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Biofluid mechanics of the human reproductive process: modelling of the complex interaction and pathway to the oocytes

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Summary

Recent revelations in the human reproductive process have fuelled much interest in this field of study. In particular, the once prevailing view of large numbers of ejaculated sperms racing towards the egg has been refuted recently. This is opposed to the current views derived from numerous clinical findings that state that only a very small number of sperms will ever enter the oviduct. It is believed that these few sperms must have been guided to make the long, tedious and obstructed journey to the egg. For a mature spermatozoon, its hyperactivated swimming motility upon capacitation plays an important role in the fertilization of a mature egg. Likewise, the female genital tract also provides guiding mechanisms to complement the survival of normal hydrodynamic profile sperms and thus promotes an eventual sperm–egg interaction. Understanding these mechanisms can be essential for the derivation of assisted conception techniques especially those *in vitro*. With the aid of computational models and simulation, suitability and effectiveness of novel assisted conception methodology can be assessed, particularly for those yet to be ready for clinical trials. This review discusses the possible bioengineering models and the mechanisms by which human spermatozoa are guided to the egg.

Keywords: Capacitation, Chemotaxis, Female genital tract, Muscular contractions, Sperm motility, Thermotaxis

Introduction

In mammals, successful conception can only occur when an ejaculated mature spermatozoon reaches a mature egg at the site of fertilization during ovulation when the egg travels from the ovary into the fallopian tube. Previously, it was a common myth that spermatozoa compete with each other towards the egg to fertilize it once they are ejaculated into the female genital tract. However, Dale & Monroy (1981) were among the first few to criticise the concept that eggs are exposed to large numbers of spermatozoa in most animal species and stress that sperm–egg ratios are usually very low, even when large numbers of sperm are produced. Moreover, other literature also suggests that there are complex mechanics that guide the sperms to the egg (Williams *et al.*, 1993; Eisenbach & Tur-Kaspa, 1999; Giojalas *et al.*, 2004). In humans, the average number of sperms per ejaculation is between the tens to hundreds of millions range. Looking at the sheer number, the likelihood of a successful sperm–egg encounter should be extremely high. On the contrary, clinical findings have shown that only about one-tenth of the sperm initially deposited in the vagina will ever reach the cervix. For those sperms that have successfully penetrated the cervical mucus, about one-tenth will reach the uterus, and about one-tenth of those, which have made it through the uterus, can enter either of the oviducts. Simply, very few of the ejaculated spermatozoa (about one out of every million spermatozoa in humans) will be successful in entering the fallopian tubes. In addition, it has been further suggested that the number of spermatozoa that can

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fertilize the egg is even smaller (Giojalas *et al.*, 2004; Luconi *et al.*, 2006).

Until recently, the description of the pathway of spermatozoon to the egg has been far from adequate. In order to acquire the ability to fertilize the egg, studies have shown that the spermatozoa must undergo a process of ripening known as capacitation (Brewis *et al.*, 2005; Aitken, 2006). This physiological change is necessary for sperm penetration of the oocyte complex and the sperm fusions with the egg. Only capacitated spermatozoa can penetrate the cumulus layer that surrounds the egg, bind the sperm receptor onto the egg coat, and undergo the acrosome reaction that enables sperm penetration through the egg coat (Bergqvist *et al.*, 2006). However, consideration must be given to the concept that all spermatozoa may not be homogeneous with respect to their fertilizing capacity, and that the egg surface may not be homogeneous with respect to sperm penetration. Simply, there may be 'hot spot' areas that allow sperm penetration while other areas are refractory (Elder & Dale, 2000). The maturating process of the spermatozoa seems correlated with the timing of ovulation in which the capacitated spermatozoa also acquire a different motility pattern termed as hyperactivation (Suarez & Ho, 2003; Luconi *et al.*, 2006). In this process, the regular sinusoidal beating pattern of the flagellum before capacitation is replaced with a high-amplitude and asymmetric bending. This is because, during the journey through the female genital tract, the ejaculated spermatozoa will encounter complex and non-Newtonian fluid environments. The sperm must be able successfully to swim through the cervical mucus, and eventually a successful sperm will penetrate the cumulus layer of the oocyte complex. The passage through these gels undoubtedly involves a combination of enzymatic activity and hydrodynamic forces (Taylor, 1951; Fulford *et al.*, 1998). Studies have suggested that the cervical mucus may block abnormal sperm from entering the uterus and/or may serve to 'preserve' the normal sperm until the optimal time for fertilization. Only a small number of normal sperm will enter the mucus and the rest that remain in the vagina will die due to the acidic pH of the latter. Essentially, those sperm that have entered the mucus can survive there for a longer period after ejaculation. Once in the cervical mucus, these sperm steadily swim upwards into the uterus over a staggered period. Thus, the cervical mucus can be seen as a sperm reservoir to be banked on if ejaculation does not take place at ovulation (Bianchi *et al.*, 2004; Dunson *et al.*, 2005). However, this is a different process in which the spermatozoa become attached to the mucosal epithelium and remain there until they have undergone capacitation at site of fertilization (Suarez & Ho, 2003).

The current literature suggests that further research needs to be instituted fully to understand the complete picture of the human conception process. With the realization that only a low number of spermatozoa can reach the egg and the slim chances that such low numbers successfully fertilize the egg by guidance mechanisms, it has attracted an increasing attention on the study of the human sperm's pathway in the female genital tract. It seems that the guiding mechanisms that allow sperm to reach the fertilization site are fundamentally coupled to fluid mechanics. In this review, the current understandings of this emerging field of study will be discussed. Particularly, this seeks to confer the different mechanics involved in the female genital tract, the importance of these guidance mechanical ingredients to the process, normal human sperm motility and possible aspects of bioengineering models or simulations to assist the understanding of the human reproductive process. Some of the information summarized in this review can only be gathered through the study of model systems from other mammalian species currently available in the literature. However, it is likely most of the mechanisms and pathways identified in these species are similar to those of humans (Turner, 2003).

Human spermatozoa

It is believed that sperm are terminally differentiated cells that are deprived of an active transcription and translation apparatus (Suarez & Pacey, 2006). Thus, they must survive in the female genital tract without the benefit of reparative mechanisms that are available to many other cells. Sperm are subjected not only to physical stresses during ejaculations and contractions of the female genital tract, but also may sustain oxidative damage. As sperm are allogenic to the female, they may encounter the defences of the female immune system meant for infectious organisms (Ohl *et al.*, 1999; Aitken, 2006; Luconi *et al.*, 2006). Ejaculated sperm must somehow use their limited resources to maintain their fertility in the face of these numerous perils. Hence, it is essential to understand the motility and structure of the human spermatozoa before feasible boundaries and assumptions can be made for potential bioengineering models.

Physiology of sperm motility

Like other mammalians, the human spermatozoa display two types of motility; activated motility as seen in freshly ejaculated sperms, and hyperactivated motility as seen in sperm at the site of fertilization as shown in Fig. 1(i) and (ii) respectively (Suarez & Ho, 2003; Luconi *et al.*, 2006). The flagellum of an activated sperm generates a symmetrical, lower-amplitude waveform that drives the sperm in a

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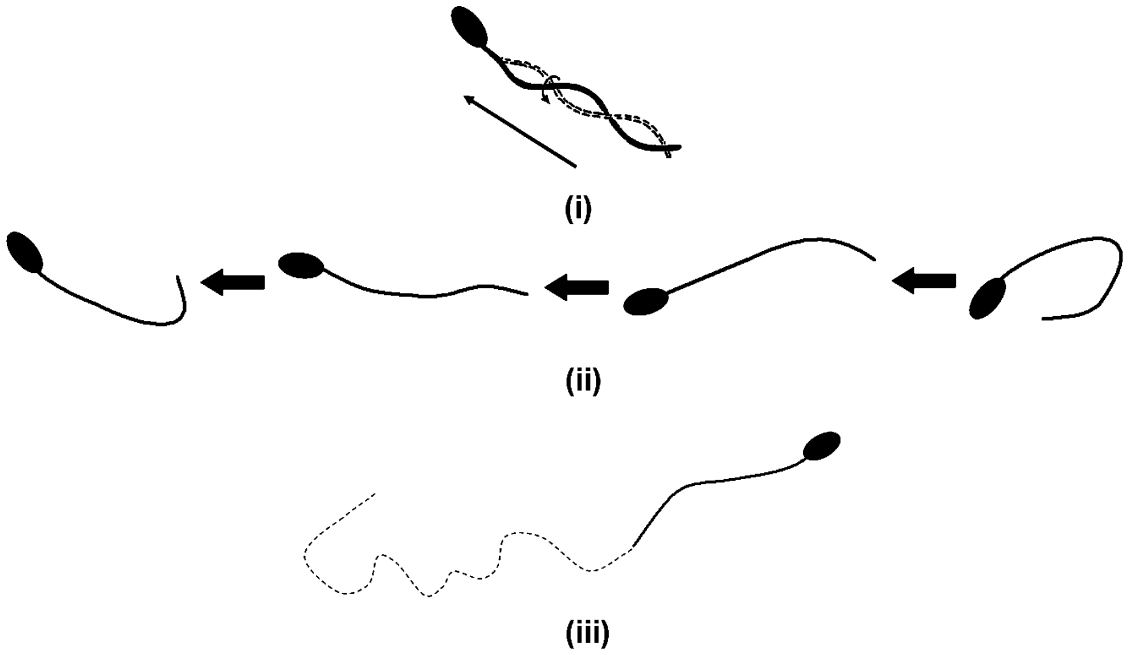

Figure 1 Spermatozoon in three possible states of motility: (i) in activated motility when freshly ejaculated in the female genital tract; (ii) after the capacitation process, the spermatozoon acquires a different motility pattern or becomes hyperactivated; and (iii) simulated spermatozoon changing its direction abruptly due to chemoattractant from the egg–cumulus complex when the regulated swimming beat ceases to exist.

reasonably straight line in relatively non-viscous media, such as seminal plasma or semen extender. Studies suggest that this form of motility aids in the propulsion of the sperm through the female genital tract (Fulford *et al.*, 1998; Suarez & Ho, 2003). It is likely that some immotile sperms do reach the oviduct and this may be due to the muscle contractions of the female genital tract. Nevertheless, most sperms that lack activated motility will fail to reach the uterotubal junction and therefore will be incapable of *in vivo* fertilization (Gaddum-Rosse, 1985). In hyperactivated motility, the pattern of the flagellar beat changes to that of a more asymmetric and higher amplitude when the sperms reach the oviduct. Unlike activated motility, hyperactivated sperm swim with vigorous and whip-like motions that give them the force that is needed to penetrate the protective membrane of the eggs in the more viscous environment of the oviduct as seen in Fig. 1(iii) (Ishijima *et al.*, 2002; Suarez & Ho, 2003; Kirichok *et al.*, 2006). It has been suggested that the flagellar beat of an activated sperm is insufficient to propel itself progressively through the oviduct. The essential physiological role of hyperactivated motility is to help the sperm to detach from the oviducal epithelium during capacitation and move progressively through the oviducal environment to reach the site of fertilization. Furthermore, hyperactivated motility may help the successful sperm penetrate into the egg (Giojalas *et al.*, 2004; Aitken, 2006). Thus, both activated and hyperactivated motilities are important for normal fertility but with each having their respective roles in the sperm's journey in the female genital tract.

Mathematical analysis of sperm motility

Analysis of sperm hydrodynamics has been well studied. This is derived primarily based on low Reynolds number slender body theory that has developed analytic and numerical solutions to the problem of flagellar propulsion in a simple viscous or Newtonian fluid. The mathematical tools that have been developed for this aspect have been biologically applied (Taylor, 1951; Katz & Berger, 1980; Andrietti & Bernardini, 1994; Fulford *et al.*, 1998). With the Reynolds numbers for sperm motion being typically small, inertia can be neglected. For derivation based on a general linear viscoelastic fluid, the governing equations, which are incompressible, are usually adopted:

$$\nabla \cdot v = 0 \tag{1}$$

$$\nabla \cdot \mathrm{T} = 0 \tag{2}$$

Where v is the velocity of the fluid and T is the stress tensor. Moreover, typical consideration of the kinematics of flagellar beat is the point at which the flagellum joins the sperm head (Taylor, 1951; Fulford *et al.*, 1998). Assume that x and y are coordinates of a system where $x = 0$ and $y = 0$ correspond

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to the translational horizontal and mean vertical displacement of this point respectively. With regards to a fixed frame, this origin has zero horizontal velocity and an oscillatory vertical velocity. The flagellar beat is then modelled by a sinusoidal wave, which passes down the flagellum, from head to tip. This can be specified by:

$$y = b\sin(kx - \omega t) \tag{3}$$

Where, t measures time, b is the amplitude of the beat, ω is the beat frequency and k is the wave number that passes down the flagellum. Generally, it is also assumed that the spermatozoon undergoes unidirectional motion. Relative to a fixed frame of reference, the velocity of the centre of the head where the head of the spermatozoon is attached to the flagellum in such a way that the centre of the head has the same velocity as the point where they join. The velocity of a material point on the flagellum relative to the fixed frame can then be derived. It is also essential to consider the tangential and normal forces of the fluid acting on the flagellum (Taylor, 1951; Katz & Berger, 1980; Fulford *et al.*, 1998). Taylor (1951) initiated this biofluid mechanic study by analysing the swimming of an infinitely long sheet undergoing small and constant amplitude oscillations in a viscous fluid with stream function (ψ):

$$\psi = -b\omega/k(1 + ky)e^{-ky}\sin(kx - \omega t) \tag{4}$$

Other researchers such as Andrietti & Bernardini (1994) have computed the velocity of propulsion and frequency of rotation of the sperm as a function of the frequency of the wave of motion travelling along the tail. They have formulated it as an integral equation that is time dependent. In their derivation, phase differences are cancelled when different values of the moment during a given period are averaged and the resulting average value of the moment (M_x) can be determined by:

$$M_x = nC_s h^2 \Omega \int_0^{\lambda} \sin^2 kx[1 + h^2k^2\cos^2(kx)]^{1/2}\,dx \tag{5}$$

Where, n is non-integer number, C_s is the tail coefficients of resistance, Ω is the velocity of rotation of the flagellar tail with respect to a fixed frame of reference, h is the radius of the flagellum, k is determined by $2\pi/\lambda$ and λ its wavelength. Using a modified resistive force theory, Fulford *et al.* (1998) also model the sperm swimming in a linear viscoelastic fluid. Their concept is based on a Fourier decomposition of the flagellar velocity, which leads to solving the Stokes flow equations with a complex viscosity. Particularly, they have suggested that the tangential forces $\delta F_{TA}(s,t)$ and normal forces $\delta F_{NO}(s,t)$ per unit length can be broken down into Fourier series:

$$\delta F_{TA}(s,t) = \sum_{m=-\infty}^{\infty} C_{TA} u_{TA}(s)\delta s e^{im\omega t} \tag{6}$$

$$\delta F_{NO}(s,t) = \sum_{m=-\infty}^{\infty} C_{NO} u_{NO}(s)\delta s e^{im\omega t} \tag{7}$$

Where, δs is an element of length on the flagellum at a distance s along the flagellum, u is the swimming velocity, denotation *TA* and *NO* are the tangential and normal component respectively.

Virtually all applications that are analysed in Newtonian fluids cannot assume to have similar outcomes as those of viscoelastic fluids. This is due to the possible qualitatively different hydrodynamics of sperm motion in both of these fluids. In particular, viscoelastic fluids exhibit stress relaxation behaviour over time. The interaction between the times required for such relaxation and the periods of applied oscillatory stresses (as for a spermatozoon flagellum) has the consequence of introducing responses to the applied stresses that are out of phase, as well as in phase with them. Thus, the force distribution created by an activated motile flagellum will be affected by fluid viscoelasticity, as will the hydrodynamic power expended. The nature of the viscoelastic behaviour will have specific consequences to the sperm, including its swimming velocity (Fulford *et al.*, 1998). Thus, the development of refined models of flagellar motility in viscoelastic media that adopt either a range description of its elastic properties or explicitly represent the elastic microstructure would present an important avenue of further investigation.

It was also long believed that the capacitation in human sperms is rather static and prolonged in time until it was recently proved otherwise. Only a small fraction of the sperm population is capacitated at any given time with the capacitated state being transient, with only about a 1 to 4 h lifespan (Luconi *et al.*, 2006). It occurs only once in the sperm's lifetime and different sperm get to this stage at staggered time frames, thus resulting in a continuous replacement of capacitated cells within the sperm population (Cohen-Dayag *et al.*, 1995; Giojalas *et al.*, 2004). Even as hyperactivated motility is often seen in association with the onset of capacitation, it has been shown that these two pathways are separate or divergent because hyperactivation and capacitation can also occur independently of one another. In clinical practices, typically only sperm with activated motility can be assessed. Unfortunately, there are no straightforward clinical methods to induce physiological hyperactivation (Cohen-Dayag *et al.*, 1995; Marquez & Suarez, 2004). Therefore, this form

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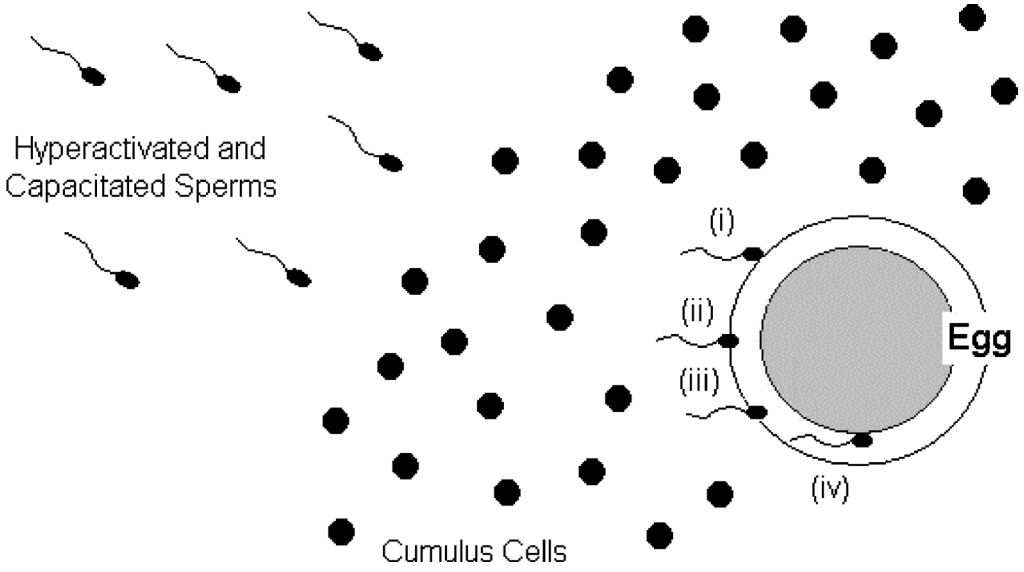

Figure 2 Only capacitated and hyperactivated spermatozoa can be attached to the egg. Sequence of the spermatozoon interaction with an egg: (i) zona pellucida (ZP) binding where receptor(s) for zona on the sperm plasma membrane interact with zona ligands to mediate sperm attachment; and (ii) induction of the acrosome reaction; (iii) sperm components on the inner acrosomal membrane interact with the ZP during zona penetration; and (iv) fusion of the sperm to the egg.

of motility is rarely examined in clinical samples. Due to this limitation, there is an inability efficiently to evaluate this function in clinical examinations. Computational simulation that is able to model this blinded process can potentially be useful as part of the assessment for new assisted conception procedures.

Structure of human spermatozoon

Understanding the physiology of a human sperm is essential to the formation of its characteristics in a simulated environment in bioengineering models. Like most normal mammalian sperm, a human spermatozoon comprises a head and tail or the flagellum that consists of a mid-piece and principal piece. For the headpiece, it plays an important role in the sperm attachment to the zona pellucida (ZP), the acellular glycoprotein coat surrounding the oocyte, and this is a crucial step in fertilization. Figure 2(i) and (ii) show that sperm binding to the ZP involves two phases. During the primary binding phase, one or more receptors for zona on the sperm plasma membrane interact with zona ligands to mediate sperm attachment and induction of the acrosome reaction. In the secondary binding phase, which takes place following the acrosome reaction, sperm components on the inner acrosomal membrane interact with the ZP during zona penetration as in Fig. 2(iii) (Tollner *et al.*, 2003; Yanagimachi, 2005). The association of sperm and ZP during primary binding occurs through two successive steps, and they are: loose attachment and tight binding, with the latter step being critical for zona penetration (Tollner *et al.*, 2003). Figure 2(iv) shows the eventual fusion of a successful spermatozoon with an egg.

The flagellar ultrastructure of a human spermatozoon (as similar to the mammalian) is also essential for its functions. Previous studies have indicated that the flagellum has an elaborate internal structure as given in Fig. 3 (Yu *et al.*, 2002; Egydio *et al.*, 2002). The midpiece can be defined by the presence of nine outer dense fibres (ODF) surrounding each of the nine outer axonemal microtubule doublets (OMD) and by a sheath of mitochondria that encloses the ODF and the axoneme. The ODF extend throughout the midpiece and into the principal piece. The mitochondrial sheath (MS) is only exclusive to the midpiece (Turner, 2003, 2006). Figure 3 shows the schematic of a cross section of the typical '9 + 2' axoneme. It consists of a central pair of singlet microtubules (CP) surrounded by nine OMD and encased by the plasma membrane. Radial spokes to a sheath surrounding the central pair connect the nine outer doublets. Sliding between pairs of outer doublets causes the bending of the axoneme. Particularly, the axoneme, which is powered by dynein molecular motors, is distributed regularly along its length and circumference (Turner, 2003; Tollner *et al.*, 2003; Aitken, 2006; Fauci & Dillon, 2006). However, there is limited knowledge about the precise nature of the spatial and temporal control mechanisms regulating the various flagellar and ciliary beats (Brokaw, 2001). Understanding this may probably bridge the gap and thus, setting a simulated platform to identify the abnormal motile sperms from a given population.

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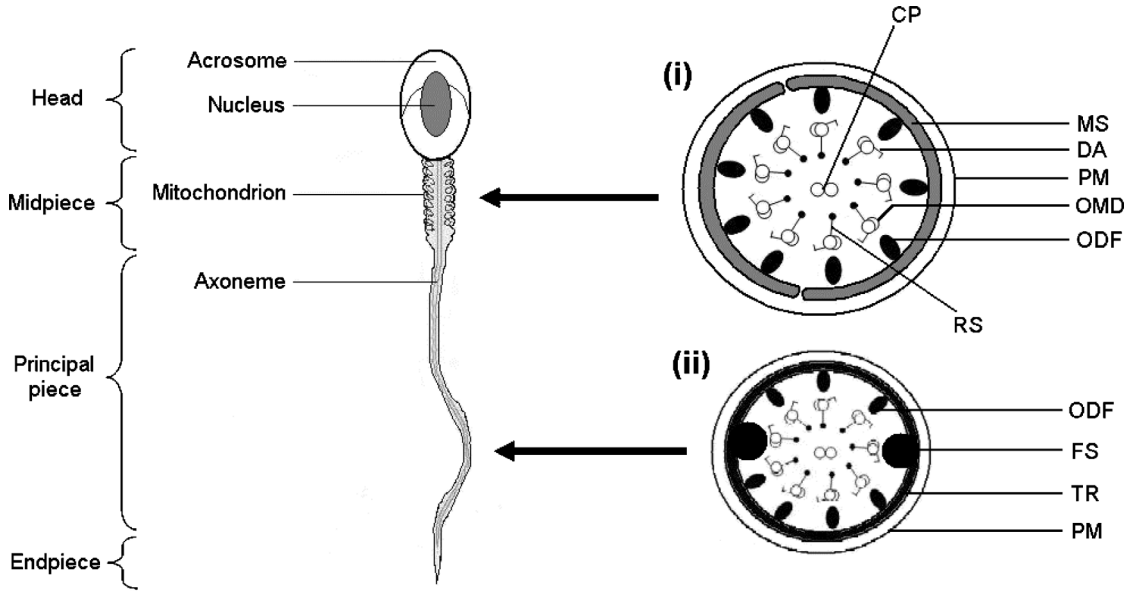

Figure 3 This image shows a typical sperm and selected ultrastructure of its flagellum: (i) Schematic cross-section of the midpiece showing the plasma membrane (PM), mitochondrial sheath (MS) surrounding the nine outer dense fibres (ODF). Besides the ODF, there are nine outer microtubule doublets (OMD) with their associated dynein arm (DA) and radial spokes (RS) and central pair of microtubule doublets (CP); and (ii) schematic cross-section of the principal piece showing the PM surrounding seven ODF. Two of the ODF are replaced by the longitudinal columns of the fibrous sheath (FS), which are connected by the transverse ribs (TR) while the axonemal components remain unchanged. The short terminal section or end-piece of the flagellum contains only the axoneme surrounded by the plasma membrane.

The principal piece of the human sperm usually extends along approximately two-thirds of the length of the flagellum. At the start of the principal piece, the MS ends and two of the ODF are replaced by the two longitudinal columns of the fibrous sheath (FS) as given in Fig. 3 (Welch *et al.*, 2000; Eddy *et al.*, 2003). Thus, the number of ODF in the principal piece is reduced from nine to seven. The columns of the FS run the length of the principal piece and are stabilized by circumferential ribs that surround the ODF. The FS is the only structure that is exclusive to the principal piece. The ODF and FS taper and eventually cease at the termination of the principal piece. The short terminal section of the flagellum is called the end-piece and contains only the axoneme surrounded by the plasma membrane. Studies have suggested that MS, ODF and FS are accessory structures that are exclusive only to the mammalian sperm flagellum including humans (Petersen *et al.*, 1999; Eddy *et al.*, 2003). There is considerable interest that focuses on how the local force production of the dynein motors is translated into the controlled and regular beating of the global structure (Fauci & Dillon, 2006; Turner, 2006). An autonomous biomechanical model needs to include an explicit representation of the force generation and activation dynamics of the dynein molecular motors, and the forces due to the passive structure of the microtubules and radial arms. These forces should then be coupled to the viscous hydrodynamics of the surrounding fluid in order to complement the bioengineered model.

Human female genital tract

Besides the intense interest and research in the human spermatozoa, the complex guidance mechanisms that exist in the female genital tract have also drawn similar level of investigational work. Passage of the sperm through the female genital tract is regulated to maximise the chance of fertilization and ensure that sperm with normal morphology and vigorous motility will eventually be the ones to succeed. Even though there is no significant difference between the numbers of spermatozoa found within the ovulatory tube compared with the non-ovulatory tube, the difference between the sperm distribution within the tubes is significant: the ovulatory tubal ampulla, where fertilization occurs, contains a significantly larger percentage of spermatozoa than those found in the contralateral ampulla (Williams *et al.*, 1993). Thus, mathematical and analytical models using a one-derivational approach may be inadequate to describe the complexity of the sperm's pathway from the site of ejaculation to the interaction with an egg. Equations that are specific to the various anatomical part of the

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female genital tract will be needed to illustrate the complete swimming patterns of the sperm. To facilitate such derivations, an understanding of the various guidance mechanisms is then importance.

Vaginal site

It has been suggested that within minutes of mating, the ejaculated human sperm may be found within the oviduct (Scott, 2000). Reliance on the sperm swimming velocity and motility alone cannot account for the distance the sperm can cover in such a short time. It has been proposed that the rapid sperm transport seen may be due to the muscular activity of the vagina, cervix and uterus (Kunz *et al.*, 1997, 2000; Suarez & Pacey, 2006). In particular, uterine peristalsis is essential for both sperm transport and fundal implantation of the embryo. It has been demonstrated that, in healthy non-pregnant females, regular and symmetric peristaltic waves from the cervix to the fundus are present during the follicular phase with increases in intensity during its later phase (Leyendecker *et al.*, 1996; Eytan & Elad, 1999). For women with fertility problems due to endometriosis, the peristaltic waves can be arrhythmic and irregular during the late follicular phase (Lyons *et al.*, 1991; Leyendecker *et al.*, 1996). By mimicking the regular and symmetric peristaltic waves, a potentially useful clinical tool for infertile women can be obtained and used to evaluate novel methodologies pertaining to this aspect of research.

Studies have proposed that the cervical mucus also assists sperm movement through the human uterine cavity. A rapid transport of sperm through the uterus by myometrial contractions can enhance their survival by propelling them past the immunological defences of the female genital tract (Eytan & Elad, 1999; Scott, 2000; Bianchi *et al.*, 2004). On the entrance to the uterus, the sperm usually outnumber the leukocytes. However, the leukocytes begin to outnumber the sperm over time and the latter may become more susceptible to the leukocytic attack. For fertilization to occur, the sperm need to pass through the uterine cavity before there is the presence of a large enough number of leukocytes (Kunz *et al.*, 1997; Suarez & Ho, 2006). Moreover, cervical mucus poses as a greater barrier to abnormal sperm that cannot swim properly or for those that have a poor hydrodynamic profile than it does to morphologically normal and vigorously motile sperm (Leyendecker *et al.*, 1996, Katz *et al.*, 1997). The findings from these studies could indicate that the cervix supports passage for normal motile sperm while hinders microbes and sperm with abnormal form or motility. Sperm may also be guided through the cervix by the microarchitecture of the cervical mucus. The main glycoproteins comprising cervical mucus are termed mucins, which are long and flexible linear molecules. The viscosity of mucus is due to the large size of mucins while elasticity results from the entanglement of the molecules (Snyder & Zaneveld, 1985; Eriksen *et al.*, 1998). Previous studies have suggested that the secretory flow in mucosal grooves causes these long molecules to be aligned and become a guidance mechanism for the sperm. It has also been demonstrated that for human sperm, they orient themselves along the long axis of threads of cervical mucus (Chretien, 2003). Thus, the sperm can swim through cervical mucus in a straighter path than they do in the seminal plasma or medium (Suarez & Pacey, 2006).

In the uterus, the spermatozoa are passively driven towards the small opening of either oviducts or fallopian tubes by waves of uterine smooth muscle contractions (Wildt *et al.*, 1998; Nishino *et al.*, 2005). This small opening at the uterotubal junction provides an additional barrier to sperm entry into the oviduct, and successful entry seems to require active sperm motility (Suarez & Ho, 2006; Eisenbach & Giojalas, 2006). The oviduct has two regions, the isthmus and the ampulla, as shown in Fig. 4. Both regions have different anatomical and physiological characteristics. However, it is worth noting that these differences are beyond the scope of this review. Spermatozoa are believed to enter the isthmus, bind strongly to the oviductal epithelium and become trapped, thus forming a reservoir. While residing in the reservoir or storage site, the spermatozoa undergo capacitation (Williams *et al.*, 1993; Cohen-Dayag *et al.*, 1995; Giojalas *et al.*, 2004). Owing to their lower affinity for the epithelium, spermatozoa that become capacitated are released from the storage site. The few spermatozoa that are released will require guidance to reach the ovulated egg successfully, which resides in the isthmic-ampullary junction of the oviduct (Wildt *et al.*, 1998; Scott, 2000; Turner, 2006).

Another interesting aspect that has been investigated is the existence of polyspermy blocking mechanisms in most mammals as well as some plants (Dale & Monroy, 1981; Wong & Wessel, 2006). Polyspermy refers to the penetration of two or more spermatozoa to a single oocyte. In most mammals, oocytes develop the ability to block polyspermy during their cycle of growth and maturation. Studies have indicated that there may be several polyspermy-blocking mechanisms, some of which become effective within a second of the first fertilization (Wang *et al.*, 2003). All other polyspermy preventive mechanisms usually become effective within minutes of fertilization. One blocking mechanism that has been well studied is the electrophysiological changes within the egg at fertilization inducing an electrical block against polyspermy. Other mechanisms can include the

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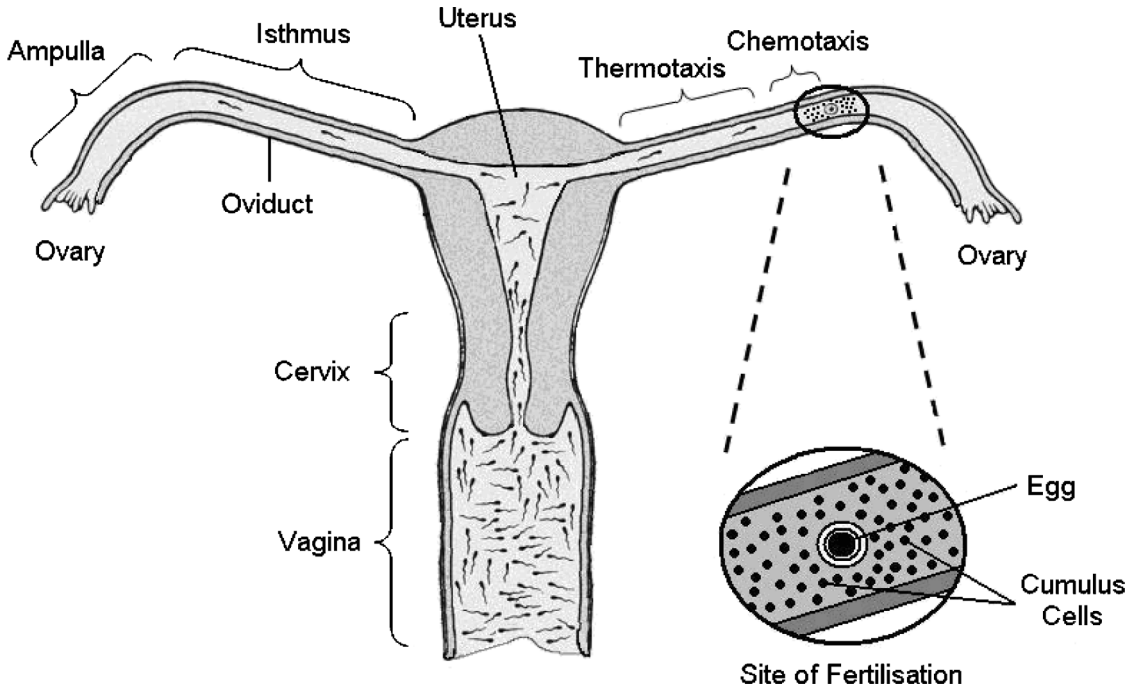

Figure 4 This diagram shows the female genital tract and its guidance mechanisms. The ejaculated spermatozoa in the vagina with their active swimming motility and passive dragging by female genital tract muscular contraction may reach the storage site in the oviduct. Only a small fraction of spermatozoa are capacitated at staggered intervals. Capacitated spermatozoa are then guided from the site of storage to the egg by a combination of chemotaxis, thermotaxis and oviductal contractions.

destruction of receptor moieties in the extracellular or vitelline envelope, the modifications of the egg plasma membrane, and the formation of a fertilization envelope or cell wall (Brawley, 1991; Wong & Wessel, 2006). It is believed that gamete fusion is correlated with the activation of many signalling cascades that contribute to the blocking of polyspermy and to egg activation. One spermatozoon alone may be capable of inducing such processes in the egg, suggesting that contributions from its membrane and/or cytoplasm are sufficient to release the egg from quiescence (Wong & Wessel, 2006).

Thermotaxis

Clinical findings have indicated that a small temperature difference exists between the sperm reservoir's site (which is relatively cooler) and the fertilization site (that is warmer) at ovulation. Even though the actual temperature difference in mated human has yet to be established, studies that have been conducted on other mammalians like rabbits and mated pigs demonstrated that there is a detectable temperature difference between the isthmus and ampulla (David *et al.*, 1972; Hunter & Nichol, 1986; Cicinelli *et al.*, 2004). These findings raise the possibility that the temperature difference serves as one of the guiding mechanisms for the spermatozoa to the site of fertilization. Particularly, the human spermatozoa have the capacity to sense small temperature differences and respond to these by thermotaxis. Furthermore, it is suggested that this difference is achieved not by an elevated temperature at the fertilization site but rather by a reduced temperature at the sperm reservoir's site (Bahat *et al.*, 2003). Only a fraction of the spermatozoa are thermotactically responsive, those that are capacitated, which is in a state of readiness for fertilising the egg. In sperm thermotaxis, only positive thermotaxis or movement towards a warmer temperature has thus far been reported (David *et al.*, 1972; Brewis *et al.*, 2005).

Present literature also suggests that there is a likelihood of an ovulation-dependent temperature gradient in the human female genital tract. This assumption is first based on studies conducted on pigs and rabbits in which the temperatures were measured. Recently, a study endorsed this possibility that the temperature in the region extending from the isthmus to the uterus decreases at ovulation in humans (Cicinelli *et al.*, 2004). The time window in humans may be much longer than in the two other mammals. It is believed that in humans there is no linkage between ovulation and sperm capacitation; therefore capacitated spermatozoa may be available for

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extended periods (Giojalas *et al.*, 2004; Bahat *et al.*, 2005). As the spermatozoa approach the vicinity of the site of fertilization by thermotaxis, they would sense another guiding mechanism within the fallopian tubes known as chemotaxis (Ralt *et al.*, 1994; Jaiswal *et al.*, 1999).

Chemotaxis

This guiding process is characterized by the directional changes in the movement towards the source of the chemoattractant. The most direct assays for the measurement of sperm chemotaxis can be based on the direction of sperm movement (Eisenbach & Tur-Kaspa, 1999; Marquez & Suarez, 2004). In essence, the behaviour of spermatozoa in response to a chemoattractant depends on their variable swimming patterns (Ralt *et al.*, 1994). In sperm chemotaxis, the observed chemo-attractant gradient originates from the egg–cumulus complex in the oviduct to guide the spermatozoa towards the egg. As in sperm thermotaxis, only capacitated spermatozoa can be chemotactically responsive (Cohen-Dayag *et al.*, 1995; Kunz *et al.*, 1997; Turner, 2006). It has been suggested that sperm chemoattractants are secreted not only prior to ovulation within the follicle but also after oocyte maturation outside the follicle. Studies have suggested that there are two possible chemoattractant origins, a mature oocyte and the surrounding cumulus cells (Sun *et al.*, 2005; Eisenbach & Giojalas, 2006).

There are other reports suggesting that chemotaxis may be due to the pH differences in the various sites of the fallopian tubes. The pH of human follicular fluid is reported to be more acidic to that of the oviductal fluid, while the pH of the uterus is thought to be less alkaline than that of the latter (Ben-Yosef *et al.*, 1996; Schuel *et al.*, 2002). This can indicate that human spermatozoa may encounter three diverse environments during their passage from the uterus to the follicle (Bastias *et al.*, 1993). An extracellular pH in the region of 7.5 is reported to be required for fertilization. This pH sensitivity is conferred on the fertilization process at least in part by the pH dependence of sperm binding to the oocyte ZP. The follicular fluid should have a pH between 7.5 and 7.7 significantly to influence the extracellular environment surrounding the oocyte at fertilization in the oviduct. It is postulated that the follicular fluid can significantly contribute to the precise environment for fertilization of oocytes after ovulation (Dale *et al.*, 1998). However, further studies are still needed to establish the reasons for the reportedly high pH in the oviduct and its possible contributions to chemotaxis.

The human spermatozoa usually swim in a curved line as determined by the pattern of their flagellar movement (Taylor, 1951; Fulford *et al.*, 1998). However, little are known about their behavioural response in a chemoattractant gradient within the oviduct. *In vitro*, when the human spermatozoa are swimming in an ascending chemoattractant gradient, they beat their flagella symmetrically and reach the chemoattractant source by maintaining the same swimming direction (Jaiswal *et al.*, 1999; Spehr *et al.*, 2004). The sperm swimming speed increases because of increased flagellar beat frequency and this phenomenon is known as chemokinesis (Ralt *et al.*, 1994; Suarez & Pacey, 2006). Conversely, when human spermatozoa are swimming away from the chemoattractant source, they will turn around abruptly because of an asymmetrical flagellar beat and then swim towards the source as shown in Fig. 1(iii) (Ralt *et al.*, 1994; Spehr *et al.*, 2004).

In vivo, the thermotaxis process complements the chemotaxis process, in that each mechanism is being functional in a region where the other seems less effective. One region is apparently between the isthmic sperm reservoir and the site of fertilization. The temperature gradient that exists at ovulation but the long-range chemotaxis may not occur due to a mixing effect of the peristaltic movements of the oviduct. In the other region, the opposite seems to hold. A measurable temperature gradient probably cannot be maintained, whereas a chemoattractant gradient appears to be very effective because of the relatively short distance and the viscoelastic milieu of the cumulus that resists the stirring action of the oviduct (Cohen-Dayag, 1995; Jaiswal *et al.*, 1998). Thus, it is reasonable to assume that the capacitated human spermatozoa are first guided by thermotaxis from the sperm reservoir towards the warmer site of fertilization. The oviductal contractions, which move liquid from the isthmus to the ampulla, may assist the swimming spermatozoa (Williams *et al.*, 1993). Present literature suggests that sperm thermotaxis and chemotaxis are long-range and short-range mechanisms that guide capacitated and 'ready-to-fertilise' spermatozoa towards the egg respectively. They occur consecutively with each in a region where the other is not functional (Bahat *et al.*, 2003). Another passive guidance mechanism is the dragging of spermatozoa by muscle contractions in the female genital tract. The existence of these sperm guidance mechanisms implies that modelling of the human spermatozoa swimming process in the female genital tract is far more complex than the once-thought competitive-race dogma. Furthermore, more advanced models that can characterize these guidance mechanisms will help examine *in vitro* assisted conception techniques whether existing or novel. Particularly, it becomes important to factor in the contributions of thermotaxis; chemotaxis and

muscle contractions in the human reproductive process whether separately or combined.

Discussion

The research interest on human sperm guidance has been limited until recent decades when it has been revolutionized. It is becoming clearer that the spermatozoa must have the ability to be actively guided to the egg. Essentially, there are new advances in the understanding of guidance mechanisms (especially thermotaxis) and their effects on the overall sperm success interaction with an egg (Bahat & Eisenbach, 2006). Obtaining this knowledge will not only increase the understanding of human fertilization but may also pave the way for potentially improved clinical applications. Specifically, this can be used in clinical procedures to obtain sperm populations that are enriched with capacitated spermatozoa *in vitro*. They can also be exploited as a diagnostic tool to assess sperm quality. In addition, these processes can possibly be used as a means of conception by interfering with the sources of infertility and effects of high maternal age (Wilding *et al.*, 2005). However, the initial step is to derive more advanced bioengineering models to better portray the biofluid mechanic forces in the sperm–egg interactions. With more precise models coupled with computational simulation, these can provide valuable insight into the biological fluid dynamic aspects of human reproduction. These can then be used as technological improvements and developments in the current or even novel assisted conception methods.

Although moderate success rates can be achieved with the present assisted conception techniques, it is unknown whether there are effects of sperm preparation or ovarian stimulation on how sperm behave or respond to the biology of the female genital tract before fertilization. Clearly, such information can be useful for those who are interested in the acquisition of knowledge and improvement of these techniques. However, there are still limited capabilities adequately to mimic the *in vitro* environment and sperm selection mechanisms of the female genital tract during natural unassisted conception. With the growing knowledge of the basic biology underlining these events *in vivo*, there are potential improvements on the assisted conception procedures. In view of this, mathematical models and bioengineered simulations can provide a viable and cheaper approach in obtaining initial findings and understanding before adopting full laboratorial experiments or clinical trials.

In regards to modelling the human reproductive process, the Reynolds number governing sperm motion is generally assumed small. Based on this assumption, the boundary element method for Stokes flow can then be applied (Taylor, 1951; Fulford *et al.*, 1998). With advanced computational packages to represent complicated geometries, like computational fluid dynamics, simulations of these bioengineered models can also be implemented (Yaniv *et al.*, 2003). For an example, the immersed boundary method that was originally developed to model two-dimensional blood flow in the heart can provide a framework that can couple the elastic dynamics of flagella, cilia, and muscular walls with a viscous and incompressible fluid (Mittal & Iaccarino, 2005). Alternatively, the grid-free method of regularized Stokeslets can be used to construct the flow field due to a distribution of regularized forces supported by the immersed boundary (Cortez *et al.*, 2005). Furthermore, there should be interest in deriving models that describe the flagellar beating of the activated, hyperactivated and chemoattractant-simulated spermatozoa in a three-dimensional swimming trajectory. Lastly, the effects and contributions of the guidance mechanisms within the female genital tract should also be incorporated to portray a more realistic understanding.

Conclusions

Over the past few decades, there have been great advances in the understanding of the fundamental mechanics of human spermatozoa motilities and the guidance mechanisms of the female genital tract. The complex interaction between the fluidic and mechanical phenomena in the human reproductive process is an interesting and essential topic in the field of biomedical engineering. Previous model examples that describe its rheological assistive behaviour have been included in this review. The challenge now is to obtain adequate knowledge of the dynamic coupling of these mechanisms in simulated yet realistic geometries to represent them. Comprehensive models that can describe the various spermatozoa motilities in the vastly varying environments in the female genital tract have the potential to provide fundamental insights to the overall reproductive process. However, more still needs to be done in order to advance further the mathematical and bioengineered models. With these acquired developments coupled with laboratorial experiments, more valuable diagnostic tools can be produced to improve the present techniques of assisted conception in this important field of research.

References

Aitken, R.J. (2006). Sperm function tests and fertility. *Int. J. Androl.* **29**, 69–75.

Andrietti, F. & Bernardini, G. (1994). The movement of spermatozoa with helical head, theoretical analysis and experimental results. *Biophys. J.* **67**, 1767–74.

https://doi.org/10.1017/S0967199408004899 Published online by Cambridge University Press

Bahat, A. & Eisenbach, M. (2006). Sperm thermotaxis. *Mol. Cell Endocrinol*. **252**, 115–9.

Bahat, A., Tur-Kaspa, I., Gakamsky, A., Giojalas, L.C., Breitbart, H. & Eisenbach, M. (2003). Thermotaxis of mammalian sperm cells, a potential navigation mechanism in the female genital tract. *Nature Med*. **9**, 149–50.

Bahat, A., Eisenbach, M. & Tur-Kaspa, I. (2005). Periovulatory increase in temperature difference within the rabbit oviduct. *Hum. Reprod*. **20**, 2118–21.

Bastias, M.C., Kamijo, H. & Osteen, K.G. (1993). Assessment of human sperm functional changes after in-vitro coincubation with cells retrieved from the human female reproductive tract. *Hum. Reprod*. **8**, 1670–77.

Ben-Yosef, D., Oron, Y. & Shalgi, R. (1996). Intracellular pH is not affected by fertilisation and the resulting calcium oscillations. *Biol. Reprod*. **55**, 461–8.

Bergqvist, A.S., Ballester, J., Johannisson, A., Hernandez, M., Lundeheim, N. & Rodriguez-Martinez, H. (2006). In vitro capacitation of bull spermatozoa by oviductal fluid and its components. *Zygote* **14**, 259–73.

Bianchi, P.G., De Agostini, A., Fournier, J., Guidetti, C., Tarozzi, N., Bizzaro, D. & Manicardi, G.C. (2004). 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**, 97–102.

Brawley, S.H. (1991). The fast block against polyspermy in fucoid algae is an electrical block. *Dev. Biol*. **144**, 94–106.

Brewis, I.A., Moore, H.D., Fraser, L.R., Holt, W.V., Baldi, E., Luconi, M., Gadella, B.M., Ford, W.C. & Harrison, R.A. (2005). Molecular mechanisms during sperm capacitation. *Hum. Fertil*. **8**, 253–61.

Brokaw, C.J. (2001). Simulating the effects of fluid viscosity on the behavior of sperm flagella. *Math. Meth. Appl. Sci*. **24**, 1351–65.

Chretien, F.C. (2003). Involvement of the glycoproteic meshwork of cervical mucus in the mechanism of sperm orientation. *Acta Obstet. Gynecol. Scand*. **82**, 449–61.

Cicinelli, E., Einer-Jensen, N., Barba, B., Luisi, D., Alfonso, R. & Tartagni, M. (2004). Blood to the cornual area of the uterus is mainly supplied from the ovarian artery in the follicular phase and from the uterine artery in the luteal phase. *Hum. Reprod*. **19**, 1003–8.

Cohen-Dayag, A., Tur-Kaspa, I., Dor, J., Mashiach, S. & Eisenbach, M. (1995). Sperm capacitation in humans is transient and correlates with chemotactic responsiveness to follicular factors. *Proc. Natl. Acad. Sci. USA* **92**, 11039–43.

Cortez, R., Fauci, L. & Medovikov, A. (2005). The method of regularized Stokeslets in three dimensions, analysis, validation and application to helical swimming. *Phys. Fluids* **17**, 031504–1-14.

Dale, B., Menezo, Y., Cohen, J., DiMatteo, L. & Wilding, M. (1998). Intracellular pH regulation in the human oocyte. *Hum. Reprod*. **13**, 964–70.

Dale, P. & Monroy, A. (1981). How is polyspermy prevented? *Gamete Res*. **4**, 151–69.

David, A., Vilensky, A. & Nathan, H. (1972). Temperature changes in the different parts of the rabbit's oviduct. *Int. J. Gynaecol. Obstet*. **10**, 52–6.

Dunson, D.B., Bigelow, J.L. & Colombo, B. (2005). Reduced fertilization rates in older men when cervical mucus is suboptimal. *Obstet. Gynecol*. **105**, 788–93.

Eddy, E.M., Toshimori, K. & O'Brien, D.A. (2003). Fibrous sheath of mammalian spermatozoa. *Microsc. Res. Tech*. **61**, 103–15.

Egydio de Carvalho, C., Tanaka, H., Iguchi, N., Ventela, S., Nojima, H. & Nishimune, Y. (2002). Molecular cloning and characterization of a complementary DNA encoding sperm tail protein SHIPPO 1. *Biol. Reprod*. **66**, 785–95.

Eisenbach, M. & Giojalas, L.C. (2006). Sperm guidance in mammals—an unpaved road to the egg. *Nat. Rev. Mol. Cell. Biol*. **7**, 276–85.

Eisenbach, M. & Tur-Kaspa, I. (1999). Do human eggs attract spermatozoa? *Bioessays* **21**, 203–10.

Elder, K. & Dale, B. (2000). *In Vitro Fertilization*, 2nd edn. Cambridge: Cambridge University Press.

Eriksen, G.V., Carlstedt, I., Uldbjerg, N. & Ernst, E. (1998). Cervical mucins affect the motility of human spermatozoa in vitro. *Fertil. Steril*. **70**, 350–4.

Eytan, O. & Elad, D. (1999). Analysis of intra-uterine fluid motion induced by uterine contractions. *Bull. Math. Biol*. **61**, 221–38.

Fauci, L. & Dillon, R. (2006). Biofluid mechanics of reproduction. *Annu. Rev. Fluid Mech*. **38**, 371–94.

Fulford, G.R., Katz, D.F. & Powell, R.L. (1998). Swimming of spermatozoa in a linear viscoelastic fluid. *Biorheology* **35**, 295–309.

Gaddum-Rosse, P. (1985). Mammalian gamete interactions, what can be gained from observations on living eggs? *Am. J. Anat*. **174**, 347–56.

Giojalas, L.C., Rovasio, R.A., Fabro, G., Gakamsky, A. & Eisenbach, M. (2004). Timing of sperm capacitation appears to be programmed according to egg availability in the female genital tract. *Fertil. Steril*. **82**, 247–9.

Hunter, R.H.F. & Nichol, R. (1986). A preovulatory temperature gradient between the isthmus and the ampulla of pig oviducts during the phase of sperm storage. *J. Reprod. Fert*. **77**, 599–606.

Ishijima, S., Baba, S.A., Mohri, H. & Suarez, S.S. (2002). Quantitative analysis of flagellar movement in hyperactivated and acrosome-reacted golden hamster spermatozoa. *Mol. Reprod. Dev*. **61**, 376–84.

Jaiswal, B.S., Cohen-Dayag, A., Tur-Kaspa, I. & Eisenbach, M. (1998). Sperm capacitation is, after all, a prerequisite for both partial and complete acrosome reaction. *FEBS Lett*. **427**, 309–13.

Jaiswal, B.S., Tur-Kaspa, I., Dor, J., Mashiach, S. & Eisenbach, M. (1999). Human sperm chemotaxis, is progesterone a chemoattractant? *Biol. Reprod*. **60**, 1314–9.

Katz, D.F. & Berger, S.A. (1980). Flagellar propulsion of human sperm in cervical mucus. *Biorheology* **17**, 169–75.

Katz, D.F., Slade, D.A. & Nakajima, S.T. (1997). Analysis of pre-ovulatory changes in cervical mucus hydration and sperm penetrability. *Adv. Contracept*. **13**, 143–51.

Kirichok, Y., Navarro, B. & Clapham, D.E. (2006). Whole-cell patch-clamp measurements of spermatozoa reveal an alkaline-activated Ca^{2+} channel. *Nature* **439**, 737–40.

Kunz, G., Beil, D., Deiniger, H., Einspanier, A., Mall, G. & Leyendecker, G. (1997). The uterine peristaltic pump. Normal and impeded sperm transport within the female genital tract. *Adv. Exp. Med. Biol*. **424**, 267–77.

Kunz, G., Beil, D., Huppert, P. & Leyendecker, G. (2000). Structural abnormalities of the uterine wall in women

with endometriosis and infertility visualized by vaginal sonography and magnetic resonance imaging. *Hum. Reprod*. **15**, 76–82.

Leyendecker, G., Kunz, G., Wildt, L., Beil, D. & Deininger, H. (1996). Uterine hyperperistalsis and dysperistalsis as dysfunctions of the mechanism of rapid sperm transport in patients with endometriosis and infertility, *Hum. Reprod*. **11**, 1542–51.

Luconi, M., Forti, G. & Baldi, E. (2006). Pathophysiology of sperm motility. *Front. Biosci*. **11**, 1433–47.

Lyons, E.A., Taylor, P.J., Zheng, X.H., Ballard, G., Levi, C.S. & Kredentser, J.V. (1991). Characterization of subendometrial myometrial contractions throughout the menstrual cycle in normal fertile women. *Fertil. Steril*. **55**, 771–4.

Marquez, B. & Suarez, S.S. (2004). Different signaling pathways in bovine sperm regulate capacitation and hyperactivation. *Biol. Reprod*. **70**, 1626–33.

Mittal, R. & Iaccarino, G. (2005). Immersed boundary methods. *Annu. Rev. Fluid. Mech*. **37**, 239–61.

Nishino, M., Togashi, K., Nakai, A., Hayakawa, K., Kanao, S., Iwasaku, K. & Fujii, S. (2005). Uterine contractions evaluated on cine MR imaging in patients with uterine leiomyomas. *Eur. J. Radiol*. **53**, 142–6.

Ohl, D.A., Menge, A.C. & Jarow, J.P. (1999). Seminal vesicle aspiration in spinal cord injured men, insight into poor sperm quality. *J. Urol*. **162**, 2048–51.

Petersen, C., Fuzesi, L. & Hoyer-Fender, S. (1999). Outer dense fibre proteins from human sperm tail, molecular cloning and expression analyses of two cDNA transcripts encoding proteins of approximately 70 kDa. *Mol. Hum. Reprod*. **5**, 627–35.

Ralt, D., Manor, M., Cohen-Dayag, A., Tur-Kaspa, I., Ben-Shlomo, I., Makler, A., Yuli, I., Dor, J., Blumberg, S. & Mashiach, S. (1994). Chemotaxis and chemokinesis of human spermatozoa to follicular factors. *Biol. Reprod.* **50**, 774–85.

Schuel, H., Burkman, L.J., Lippes, J., Crickard, K., Forester, E., Piomelli, D. & Giuffrida, A. (2002). *N*-Acylethanolamines in human reproductive fluids. *Chem. Phys. Lipids* **121**, 211–27.

Scott, M.A. (2000). A glimpse at sperm function in vivo, sperm transport and epithelial interaction in the female reproductive tract. *Anim. Reprod. Sci*. **60–1**, 337–48.

Snyder, M.G. & Zaneveld, L.J. (1985). Treatment of cervical mucus with lectins, effect on sperm migration. *Fertil. Steril.* **44**, 633–7.

Spehr, M., Schwane, K., Riffell, J.A., Barbour, J., Zimmer, R.K., Neuhaus, E.M. & Hatt, H. (2004). Particulate adenylate cyclase plays a key role in human sperm olfactory receptor-mediated chemotaxis. *J. Biol. Chem*. **279**, 40194–203.

Suarez, S.S. & Ho, H.C. (2003). Hyperactivation of mammalian sperm. *Cell. Mol. Biol*. **49**, 351–6.

Suarez, S.S. & Pacey, A.A. (2006). Sperm transport in the female reproductive tract. *Hum. Reprod. Update* **12**, 23–37.

Sun, F., Bahat, A., Gakamsky, A., Girsh, E., Katz, N., Giojalas, L.C., Tur-Kaspa, I. & Eisenbach, M. (2005). Human sperm chemotaxis, both the oocyte and its surrounding cumulus cells secrete sperm chemoattractants. *Hum. Reprod*. **20**, 761–77.

Taylor, G.I. (1951). Analysis of the swimming of microscopic organisms. *Proc. R. Soc. Lond. A* **209**, 447–61.

Tollner, T.L., Yudin, A.I., Cherr, G.N. & Overstreet, J.W. (2003). Real-time observations of individual macaque sperm undergoing tight binding and the acrosome reaction on the zona pellucida. *Biol. Reprod*. **68**, 664–72.

Turner, R.M. (2003). Tales from the tail, what do we really know about sperm motility? *J. Androl.* **24**, 790–803.

Turner, R.M. (2006). Moving to the beat, a review of mammalian sperm motility regulation. *Reprod. Fertil. Dev.* **18**, 25–38.

Wang, W.H., Day, B.N. & Wu, G.M. (2003). How does polyspermy happen in mammalian oocytes? *Microsc. Res. Tech*. **61**, 335–41.

Welch, J.E., Brown, P.L., O'Brien, D.A., Magyar, P.L., Bunch, D.O., Mori, C. & Eddy, E.M. (2000). Human glyceraldehyde 3-phosphate dehydrogenase-2 gene is expressed specifically in spermatogenic cells. *J. Androl*. **21**, 328–38.

Wilding, M., Di Matteo, L. & Dale, B. (2005). The maternal age effect, a hypothesis based on oxidative phosphorylation. *Zygote* **13**, 317–23.

Wildt, L., Kissler, S., Licht, P. & Becker, W. (1998). Sperm transport in the human female genital tract and its modulation by oxytocin as assessed by hysterosalpingoscintigraphy, hysterosonography, electrohysterography and Doppler sonography. *Hum. Reprod. Update* **4**, 655–66.

Williams, M., Hill, C.J., Scudamore, I., Dunphy, B., Cooke, I.D. & Barratt, C.L. (1993). Sperm numbers and distribution within the human fallopian tube around ovulation. *Hum. Reprod*. **8**, 2019–26.

Wong, J.L. & Wessel, G.M. (2006). Defending the zygote, search for the ancestral animal block to polyspermy. *Curr. Top. Dev. Biol*. **72**, 1–151.

Yanagimachi, R. (2005). Intracytoplasmic injection of spermatozoa and spermatogenic cells, its biology and applications in humans and animals. *Reprod. Biomed. Online* **10**, 247–88.

Yaniv, S., Elad, D., Jaffa, A.J. & Eytan, O. (2003). Biofluid aspects of embryo transfer. *Ann. Biomed. Eng*. **31**, 1255–62.

Yu, Y., Oko, R. & Miranda-Vizuete, A. (2002). Developmental expression of spermatid-specific thioredoxin-1 protein, transient association to the longitudinal columns of the fibrous sheath during sperm tail formation. *Biol. Reprod*. **67**, 1546–54.