Minireview

The human spermatozoon – a stripped down but refined machine

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Abstract

A recent paper in *BMC Developmental Biology* describes the development of the annulus of the mouse sperm cell, but much remains to be learnt about sperm cells despite their importance in human fertility.

The global health problems of infertility and sperm dysfunction

In a world in which population growth and its subsequent control seem to be critical problems, it is counterintuitive to think that one in six couples are subfertile (still trying for a child after over 1 year of unprotected intercourse). In fact, approximately 80 million couples throughout the world are subfertile [1]. What is perhaps more surprising is that male infertility accounts for at least 50% of these cases and that sperm dysfunction (sperm being present in the ejaculate but lacking 'normal' function) is the single most common cause, affecting approximately one in 20 men. This is a high proportion of the population compared with other prevalent diseases; male subfertility is thus a very significant global problem and, most worryingly, reports suggest that its prevalence is increasing.

A recent report by Guan and colleagues in *BMC Developmental Biology* [2] describes the development of a specific but relatively poorly studied structure, the annulus, in the formation of the mature spermatozoon. The annulus is an electron dense ring structure at the junction of the mid piece and principal piece. Its function is not clearly established but it may constitute a diffusion barrier between the two compartments and/or facilitate mitochondria migration and alignment along the axoneme. This study adds to what is a remarkably small amount of knowledge about the human sperm cell. Here, we review what we know about this highly specialized cell, the spermatozoon, and what treatments are available for men with sperm dysfunction.

Unfortunately, no treatments are available for sperm dysfunction except for those that simply bring the sperm closer to the egg. Despite the claims of a number of authors, there is no drug a man can take to improve sperm function. The only realistic treatment option for the man is assisted conception (one of the treatments that are collectively termed assisted reproductive technology or ART), which in the majority of cases consists of *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI). IVF is used if there are sufficient sperm available to add to eggs in culture (about 500,000 sperm at about 50,000 per egg). ICSI, which is by far the more common technique, is generally used when only a few sperm (<2-3×10^6) are seen in the ejaculate and they are perceived to be too weak or abnormal to penetrate the outer layers of an egg. A single spermatozoon is then selected by the embryologist and injected directly into the cytoplasm of the egg. Both these techniques are expensive (an average of about €7,000 per cycle in the UK), they are invasive, they have limited success (in the overwhelming majority of European Union countries, fewer than 25% of cycles started produce a birth [3]), they carry significant risks and they are not widely available. However, the number of ART cycles is increasing in all areas of the world and shows no signs of leveling off, probably because there are no alternative treatments and also because couples are leaving it later to try for children and ignoring the negative consequences of age on gamete quality in men and women.

The composition of the spermatozoon

Developing effective rational non-ART therapy for sperm dysfunction requires a clear understanding of the biological, genetic, cellular and molecular mechanisms of the production (spermatogenesis) and function of the normal spermatozoon. However, until recently, progress has been painfully slow. In part this is due to the complexity and specialized function of the mature sperm, which is dramatically different from all other cells in the body (Figure 1). Sperm cells are characterized by their lack of physiologically active transcription or translation, their rapid motility, their tightly condensed DNA (in an almost crystalline state, packaged by protamines) and the fact that they are produced in excessive numbers (a man produces about 1,000 sperm every heartbeat and only one is used per conception). Although such attributes make the cells
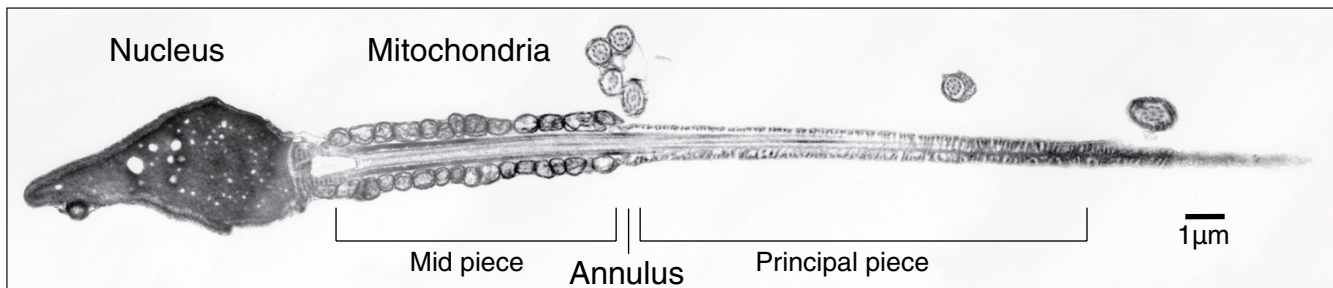
Figure 1

Montage transmission electron micrograph of a human sperm cell. The cell has a compact nucleus, conspicuous mitochondria, no endoplasmic reticulum, minimal cytoplasm and a large tail (about 45 μm in length). Superfluous cytoplasm and associated machinery is jettisoned when the sperm emerges from the testis, leaving a 'stripped down', minimalist cell.

particularly interesting to study, they present challenges to the use of traditional methods of cell and molecular biology, such as transfection and gene/protein expression patterns.

In addition, a bedrock tool of understanding – knockout mice – has limited use in this context because (i) there is significant redundancy in the reproductive process, (ii) the pathology of knockouts, although similar to that seen in men with sperm dysfunction, is often not the same, and (iii) fertilization in humans has several very specific differences from that in mice. Consequently, successful examples of identifying gene defects in subfertile men by screening for genes knocked out in mice are rare. Usually no mutations in a gene of interest are found. For example, knockout mice lacking the *Csnk2a2* gene (which encodes casein kinase IIa) have round-headed sperm similar to those seen in subfertile men with globozoospermia, but when six such men were tested for mutations in the human homolog of the gene, no mutations were found [4]. Thus, alternative strategies are required. Recently, breakthrough results using other techniques have appeared in three areas: the composition of the spermatozoon, the packaging of sperm DNA and the chemotaxis that leads the sperm to the egg.

The sperm toolkit
With no physiologically active transcription and translation, spermatozoa are ideal cells to study from a proteomic perspective – a true proteome can be established [5]. The first indication of the complete sperm toolkit – the human sperm proteome – is now appearing [6]. This includes some surprising findings, such as that sperm have a complete proteasome (used for degrading proteins for reuse), which is at odds with the idea of a cell type that does not require protein turnover. Data will probably be available on specific regions of the cell, such as tail proteins, membrane proteins and nuclear complexes, which together will allow a comprehensive first draft of the human sperm proteome. Comparing this with the proteomes of sperm from other species will help to answer

fundamental questions, such as what basic machinery is necessary to make a functionally mature male gamete.

Proteomics also provides the opportunity to examine key dynamic processes involving post-translational protein modifications, about which we know almost nothing; for example, the development of fertilizing capacity (capacitation). Key kinases are only just being identified, and the first details of the identity and dynamics of proteins phosphorylated during human sperm capacitation have now emerged [7]. Such studies will not be confined to phosphorylation, as the first, rough draft of the human sperm *S*-nitroso proteome is now available, which shows that over 240 proteins undergo post-translational modification (*S*-nitrosylation) in response to stimulation with nitric oxide, which enhances sperm motility [8].

Proteomic studies such as these [5-8] will allow a comprehensive and unbiased comparison between the normal spermatozoon and abnormal or dysfunctional cells, providing insights into critical aspects of sperm function and dysfunction. However, current case reports are limited by rather crude and unconvincing clinical diagnosis, for example 'poor' motility (which encompasses many causes), combined with unsophisticated proteomics and bioinformatics. Therefore, only a glimpse of the richness of the data has yet been revealed. With refined diagnosis and more sophisticated proteomics, such as quantitative labeling, our understanding will increase substantially.

Packaging of the DNA and the consequences of damage to sperm DNA
The chromatin packaging in the mature spermatozoon is very different from that of somatic cells – it is very tightly packed, and the DNA is resistant to nucleases and sonication and cannot repair itself. The DNA is arranged into a toroid structure in which protamines account for at least 90% of the chromatin and histones for about 10%. Abnormalities in packaging, such as abnormal ratios between protamines 1 and 2, have been known for some
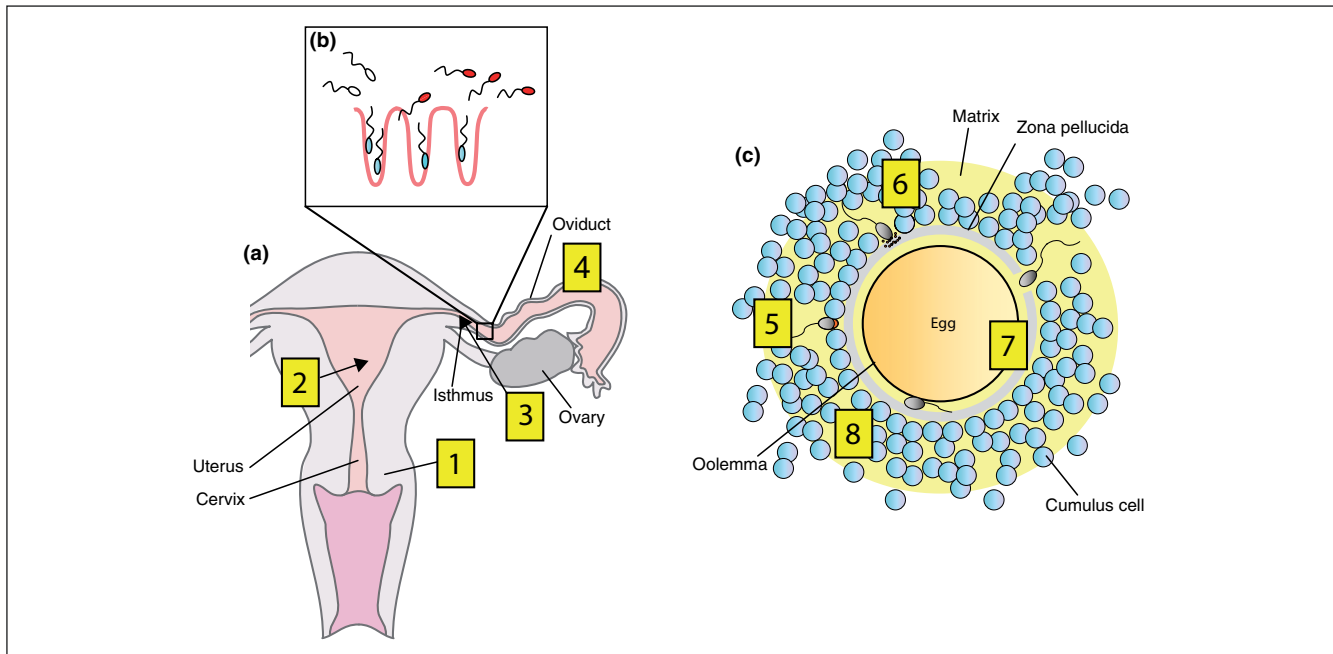
Figure 2

The remarkable journey of the sperm to the egg. **(a)** After intercourse, sperm enter the cervical mucus (1), where they begin capacitation. During passage to the uterus (2), the sperm induce a host reaction. Leukocytes, which outnumber the sperm 100:1, engulf normal and abnormal spermatozoa. In humans only one in 14,000,000 ejaculated human sperm reach the oviduct. Experiments in non-human mammals have shown that the oviduct (particularly the isthmus, where the duct is narrowest) (3) acts as a sperm store, potentially involving intimate contact between the sperm and the epithelial surface. Fertilization occurs in the oviduct (4). **(b)** *In vitro* experiments suggest that intracellular Ca^{2+} is low in the sperm that are attached to the isthmus epithelium (dark blue), and that this maintains their longevity and function. Before ovulation, the sperm detach, possibly as a result of the expression of hyperactivated motility. Detached cells (red) have higher intracellular Ca^{2+} levels and more vigorous motility and find the egg by responding to chemotactic cues (not yet identified in humans). **(c)** When the sperm reach the egg, hyperactivation enables penetration of the surrounding layer of cumulus cells (light blue) embedded in matrix (yellow) and the sperm attaches to the zona pellucida (5). Upon attachment the sperm undergoes the acrosome reaction, in response to binding of the zona pellucida (6), which, combined with hyperactivated motility, permits penetration of the zona (7) and fusion with the egg membrane (oolemma) (8). Adapted from [12].

time to have adverse reproductive consequences, such as reduced success at ART. However, surprisingly little is known about the histone fraction, which was previously presumed to be a 'leftover' from remodeling during spermiogenesis (the last stages of spermatogenesis when the cell is transformed from a round haploid cell to a motile cell). Recent data have changed our perception and suggested a bias in gene localization, with the histone-containing regions containing significantly more genes encoding proteins involved in embryogenesis than other regions [9]. In addition, the histones are susceptible to a myriad of potential epigenetic changes through the histone code, the normality of which could easily be disturbed in dysfunctional cells. This is a level of complexity in the sperm cell that has not yet been fully appreciated.

Of critical importance to men with sperm dysfunction is the unambiguous relationship between poor-quality sperm and high levels of DNA damage in the cells. This has minimal relevance for natural conception as damaged cells are unable to fertilize, but with ICSI cells with high levels of DNA damage are often the only ones available and are thus used for injection. Clinical data from many ART programs using a variety of DNA-damage assays show a strong negative correlation between DNA damage in the sperm and rates of embryo development and implantation and, importantly, a positive correlation with levels of miscarriage [10]. However, the longer term consequences of using damaged cells, such as the health of offspring, are unknown. Data from mice suggest that caution is needed, as using DNA-damaged sperm for ICSI was associated with pathological changes in the resultant adult offspring, such as changes in behavior, higher incidence of cancer and premature aging [11].

We are only at the beginning of our understanding of sperm chromatin and DNA and, when there is damage, we do not know the point in the lifecycle of the cell at which the damage originates, its causes (for example whether it is oxidative in nature) or the nature of the damage (for example, single- and/or double-strand breaks and/or DNA crosslinking). Other basic questions remain unanswered.

Does the origin and nature of the damage suggest less or more severe consequences? Can the egg repair the damage and, if so, is there a threshold of damage above which it can no longer do so? This is an unusual field in which the clinical data are relatively clear and consistent but have yet to be accompanied with high quality scientific studies.

Searching for the egg

Sperm motility is essential for natural fertility, and one key way to understand the cellular and molecular mechanism of motility is to document the response of the individual cell during chemotaxis (Figure 2). This process, once thought to be the preserve of sea urchins and star fish, has now been convincingly demonstrated in humans using both artificial factors and egg-derived ones, such as progesterone. With the advent of high-speed single-cell imaging combined with controlled uncaging of fluorescent molecules, the complex kinetics and signaling systems involved in sperm motility are now beginning to be explored. In humans, although cAMP, cGMP and calcium are known to be important regulatory signals, the timing of events is elusive. It is unlikely to be as simple as an increase in cellular calcium resulting in a change in direction following increased flagellar asymmetry. Potentially, the development of hyperactivation (an excited erratic movement of the sperm cell associated with capacitation) may be a good model for studying the calcium response of cells [12], particularly the involvement of the release of calcium from stores and its association with pH-sensitive calcium channels (CatSper channels). This is clinically relevant, as failure to undergo hyperactivation is significantly associated with reduced fertilization success.

A fascinating aspect of internal fertilization in humans is that sperm operate in a highly complex, mucus-based environment, with different viscoelastic properties and fluid mechanics from those of the simple media that are universally used to study motility. For example, sperm preferentially swim along surfaces and thus might crawl, rather than swim, up the female reproductive tract. As a result, experiments modeling and examining sperm behavior in these physiologically relevant environments are essential for obtaining an accurate analysis, as recently demonstrated by Smith and colleagues [13].

In summary, there is an urgent need to develop a more detailed understanding of the physiological, biochemical and molecular functioning of the human sperm cell. We can use this knowledge as a platform to improve the diagnosis of male infertility with, for example, robust biomarkers and the development of non-ART-based therapies. The tools at our disposal have never been more accessible, powerful and sophisticated, so, if they are used wisely, it is likely that rapid progress will be made in the very near future. Perhaps then a structure such as the annulus – which has been known for over 100 years – will reveal its secrets.

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