 19, 7, 1562–9.
- Hull MG et al (1985) Population study of causes, treatment and outcome of infertility. *BMJ* 291, 1693–1697
- Hunter RHF, Peterson HH and Greve T (1999) Ovarian follicular fluid, progesterone and Ca²⁺ ion influences on sperm release from the Fallopian tube reservoir. *Mol Reprod Dev* 54, 283–291
- Huszar G, Ozenci CC, Cayli S, Zavacki Z, Hansch E and Vigue L (2003) Hyaluronic acid binding by human sperm indicates cellular maturity, viability and unreacted acrosomal status. *Fertil Steril* 79 (Suppl 3), 1616–1624.
- Ikawa M, Wada I, Kominami K, Watanabe D, Toshimori K, Nishimune Y and Okabe M (1997) The putative chaperone calyculin is required for sperm fertility. *Nature* 387,607–611.
- Irvine S et al (1996) Evidence of deteriorating semen quality in the United Kingdom: birth cohort study in 577 men in Scotland over 11 years. *BMJ*, 312, 467–471
- Jansen RPS (1980) Cyclic changes on the human Fallopian tubes isthmus and their functional importance. *Am J Obstet Gynecol* 136,292–308.
- Jensen TS, Bjorge L, Wollen A-L and Ulstein M (1995) Identification of the complement regulatory proteins CD46, CD55 and CD59 in human Fallopian tube, endometrium and cervical mucosa and secretion. *Am J Reprod Immunol* 34, 1–9
- Jorgensen N et al (2001) Regional differences in semen quality in Europe. *Human Reprod*, 16, 1012–1019
- Katz DF, Morales P, Samuels SJ and Overstreet JW (1990) Mechanisms of filtration of morphologically abnormal human sperm by cervical mucus. *Fertil Steril* 54, 513–516
- Katz DF, Slade DA and Nakajima ST (1997) Analysis of preovulatory changes in cervical mucus hydration and sperm penetrability. *Adv Contracep* 13, 143–151
- Kervancioglu ME, Djahanbakhch O and Aitken RJ (1994) Epithelial cell coculture and the induction of sperm capacitation. *Fertil Steril* 61,1103–1108.
- Krege JH, John SW, Langenbach LL, Hodgins JB, Hagaman JR, Bachman ES, Jennette JC, O'Brien DA and Smithies O (1995) Male-female differences in fertility and blood pressure in ACE-deficient mice. *Nature* 375,146–148.
- Kruger TF, Menkveld R, Stander FSH, Lombard CJ, Van Der Merwe JP, Van Zyl JA, Smith K (1986) Sperm morphologic features as a prognostic factor in in vitro fertilization. *Fertil Steril*, 46, 1118–1123.
- Kunz G, Beil D, Deininger H, Wildt L and Leyendecker G (1996) The dynamics of rapid sperm transport through the female genital tract: evidence from vaginal sonography of uterine peristalsis and hysterosalpingoscintigraphy. *Hum Reprod* 11, 627–632
- Kura T and Nakashima Y (2000) Conditions for the evolution of solid sperm classes. *Evolution*, 54, 1, 72–80
- Holt WW (2005) Is quality assurance in semen analysis still really necessary? A spermatologist's viewpoint. *Human Reproduction*, 20, 2, 2983–2986
- Holt WW and Van Look KJW (2004) Concepts in sperm heterogeneity, sperm selection and sperm competition as biological foundations for laboratory tests of semen quality. *Reproduction*, 127, 527–535
- Lefebvre R and Suarez SS (1996) Effect of capacitation on bull sperm binding to homologous oviductal epithelium. *Biol Reprod* 54,575–582.
- Licht M (1998) Retrospektive Untersuchung der zwischen 1956 und 1995 in der Abteilung für Andrologie des Universitätskrankenhauses Hamburg-Eppendorf erhobenen Spermogramme. Dissertation, Hamburg: Fachbereich Medizin, Universität Hamburg.
- Lin Y, Mahan K, Lathrop WF, Myles DG and Primakoff P (1994) A hyaluronidase activity of the sperm plasma membrane protein PH-20 enables sperm to penetrate the cumulus cell layer surrounding the egg. *J Cell Biol* 125, 1157–1163
- Lyons EA, Taylor PJ, Zheng XH, Ballard G, Levi CS and Kredentser JV (1991) Characterization of subendometrial myometrial contractions throughout the menstrual cycle in normal fertile women. *Fertil Steril* 55,771–774
- Mahmoud AI and Parrish JJ (1996) Oviduct fluid and heparin induce similar surface changes in bovine sperm during capacitation. *Mol Reprod Dev* 43,554–560.
- Matthur S, Rosenlund C, Carloton M, Caldwell J, Barber M, Rust PF and Williamson HO (1988) Studies on sperm survival and motility in the presence of cytotoxic sperm antibodies. *Am J Reprod Immunol Microbiol* 17, 41–47
- Meizel S (2004) The sperm, a neuron with a tail: 'neuronal' receptors in mammalian sperm. *Biol Rev Camb Phil Soc* 79, 713–732
- Metz KG and Mastroianni L Jr (1979) Tubal pregnancy subsequent to transperitoneal migration of spermatozoa. *Obstet Gynecol Surv* 34, 554–560.
- Morales P, Roco M and Vigil P (1993) Human cervical mucus: relationship between biochemical characteristics and ability to allow migration of spermatozoa. *Hum Reprod* 8, 78–83
- Mortimer D and Menkveld R (2001) Sperm morphology assessment – historical perspectives and current opinions. *J Androl*, 22, 2, 192–205
- Mortimer ST and Swan MA (1995) Variable kinematics of capacitating human spermatozoa. *Hum Reprod* 10,3178–3182
- Mortimer D and Templeton AA (1982) Sperm transport in the human female reproductive tract in relation to semen analysis characteristics and time of ovulation. *J Reprod Fertil* 64,401–408.
- Moyer DL, Rimdusit S and Mishell DR Jr (1970) Sperm distribution and degradation in the human female reproductive tract. *Obstet Gynecol* 35, 831–840.
- Nakanishi T, Isotani A, Yamaguchi R, Ikawa M, Baba T, Suarez SS and Okabe M (2004) Selective passage through the uterotubal junction of sperm from a mixed population produced by chimeras of calyculin-knockout and wild-type male mice. *Biol Reprod* 71,959–965.
- Ohmura et al (1999) *J Biol Chem* 274 29426
- Oliveira RG, Romasi L, Rovasio RA and Giojalas LC (1999) Increased velocity and induction of chemotactic response in mouse spermatozoa by follicular and oviductal fluids. *J Reprod Fertil* 115, 23–27
- Olsen GW, Rose CE, Bodner KM, Lipshultz LI, Ramlow JM (1995)
- Overstreet JW, Coats C, Katz DF and Hanson FW (1980) The importance of seminal plasma for sperm penetration of cervical mucus. *Fertil Steril* 34, 569–572
- Overstreet JW and Cooper GW (1978) Sperm transport in the reproductive tract of the female rabbit. I. The rapid transit phase of transport. *Biol Reprod* 19,101–114.
- Overstreet GW and Katz DF (1977) Sperm transport and selection in the female tract. In *Development in Mammals*, 2, 31–66. Ed Johnson MH. Elsevier/North-Holland, Amsterdam
- Pacey AA, Davies N, Warren MA, Barratt CL and Cooke ID (1995a) Hyperactivation may assist human spermatozoa to detach from intimate association with the endosalpinx. *Hum Reprod* 10,2603–2609.
- Pacey AA, Hill CJ, Scudamore IW, Warren MA, Barratt CLR and Cooke ID (1995b) The interaction in vitro of human spermatozoa with epithelial cells from the human uterine (Fallopian) tube. *Hum Reprod* 10,360–366.
- Pandya I and Cohen J (1985) The leucocytic reaction of hum-421
- Parker GA (1970) Sperm competition and its evolutionary consequences in the insects. *Biolog Rev*, 45, 525–567
- Parker GA (1998) Sperm competition and the evolution of ejaculates: towards a theory base. In *sperm competition and sexual selection* (p3-54) (Birkhead, TR and Moller AD (eds))
- Penfold, LM, Holt, C, Holt, WV, Welch, GR, Cran, DG and Johnson, LA 1998 Comparative motility of X and Y chromosome bearing sperm separated on the basis of DNA content by flow cytometry. *Mol. Reprod. Dev.* 50, 323–327.
- Polge C, Salamon S and Wilmut I (1970) Fertilising capacity of frozen boar semen following surgical insemination. *Vet Rec* 87,424–428.
- Pound N, Shackelford TK and Goetz AT (2006) Sperm competition in humans from Sperm competition in humans: classic and contemporary readings, published by Springer Science and Business Media, Inc
- Primakoff et al (2000) *Trends Genet.* 16, 83
- Quill TA, Sugden SA, Rossi KL, Doolittle LK, Hammer RE and Garbers DL (2003) Hyperactivated sperm motility driven by CatSper2 is required for fertilization. *Proc Natl Acad Sci USA* 100,14869–14874.
- Ralt D, Goldenberg M, Fetterolf P, Thompson D, Dor J, Mashiachi S, Garbers DL and Eisenbach M (1991) Sperm attraction to a follicular fluid factor(s) correlates with human egg fertilizability. *Proc Natl Acad Sci USA* 88,2840–2844.
- Ralt D, Manor M, Cohen-Dayag A, Tur-Kaspa I, Ben-Shlomo I, Makler A, Yuli I, Dor J, Blumberg S, Mashiach S et al. (1994) Chemotaxis and chemokinesis of human spermatozoa to follicular factors. *Biol Reprod* 50,774–785.
- Rasweiler JJ (1987) Prolonged receptivity to the male and the fate of spermatozoa in the female black mastiff bat, *Molossus ater*. *J Reprod Fertil* 79,643–654.
- Reeve L, Lashen H and Pacey AA (2005) Endometriosis affects sperm-endosalpigeal interactions. *Hum Reprod* 20,448–451.
- Reeve L, Ledger WL and Pacey AA (2003) Does the Arg-Gly-Asp (RGD) adhesion sequence play a role in mediating sperm interaction with the human endosalpinx? *Hum Reprod* 18,1461–1468.
- Roberts, SJ (1986) *Veterinary Obstetrics and Genital Diseases*, 3rd edn. Stephen Roberts, Woodstock, VT
- Robl JM and Dziuk PJ (1988) Comparison of heterospermic and homospermic inseminations as measures of male fertility. *J Exp Zool* 245, 97–101

- Rodriguez-Martinez H, Nicander L, Viring S, Einarsson S and Larsson K (1990) Ultrastructure of the uterotubal junction in preovulatory pigs. *Anat Histol Embryol* 19,16–36.
- Rubenstein BB, Strauss H, Lazarus ML and Hankin H (1951) Sperm survival in women. *Fertil Steril* 2,15–19.
- Russell LD, Russell JA, Macgregor GR and Meistrich ML (1991) Linkage of manchette microtubules to the nuclear-envelope and observations of the role of the manchette in nuclear shaping during spermatogenesis in rodents. *Am J Anat* 192, 97–120
- Ryu H-M, Lin WW, Lamb DJ, Chuang W, Lipshultz LI and Bischoff FZ (2001) Increased chromosome X, Y and 18 nondisjunction in sperm from infertile patients that were identified as normal by strict morphology: implication for intracytoplasmic sperm injection. *Fert Steril* 76, 879–883
- Seuanez, H (1981) Morphology and DNA quantitation of human and great ape spermatozoa. *A J Primatol*, 1, 2, 193–202
- Shalgi R, Smith TT and Yanagimachi R (1992) A quantitative comparison of the passage of capacitated and uncapacitated hamster spermatozoa through the uterotubal junction. *Biol Reprod* 46,419–424.
- Sharpe RM and Irvine, DS (2004) How strong is the evidence of a link between environmental chemicals and adverse effects on human reproductive health? *BMJ* 328, 447-451
- Smallcombe A and Tyler KR (1980) Semen-elicited accumulation of antibodies and leucocytes in the rabbit female tract. *Experientia* 36, 88–89
- Sobrero aJ and MacLeod J (1962) The immediate postcoital test. *Fertil Steril* 13, 184–189
- Spehr M, Gisselmann G, Poplawski A, Riffell JA, Wetzel CH, Zimmer RK and Hatt H (2003) Identification of a testicular odorant receptor mediating human sperm chemotaxis. *Science* 299,2054–2058.
- Spehr M, Schwane K, Riffell JA, Barbour J, Zimmer RK, Neuhaus EM and Hatt H (2004) Particulate adenylate cyclase plays a key role in human sperm olfactory receptor-mediated chemotaxis. *J Biol Chem* 279,40194–40203.
- Stahlberg R, Harlizius B, Weitze KF and Waberski D (2000) Identification of embryo paternity using polymorphic DNA markers to assess fertilising capacity of spermatozoa after heterospermic insemination in boars. *Theriogenology* 53, 1365–1373
- Stewart DL, Spooner RL, Bennett GH, Beatty RA and Hancock JL (1974) A second experiment with heterospermic insemination in cattle. *J Reprod Fertil* 36, 107–116
- Storgaard L et al (2003) Does smoking during pregnancy affect sons' sperm counts? *Epidemiol*, 14, 278–286
- Suarez SS and Dai XB (1992) Hyperactivation enhances mouse sperm capacity for penetrating viscoelastic media. *Biol Reprod* 46,686–691.
- Suarez SS, Katz DF, Owen DH, Andrew JB and Powell RL (1991b) Evidence for the function of hyperactivated motility in mammalian sperm. *Biol Reprod* 44,375–381.
- Suarez SS and Oliphant G (1982) The interaction of rabbit spermatozoa and serum complement proteins. *Biol Reprod* 27, 473–483
- Suarez SS and Osman RA (1987) Initiation of hyperactivated flagellar bending in mouse sperm within the female reproductive tract. *Biol Reprod* 36, 1191–1198.
- Suarez SS and Pacey AA (2006) Sperm transport in the female reproductive tract. *Hum. Reprod* 12, 23–37.
- Sun F, Bahat A, Gakamsky A, Girsh E, Katz N, Giojalas LC, Tur-Kaspa I and Eisenbach M (2005) Human sperm chemotaxis: both the oocyte and its surrounding cumulus cells secrete sperm chemoattractants. *Hum Reprod*, 20, 3, 761–767
- Swan (2003) Do environmental agents affect semen quality? *Epidemiol* 14, 3
- Swan SH, Elkin EP, Fenster L (1997) Have sperm densities declined: a reanalysis of global trend data. *Environ Health Perspect*, 105, 1228–1232
- Swan SH, Elkin EP, Fenster L (2000) The question of declining sperm density revisited: an analysis of 101 studies published 1934–1996. *Environ Health Perspect*, 108, 961–966
- Swan, SH (2006) Semen quality in fertile US men in relation to geographical area and pesticide exposure. *Int J Androl*, 29, 1, 62–8
- Taylor NJ (1982) Investigation of sperm-induced cervical leukocytosis by a double mating study in rabbits. *J Reprod Fertil* 66, 157–160
- Thierfelder W et al (1999) Abnahme der Spermaqualität bei gesunden Männern aus ungewollt kinderlosen Partnerschaften. *Bundesgesundheitsblatt-Gesundheitsforschung-Gesundheitsschutz*, 6, 471–478
- Thompson LA, Barratt CLR, Bolton AE and Cooke ID (1992) The leukocytic reaction of the human uterine cervix. *Am J Reprod Immunol* 28,85–89.
- Tyler KR (1977) Histological changes in the cervix of the rabbit after coitus *J Reprod Fertil* 49, 341-345
- Wilcox, AJ, Weinberg CR and Baird DD (1995) Timing of sexual intercourse in relation to ovulation. Effects on the probability of conception, survival of pregnancy, and sex of the baby. *N Engl J Med* 333, 1517-1521.
- Wilding M, Di Matteo L, Dale B. (2005) The maternal age effect: a hypothesis based on oxidative phosphorylation. *Zygote* Nov;13(4):317–23.
- Williams M, Hill CJ, Scudamore I, Dunphy B, Cooke ID and Barratt CLR (1993) Sperm numbers and distribution within the human Fallopian tube around ovulation. *Hum Reprod* 8, 2019–2026
- Yamagata K, Nakanishi T, Ikawa M, Yamaguchi R, Moss SB and Okabe M (2002) Sperm from the calmeglin-deficient mouse have normal abilities for binding and fusion to the egg plasma membrane. *Dev Biol* 250, 348–357.
- Yanagimachi R and Chang MC (1963) Sperm ascent through the oviduct of the hamster and rabbit in relation to the time of ovulation. *J Reprod Fertil* 6,413–420.
- Yanagimachi R (1994) In Knobil E and Neill JD (eds) *The Physiology of Reproduction*, 3rd Ed. Raven Pres, Ltd, New York, 189–317
- Yudin AI, Hanson FW and Katz DF (1989) Human cervical mucus and its interaction with sperm: a fine-structural view. *Biol Reprod* 40, 661-671
- Zhu J, Barratt CL, Lippes J, Pacey AA and Cooke ID (1994) The sequential effects of human cervical mucus, oviductal fluid and follicular fluid on sperm function. *Fertil Steril* 61, 1129–1135
- Zhu GZ et al (2002) *Development* 129, 1995