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REVIEW

Do sperm possess a molecular passport? Mechanistic insights into sperm selection in the female reproductive tract

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ABSTRACT: Most male mammals produce far more spermatozoa on a daily basis than is strictly necessary for reproduction and females have evolved mechanisms that prevent all but a small minority from reaching the vicinity of their oocytes. One potential explanation for the stringent selection is that females have developed these mechanisms as a way of avoiding polyspermy as well as exercising post-copulatory choice over the characteristics of the fertilizing spermatozoon. Relatively little is known about how these processes would operate, but here we use evidence from biochemical, molecular and genetic studies of sperm transport in support of a hypothesis proposing that the female reproductive tract can read and interpret a spermatozoon's 'molecular passport' or genetic signature. Such a signature would permit only a highly selected sperm population to reach and fertilize the oocyte. Moreover, the selection criteria might not only be concerned with successful fertilizing ability, but could also be tailored to suit the genetic qualities of individual females.

Key words: female reproductive tract / sperm competition / sperm selection

Introduction

In the early 1970s, Parker formally proposed (Parker, 1970) the hypothesis that because females of many species frequently accept matings by more than one male, rivalry between males, and between their ejaculates, would lead to sperm competition. In this scenario, the ability to produce and ejaculate larger numbers of spermatozoa than competing males represents an advantage in terms of breeding success. In principle, this is similar to a raffle, where the probability of winning is increased by purchasing more tickets. Unlike a fair raffle, however, the outcome of sperm competition can be biased if males develop specific behaviours that reduce the effectiveness of their rival's mating ability (Parker *et al.*, 2010). Thus, the last male to mate with a particular female might possess an advantage in terms of sperm transport within the female reproductive tract (Jones *et al.*, 2002), especially if the last male is able to remove previous ejaculates from the female reproductive or inactivate their spermatozoa. Sperm competition has impacted upon the evolution of phenotypic traits of spermatozoa such as head shape, midpiece volume (Anderson *et al.*, 2005) and flagellar length (Bauer and Breed, 2006; Kleven *et al.*, 2008), with consequential effects on swimming velocity (Lupold *et al.*, 2009) and ability to fertilize oocytes (Fitzpatrick and Lupold, 2014; Ramm, 2014; Ramm and Scharer, 2014). As sperm shape, size and the rate of sperm production can only change by modifying the process of spermatogenesis, sperm competition has exerted an important evolutionary pressure on testis size and function (Hosken and Ward, 2001). It therefore follows that the complex mechanisms controlling spermatogenesis must, unarguably, have been affected by sperm competition (White-Cooper and Bausek, 2010). Indeed, molecular evidence shows that genes involved in spermatogenesis are among the most rapidly evolving of the reproductive axis (Swanson *et al.*, 2001; Swanson and Vacquier, 2002; Good *et al.*, 2011; Carnahan-Craig and Jensen-Seaman, 2014; Dhole and Servedio, 2014).

The strong evolutionary influence of sperm competition is countered to some extent by the evident post-copulatory ability of females to 'choose' which spermatozoa are allowed to reach and fertilize the oocyte (Fitzpatrick and Lupold, 2014). This ability, widely known as 'cryptic female choice' (Birkhead, 1998), may involve selecting between different spermatozoa from an individual male (Immler, 2008) or between spermatozoa from different males. These scenarios imply that the female reproductive tract employs molecular recognition mechanisms to obtain information about the individual spermatozoon, or the individual male, otherwise selective processes would not function. The existence of cryptic female choice in diverse species of insects, birds, reptiles and mammals has been widely researched and recognized (Bloch Qazi, 2003; Hosken and Stockley, 2003; Jennions and Petrie, 2000; Bussiere *et al.*, 2006; Briceno and Eberhard, 2009), but, the mechanistic basis for sperm selection in

mammals is still obscure. Our purpose in writing this review is to examine this topic in the light of emerging evidence about sperm–female tract interactions in mammals. This mechanistic approach aims to complement widely studied and reviewed evolutionary implications of cryptic female choice, and provide insights about 'how' the choices are made (proximate explanations, *sensu*; Ramm, 2014) rather than why the choices are made.

Sperm selection as a response to polyspermy

While relatively few spermatozoa may, strictly speaking, be needed to effect fertilization, a certain minimum quantity has to be inseminated to ensure that sufficient will pass through the cervix and uterine horns in order to reach the oviducts and ultimately interact with the oocytes. Studies of artificial insemination in various species, including humans, cattle, pigs and sheep, have established that there are minimum thresholds for the quantity of spermatozoa inseminated (Den Daas *et al.*, 1998; Achard *et al.*, 2005), and have shown that conception rates typically increase with higher sperm doses, eventually reaching a maximum where further increases have no further influence. In a study of human fertility (Achard *et al.*, 2005), the conception rate fell from 40 to 24% per cycle when fewer than 1.5 million spermatozoa were inseminated. Similarly, the achievement of maximum calving rates in dairy cattle requires the insemination of between 1 and 11 million spermatozoa (Den Daas *et al.*, 1998); lower numbers usually result in significantly poorer fertility. Artificial insemination outcomes in pigs are minimal unless sperm numbers exceed 1 billion (Flowers and Esbenshade, 1993; Flowers, 1997), also reflecting the need for a minimum number of functionally competent spermatozoa. These observations set the scene for a consideration of sperm selection processes in mammals, which clearly have to operate against a background that involves a high rate of loss of inseminated spermatozoa. It seems likely that these initially high numbers of spermatozoa are required in order for sufficient numbers to reach the vicinity of the utero-tubal junction (UTJ) prior to entering the oviducts.

The cervix and UTJ present spermatozoa with physical, chemical and anatomical barriers that control their progress (Martyn *et al.*, 2014; Yaniz *et al.*, 2014) and most spermatozoa (>99%) in an ejaculate fail to breach these obstacles (Fig. 1). Blind-ended cervical crypts can block sperm transport, but conversely may also allow cohorts of live spermatozoa to be stored for several days before continuing. The potential duration of fertile life of stored spermatozoa has been estimated as 5 days in humans (Croxatto, 2002) and 6–9 days in domestic dogs (Concannon *et al.*, 1983; England *et al.*, 2006). Because the physical nature of the cervical mucus is under hormonal control during the reproductive cycle, it can both facilitate and inhibit sperm transport towards the uterus and oviduct. In fact, functional incompatibility between spermatozoa and cervical mucus can be an important cause of infertility; this has been widely recognized in human clinical medicine where the 'post-coital' test has been widely used to test for pathological incompatibility between spermatozoa and mucus (Barratt *et al.*, 1992).

Paradoxically, if the female reproductive tract allowed too many spermatozoa to reach the oviduct, there would be a heightened risk of polyspermy (where individual oocytes are simultaneously penetrated by two or more spermatozoa) and embryonic death would ensue. The female reproductive tract has consequently developed at least three mechanisms for the prevention of polyspermy. These involve: (i) selectively preventing the passage of spermatozoa through the UTJ unless they express certain membrane proteins on their surfaces (calmegin, calreticulin, ADAM1a, 2, 3 and angiotensin-converting enzyme, among others) (Cho *et al.*, 1998; Ikawa *et al.*, 2001, 2011; Shen *et al.*, 2013); (ii) hardening the zona pellucida through the action of oviductal proteins (e.g. oviduct-specific glycoprotein and heparin-like glycosaminoglycans), making it resistant to hydrolytic enzymes and turning it into a selective barrier that inhibits sperm progress towards the oolemma (Coy *et al.*, 2008; Coy and Aviles, 2010); and (iii) deploying intracytoplasmic hydrolytic enzymes sequestered in cortical granules situated beneath the oocyte plasma membrane to harden the zona pellucida still further after entry of the first, fertilizing spermatozoon (Puppo *et al.*, 2008; Gadella and Evans, 2011).

Ensuring that sufficient competent spermatozoa reach the vicinity of the oocyte, while simultaneously restricting the numbers and preventing polyspermy requires delicate balancing mechanisms, but also provides scope for females to exercise a degree of choice. Compelling evidence for the existence of mechanisms that provide such choice has been provided by the use of heterospermic artificial inseminations. Heterospermic insemination (HI) outcomes can be highly skewed even when all of the males involved are known to produce good quality spermatozoa that are highly fertile when inseminated on their own. Controlled experiments conducted mainly on farm animals have demonstrated unequivocally that the female reproductive tract can significantly skew the outcome of artificial inseminations carried out by inseminating females with mixed semen samples containing balanced sperm numbers taken from two or more males (Robl and Dziuk, 1988; Dziuk, 1996). This approach to fertility estimation eliminates female effects, such as the timing of insemination relative to ovulation, and also eliminates the influence of relative sperm numbers. In some studies, the skewed fertilization rates are as high as 97% in favour of one semen sample over another (Kasimanickam *et al.*, 2006). In this particular study of bovine fertility, total progressive motility was positively correlated with the degree of skew, but none of the other usual measures of sperm quality was significantly correlated. In a similar vein, a heterospermic study carried out with semen from two boars (Stahlberg *et al.*, 2000) found a 70 to 30% ratio of embryo paternity (95 embryos from 11 females were tested for paternity). When the same semen samples were used for homospermic inseminations, there was no difference in the fertilization rates. It is of interest, however, that the skewed fertilization outcome of the HIs in this study was mirrored by the relative mean numbers of accessory spermatozoa associated with zonae pellucidae after homospermic insemination (21.8 versus 52.4). This indicates that the skew was caused by differences in the relative ability of spermatozoa to access the oocytes, implicating selective sperm transport as the likely limiting factor.

Evidence of this nature implies that sperm selection *in vivo* is based on a complex molecular dialogue between the spermatozoa and the female reproductive tract. In this article, we propose that this idea, which we term the 'molecular sperm passport hypothesis', has some merit in explaining some of the reproductive skews (including sex ratio skews) that occur under natural, as well as experimental, mating conditions (Clutton-Brock and Iason, 1986; Clutton-Brock *et al.*, 1986; Berger, 1995).

Reproductive skews that arise through the intrinsic genetic properties of single spermatozoa are pertinent to this discussion. Detailed studies of

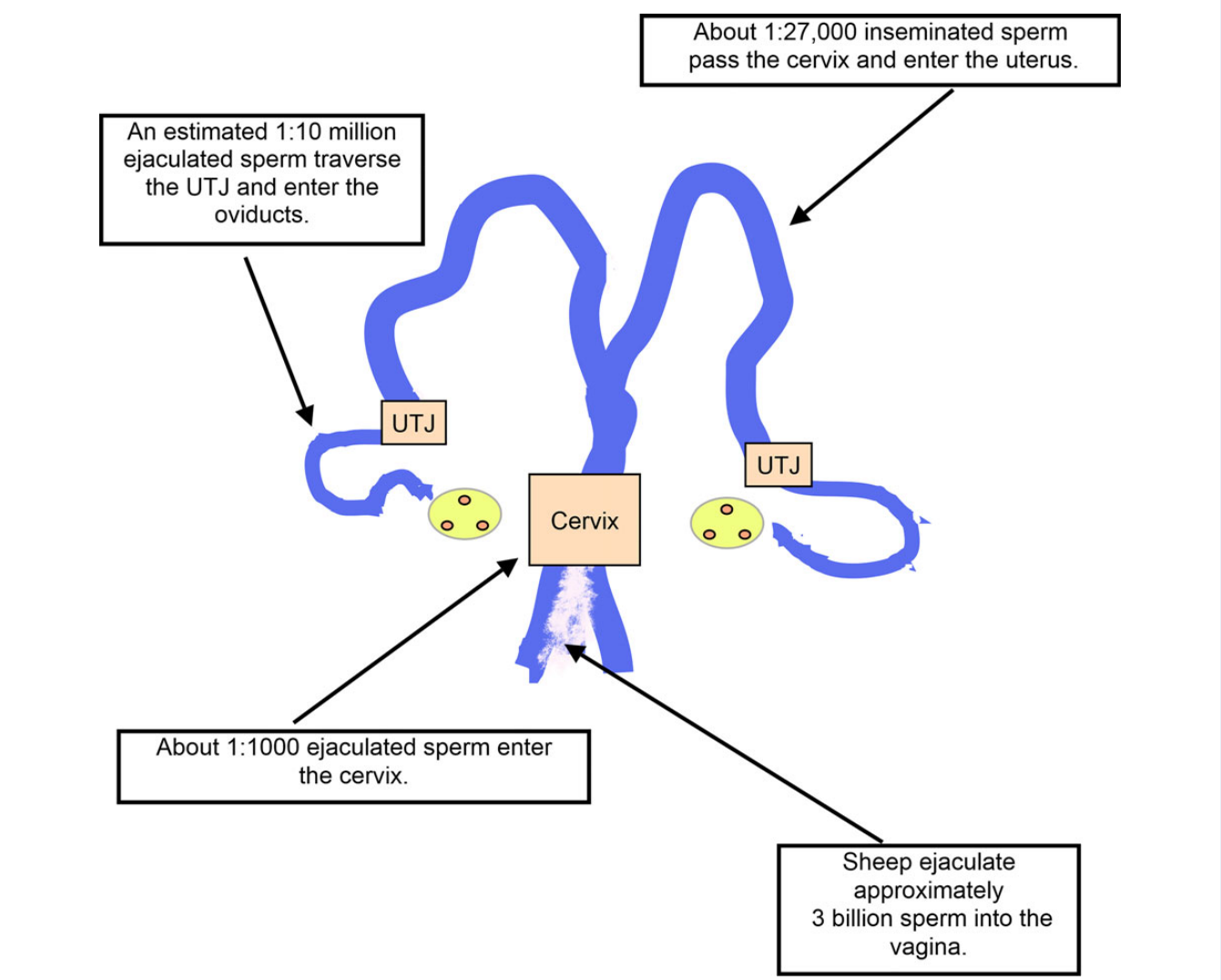

Figure 1 While artificial insemination was still being developed as a practical tool for breeding agricultural species, researchers evaluated the efficiency of sperm transport within the female reproductive tract. Several important studies tracked the time course of sperm transport through the complex anatomy of the female reproductive tract. Such studies typically involved the experimental insemination of oestrous females with known numbers of spermatozoa; the females were slaughtered at intervals after insemination whereupon the reproductive tracts were flushed and the different anatomical regions were examined for the presence of spermatozoa. Data from sheep (summarized in Fig. 1) and pigs showed that only about 1:10 million of the inseminated spermatozoa managed to reach the oviducts (First *et al.*, 1968; Hawk *et al.*, 1978) forming a functional sperm reservoir. Similarly, human studies have revealed that there are ~1000 spermatozoa within the Fallopian tube 8–15 h after coitus (Croxatto, 2002). The sperm reservoir is established during the first few hours after insemination, declines after ovulation (Rodriguez-Martinez *et al.*, 2005; Rodriguez-Martinez, 2007; Hunter, 2012; Rijsselaere *et al.*, 2014) and contains a highly selected sperm population.

the mouse t-haplotype (Olds-Clarke and Johnson, 1993; Olds-Clarke, 1996) have revealed that genetically distinct spermatozoa produced within a single testis can engage in a form of competition that results in the non-Mendelian inheritance of one genotype. This model, which has been reviewed extensively (Olds-Clarke, 1996), concerns the well-established observation that sperm motility is severely compromised in homozygous mice carrying t-haplotypes on both alleles of chromosome 17. In contrast, the heterozygous mice, which are identical in all other respects, produce spermatozoa with superior motility while wild-type spermatozoa show normal motility. Experimentally, it has been shown that spermatozoa from homozygous mice are virtually incapable of reaching the oviduct (Olds-Clarke, 1991), but that spermatozoa from the heterozygotes can, in fact, enter the oviduct and fertilize oocytes. These observations have been explained on the basis that there are two tightly linked, and post-meiotically expressed, genetic factors known, respectively, as the T-complex transmission ratio distorters (TCD) and the T-complex responder (TCR). The TCD causes the defective flagellar action and the TCR rescues flagellar action within the same cell, and moreover confers a selective fertilization advantage to the t-haplotype spermatozoa (Herrmann *et al.*, 1999).

These observations call into question the widely accepted dogma that intercellular bridges, which form cytoplasmic connections between

spermatocytes and spermatids during spermatogenesis, allow unrestricted sharing of post-meiotically expressed transcripts between daughter cells. In the presence of unrestricted transcript sharing, the TCR would rescue motility in all of the spermatozoa formed within a single clonal syncytium of spermatids, but this is not the case. Two lines of evidence (for review, see Ellis *et al.*, 2011) support the hypothesis that transcripts are confined within individual spermatids, possibly by linkage to cytoskeletal proteins. Véron *et al.* (2009) demonstrated that transcripts are retained within individual spermatids by mRNA-tethering rather than being shared among members of the post-meiotic syncytium. Furthermore, Martin-DeLeon *et al.* (2005) showed that in males carrying different alleles of the Spam1 hyaluronidase gene, transcript compartmentation within spermatids produced biochemically and functionally different sperm populations, and resulted in transmission ratio distortion. The experimental confirmation that transcripts are not always shared across intercellular bridges suggests that this is highly likely to be a conserved process, responsible for non-Mendelian inheritance across species. Taking this argument a step further suggests that this mechanism could be important in sperm selection, sexual selection (whereby it might exert significant influence on cryptic female choice) and ultimately in the evolutionary history and future of a species.

Sperm recognition mechanisms within the female reproductive tract

Although some mammalian species are able to interbreed with closely related species, an experiment aimed at investigating whether the UTJ is able to impede transit of 'foreign' spermatozoa suggested that this is the case. When female hamsters were artificially inseminated with live and motile rat, mouse, guinea pig and rabbit spermatozoa, as well as immotile hamster spermatozoa, all types could pass through the UTJ, but only in small numbers compared with live hamster spermatozoa (Smith *et al.*, 1988). Similarly, experiments in three species of vesper mice (Calomys; Rodentia, Cricetidae) (Roldan *et al.*, 1985) showed that homologous inseminations were more successful at producing embryos than heterologous inseminations. Discrimination of spermatozoa by the UTJ on the basis of their head shape and motility is also important in the control of sperm transport (Krzanowska *et al.*, 1995).

A novel experimental study of sperm transport in the red spotted newt (*Notophthalmus uiridescens*) (Hardy and Dent, 1986) demonstrated that entry of spermatozoa into the spermathecae is a selective process. These researchers placed rabbit spermatozoa into the cloacal regions of four female newts and compared the outcomes with parallel experiments undertaken with newt spermatozoa. Interestingly, the rabbit spermatozoa were unable to enter the spermathecae, despite displaying vigorous motility, while the conspecific spermatozoa were able to do so. Despite the highly unusual experimental design, this experiment clearly showed that entry into spermathecae must be controlled by recognition systems operated by cell–cell interactions involving the spermatozoa and the spermathecal cells.

The extent of skew in cattle HI experiments was highly and negatively correlated with the DNA fragmentation rate (%DFI) in the semen samples ($r = -0.87$: $P < 0.005$), while plasma membrane integrity (%PMI) was positively correlated ($r = 0.87$: $P < 0.005$) with the extent of skew (Kasimanickam *et al.*, 2006). This study is of interest because it highlights the possibility that the female reproductive tract rejects membrane-damaged spermatozoa, but is then able to discriminate between the remaining intact cells on the basis of their DNA integrity. If this is true, it implies that membrane-intact spermatozoa somehow express membrane surface information about their DNA status. Evidence that human cervical mucus acts as a filter capable of reducing the proportion of spermatozoa carrying fragmented DNA (Bianchi *et al.*, 2004) supports the idea that the sperm surface mirrors sperm DNA status. Of itself, DNA fragmentation status would provide a rather crude estimate of genetic quality because it is primarily a measure of DNA damage. However, eliminating spermatozoa because their DNA had been damaged during spermatogenesis or maturation in the epididymis seems eminently sensible because the defective cells might nevertheless possess the capacity to reach and fertilize oocytes, resulting in poor quality embryos. In fact, some direct cell-by-cell correlations of human sperm morphology (Mangiarini *et al.*, 2013) and DNA fragmentation have indicated that poor morphology is correlated with poor DNA status.

Clues about the relationship between sperm surface characteristics and sperm quality are available from human clinical research, where laboratory techniques aimed at selecting the best, most fertile spermatozoa are used to maximize fertilization outcomes for infertile couples. A recent systematic review (Said and Land, 2011) of such methods is informative because the discriminatory properties of the sperm surface were examined in the context of other sperm properties, including DNA fragmentation status. A selection method based largely on electrophoresis and electronegative charge was found to produce sperm populations enriched in DNA-intact spermatozoa in the three cited studies that employed DNA assessment (Ainsworth *et al.*, 2005; Chan *et al.*, 2006; Razavi *et al.*, 2010). The main source of negative charge on the sperm plasma membrane has been attributed to a specific GPI-anchored glycoprotein, CD52 (Schröter *et al.*, 1999), which contains highly sialylated polylactosamine-containing carbohydrate chains. Increased sperm surface negative charge is a correlate of sperm maturation in the epididymis and therefore this selection method is likely to be acting as a filter for mature versus immature spermatozoa. Moreover, since chromatin cross-linking and nuclear stabilization is another correlate of epididymal sperm maturation (Calvin and Bedford, 1971), the negative charge seems to be further useful source of information about sperm quality. Ability to interact with hyaluronic acid is also a marker of sperm maturation that has been correlated with sperm DNA integrity (Yagci *et al.*, 2010) and is used as a laboratory method for sperm selection. Hyaluronic acid is found extensively in the female reproductive tract, including cervical mucus, oviductal fluid and the cumulus cells that surround the oocyte. A recent study (Liu *et al.*, 2014) has shown that human spermatozoa expressing the hyaluronic acid receptor (CD44) display better plasma membrane structure, mitochondrial membrane potential, fertilizing potential and maturation characteristics than their counterparts that lack CD44. Thus, CD44 is a putative signal of sperm quality that is displayed on the sperm surface and is available to be read by other cells in the female reproductive tract. These considerations strongly suggest that the female reproductive tract is capable of using information available on the cell surface to accept or reject individual spermatozoa during their progress towards the oocytes. Such a sophisticated level of discrimination is not unprecedented in other taxonomic groups.

Hyaluronic acid has been also been found to assist with the formation of the oviductal sperm reservoir (Rodriguez-Martinez *et al.*, 2001; Liberda *et al.*, 2006); this is of particular interest because the sperm

reservoir is a site that collects spermatozoa that have already traversed the UTJ, then selectively releases them so they can continue their progress towards the oocyte. Once the spermatozoa gain access to the oviductal isthmus, they exert some control over their own environment. This was first noted in experiments (Ellington *et al.*, 1993) showing that if cultured oviductal epithelial cells are co-incubated with spermatozoa, the epithelial cells respond by the *de novo* synthesis of proteins. Several follow-up studies in mice and pigs (Fazeli *et al.*, 2004; Georgiou *et al.*, 2007; Yeste *et al.*, 2014) have lent support to these findings. Heat-shock proteins (HSPs) are prominent among the proteins up-regulated by spermatozoa (Elliott *et al.*, 2009; Holt and Fazeli, 2010). While HSPs are usually found within the cell cytoplasm, oviductal cells secrete them into the luminal fluid where they are then able to interact with the sperm surface and exert some control over their membrane properties. Extracellular HSPA8 induces fluidization of the sperm plasma membrane (Moein-Vaziri *et al.*, 2014), enhances the oviduct-binding ability of exposed spermatozoa and even reduces polyspermy in pig IVF. These findings support the concept that having initially selected a sperm population that is suitable for fertilization, the female reproductive tract has also developed mechanisms to ensure that the spermatozoa can be stored until they are needed.

If sperm qualities are only viewed in terms of plasma membrane biochemistry, they fail to emphasize the importance of dynamic sperm signalling responses. During their journey from the cervix through the uterine horns, towards the oviduct and ultimately to the oocyte, the spermatozoa must travel through fluids of varying viscosity and different ionic and hormonal composition, and encountering different collections of macromolecules. Many of these molecules in the environment have the capacity to interact with specific sperm receptors and modulate the different cell signalling pathways that control the physiological state of the spermatozoa (Tapia *et al.*, 2012). Capacitation status is controlled by signalling pathways that modulate intracellular pH and calcium concentration; bicarbonate controls sperm motility (Holt and Harrison, 2002) and membrane lipid architecture (Harrison, 2004), while hyperactivated motility is modified by the presence of progesterone (Armon and Eisenbach, 2011; Arnoult *et al.*, 2011; Lishko *et al.*, 2011; Strunker *et al.*, 2011).

Investigations of chemotactic mechanisms that guide spermatozoa towards the oocyte(s) have identified progesterone as a significant molecular signal that modifies sperm flagellar activity (Oren-Benaroya *et al.*, 2008; Teves *et al.*, 2009; Armon and Eisenbach, 2011; Blengini *et al.*, 2011; Guidobaldi *et al.*, 2012). The action of progesterone is elicited via its receptor, CatSper (Arnoult *et al.*, 2011; Lishko *et al.*, 2011; Strunker *et al.*, 2011), and the consequent modulation of intracellular calcium concentrations that change flagellar activity (Kaupp *et al.*, 2008). Two recent studies (Zuccarello *et al.*, 2011; Caballero-Campo *et al.*, 2014) have demonstrated that sperm chemotaxis is modulated not only by progesterone but also via chemokine–receptor interactions involving factors produced by oocytes, granulosa cells and endometrial cells. One of the studies (Zuccarello *et al.*, 2011) focused on the interaction between CXCR4 (chemokine CXC motif receptor 4) present in human spermatozoa and SDF1 (chemokine stromal cell-derived factor-1), a member of the CXC chemokine family also known as CXCL12. It is of specific interest in the context of this review that $<$30% of live human spermatozoa express CXCR4 (Kim *et al.*, 1999; Zuccarello *et al.*, 2011), meaning that $\sim$70% of spermatozoa would be unresponsive to chemical signals emanating from granulosa cells and oocytes. The other study (Caballero-Campo *et al.*, 2014) identified CCR6, a chemokine receptor common to several chemoattractant peptides (Yang *et al.*, 1999), in human and mouse spermatozoa and also noted that the protein was not detected in every cell. Moreover, it was observed that CCR6 expression on the sperm surface was more intense after capacitation. The existence of this chemotactic interaction mechanism, together with differential expression of appropriate receptors between spermatozoa, is compatible with the concept of a molecular passport for spermatozoa based on between-sperm differences. That chemotaxis is stimulated by several different mechanisms is, however, something of a puzzle: do the separate mechanisms operate synergistically or do they perhaps indicate the existence of an exceedingly sensitive and discriminatory sperm selection system? Interactions between chemokine receptors and one specific β-defensin (DEFB126), which is distributed along the human sperm surface (Tollner *et al.*, 2011) and together with CD52 contributes much of the sperm surface sialic acids, have recently also been implicated for their importance in the progression of spermatozoa through viscous media, widely used as substitutes for cervical mucus (Dorin and Barratt, 2014). Despite having normal semen parameters and sperm motility, spermatozoa from men with the DEFB126 mutation showed 84% reduction in the rate of penetration through hyaluronic acid gel, coupled with lowered conception rates.

Sperm penetration through the zona pellucida becomes more difficult once oocytes have been in contact with oviductal fluid (Coy *et al.*, 2002, 2008), emphasizing the importance of sperm hyperactivation for developing the degree of mechanical thrust needed to penetrate the hardened zona pellucida. In terms of sperm selection, the principle that every component of a signalling pathway has to be present within the spermatozoon is even valid at the final stages of oocyte activation, once zona and oolemmal penetration has occurred. Recent research into oocyte activation has revealed that unless the fertilizing spermatozoon transfers a cytoplasmic form of phospholipase C ζ into the oocyte, embryo development does not occur normally because the requisite periodic and characteristic calcium waves are not generated within the zygote (Swann and Lai, 2013; Nikiforaki *et al.*, 2014). Viewed in this way, it is apparent that if spermatogenesis is defective, the resultant molecular errors are likely to prevent an individual spermatozoon from ever reaching the oocyte.

The subtlety of sperm selection

While it may be relatively straightforward to convey information about sperm maturation status and DNA integrity, it seems that females might be rather more sophisticated in their selection criteria. As an extension to our molecular passport hypothesis, we suggest that females could be searching for male gametes that best match their own genetic attributes, or that suit environmental conditions pertaining at any given time. A recent study (Ghaderi *et al.*, 2011) provides a link between sexual selection and immune function in humans. Human sperm surfaces lack a specific form of sialic acid (*N*-glycolylneuraminic acid; Neu5Gc), but humans nevertheless produce circulating antibodies against Neu5Gc that enter the female reproductive tract and inactivate any incompatible spermatozoa. This exemplifies the principle that females can target paternal antigens and use the immune system to facilitate selection (Dorus *et al.*, 2012). Immunological studies of sperm transport have also shown that once a cohort of spermatozoa reaches the uterus, they induce the 'post-mating inflammatory response' which

prompts an influx of neutrophils into the uterine lumen (Schuberth *et al.*, 2008). This provides another level of sperm selection mediated by direct cell–cell interactions.

The major histocompatibility system (MHC) has been widely researched for its relevance to reproduction and it is likely that mechanisms exist to identify and select the best match between maternal and paternal MHC characteristics (Ziegler *et al.*, 2005; Milinski, 2014). Mammalian species, including humans (Wedekind and Furi, 1997; Wedekind and Penn, 2000), are known to use olfactory cues to distinguish MHC-genotypes in the context of mate choice, but it is not yet clear whether MHC sensing also operates at the gametic level in mammals and birds. Recent studies in Jungle fowl (Gillingham *et al.*, 2009) have shown that males increase the size of their ejaculate when mating with MHC-dissimilar females. Moreover, further investigations revealed that under natural mating conditions, the number of spermatozoa reaching the oocyte surface (the perivitelline envelope) was affected by the male–female MHC combination; sperm numbers were higher when female MHC genotype differed from that of the males (Lovlie *et al.*, 2013). The authors attributed these effects to physiological mechanisms operating after copulation and thereby affecting cryptic female choice. Interestingly, the effects of MHC on differential sperm transport were not detectable in parallel experiments conducted using artificial insemination, possibly indicating that females relied upon male phenotypic and behavioural cues to influence sperm transport. Similar effects have been noted in sand lizards (Olsson *et al.*, 2004) and experiments in mice have shown that seminal plasma components significantly improve a female's immune tolerance to 'foreign' spermatozoa by their actions on uterine physiology (Robertson *et al.*, 2009).

Spermatozoa within all ejaculates from individual males are already known to differ genetically in one fundamental respect. There are two equally sized populations of mammalian spermatozoa, each carrying different sex-determining chromosomes (X and Y), and although it is widely known that females can bias the sex ratio of their offspring (Clutton-Brock and Iason, 1986; James, 2009), no satisfactory mechanistic explanation for this has yet been discovered. A recent study in pigs provided an important mechanistic insight into this phenomenon by demonstrating that the sperm-induced gene expression patterns within the oviduct differed significantly according to whether the inseminated sperm populations contained the X- or Y-chromosome (Almiñana *et al.*, 2014). In this study, sex-sorted X- and Y-bearing spermatozoa were inseminated separately into the right and left oviducts of the same females. Gene expression responses by the oviductal cells were examined using microarrays and it was found that 501 of 24 123 probes were significantly changed by the presence of Y-bearing spermatozoa in relation to the X-bearing sperm population. Of the 501 transcripts, 271 (54.1%) were down-regulated and 230 transcripts (45.9%) were up-regulated when the Y chromosome-bearing spermatozoa were present in the oviduct. Although this was an artificial and experimental study, these findings demonstrate that oviductal cells can distinguish between sperm genotypes, presumably using mechanisms that involve reading information from the sperm surface.

Although the mechanisms responsible for such differential and profound responses to sperm phenotype remain largely unknown, there are many potentially relevant and accessible candidates that might be responsible. These include responses to external signalling molecules, the presence of cell surface markers such as ubiquitin (Sutovsky *et al.*, 2001) and the complex arrays of carbohydrates that constitute the 'glycome' (Kuo *et al.*, 2009; Pang *et al.*, 2009; Kadirvel *et al.*, 2012; Silva *et al.*, 2014). Some important sperm surface modifications are attributable to post-translational mechanisms that modify the existing proteins. Phosphorylation has been extensively studied in spermatozoa and has been shown to regulate multiple processes, including motility and capacitation (Visconti, 2009), and numerous human sperm proteins have been identified as targets of nitrosylation (Lefievre *et al.*, 2007). Sumoylation, whereby sperm proteins are modified by 'small ubiquitin-like modifiers' (SUMO), is another important regulatory mechanism in germ cells. A recent combined proteomic and microscopic analysis of human spermatozoa (Vigodner *et al.*, 2013) demonstrated increased levels of sumoylation in defective spermatozoa, such as those with two-tails, curled tails and abnormal heads. In addition, there is emerging evidence that each individual spermatozoon contains its own population of microRNAs (Curry *et al.*, 2011; Das *et al.*, 2013; Salas-Huetos *et al.*, 2014). These are RNA sequences of ~20 bases (Hausser and Zavolan, 2014), which are known to be powerful modulators of gene expression. As the various microRNA species adopt unique three-dimensional shapes, they are important candidates in any mechanism that involves selection. Little information is currently available about their role in sperm selection *in vivo*, but evidence from clinical studies supports the hypothesis that microRNAs can make the difference between successful and unsuccessful infertility treatment (Garrido *et al.*, 2009, 2013; Garcia-Herrero *et al.*, 2011). Correlations between the fertility of artificially inseminated bull semen and the nature of its microRNA content have also been demonstrated (Kasimanickam *et al.*, 2012).

Implications for clinical translation

The evidence presented above supports the view that females have evolved mechanisms for the selection of spermatozoa (summarized in Fig. 2) that might provide them with genetic benefits. This scenario has been the subject of intensive research in evolutionary biology for many years and, on the whole, experiments undertaken with a variety of taxonomic groups have lent credence to the widespread importance of these mechanisms. Here, we have aimed to demonstrate the complexity and selectivity of sperm transport mechanisms in mammals and to emphasize the potential mechanisms that may be involved.

Having gathered evidence that demonstrates the sophistication of sperm selection processes within the female reproductive tract, we are forced to wonder whether bypassing these mechanisms during modern infertility treatments such as intracytoplasmic sperm injection (ICSI) will eventually produce adverse outcomes. At the present time, choosing one individual spermatozoon for ICSI, out of the many that are available, seems to be a matter of chance, although some laboratories are striving to develop and use selection techniques (Said and Land, 2011). Although the effectiveness of assisted reproductive technologies for overcoming infertility is not in doubt, increased incidences of genomic imprinting-related disorders are a cause for concern (Le Bouc *et al.*, 2010; Lazaraviciute *et al.*, 2014). These disorders, which are attributable to changes in DNA methylation at specific loci, may not be caused by ICSI alone but by the many separate treatment steps that potentially affect the periconception environment. In fact, some have argued that epigenetic changes can be repaired to some extent (Rajender *et al.*, 2011) and are less of a concern than other genetic problems identifiable by

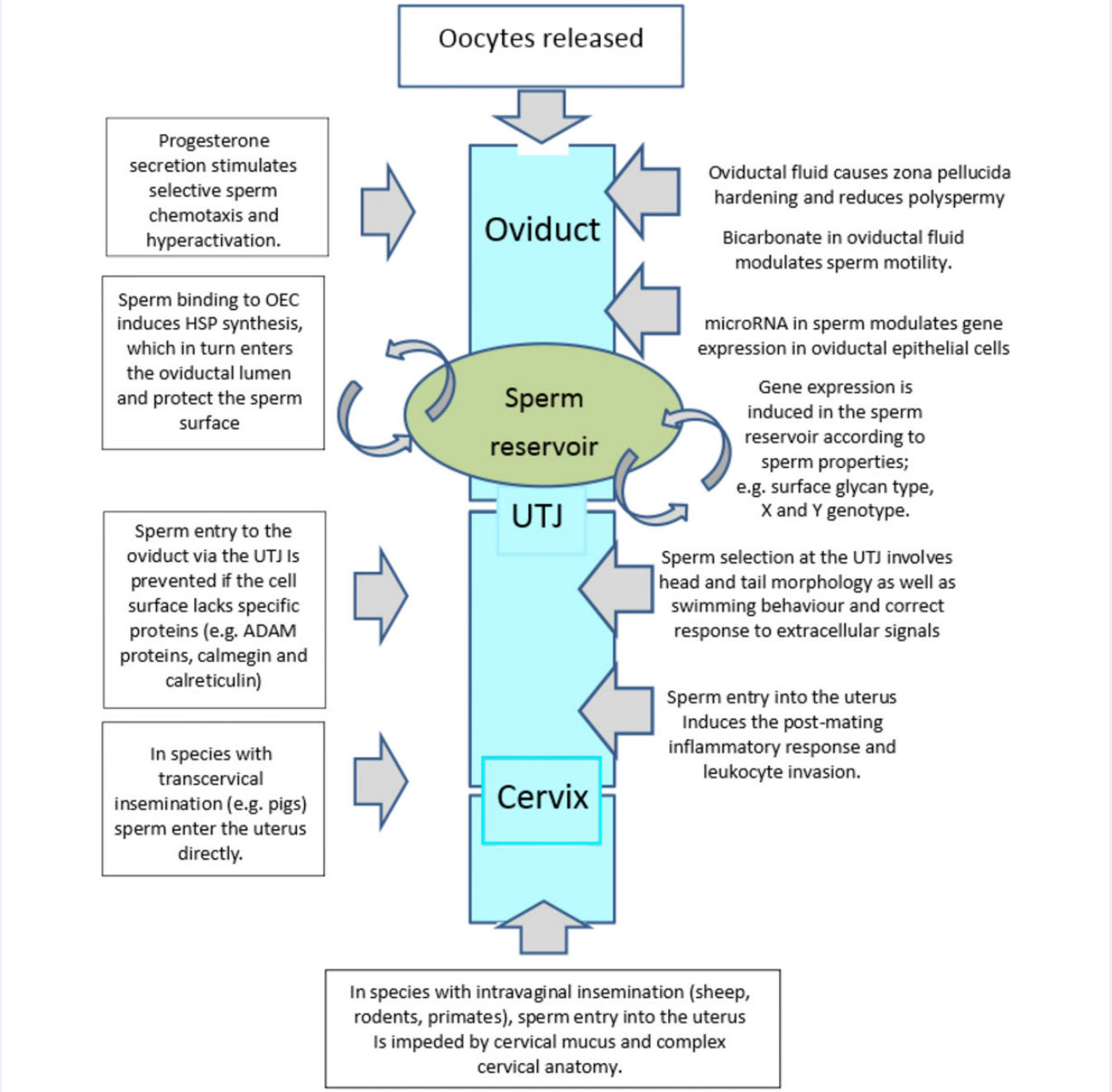

Figure 2 Schematic diagram highlighting some of the selective processes that act as sperm quality filters within the female reproductive tract.

chromosomal screening (Patrat *et al.*, 2010). These contradictory views will eventually be resolved, especially when babies born after IVF and ICSI are old enough to allow the significance of developmental programming effects (Bateson *et al.*, 2014) to be more precisely appreciated.

We conclude this article by re-emphasizing that there are important scientific and clinical benefits to be gained from investigating the molecular characteristics of spermatozoa in relation to fertility. We envisage that the availability of advanced high throughput technologies such as genomics, proteomics and glycan arrays will shortly transform our understanding of sperm biology and enable the development of a new generation of semen assessment protocols. Some progress with such approaches has already been reported (Garrido *et al.*, 2009, 2013; Garcia-Herrero *et al.*, 2011). Similarly, approaching the issue of sperm quality assessment from a molecular perspective will provide novel scientific information about sperm quality in relation to evolutionary biology.

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Conflict of interest

None declared.

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