# Harnessing the biology of the oviduct for the benefit of artificial insemination

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Spermatozoa fulfil a single role, namely achieving syngamy by transporting the haploid genome to their counterpart gamete, the oocyte. Simple as this may seem, it is fraught with many difficulties, especially in the face of biological processes that enable females to select spermatozoa after they have mated multiply with several males. Conversely, the female reproductive tract sequesters a privileged sperm subpopulation in the oviductal isthmus for variable periods of time, releasing them when the time is opportune for fertilisation. Recent studies of sperm transport in the female reproductive tract suggest that these phenomena involve signalling dialogues between spermatozoa and the female reproductive tract environment. Opportunities for mutual signalling are immense but have received relatively little attention. The oviduct is an organ of crucial significance in modulating sperm function and may be one of the most important sites for determining many aspects of sperm selection and competition. The oviductal environment possesses the potential for enhancing sperm survival, suppressing and activating sperm motility as required, and responds to the arrival of spermatozoa by producing novel proteins. While the biological nature of the sperm-oviduct dialogue is interesting for its own sake, the mechanisms that govern these processes offer opportunities for the improvement of artificial insemination procedures. If oviductal proteins enhance sperm survival, they offer opportunities for the development of long-life semen diluents. Conversely, if we understood the basis of sperm selection we may be able to concentrate on identifying and using only the best sperm subpopulations for improved animal breeding efficiency.

## Introduction

Artificial insemination (AI) is now a fundamental technology for breeding domestic animals, in human infertility treatments and in wildlife conservation programmes for breeding threatened species. The agricultural application of AI is mainly focused upon the management and im-

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provement of breed genetics, where traits that are identified as desirable can be efficiently distributed through the use of diluted and/or frozen semen. However, current methods for short and long term semen preservation severely compromise the sperm's survival, both in the extenders and in the female reproductive tract, and hence limit the successful application of the technique.

Protection and maintenance of spermatozoa is a natural function of the mammalian oviduct and it would seem obvious that elucidating the mechanisms involved might help identify components or functions of the oviduct that could usefully be harnessed for semen preservation. Of course, the oviduct is also tasked with preparing spermatozoa for fertilisation, a destabilising process which might be expected to enhance sperm death through premature capacitation and acrosome reactions. Under natural conditions this degenerative outcome tends to be suppressed until an appropriate time for fertilisation, implying that the oviduct exerts subtle control over sperm function and achieves a balance between storage and stability on the one hand and activation on the other. We are still ignorant of the way in which this is achieved, even though the topic has been studied for many years. This has partly been due to the difficulty of developing appropriate laboratory models and *in vitro* approaches; it is inevitable that such model systems are relatively poor substitutes for the real *in vivo* oviduct, and it has generally been the case that logically directed studies of a living system have been unable to monitor multiple cellular events and processes simultaneously. Modern high throughput techniques in cell biology, especially genomics and proteomics, are now changing the way such studies can be undertaken and therefore we expect to see a transformation of this field in the very near future.

Given the developing status of this field, the aims of this review will be to examine our current knowledge of the way in which spermatozoa and oviduct mutually interact and to provide a critical appraisal of the investigative techniques typically employed in these investigations.

## Biological background

Sperm storage in the female reproductive tract has been extensively documented in a range of vertebrates that includes sharks (Pratt and Tanaka, 1994), salamanders (Sever and Brizzi, 1998) snakes and turtles (Gist and Jones, 1987; Galbraith 1993) as well as many birds and mammals. Some of these species store spermatozoa for several months or even years. The evolutionary development of this remarkable ability provides an interesting parallel to the aims and intentions of biotechnologists who wish to extend the lifespan of spermatozoa, either in a bottle before insemination or within the female reproductive tract after insemination. In both instances the motivation is to uncouple the synchrony between insemination and ovulation. If afforded this capability, animal breeders would be able to inseminate females without worrying too much about accuracy of ovulation prediction, or at the very least would be able to relax the synchrony by several hours without compromising fertility.

In birds, spermatozoa are stored in sperm storage tubules (Bakst, 1993; Birkhead, 1998; Bakst and Vinyard, 2002) and can remain there for several weeks (6-42 days). Prior to fertilisation the spermatozoa relocate to the infundibular region of the oviduct in readiness to meet newly ovulated eggs. Some reptiles store spermatozoa for very long periods indeed; estimates range from several months to several years (Galbraith, 1993). Although these long storage periods allow the females to retain spermatozoa from one season to another, it is also of relevance that some species have succeeded in dissociating the need for synchrony between ovulation and mating. Sperm storage occurs in several different regions within the female reproductive tract,

ranging from sacs in the infundibular region of the oviduct to crypts in the mucosa of the uterovaginal junction (Gist and Congdon, 1998). Some of these sperm storage regions are ducts of oviductal glands or crypt-like extensions of the oviductal epithelium at the base of mucosal folds (Gist and Jones, 1987; Almeida-Santos *et al.*, 2004). Use of modern genetic markers has confirmed that stored spermatozoa are indeed used for fertilisation, even though other matings may have occurred during the intervening period (Pearse *et al.*, 2001).

Although sperm storage in these species has been described extensively, and its adaptive significance interpreted, few studies have addressed the physiological mechanisms that may be involved. Breque *et al.* (2003) observed that avian semen contains a cocktail of substances, including high concentrations of vitamins C and E, plus glutathione, which could protect spermatozoa by inhibiting lipid peroxidation. These authors presented evidence that the sperm storage sites in female birds possess intrinsic antioxidant capabilities owing to elevated activities of glutathione peroxidase and superoxide dismutase. They supported their views by arguing that sperm storage and fertilising abilities can also be improved by supplementation of the diet with extra vitamin E and selenium. A slightly different perspective was provided by Zaniboni and Bakst, (2004) who demonstrated the presence of aquaporins in avian sperm storage tubules; as these proteins form water channels in cell membranes, these authors proposed that active water exchange between spermatozoa and the storage gland environment might help in the maintenance of sperm viability.

Mammals are also capable of sperm storage in the female reproductive tract, although for shorter periods of time. Sperm storage is particularly well documented in some bat species. Matings take place in the late summer and spermatozoa are stored until fertilisation occurs several months later during the following spring (Racey, 1979; Racey *et al*, 1987; Racey and Potts, 1970). Morphological observations of sperm disposition within the bat female reproductive tract have revealed that direct contact is frequently established between the uterine epithelium and the sperm head plasma membrane (Wimsatt *et al.*, 1966; Sharifi *et al.*, 2004). These are such intimate contacts that they resemble the interactions between sperm heads and the Sertoli cells of the testis. Nevertheless, in a uterus distended with spermatozoa, the population bound to epithelial cells may represent only a minority of the cells actually present. This casts some doubt on whether the interaction with epithelial cells is essential, or whether the cell-cell interactions are dynamically unstable thus allowing all spermatozoa to reside at the epithelial surface for short periods of time.

Many other mammalian species are able to store spermatozoa, although for periods of days or weeks rather than months. Pioneering experimental insemination studies by Hammond and Asdell (1926) demonstrated that rabbit spermatozoa are capable of surviving in the female reproductive tract for 20-30 hours; for these experiments, insemination was performed without inducing ovulation. A short period of sperm storage in the isthmus region of the oviduct has since been noted in many species (e.g. sheep, Hunter and Nichol, 1983; cow, Lefebvre *et al.*, 1995; pig, Hunter *et al.*, 1984; mare, Boyle *et al.*, 1987; several rodents and marsupials; Taggart and Temple-Smith, 1991; Bedford and Breed, 1994; Esponda and Moreno, 1998).

Although various bat species have adopted their own idiosyncratic means of achieving sperm storage, the other mammals seem to have adopted broadly similar strategies for achieving this effect. Spermatozoa reach the oviductal isthmus having negotiated their way through the uterotubal junction, whereupon they become associated with epithelial cells and a sperm reservoir is formed (for reviews, see Suarez, 1998; Topfer-Petersen *et al.*, 2002). There is some dispute as to the nature of this association; while some authors have reported the establishment of specific sperm-epithelial binding interactions mediated via oligosaccharide groups, others consider that spermatozoa merely become entrapped in oviductal secretions. Prior to ovulation, sperm transport is also physically inhibited; Hunter (2002) commented that viscous glycoproteins form a

mucus-like plug which almost blocks the distal portion of the isthmus and probably serves several functions, especially the prevention of uterine and ampullary fluids from reaching the sperm reservoir.

The formation of the oviductal sperm reservoir in mammals seems to be important in maintaining sperm viability for a few hours or days. However, unlike the bats, other mammals do not seem to have developed elaborate and semi-permanent sperm-epithelial membrane specialisations, although contact between spermatozoa and epithelial surfaces sometimes, but not always, in crypts, does indeed occur. The nature of the storage mechanism in mammals and other taxa has so far remained elusive; however, the simple physical resemblance between sperm-oviduct interactions in mammals and some of the amphibians and reptiles provides encouragement that eventually general underlying principles might be uncovered. Given that spermatozoa lack the cellular machinery required for *de novo* protein synthesis, it seems unlikely that wholesale renewal and replacement of sperm components might occur during the storage period. However, in the absence of such replacement the spermatozoa must be vulnerable to damage and degeneration, possibly from oxidative metabolic processes and their products. It therefore seems a reasonable working hypothesis that the important mechanisms may focus on reducing these deleterious effects. Moreover, because the oviductal environment must be largely under epithelial control, it follows that studying the physiology and biochemistry of the epithelial cells must be a sensible starting point for investigating the mechanisms of sperm storage.

Epithelial cell activities may affect closely associated cells in many ways. Not only do they secrete newly synthesised proteins and small peptides into the fluid environment, but they control the ionic composition of the fluid. This is achieved by balancing fluid uptake and export as well as by manipulating ion transport across the apical epithelial cell plasma membrane. Furthermore, while some of the proteins will be soluble in tubal fluid others will become exposed at the epithelial cell surface and will only make direct contact with spermatozoa if they are in close apposition. It is feasible to propose that some of these membrane proteins may participate in cell-cell communication, acting to modulate aspects of sperm metabolism and function. A considerable amount of research has already been undertaken in many of these areas and the interested reader should consult a wealth of previous reviews in this topic (for example: Hunter, 2001; Rodriguez-Martinez *et al.*, 2001; Killian, 2004; Rodriguez-Martinez *et al.*, 2005).

## Methodological approaches to sperm-oviduct studies

Several different approaches to the physiological study of sperm-oviduct interactions have been pursued. Given that the interaction is difficult to study *in situ*, any *in vitro* system will possess strengths and weaknesses. It is worth reviewing these briefly as context against which to judge current progress. Some groups have developed methods for obtaining oviduct secretions directly from the *in situ* oviduct by the use of indwelling catheters (for review, see Killian, 2004) and have both analysed the constituent composition of these fluids and tested their effects on fertilisation and embryonic development. An obvious advantage of this approach is that the nature of the secretions can be correlated accurately with the stage of the reproductive cycle. Achieving this precision of control by *in vitro* methodology is considerably more problematic. Others have adopted a different approach, namely that of culturing oviductal cells *in vitro*, and then either observing how spermatozoa interact with the cells (Pacey *et al.*, 1995; Fazeli *et al.*, 1997, 1999; Petrunkina *et al.*, 2001) or recovering conditioned culture medium for testing sperm responses (Kouba *et al.*, 2000). The considerable variety of culture systems employed in

such studies poses some problems for the interpretation of data. Different systems vary in sophistication; some researchers have used confluent monolayer cultures of isolated oviductal epithelial cells (OEC) while others have been at pains to develop more sophisticated systems in which the epithelial cells retain their natural polarity (Leese *et al.*, 2001). Still others have argued that direct contact between spermatozoa and the oviductal surface is critical in modulating sperm survival and that this is therefore the interaction of greatest interest (Smith and Nothnick, 1997; Elliott *et al.*, 2001a,b; Fazeli *et al.*, 2003).

This disparity of approaches has produced a massive amount of complementary but somewhat confusing evidence about ways in which sperm physiology is controlled by the oviduct. Examination of the literature reveals not only species differences in the way that spermatozoa respond, but also conflicting views on whether capacitation is enhanced or retarded, motility suppressed or activated, and even dispute about whether sperm actually bind to the oviductal surface or not. We have favoured the investigation of mechanisms that involve direct contact, partly because it allows some simplification of the experimental approach. A few comments about interpreting these data may be useful at this point.

The mammalian uterotubal junction acts as a selective barrier to sperm transport; various different types of study have indicated that spermatozoa must be physically intact (i.e. possess intact plasma membrane and acrosome; Esponda and Moreno, 1998), functionally competent at the molecular level (Nakanishi *et al.*, 2004) and appropriately motile. In the latter context it is important to mention the impressive body of literature derived from studies of the *t*-haplotype mouse (Olds-Clarke and Johnson, 1993; Herrmann *et al.*, 1999). In these mice, specific mutations of the *t*-locus cause defective flagellar function that produces transmission ratio distortion in the next generation. This has been attributed to the relative inability of the defective spermatozoa to enter and traverse the uterotubal junction and reach the oviductal isthmus. Selectivity based on other sperm properties, for example the presence or absence of fertilin-ß (Cho *et al.*, 1998), has also been observed. Such rigorous selection means that spermatozoa in the oviductal reservoir will probably also be optimised for storage within a protected site; as a result of their biochemical and structural integrity they probably produce minimal amounts of deleterious free radicals that might cause degeneration of both themselves and their environment.

As spermatozoa in the oviductal reservoir are thus preselected for their stability and integrity, it is likely that continued stabilisation is all that is necessary for further short-term ($<48$h) storage. Contact between spermatozoa and the oviductal epithelial surface seems to be a constant theme across many mammalian species, from mice (Esponda and Moreno, 1998) to marsupials (Bedford and Breed, 1994), so it is reasonable to believe that physical proximity of plasma membranes is involved in the survival mechanism. Although there is much evidence to suggest that oviductal secretions affect capacitation, and even the incidence of polyspermy at fertilisation (Dubuc and Sirard, 1995; Bureau *et al.*, 2000), these effects may be independent of sperm survival in the isthmus and probably act to control sperm function in the ampulla, where fertilisation actually takes place.

## Direct cell-cell contact and sperm survival

For investigators, the study of direct contact between sperm and oviduct poses a number of problems as well as advantages. The main disadvantage is that attempts to elucidate the biochemical interactions almost inevitably involve deconstruction of the *in vivo* system into what the investigator believes are important component parts; significant uncertainty is associated with the interpretation of data from such studies as we discuss below. From this perspective the

study of oviductal fluid and its interactions with spermatozoa is less problematic as a research method.

A simple but important source of experimental uncertainty that arises whenever an experiment is set up to investigate sperm-oviductal interactions *in vitro* is the quality and preparation of spermatozoa to be used. The investigator should probably aim to prepare a subset of high quality spermatozoa for experimental use, thus mimicking the selective activities of the uterotubal junction. This can be achieved to some extent by using selective methods such as Percoll gradient centrifugation or swim-up. Non-selective methods such as washing in a defined medium are probably inadvisable for such experiments on the grounds that they are unphysiological. The use of swim-up separation is also likely to be physiologically inappropriate for many species because it involves exposing the spermatozoa to media typically designed to induce capacitation. Given that oviductal epithelial cells preferentially bind uncapacitated, rather than capacitated, spermatozoa (Fazeli *et al.*, 1999), this sperm preparation method will tend to result in sperm- oviduct membrane binding interactions that may not be relevant. In this context it is also worthwhile considering that where the number of spermatozoa binding to the oviduct has been estimated by *in vivo* studies, it is surprisingly low in comparison to the numbers inseminated. Mburu *et al.* (1996) estimated that, in pigs, the uterotubal junction and lower isthmus collectively contained roughly 3000 spermatozoa prior to ovulation; Hunter's estimates of 4000-5000 are almost identical (Hunter, 2002). Compared with the number of spermatozoa in a whole ejaculate, around 300 ml of fluid containing 300 million sperm/ml (a total of about 90 x $10^9$), it is easy to see that potentially massive sperm selection pressures are being imposed. As with the mouse studies reported by Esponda and Moreno (1998), Mburu *et al*, (1996) found by scanning electron microscopy that pig spermatozoa in the oviductal isthmus possessed intact plasma membranes and acrosomes, confirming the view that this is a stringently selected population. In the light of this discussion it is apparent that Percoll (or similar) sperm separation is currently the best experimental approach; however, the development of more sophisticated sperm selection methods holds considerable promise for such experiments (for review, see Suh *et al.*, 2003).

Numerous research groups have studied the interaction of spermatozoa with cultured oviduct cells *in vitro*, using monolayers, explants and freely suspended cells (Thomas *et al.*, 1995; Dobrinski *et al.*, 1997; Green *et al.*, 2001; Bosch *et al.*, 2001; Gualtieri *et al.*, 2005). This methodology has demonstrated that the cultured cells retain their ability to enhance sperm survival for periods up to several days and that control cells of non-reproductive origin, e.g. kidney, duodenum and lung, cannot match this ability. These experiments have also shown that the initial sperm binding event is mediated via specific oligosaccharide residues; in pigs, the appropriate carbohydrate recognition molecules are synthesised in the seminal vesicles and prostate and transferred to the sperm surface via seminal plasma (Topfer-Petersen *et al.*, 2002). This initial binding is reminiscent of the sperm-oocyte interaction, where primary and secondary binding events take place. It is feasible that the sperm-oviduct interactions also involve secondary binding in which other receptors and ligands are able to express their functional importance.

Smith and Nothnick (1997) were among the first to demonstrate that direct contact with apical plasma membrane (APM) vesicles prepared from the rabbit oviduct are able to enhance sperm viability *in vitro*. Interestingly, they were also able to show that the effect was a specific property of the oviduct, other types of epithelia being unable to serve as substitutes. Moreover, their experiments demonstrated that the epithelial plasma membrane vesicles alone were sufficient for this effect to occur. *De novo* protein synthesis by epithelial cells can therefore be ruled out as an essential step in the mediation of sperm survival, although other studies have

shown that sperm binding nevertheless induces new gene expression (Thomas *et al.*, 1995; Fazeli *et al.*, 2004).

Careful consideration of Smith and Nothnick's (1997) demonstration that membrane vesicles prepared from the oviduct had the ability to enhance sperm survival suggests that regulatory receptor-ligand interactions are probably being recruited to control sperm function. Spermatozoa are well known to possess myriads of membrane receptors, most whose functions are at best still unclear; this led Meizel (2004) to call them "neurones with a tail". Capacitation and the acrosome reaction have been identified as being promoted by receptor-ligand interactions, so it is conceivable that inhibition of capacitation and of the acrosome reaction are controlled in the same way, and that appropriate ligands are exposed within the isolated membrane fractions. This hypothesis was investigated further by Fazeli *et al.*, (2003) who, in an effort to focus on the membrane proteins in more detail, prepared soluble fractions of porcine APM (sAPM) and showed that these also possessed the ability to prolong the survival of boar spermatozoa *in vitro*. In these studies the sAPM was derived from the membrane vesicles by extraction with a high concentration (1M) of sodium chloride. As this treatment corresponds to the textbook definition of peripheral membrane proteins, being tightly bound to a membrane but not integrated into its structure, these findings support the hypothesis that direct contact with spermatozoa is particularly important in maintaining sperm viability and integrity *in vivo*. Based on these results we are currently examining sperm-membrane protein interactions in more detail to identify the most significant receptors and ligands.

The interactions of bovine spermatozoa with oviductal cell apical plasma membrane components have also been studied in considerable detail by Boilard *et al.*, (2002). These authors, in addition to confirming that the interaction prolongs sperm viability, showed that the isolated membrane fraction was capable of controlling intracellular calcium concentrations and preventing individual spermatozoa from reaching lethal calcium levels. More recently these authors have also shown, by the use of radiolabelled membrane fractions, that the spermatozoa engage in tight binding with several membrane proteins; one of these was identified as a heat shock protein (HSP60) and another as glucose-regulated protein 78 (GRP78). A possible role of heat shock proteins in the enhancement of sperm viability is to be taken seriously as these proteins engage in protein repair processes when cells are exposed to stresses. They have been identified in other studies of the oviduct, and are therefore credible participants in the sperm-oviduct interaction. Nevertheless, the technical considerations outlined above should be taken into account when interpreting these results. The spermatozoa used in the experiments were not selected for integrity or quality, and had in fact been cryopreserved, thus changing their surface characteristics. Furthermore, there is a possibility that the radiolabelled proteins could have originated on the cytoplasmic face of the apical plasma membrane, thus not normally engaging in sperm-oviduct interactions.

## Oviductal regulation of sperm motility and capacitation

Several recent studies have provided evidence that sperm-oviduct interactions result in signal transduction processes that control capacitation. A study by Gualtieri *et al.*, (2005) illustrated two significant aspects of this interaction, namely that when bull spermatozoa are bound to oviductal cell monolayers *in vitro* they exhibit both low intracellular calcium concentrations and low levels of tyrosine phosphorylation of proteins. Elevation of intracellular calcium concentration and tyrosine phosphorylation are two widely recognised indications of capacitation, and so it is also significant that in their study Gualtieri *et al.*, (2005) also showed that sperm release from the oviductal surface involved both processes. Comparable data, derived from

studies of porcine and equine oviductal cells, have been also been reported previously (Dobrinski *et al.*, 1997; Topfer-Petersen *et al.*, 2002).

In similar vein, significant downregulation of another crucial aspect of sperm function, namely motility, has also been reported by a number of authors (e.g.; Overstreet and Cooper, 1975; Grippo *et al.*, 1995). In our laboratory we are currently using soluble APM preparations to see whether this involves specific ligand-receptor interactions. Boar sperm motility is unusually sensitive to environmental effects, especially the presence of bicarbonate, and much has been written about it in relation to fluidisation of the plasma membrane, the rapid elevation of intracellular cAMP concentration (Harrison *et al.*, 1993a,b; Harrison *et al.*, 1996), protein kinase activation (Harrison, 2004) and the way in which boar sperm motility increases rapidly *in vitro* (< 2 min) when bicarbonate is added to suspensions (Holt and Harrison, 2002). At face value this last observation, which is indeed very striking (Fig. 1), appears at variance with reports of motility suppression in the oviduct, where bicarbonate concentration is reportedly rather high (approximately 35mM; Rodriguez-Martinez *et al.*, 2005).

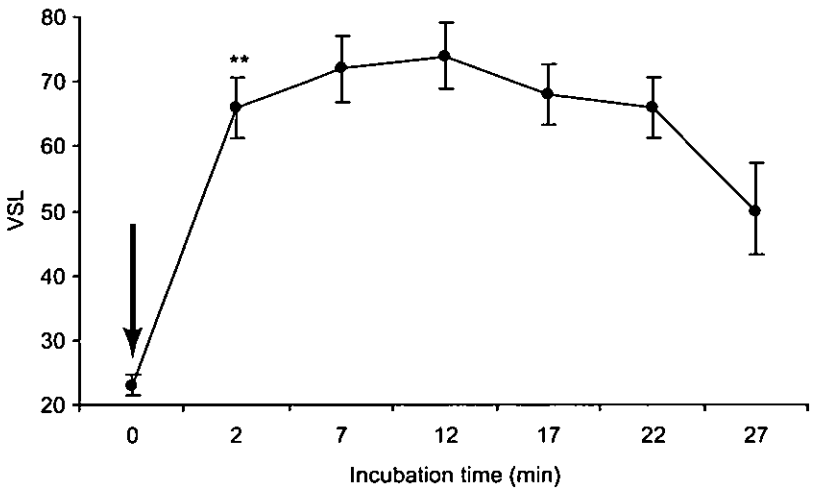


**Fig. 1.** Illustration of bicarbonate-induced increase in linear velocity of boar spermatozoa. Percoll washed spermatozoa from a single ejaculate were pre-incubated in Tyrode's medium (lacking bicarbonate) for 10 min before the addition of 15 mM bicarbonate/$CO_2$. Subsamples were taken for videorecordings of motility shortly before ("arrow") and at intervals after bicarbonate addition. Controls supplemented with appropriate amounts of NaCl were sampled 27 minutes after the addition of 15 mM bicarbonate/$CO_2$ (data not shown). Motility parameter values were obtained by analysis of individual sperm tracks using the Hobson Sperm Tracker (For detailed method, see Holt and Harrison, 2002). The graph shows the mean straight-line velocity (VSL) for all the motile sperm analysed at each time point (200 cells per time point); the bars show the 95% confidence intervals of the means. Significantly increased VSL is apparent 2 minutes after bicarbonate addition but it then reaches a maximum at 12 minutes and velocity declines thereafter (**: different from "zero-time" value, $P < 0.005$).

In keeping with the arguments presented above, we hypothesise that oviductal components can interact with uncapacitated spermatozoa to suppress their motility, despite the presence of bicarbonate. Our preliminary data (Satake *et al.*, 2005) supports this hypothesis, showing that the soluble APM preparation described above is indeed capable of reducing the motility activa-

tion response (Fig. 2). Sperm motility was measured quantitatively using computerised semen assessment technology (CASA), and it is clear that when Percoll-washed boar spermatozoa are stimulated with 15mM bicarbonate/$CO_2$, the inclusion of 100$\mu$g/ml sAPM can reduce the degree of stimulation. The same abstract also describes an accompanying experiment establishing that the effect is not achieved simply by blocking the uptake of bicarbonate by spermatozoa, because intracellular pH became elevated in tandem with incubation time. These results are consistent with the modulation of signal transduction pathway(s), but at present we cannot be certain of the pathways involved.

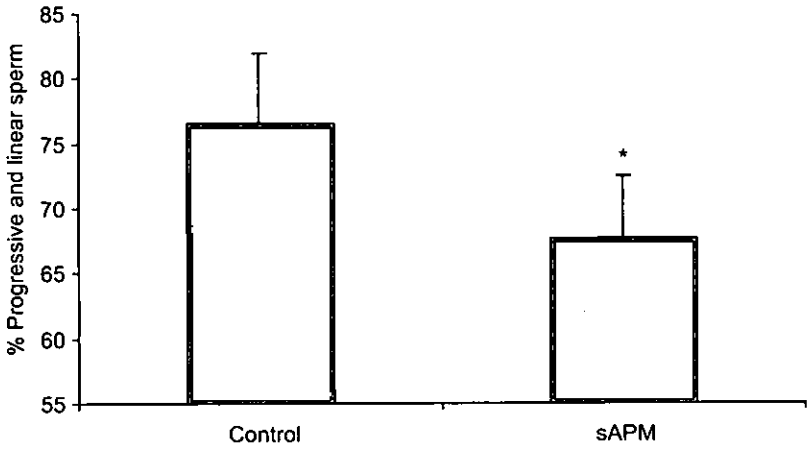


**Fig. 2.** Using the same methodology as described for Figure 1, the stimulation effect of bicarbonate was tested on 9 separate samples of washed boar semen and compared with equivalent effects in paired samples treated with 100 $\mu$g/ml sAPM (solubilised apical plasma membrane fraction; Fazeli *et al.*, 2000). Analysis of the sperm motility response was undertaken using cluster analysis of four kinematic parameters to examine sperm subpopulation responses. Inclusion of sAPM in the experimental medium reduced the bicarbonate-induced sperm activation response, characterised in this graph as the proportion of linear and progressive spermatozoa observed after activation (ANOVA; $F_{1/148}$; $P = 0.024$).

Some recent evidence about the suppressive control of boar sperm motility is, however, pertinent in this respect. Recently Aparicio *et al.* (2005) demonstrated that boar sperm motility can be downregulated via the phosphatidylinositol 3-kinase (PI-3 kinase) signal transduction pathway, which appears to counterbalance activation via the bicarbonate-sensitive, protein kinase A-stimulated pathway. These two pathways, if differentially active in the oviduct, are certainly capable of positively and negatively controlling boar sperm motility.

## Prospects for the exploitation of oviductal function for biotechnology

In the discussion above, we have deliberately concentrated on the natural mechanisms that enhance sperm survival in the oviductal reservoir by turning off a number of their functions. An extensive literature shows that the suppression of sperm function is reversed around the time of ovulation, with the expression of activated and hyperactivated motility, changes in membrane lipid architecture and fluidity, upregulation of protein phosphorylation and even the possibility

that spermatozoa become sensitive to chemotactic signals emanating from oocytes (Eisenbach, 1999). Switching from one state to another is a physiological response, possibly dominated by the endocrine background, but also modulated by the spermatozoa themselves which stimulate *de novo* protein synthesis within the oviduct (Ellington *et al.*, 1993; Thomas *et al.*, 1995; Fazeli *et al.*, 2004).

The oviductal system in mammals appears to offer a variety of opportunities that may be open to exploitation for improving sperm survival and artificial insemination. Most seem to be based around signal transduction activities that are an evident feature of sperm-oviduct interactions. Emerging evidence that sperm metabolism, capacitation and motility can be turned off and on as required, suggests that, in principle, the prospects for long-term sperm survival seem very promising. Combining this fundamental level of control with the use of anti-oxidants, some of which are already known to operate within the oviduct (e.g. catalase; Lapointe *et al.*, 1998), could very well see the extension of *in vitro* sperm lifespan many days beyond that currently achievable. Although this is still rather speculative, it nevertheless represents informed speculation and is more soundly based than the mainly empirical approach to sperm diluent development that has traditionally been employed.

Although oviductal proteins appear to be the important modulators of sperm function, biosecurity and practicality will dictate that for commercial purposes it will not be possible to isolate and use oviductal proteins from slaughtered animals as sources of materials. This has important implications for research, because we must not only determine the nature of the biological processes that control sperm function, we will have to identify the relevant receptors and ligands so that modulators can be synthesised and produced on a large scale. Although we are still at the first stage, advances in proteomics, crystallography and molecular modelling should enable us to progress to the second stage once we have a series of confirmed targets. This is very much the domain of the new "Biotech" industries, which depend on intellectual property rights for survival; further significant progress may therefore lie in the hands of commercial enterprises rather than academic scientists.

Besides seeking ways to extend the lifespan of all spermatozoa, logically we should also imitate the oviduct and try to select the best subpopulation for long-term storage. As a minimum, the oviduct is selecting spermatozoa on the basis of cellular integrity and motility; exclusion of damaged cells may help to minimise the production of free radicals and the release of lytic enzymes from damaged acrosomes. At present, busy centres process semen on an industrial scale and so the introduction of sperm selection procedures may seem highly inconvenient. However, with the gradual introduction of deep intrauterine insemination procedures that require smaller insemination volumes and fewer sperm, selecting high quality sperm may be less of a technical imposition.

## Conclusions

In this review we have demonstrated that aspects of oviductal function concerned with the maintenance of sperm viability are remarkably conserved across a range of vertebrates, and uncouple the synchrony between insemination timing and ovulation. Given the extremely long storage periods accomplished by some species, especially reptiles, it is clear that important mechanisms for the prolongation of sperm viability operate successfully and outperform any semen diluent technologies developed to date. Although insufficient data is available about cell signalling pathways and capacitation in species such as reptiles, analysis of the literature implies that this level of performance is likely to be achieved through cell signalling pathways that tend to prevent both fertilisation and premature cell death. Sperm motility sup-

pression is a common feature of natural sperm storage mechanisms; if there is no immediate requirement for sperm motility then it makes energetic sense to suppress it, together with the associated requirement for substrates. Intuitively this seems to be a sensible policy for the design of long-life semen diluents. Indeed, anyone who examines boar spermatozoa in BTS medium will observe that their motility is somewhat poor. Unfortunately, this principle is poorly understood by some AI practitioners, who mistakenly equate the poor motility with poor quality.

We have not addressed the likelihood that sperm repair mechanisms might also be in operation, but this should not be overlooked. Recent evidence that boar sperm quality may be correlated with the presence of chaperonins and heat shock proteins (Huang *et al.*, 1999, 2000) coupled with the identification of HSP60, one of the oviduct membrane components that bind avidly to spermatozoa (Boilard *et al.*, 2004), provides food for thought. These proteins exist for their protective abilities and it would be unsurprising if they were significant players in the maintenance of sperm viability.

## Acknowledgements

We thank Defra, BBSRC, Genus Breeding Ltd and Sygen International Plc for their financial support.

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