Sperm Storage in the Female Reproductive Tract

William V. Holt* and Alireza Fazeli

Academic Unit of Reproductive and Developmental Medicine, University of Sheffield, Sheffield S10 2SF, United Kingdom; email: Bill.holt@sheffield.ac.uk; A.Fazeli@sheffield.ac.uk

Annu. Rev. Anim. Biosci. 2016. 4:291–310

First published online as a Review in Advance on November 2, 2015

The *Annual Review of Animal Biosciences* is online at animal.annualreviews.org

This article's doi: 10.1146/annurev-animal-021815-111350

*Corresponding author.

Keywords

sperm storage tubules, heat shock proteins, bats, reptiles, birds, mammals

Abstract

The capacity for sperm storage within the female reproductive tract occurs widely across all groups of vertebrate species and is exceptionally well developed in some reptiles (maximum duration seven years) and fishes (maximum duration >1 year). Although there are many reports on both the occurrence of female sperm storage in diverse species and its adaptive benefits, few studies have been directed toward explaining the mechanisms involved. In this article we review recent findings in birds and mammals in an effort to develop hypotheses that could be translated into research applications in animal breeding technologies. There are pockets of evidence to suggest that the local epithelial cells, sometimes arranged as sperm storage tubules, can respond to spermatozoa by producing heat shock proteins as well as providing an environment rich in antioxidants. Moreover, the local immune system seems to tolerate the arrival of spermatozoa, while retaining the ability to combat the arrival of infectious microorganisms.

INTRODUCTION

The capacity for prolonged sperm storage within the female reproductive tract occurs widely across all groups of vertebrate species. With the exception of bats, which can store spermatozoa for several months, most mammals store spermatozoa for shorter periods (2–5 days in many species, but up to 9 and 15 days in dogs and some marsupials, respectively). Some avian species possess more effective sperm storage abilities that can support sperm fertility for several weeks, and some reptiles and fishes have even improved upon this and can store fertile spermatozoa for more than 1–2 years. The adaptive benefits of sperm storage (i.e., why does it happen?) have been discussed previously (1) in the context of sexual selection and social systems, but until recently, relatively few studies have been directed toward understanding and explaining the mechanisms by which the female tract is able to promote sperm survival.

Attempts to store spermatozoa at body temperature in the laboratory, without resorting to cryopreservation, typically reach their limit after two to three days. Nevertheless, diverse species clearly have invented natural and highly effective solutions to this problem, and it is surprising that despite many years of research the scientific community has been unable to match them. If different species have evolved their own idiosyncratic solutions, multiple mechanisms should be waiting to be discovered. However, if there is, in fact, only one simple and common mechanism that is readily accessible to diverse groups of species, it follows that the mechanism should be more easily discoverable in the laboratory. Although we recognize that studies on wild species are usually neither practically nor ethically easy to undertake, there is clearly a huge and largely unexplored field to be investigated. This review aims to examine the comparative literature on sperm storage in nature in an attempt to identify clues about the possible modes of action and thereby suggest potentially useful avenues of research that might translate into novel practical approaches for sperm storage in vitro.

Detailed tables documenting sperm storage abilities in diverse vertebrate species have been published previously (1–3) and therefore are not reproduced here. Instead, we represent roughly the same information in **Figure 1**, where it has been combined with a simplified version of an evolutionary tree showing how the various species groups are interrelated. The figure shows that these relationships are far from straightforward and that sperm storage ability may have been gained, lost, and regained during evolution. That it has not been gained once and then retained permanently by a particular lineage underlines the principle that sperm storage mechanisms should be amenable to elucidation, given the sophisticated analytical tools that are available today.

However, the evolutionary tree itself may provide a few basic clues when the microanatomy of sperm storage is taken into consideration. The divergence of birds and mammals is estimated to have occurred roughly 300 million years ago (4) and predates the divergence of birds and reptiles. It is therefore perhaps instructive to see that sperm storage in the female reproductive tract of birds, some reptiles, and some sharks (5) occurs largely within specialized sperm storage tubules (SSTs); this is in contrast to the situation in mammals, which do not possess SSTs. However, the lack of SSTs in most anuran amphibians [except *Ascaphus truei* (6, 7)] and the possession of spermathecae, an alternative sperm storage structure, in the caecilians (8) are consistent with the earlier divergence of the bird-reptile lineage from the amphibians. Many mammals have managed short-term sperm storage largely without specialized tubules or spermathecae, although sperm storage crypts have been reported in some mammalian groups, especially marsupials and insectivores (9–11). In contrast with the situation in most mammals, some sharks have developed an extraordinary capability whereby spermatozoa can be stored in the female reproductive tract for more than a year (12). This has occasionally caused some confusion because it is also apparent that sharks can reproduce by parthenogenesis in the absence of any male contact (13, 14).

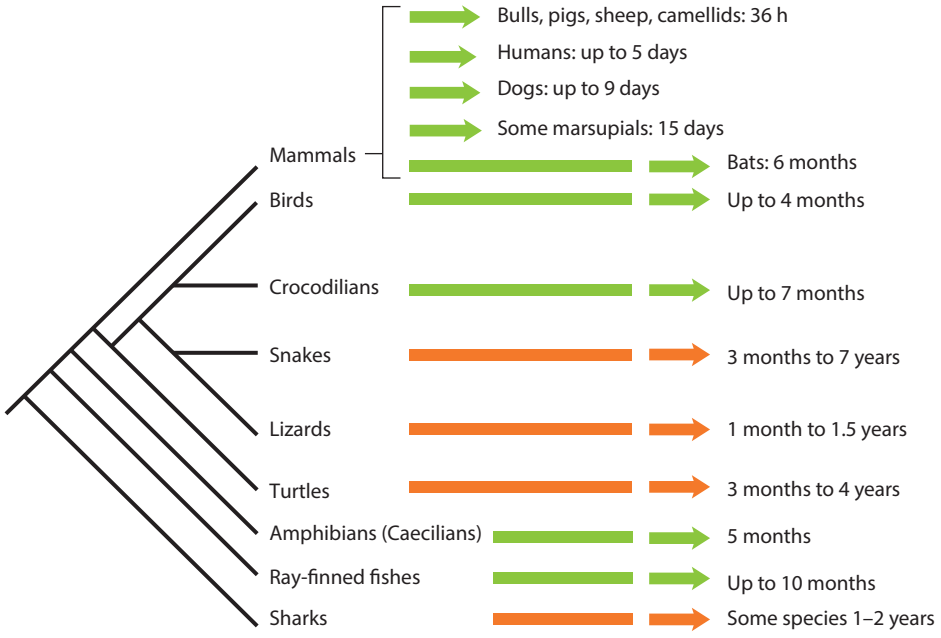

Figure 1

Highly summarized schematic diagram showing evolutionary relationships between groups of species and durations of sperm storage in the female reproductive tract.

Although microanatomical similarities relevant to sperm storage can be discerned between species and clades, sperm storage in the female reproductive tract has clearly developed in a variety of directions. Many species, especially mammals, store spermatozoa in the vagina, cervix, uterus, or oviduct, whereas birds and some reptiles typically possess blind-ended SSTs that maintain sperm viability for variable periods prior to fertilization. These are not hard and fast rules, however. For instance, some marsupials have evolved sperm storage crypts in the uterus and oviducts (15), and some reptiles store spermatozoa in close apposition, but not attached, to epithelial cells (16, 17). Moreover, although we commented upon sharks in general in the paragraph above, it should be noted that, as a group, fishes present a bewildering array of mechanisms. These include direct storage within ovarian follicles (18, 19), which are also the sites of fertilization, and even some mating strategies that involve multiple copulations with immature females and subsequent sperm storage until the females become mature and ovulate several months after mating (20, 21). These observations indicate that whereas explaining sperm storage in terms of microanatomical considerations has some value within related groups of species, it may be unwise to draw unwarranted and oversimplified conclusions from highly divergent groups of species.

Descriptions of sperm storage in the female reproductive tract are almost invariably accompanied by morphological observations showing the spermatozoa within their storage sites. Many of these reports include the application of traditional histological staining techniques in the expectation that they will provide explanatory information about the sperm survival mechanisms. Staining methods such as the periodic acid–Schiff technique, which detects neutral mucopolysaccharides and glycogen; the Alcian blue technique, which detects acidic mucopolysaccharides; and the Sudan Black method for detecting lipids have been used in several histological studies [e.g.,

of bats (22), sharks (5), rattlesnakes (23), and blue mouth rockfish (24)]. Although the epithelial cells typically stain positively with these approaches, the outcome is relatively uninformative because epithelial cells from many sources, e.g., the trachea and intestine, are also known to produce these secretions. Enzyme histochemistry has also been used, and outcomes typically reveal the presence of acid phosphatase in epithelia; once again, this is so general that metabolic inferences are unjustified. Although there is no doubt that the environment provided by these epithelia must actively prolong the life of the spermatozoa, it is unfortunate that the older studies are relatively unhelpful in terms of mechanistic explanation. Ideas about spermatozoa being sustained or nourished within the female reproductive tract are very common in the older literature, and in their time they would have seemed entirely logical. However, more recent advances in sperm biology and physiology would tend to suggest that these views are too simplistic. A more likely scenario is that the female reproductive tract provides a sophisticated environment that interacts with the spermatozoa via signal transduction pathways, possibly reducing their activity and protecting against harmful reactive oxygen species (ROS) and inappropriate immune threats (25, 26).

PRELIMINARY INVESTIGATIONS USING CELL CULTURE

Despite predating the era of proteomics and genomics by several decades, a series of intriguing experiments published by Ashizawa and colleagues between 1976 and 1985 (27–31) showed that fowl sperm survival in vitro at 38°C and 41°C was improved significantly by coculture with a variety of cell types of both reproductive [oviductal, shell gland, utero-vaginal junction (UVJ), and HeLa cells] and nonreproductive BHK-21 (hamster kidney cells) origin. Remarkably, these authors also tested the survival of ram, bull, and boar spermatozoa in the presence and absence of the cultured cells and found that HeLa and BHK-21 cells prolonged the sperm survival two- or threefold (29). Under the same conditions, fowl sperm survival time was considerably longer than that for the mammalian spermatozoa (18–27 h for ram, bull, and boar spermatozoa compared with 120 h for fowl spermatozoa). Subsequent investigations revealed that cultured chick embryonic skeletal muscle cells produce low molecular weight substances (<10 kDa) with the capacity to prolong fowl sperm survival and motility in vitro (31). Parallel studies also showed that diffusible molecules (>12 kDa) present in conditioned media obtained from hen oviductal cell cultures could prolong the motility of chicken spermatozoa in vitro (32), although less effectively than the cultured cells themselves.

Beneficial effects of homologous coculture systems, using epithelial cells and spermatozoa from the same species, were also demonstrated in many studies undertaken in the same era (33, 34), thus establishing the general principle that spermatozoa in culture survive best when in the presence of somatic cells. Ashizawa's studies are of special interest because they indicate a certain degree of flexibility in terms of species specificity between epithelial cells and the support they provide to the spermatozoa. The observation that both HeLa cells, which originated from a human cervical cancer, and hamster kidney cells are able to prolong fowl sperm survival in culture leads to the conclusion that the mechanisms involved may be ancient, predating the evolutionary divergence of mammals and birds. Therefore, the biochemistry and physiology of SSTs in birds and reptiles likely also share functional characteristics.

The advent of genomic and proteomic techniques has meant that more recent studies of sperm storage have been better suited to functional interpretation. Therefore, we focus most of this review on the detailed observations that have emerged in the past 10–15 years in domestic species, drawing upon information from other species groups where appropriate.

SPERM STORAGE IN BIRDS

There is a considerable body of evidence about the transport and fate of spermatozoa in both domestic poultry (35–37) and wild birds (38, 39). After insemination, a selected cohort of high-quality spermatozoa collects within SSTs situated close to the UVJ (for reviews, see References 40 and 41) and can remain in situ for several weeks before they eventually proceed toward the oocytes for fertilization. When the SSTs are dissected and viewed by light microscopy, the live spermatozoa are clearly visible (42). Comparative differences in sperm storage capacity between species and breeds and the duration of storage have been attributed to the relative number of SSTs present in the reproductive tract (43, 44). Experimental studies have shown that spermatozoa from sequential inseminations do not mix but are segregated into different SSTs (37). This effect has been implicated in the operation of sperm competition mechanisms but is only one of many factors influencing relative fertility between individual males (45, 46).

The experiments cited above showing that avian sperm life span could be extended through coculture with various epithelial cell types inferred strongly that the effects must be mediated by somatic cell products. The specialized in vivo organization of epithelial cells into blind-ended tubules would undoubtedly assist sperm storage by confining the diffusible products to the immediate locality, therefore allowing the concentration of these products to be controlled and not wasted. More recent genomic and proteomic studies have allowed some of these diffusible products to be identified and significantly have shown that the colonization of SSTs by spermatozoa induces their upregulation (25, 47–50). The molecules in question have so far been identified as avidin, avidin-related protein-2, progesterone receptor, and transforming growth factor-β (TGF-β) and its receptors. Tentative identification of upregulated molecules in a study of turkey insemination responses employing SAGE [serial analysis of gene expression (50)] included cytoskeletal proteins, metabolic enzymes, membrane transport proteins, and heat shock protein 90 (hsp90). Functionally, these observations point largely toward local immune suppression (TGF-β) and the control of gene expression [progesterone receptors not only modulate steroid function but act in tandem with many signaling pathway components and chaperone proteins, including hsp90 (51)]. In a separate study, insemination in chickens also caused upregulation of interleukin-1β and lipopolysaccharide-induced TNF factor in the avian vagina (47), but explicitly not in the oviduct. Although highly localized immune suppression may be required to prevent destruction of spermatozoa in SSTs, immune function within the oviduct must be maintained to prevent microbial infections. Eleven avian β-defensins have been identified as functionally important within the hen oviduct and vagina (52), and five different subtypes are upregulated in response to the administration of lipopolysaccharide, which is considered to be a surrogate for bacterial infection. Coincidentally, recent investigations of the human reproductive tract have shown that a specific form of β-defensin (DEFB114), which also neutralizes lipopolysaccharide activity, supports the prolongation of human sperm motility in vitro at 37°C (53). Although these are independent and unrelated observations, they suggest that future research into sperm survival might profitably explore the role of defensins.

Although spermatozoa can be stored for prolonged periods in the avian SSTs, they are eventually required to escape the confinement of these narrow tubules and ascend the reproductive tract toward the site of fertilization. Suggested sperm release mechanisms have attempted to accommodate the view that the stored spermatozoa show continuous motility and would therefore always tend to swim forward toward the blind end of the SST (54). This model, proposed by David Froman, suggested that the SSTs maintain a continuous outflow of luminal fluid, and therefore any reduction in sperm motility would sweep them out of the tubules. Other studies have shown that flagellar activity during sperm storage in SSTs is actually reduced (55) but is

then stimulated by the progesterone-induced upregulation of heat shock protein 70 (hsp70) in the peri-ovulatory oviduct (56). The peri-ovulatory period is characterized by increased concentrations of circulating progesterone, which has been identified experimentally as the signal for sperm release (55). Progesterone injections cause sperm release within one hour from the UVJ of laying hens, and the UVJ was shown to be rich in progesterone receptors. The motility stimulation occurring around this time would be helpful not only for escaping the SSTs but also for making progress toward the oocytes for fertilization. Sperm motility control in chicken, turkey, and other avian spermatozoa has been a topic of wide research interest, partly because they exhibit reversible, temperature-dependent motility suppression. These studies show that the motility control is exerted via the interplay of flagellar protein kinases and phosphatases (57–59), interactions with intracellular calcium, and phosphatidyl inositol 3-kinase signaling (60).

Even though theories of sperm-SST interactions no longer need to posit a role for continuous outflow of luminal fluid, active water transport in SSTs seems to be very important, as evidenced by the presence of aquaporins in the epithelial cells lining the SSTs (61). In addition, SST functions such as sperm release are also likely to be controlled via neural factors. Immunohistochemical studies have revealed that, like other regions of the oviduct, they are richly innervated (62) and therefore subject to the influences of smooth muscle contraction and relaxation.

This discussion has summarized a great deal of recent research showing how sperm storage in the avian female reproductive tract is modulated by the dynamic control of gene expression under the influence of the endocrine system. Sperm motility is suppressed during storage and then restored when needed for fertilization, whereas immune function has to be controlled in a highly localized manner. Because sperm membranes are rich in polyunsaturated fatty acids and therefore vulnerable to ROS-induced damage, the reproductive tract has also developed antioxidant systems involving ascorbic acid, glutathione, and superoxide dismutase (63). These interactions are summarized schematically in **Figure 2**. Research into the formulation of new and effective avian sperm diluents for the artificial insemination industry should seek to capitalize on all of this new knowledge.

SPERM STORAGE IN MAMMALS

Influences of the female reproductive tract on sperm storage and functionality have fascinated biologists for many years, especially in the context of sexual selection and sperm competition (64–66). The female reproductive tract appears to act as a gatekeeper that allows the passage of only a small minority of spermatozoa out of the millions that are contained within ejaculates; the criteria for selection are still controversial (67). Regardless of the sperm selection criteria, most female mammals appear to possess an innate ability to collect and store several thousands of the ejaculated spermatozoa for a few hours or days, especially if ejaculation preceded ovulation. Sites of sperm storage in the female reproductive tract are species-dependent and include the vagina, cervix, uterus, utero-tubal junction (UTJ), and oviductal isthmus (for reviews, see References 68 and 69).

ROS are widely recognized as important causes of in vitro–induced sperm damage (70), and there is a long history of adding antioxidants such as glutathione (71), vitamin C (72, 73), and other compounds to sperm extenders in an effort to improve sperm survival. Not surprisingly, oxidative damage is also a potential problem for spermatozoa being stored within the female reproductive tract, and mammals, like birds, apparently have evolved natural antioxidant mechanisms to combat the problem. Superoxide dismutase (74) and glutathione peroxidase (75) are effective antioxidant enzymes expressed in the bovine oviduct. Superoxide dismutase was also found in uterine flushings obtained from female dogs (76) and exerted beneficial effects on sperm survival when incubated in

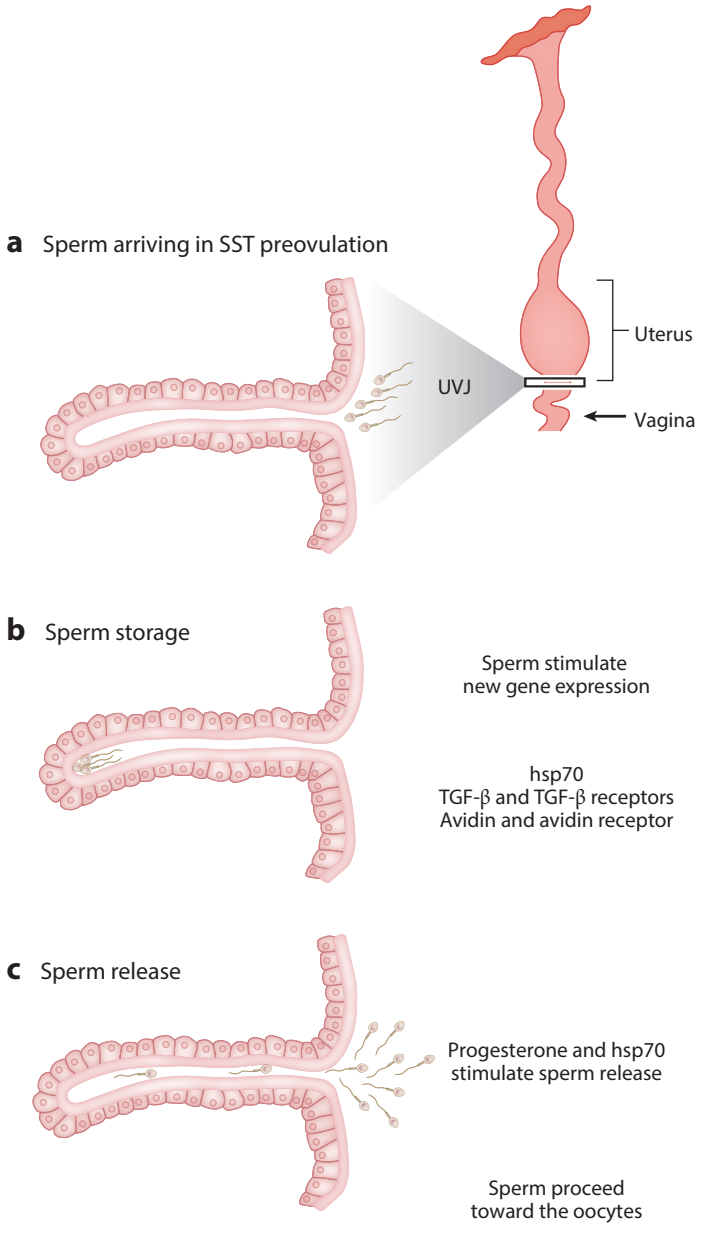

Figure 2

Diagram summarizing the main events that occur during sperm storage in the female reproductive tract of birds. (*a*) After copulation, spermatozoa enter sperm storage tubules (SSTs) located at the utero-vaginal junction (UVJ) of the reproductive tract. These are rich in antioxidants and provide an immunosuppressive environment. (*b*) Spermatozoa stimulate de novo gene expression (TGF-β, TGF-β receptors, progesterone receptor, avidin, avidin-related protein-2, interleukin 1-β, and lipopolysaccharide-induced TNF factor) by SST epithelial cells. (*c*) Sperm release is stimulated by progesterone and heat shock protein 70 (hsp70), whereupon the sperm continue their progress toward the oocyte.

culture media. Similarly, human spermatozoa were protected from ROS-induced damage when incubated with epithelial cell membrane proteins obtained from the Fallopian tube.

Species-specific sperm storage mechanisms have been moulded by anatomical sites of sperm deposition, social mating systems, and physiological diversity, and it is not possible to regard all mammals as a single group. Differences occur even between closely related species; for example, temperate pipistrelle bats can store spermatozoa in the uterus over winter (22) when body temperature falls to approximately 4°C, but the tropical bat *Scotophilus heathii* stores spermatozoa over winter when the body temperature falls only as low as 20–28°C (77). A group of small carnivorous Australian marsupials (*Antechinus* spp.) exhibit an extreme reliance on successful sperm storage for their survival as a species. Mating is followed by the death of all males in the population (78, 79), and the females sequester the spermatozoa in oviductal crypts for a short period prior to ovulation and fertilization. Both female dogs (bitches) (80) and horses (81) can store spermatozoa if mating occurs two to six days prior to ovulation.

The microanatomy of sperm storage reservoirs varies between species, involving the entrapment of spermatozoa by viscous mucus inside the lumen of the reproductive tract, sequestration within deep folds in the oviductal epithelium (82), alignment of sperm heads facing the epithelial surface (22, 83, 84), and/or the capture of spermatozoa within uterine or oviductal crypts (85–87). The spermatozoa often become physically bound to epithelial cells through interactions involving oligosaccharides (88) and/or sulphated glycosaminoglycans (89, 90), and in the extreme case of the little brown bat *Myotis lucifugus*, the sperm heads burrow deep into epithelial cell indentations (91).

Such considerable diversity means that it is far from easy to draw general mechanistic conclusions about sperm storage in the female reproductive tract. However, several investigations of prolonged sperm survival showed that membrane fractions and protein extracts isolated from oviductal epithelial cells (OEC) could support sperm viability in vitro (92–95). Efforts to identify a subset of OEC membrane proteins that bound specifically to the surface of boar spermatozoa (95) demonstrated the presence, among others, of a group of heat shock proteins [hsp70 kDa 1A, hsp90, hspA8 (formerly known as hsc70), and glucose regulated protein 78 (GRP78)]. Other studies focused on bovine sperm-OEC interactions also identified GRP78 and hsp60 for their sperm-binding ability (96, 97). Subsequent experiments on in vitro sperm survival in the presence of recombinant hspA8 showed convincingly that this protein could extend the life spans of boar, bull, and ram spermatozoa under culture conditions and even when added to sperm extenders (93, 98, 99). HspA8 has been shown to exert its effects immediately upon contact with the sperm plasma membrane by increasing membrane fluidity, as measured by fluorescent recovery and photobleaching (99). In this study, the membrane fluidization and protective effects were shown to be (*a*) dependent on the presence of sperm plasma membrane cholesterol, (*b*) reduced by incubation in a capacitating environment, and (*c*) reversibly restored by depleting and then replenishing membrane cholesterol through incubating spermatozoa with cholesterol-loaded cyclodextrins. In addition, sperm exposure to hspA8 induced a decrease in mitochondrial activity.

The heat shock proteins are multifunctional molecules that occur in multiple taxonomic groups and species, including bacteria, plants, and higher vertebrates. Moreover, hspA8 is an exceptionally well-conserved member of the hsp70 family (100). Until relatively recently, it was believed that mammalian heat shock proteins were exclusively intracellular molecules and that they were present only in extracellular compartments in pathological conditions such as necrotic cell death. However, extensive evidence now supports the view that stress proteins can be released under nonpathological conditions and exert protective roles. For example, early research demonstrated the transfer of hspA1A (hsp70) and hspA8 from adjacent glial cells to the squid giant axon (101) and showed that the exogenous hspA1A enhanced the stress tolerance of the neuronal cells. Like other members of the hsp70 family of chaperone proteins, hspA8 interacts with multiple targets

through a mechanism that involves an ATP binding and hydrolysis cycle. Investigation of the binding partners has yet to be undertaken in the context of sperm survival, especially as there are some interesting hints that this would be a profitable line of research. For example, the survival factor B-cell lymphoma factor 2 (Bcl-2), which is upregulated in epithelial cells of the UTJ during sperm storage in the female *S. heathii*, and whose expression has also been detected in mouse embryos (102) and bovine OEC (103), is one of hspA8's molecular binding partners (104).

Because the oviduct is potentially vulnerable to risks of microbial infection, it has developed immune mechanisms to protect itself (105). The spermatozoa represent nonself entities, and so it is reasonable to suppose that they would be challenged and destroyed when they enter the female reproductive tract. However, the situation is more complex than this. The immune system is evidently modulated in favor of spermatozoa; for example, phagocytosis of spermatozoa by polymorphonuclear leukocytes (PMN) in vitro is suppressed by acid glycoprotein-1, which is a major acute phase immunomodulatory protein produced mainly in the liver (106), by a mechanism that reduces superoxide production by PMN in the presence of spermatozoa. The complexity of interactions between spermatozoa and uterine epithelial cells was highlighted in studies of the pig reproductive tract, where it was apparent that a cohort of intact and noncapacitated spermatozoa received protection from phagocytosis by PMN, while damaged, capacitated, and moribund spermatozoa were destroyed (26, 107–109). Sperm interactions with the reproductive tract are also modulated by β-defensins, which are a group of antimicrobial peptides, some of which directly affect sperm function (110).

As discussed above in relation to birds, it is apparent from studies in mares, mice, and pigs that when spermatozoa enter the reproductive tract they induce changes in their own environment, and it is significant that some of these changes are associated with sperm protection and survival. Evidence from interactions between spermatozoa and cultured equine OEC first demonstrated this effect, when the de novo synthesis of proteins was detected (111). Later genomic experiments in mice (112) identified the upregulation of adrenomedullin and prostaglandin endoperoxidase synthase-2 transcripts in response to the arrival of spermatozoa, and proteomic studies of pig oviduct responses to spermatozoa detected the upregulation of approximately 20 proteins in oviductal fluid (113, 114). A significant number of heat shock proteins are among the molecules upregulated by the arrival of spermatozoa in the oviduct; in vitro studies of sperm-OEC interactions showed that hsp90AA1, hspA5, and hspA8 were all upregulated within 3–6 h (115). One important finding from this particular study was the requirement for physical contact between spermatozoa and OEC. Upregulation of heat shock proteins was not induced if the spermatozoa were prevented direct access to the OEC surface, which might explain the commonly observed regular alignment of sperm heads against the oviductal epithelium in many mammalian species.

The direct interaction of spermatozoa and OEC has also been shown to reduce intracellular calcium concentrations in spermatozoa (86, 116, 117), thus helping to prevent capacitation and the premature acrosome reaction, both of which curtail sperm survival. As with bird spermatozoa in SSTs, mammalian sperm motility tends to be downregulated within the oviductal environment. Overstreet & Cooper (118) first observed this in rabbits, attributing the effect to high local concentrations of potassium, but more recent research suggests that interactions with OEC modulate the action of sperm flagellae through multiple signaling pathways involving adenylyl cyclase, the control of protein phosphorylation, and phosphatidyl inositol 3-kinase signaling (119–121). When porcine oviductal fluid collected from follicular phase reproductive tracts was separated into two fractions (> and <100 kDa), the lower molecular weight fraction inhibited the expected sperm motility stimulation (122) normally elicited by bicarbonate (123). This was consistent with other studies showing inhibition of bicarbonate-induced boar sperm motility stimulation by bovine recombinant hspA8 (124) and a soluble protein fraction derived from the apical plasma

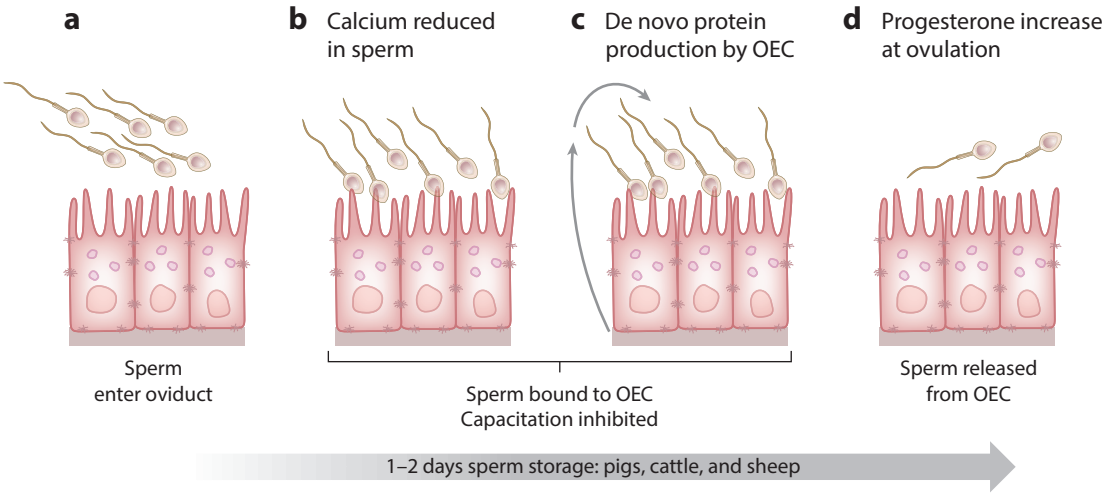

Figure 3

Diagram summarizing the main events that occur during oviductal sperm storage in pigs, cattle, and sheep. (*a*) Spermatozoa enter the oviductal isthmus via the utero-tubal junction. (*b*) Some spermatozoa bind to oviductal epithelial cell (OEC) surfaces, whereupon their motility and intracellular calcium concentrations are reduced; capacitation is also inhibited. (*c*) The direct contact between spermatozoa and epithelial cells induces de novo gene expression and protein synthesis. Multiple proteins, including heat shock proteins, are secreted into the oviductal lumen, where they protect sperm membranes and facilitate sperm storage. (*d*) Increased peri-ovulatory progesterone production induces spermatozoa to escape and resume their progress toward the oocyte(s).

membranes of OEC (125). These results are significant because oviductal fluid is so rich (39–90 mM) in bicarbonate (126) that the spermatozoa would normally be expected to become capacitated and hyperactivated rather quickly, thus shortening their life span unless appropriate counteractive influences existed. Oviductal fluid in mammals contains a highly complex mixture of proteins that affect sperm survival, capacitation, and behavior (127), and studying individual components, although informative, may not truly represent the situation in vivo. For example, although oviduct-specific glycoprotein, also known as oviductin, stimulates both the acrosome reaction (128) and sperm-oocyte binding, it has also been shown to reduce sperm motility and has even been regarded as a functional mediator of negative sperm selection (129). A detailed discussion of the individual oviductal proteins involved in sperm-oviduct interactions is outside the scope of this review, and the reader is directed toward other recent reviews that have addressed this issue (89, 130–132). Some of the significant aspects of sperm storage in domestic mammals are summarized in **Figure 3**.

The dynamic interplay between oviductal cell secretions, sperm-OEC binding, ionic composition, and the changing circulatory steroid concentrations mediated by the ovaries creates multiple opportunities for the subtle control of sperm behavior. There is considerable evidence that sperm storage within the mammalian reproductive tract is not merely an adaptation that helps to optimize the chances of sperm-egg interactions but is involved in sperm selection (reviewed in References 67 and 133). Ejaculated semen contains cohorts or subpopulations of spermatozoa that vary in many respects, ranging from shape and size to DNA fragmentation status and responsiveness to signaling molecules. The controlled storage and release of such sperm subpopulations over different time frames may therefore be critically important in the determination of skewed paternity outcomes following inseminations with mixed semen samples. Such situations are well known from studies of sperm competition (134), cryptic female choice (135), and heterospermic insemination (136, 137).

Sperm Storage in the Bat, *Scotophilus heathii*

In their review of reproductive delays, Orr & Zuk (134) identified 24 bat species that have been reported to store spermatozoa for periods of <30 to 225 days; interestingly, their earliest citations date from the 1930s and 1940s. Unfortunately, however, detailed mechanistic studies of delayed ovulation and sperm survival have been carried out in only a single species, the greater Asiatic yellow bat (*S. heathii*). The insights gained from an extended research program on this tropical bat are of significant interest, although it is recognized that they may not represent a universal mechanism adopted by all bats that exhibit sperm storage within the female tract.

In *S. heathii*, spermatozoa are stored in the uterus, UTJ, and oviducts after mating in early winter until ovulation takes place in late February or early March (138, 139). Prior to the sperm storage period, the females increase their body mass by 150%, mainly owing to heavy accumulation of white adipose tissue (140), and subsequently the ovaries produce high levels of the circulatory androgens androstenedione and testosterone (141). These effects are believed to be responsible for suppressing ovulation. In conjunction with the high androgen levels, the oviductal epithelia express androgen receptors and androgen binding protein (142). The high androgen levels are stimulated by enhanced insulin production by the ovarian thecal and interstitial cells (143) and are accompanied by a decline in blood glucose and carnitine (77). The expression of glucose transporters (GLUT3 and GLUT5) at the UTJ also declines during the sperm storage period, but the concentration of carnitine and the expression of carnitine transporter (OCTN2) and hormone-sensitive lipase at the UTJ increase (77, 144). These results suggest that sperm storage is enhanced by decreasing the local glucose concentration and increasing the amount of free fatty acids.

The same research group (145) also showed that during sperm storage, the UTJ expresses Bcl-2, which, together with a family of related cytoplasmic proteins, is a key regulator of apoptosis (for reviews see References 146 and 147). In the context of sperm storage mechanisms, the Bcl-2 would be acting as a survival factor with antiapoptotic activity. Moreover, the Bcl-2 expression is testosterone dependent and was reduced by the experimental administration of antiandrogen. The same authors also showed that the UTJ expresses caspase 3, a proapoptotic factor (148), and observed modulation of gene expression in relation to the sperm storage events.

The impressive body of work on sperm storage in *S. heathii* is an excellent example of the benefits obtained by carrying out wide-ranging and integrated reproductive studies in a particular species. The main lesson from this work is that instead of sperm storage mechanisms being viewed as localized modifications within a specific region of the reproductive tract, the results emphasize that the entire physiology of the female is involved. The pituitary and hypothalamus are classically known to be involved in controlling responses to seasonality, and in this species the responses include ovarian function, control of appetite, control of hibernation, and control of adipose tissue function. It is not yet clear whether these adaptations have also been adopted by other bats, or even by other sperm-storing taxonomic groups, such as reptiles; we must await the generation of further research data.

CONCLUSIONS

What can we learn from comparative studies of sperm storage mechanisms? The most valuable clues have come from studies of birds and a few mammals in which immune suppression, the presence of antioxidant systems, and metabolic suppression are important. The de novo induction and synthesis of heat shock proteins during sperm storage are now known to favor sperm survival in mammals, but although this is probably also true in other taxonomic groups, there is insufficient evidence to support this view. Reduced temperature, which is the cornerstone of sperm storage in the artificial insemination industry, must be less important than we think because in vivo sperm

storage often takes place at physiological temperatures. In terms of biotechnology, prolonging sperm function is becoming increasingly important as new breeding paradigms emerge. Sperm sexing by flow sorting produces two sperm populations of limited size, bearing the X and Y chromosomes, respectively. These samples can be maintained at ambient temperature for a matter of hours or can be cryopreserved if long-term storage is needed. Flow sorting currently has a relatively low throughput, but as this technology becomes more efficient and larger sperm samples are produced, the wide geographical distribution of nonfrozen samples will become increasingly important. Keeping the spermatozoa alive and functional without subjecting them to cooling and cold shock would allow breeding companies to trade over longer distances, even when using small sperm numbers. The use of artificial insemination as an adjunct to conservation breeding programs would also benefit from advances in such technologies, especially with respect to species for which there is currently no reliable (or even unreliable) sperm cryopreservation method [e.g., wallabies and koalas (149, 150)].

SUMMARY POINTS

1. Sperm storage in the female reproductive tract is widespread across many taxonomic groups, including insects, fishes, amphibians, reptiles, birds, and mammals. However, in some groups of species the ability to store spermatozoa appears to have evolved, disappeared, and then been regained. This suggests that, in principle, the underlying processes actually exist in most species but must be coordinated appropriately for sperm survival to be enhanced.
2. Some birds and reptiles have evolved microanatomical adaptations of the female reproductive tract known as sperm storage tubules. These are microscopic, blind-ended, epithelial-cell-lined tubules that are large enough to accommodate groups of spermatozoa. The spermatozoa are usually arranged inside the tubules with their heads pointing toward the blind end, and they remain in place between the time of copulation and the peri-ovulatory period. The sperm storage tubules are rich in antioxidants that would assist with prolonged sperm survival.
3. Some studies have shown that sperm release from the sperm storage tubules coincides with increased progesterone production around the time of ovulation. Sperm mobilization is also stimulated by the production of heat shock protein 70 around the time of ovulation.
4. Significantly, when the spermatozoa reach the sperm storage tubules they stimulate de novo gene expression (observed through a genomic study in turkeys). The nature of the expressed genes is still poorly known but includes avidin, which is a biotin-binding protein found in egg white.
5. Most mammals do not store spermatozoa for very long periods, but many species of bat have nevertheless evolved this ability. Bat spermatozoa are not stored within sperm storage tubules, but many become regularly arranged within the uterus, utero-tubal junction, and oviducts, with their heads in close apposition to epithelial cells.
6. The mechanism of sperm storage has been studied in detail in one tropical bat species (*Scotophilus heathii*). Delayed ovulation over winter is accompanied by high androgen levels, and the epithelial cells adjacent to the stored spermatozoa express the Bcl-2 gene, which is known to enhance cell survival and inhibit apoptosis.

7. Spermatozoa from other mammalian species also exhibit the intimate sperm–epithelial cell apposition, and a few species have also evolved specialized epithelial crypts that sequester the spermatozoa. In vivo studies in mice and in vitro studies of equine and porcine oviductal cells have shown that interaction with spermatozoa stimulates de novo expression of multiple genes, including several heat shock proteins.
8. Experimental studies have demonstrated that one particular 70-kDa heat shock protein (hspA8) can be used to enhance sperm survival in vitro, even when added to semen diluents at low (μg/ml) concentrations.

FUTURE ISSUES

1. The data gleaned thus far from studies of birds and mammals offer complementary clues about the mechanisms of sperm survival that future researchers could exploit. For example, de novo gene expression in response to spermatozoa occurs in both groups, but although the outcomes in mammals have identified the importance of heat shock proteins, there is very limited information about heat shock protein production by the sperm storage tubules.
2. Understanding of the significance of heat shock proteins in mammalian sperm survival is still at an early stage. Given the complex roles and interactions of these multifunctional chaperone proteins, considerable technological and practical benefits likely would accrue from testing sperm survival responses to combinations of heat shock proteins and their binding partners.
3. Understanding the innate immune system in greater detail would be a fruitful direction for future research. It is apparent that spermatozoa are treated as privileged cells in an environment that is highly adapted to combat invasions of microorganisms.
4. Modulation of the immune response so that spermatozoa are destroyed instead of being maintained would also be useful for the development of new approaches to contraception and pest control.
5. Although it is technically and ethically difficult to study the molecular and physiological mechanisms of sperm storage in wild species, such as snakes, crocodiles, sharks, and turtles, some of these species would provide invaluable clues. Although research opportunities are mostly opportunistic, it is feasible to use modern immunohistochemical methods for the localization of key proteins and peptides in thin sections from formalin-fixed tissues. The main obstacle here is the establishment of good working relationships with biologists who work regularly with such species.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

1. This review focuses on the adaptive significance of sperm storage and includes detailed tables of sperm storage data across different taxonomic groups.

1. **Birkhead TR, Møller AP. 1993. Sexual selection and the temporal separation of reproductive events: sperm storage data from reptiles, birds and mammals. *Biol. J. Linn. Soc.* 50:295–311**
2. Holt WV, Lloyd RE. 2010. Sperm storage in the vertebrate female reproductive tract: How does it work so well? *Theriogenology* 73:713–22
3. Howarth B Jr. 1974. Sperm storage: as a function of the female reproductive tract. In *The Oviduct and Its Functions*, ed. AD Johnson, CW Foley, pp. 237–70. New York: Academic
4. Hedges SB, Dudley J, Kumar S. 2006. TimeTree: a public knowledge-base of divergence times among organisms. *Bioinformatics* 22:2971–72
5. Storrie MT, Walker TI, Laurenson LJ, Hamlett WC. 2008. Microscopic organization of the sperm storage tubules in the oviducal gland of the female gummy shark (*Mustelus antarcticus*), with observations on sperm distribution and storage. *J. Morphol.* 269:1308–24
6. Sever DM. 2002. Female sperm storage in amphibians. *J. Exp. Zool.* 292:165–79
7. Sever DM, Moriarty EC, Rania LC, Hamlett WC. 2001. Sperm storage in the oviduct of the internal fertilizing frog *Ascaphus truei*. *J. Morphol.* 248:1–21
8. Kuehnel S, Kupfer A. 2012. Sperm storage in caecilian amphibians. *Front. Zool.* 9:12
9. Bedford JM, Mock OB, Phillips DM. 1997. Unusual ampullary sperm crypts, and behavior and role of the cumulus oophorus, in the oviduct of the least shrew, *Cryptotis parva*. *Biol. Reprod.* 56:1255–67
10. Bedford JM, Cooper GW, Phillips DM, Dryden GL. 1994. Distinctive features of the gametes and reproductive tracts of the Asian musk shrew, *Suncus murinus*. *Biol. Reprod.* 50:820–34
11. Bedford JM, Breed WG. 1994. Regulated storage and subsequent transformation of spermatozoa in the Fallopian tubes of an Australian marsupial, *Sminthopsis crassicaudata*. *Biol. Reprod.* 50:845–54
12. Pratt HL Jr. 1993. The storage of spermatozoa in the oviducal glands of western North Atlantic sharks. *Environ. Biol. Fishes* 38:139–49
13. Edwards RG. 2007. The significance of parthenogenetic virgin mothers in bonnethead sharks and mice. *Reprod. Biomed. Online* 15:12–15
14. Chapman DD, Shivji MS, Louis E, Sommer J, Fletcher H, Prodohl PA. 2007. Virgin birth in a hammerhead shark. *Biol. Lett.* 3:425–27
15. Taggart DA, Temple-Smith PD. 1991. Transport and storage of spermatozoa in the female reproductive tract of the brown marsupial mouse, *Antechinus stuartii* (Dasyuridae). *J. Reprod. Fertil.* 93:97–110
16. Han XK, Li ZL, Li MY, Bao HJ, Hei NN, Chen QS. 2008. Ultrastructure of anterior uterus of the oviduct and the stored sperm in female soft-shelled turtle, *Trionyx sinensis*. *Anat. Rec.* 291:335–51
17. Han XK, Zhang L, Li MY, Bao HJ, Hei NN, Chen QS. 2008. Seasonal changes of sperm storage and correlative structures in male and female soft-shelled turtles, *Trionyx sinensis*. *Anim. Reprod. Sci.* 108:435–45
18. Vila S, Sàbat M, Hernandez MR, Muñoz M. 2007. Intraovarian sperm storage in *Helicolenus dactylopterus dactylopterus*: fertilization, crypt formation and maintenance of stored sperm. *Raffles Bull. Zool.* 14(Suppl.):21–27
19. Chiarini-Garcia H, Vieira FO, Godinho HP. 2014. Morphofunctional changes of female germinal epithelium to support spermatozoa along the annual reproductive cycle in an inseminating catfish (*Trachelyopterus galeatus*, Auchenipteridae). *J. Morphol.* 275:65–75
20. Warner RR, Harlan RK. 1982. Sperm competition and sperm storage as determinants of sexual dimorphism in the dwarf surfperch, *Micrometrus minimus*. *Evolution* 36:44–55
21. Darling JDS, Noble ML, Shaw E. 1980. Reproductive strategies in the surfperches: multiple insemination in natural-populations of the shiner perch, *Cymatogaster aggregata*. *Evolution* 34:271–77
22. Racey PA, Potts DM. 1970. Relationship between stored spermatozoa and the uterine epithelium in the pipistrelle bat (*Pipistrellus pipistrellus*). *J. Reprod. Fertil.* 22:57–63
23. Almeida-Santos SM, Salomao MG. 1997. Long-term sperm storage in the neotropical rattlesnake *Crotalus durissus terrificus* (Viperidae: Crotalinae). *Jpn. J. Herpetol.* 17:46–52
24. Muñoz M, Koya Y, Casadevall M. 2002. Histochemical analysis of sperm storage in *Helicolenus dactylopterus dactylopterus* (Teleostei: Scorpaenidae). *J. Exp. Zool.* 292:156–64

25. Das SC, Isobe N, Nishibori M, Yoshimura Y. 2006. Expression of transforming growth factor-β isoforms and their receptors in utero-vaginal junction of hen oviduct in presence or absence of resident sperm with reference to sperm storage. *Reproduction* 132:781–90
26. Schuberth HJ, Taylor U, Zerbe H, Waberski D, Hunter R, Rath D. 2008. Immunological responses to semen in the female genital tract. *Theriogenology* 70:1174–81
27. Ashizawa K, Nishiyama H, Nagae T. 1976. Effects of oviducal cells on the survival and fertilizing ability of fowl spermatozoa. *J. Reprod. Fertil.* 47:305–11
28. Ashizawa K, Nishiyama H. 1977. Effects of various cultured cells on the survival and fertilizing ability of fowl spermatozoa. *J. Reprod. Fertil.* 49:405–7
29. Ashizawa K, Tokudome Y, Okauchi K, Nishiyama H. 1982. Effects of HeLa and BHK-21 cells on the survival of fowl, bull, ram and boar spermatozoa in vitro. *J. Reprod. Fertil.* 66:663–66
30. Ashizawa K, Nishiyama H. 1983. Prolonged survival of fowl spermatozoa in the oviducal tissues in organ culture. *Br. Poult. Sci.* 24:27–32
31. Ashizawa K, Tamiya E, Okauchi K, Nishiyama H. 1985. Prolonged motility of fowl spermatozoa in vitro due to a low-molecular weight factor(s) released from cultured embryonic cells. *Anim. Reprod. Sci.* 9:181–88
32. Fujihara N, Koga O. 1982. Factor(s) influencing the survival of cock spermatozoa in vitro. *Can. J. Anim. Sci.* 62:951–53
33. Yeung WSB, Ng VKH, Lau EYL, Ho PC. 1994. Human oviductal cells and their conditioned medium maintain the motility and hyperactivation of human spermatozoa in vitro. *Hum. Reprod.* 9:656–60
34. Dubuc A, Sirard MA. 1995. Effect of coculturing spermatozoa with oviductal cells on the incidence of polyspermy in pig in vitro fertilization. *Mol. Reprod. Dev.* 41:360–67
35. Bakst MR. 1983. Fate of turkey spermatozoa after intrainfundibular and intramagnal inseminations. *J. Reprod. Fertil.* 67:315–17
36. Brillard JP, Bakst MR. 1990. Quantification of spermatozoa in the sperm-storage tubules of turkey hens and the relation to sperm numbers in the perivitelline layer of eggs. *Biol. Reprod.* 43:271–75
37. King LM, Brillard JP, Garrett WM, Bakst MR, Donoghue AM. 2002. Segregation of spermatozoa within sperm storage tubules of fowl and turkey hens. *Reproduction* 123:79–86
38. Briskie JV. 1994. Seasonal patterns of sperm storage in the yellow-headed blackbird *Xanthocephalus xanthocephalus*. *Ibis* 136:323–30
39. Malecki IA, Cloete SWP, Gertenbach WD, Martins GB. 2004. Sperm storage and duration of fertility in female ostriches (*Struthio camelus*). *S. Afr. J. Anim. Sci.* 34:158–65
40. Das SC, Isobe N, Yoshimura Y. 2008. Mechanism of prolonged sperm storage and sperm survivability in hen oviduct: a review. *Am. J. Reprod. Immunol.* 60:477–81
41. Sasanami T, Matsuzaki M, Mizushima S, Hiyamm G. 2013. Sperm storage in the female reproductive tract in birds. *J. Reprod. Dev.* 59:334–38
42. Bakst MR. 1998. Structure of the avian oviduct with emphasis on sperm storage in poultry. *J. Exp. Zool.* 282:618–26
43. Bakst MR, Donoghue AM, Yoho DE, Moyle JR, Whipple SM, et al. 2010. Comparisons of sperm storage tubule distribution and number in 4 strains of mature broiler breeders and in turkey hens before and after the onset of photostimulation. *Poultry Sci.* 89:986–92
44. Birkhead TR, Møller AP. 1992. Numbers and size of sperm storage tubules and the duration of sperm storage in birds—a comparative study. *Biol. J. Linn. Soc.* 45:363–72
45. Birkhead TR, Fletcher F. 1998. Sperm transport in the reproductive tract of female zebra finches (*Taeniopygia guttata*). *J. Reprod. Fertil.* 114:141–45
46. Birkhead TR, Martinez JG, Burke T, Froman DP. 1999. Sperm mobility determines the outcome of sperm competition in the domestic fowl. *Proc. Biol. Sci.* 266:1759–64
47. Das SC, Isobe N, Yoshimura Y. 2009. Changes in the expression of interleukin-1β and lipopolysaccharide-induced TNF factor in the oviduct of laying hens in response to artificial insemination. *Reproduction* 137:527–36
48. Das SC, Isobe N, Yoshimura Y. 2010. Analysis of changes in the expression of transforming growth factor-βs in the utero-vaginal junction of hen oviduct in response to sperm concerning their significance in sperm survivability. *J. Poult. Sci.* 47:326–32

42. Murray Bakst has made a major contribution to understanding the physiology and function of specialized sperm storage tubules in poultry, and many of the references not cited here are worth reading in detail.

49. Foye-Jackson OT, Long JA, Bakst MR, Blomberg LA, Akuffo VG, et al. 2011. Oviductal expression of avidin, avidin-related protein-2, and progesterone receptor in turkey hens in relation to sperm storage: effects of oviduct tissue type, sperm presence, and turkey line. *Poult. Sci.* 90:1539–47

50. Long EL, Sonstegard TS, Long JA, Van Tassell CP, Zuelke KA. 2003. Serial analysis of gene expression in turkey sperm storage tubules in the presence and absence of resident sperm. *Biol. Reprod.* 69:469–74

50. The first in vivo demonstration using genomic technology illustrating that when spermatozoa reach the sperm storage tubules in an avian species (turkey) they elicit de novo gene expression.

51. Sanchez ER. 2012. Chaperoning steroidal physiology: lessons from mouse genetic models of Hsp90 and its cochaperones. *Biochim. Biophys. Acta* 1823:722–29
52. Mageed AM, Isobe N, Yoshimura Y. 2008. Expression of avian β-defensins in the oviduct and effects of lipopolysaccharide on their expression in the vagina of hens. *Poult. Sci.* 87:979–84
53. Yu HG, Dong J, Gu YH, Liu HY, Xin AJ, et al. 2013. The novel human β-defensin 114 regulates lipopolysaccharide (LPS)-mediated inflammation and protects sperm from motility loss. *J. Biol. Chem.* 288:12270–82
54. Froman D. 2003. Deduction of a model for sperm storage in the oviduct of the domestic fowl (*Gallus domesticus*). *Biol. Reprod.* 69:248–53
55. Ito T, Yoshizaki N, Tokumoto T, Ono H, Yoshimura T, et al. 2011. Progesterone is a sperm-releasing factor from the sperm-storage tubules in birds. *Endocrinology* 152:3952–62
56. Hiyama G, Matsuzaki M, Mizushima S, Dohra H, Ikegami K, et al. 2014. Sperm activation by heat shock protein 70 supports the migration of sperm released from sperm storage tubules in Japanese quail (*Coturnix japonica*). *Reproduction* 147:167–78
57. Nguyen TMD, Alves S, Grasseau I, Metayer-Coustard S, Praud C, et al. 2014. Central role of 5′-AMP-activated protein kinase in chicken sperm functions. *Biol. Reprod.* 91:121
58. Ashizawa K, Oyama N, Katayama S, Narumi K, Tatemoto H, Tsuzuki Y. 2013. Regulation of fowl sperm motility: evidence for the indirect, but not direct, involvement of dynein-ATPase activity on the reversible temperature-dependent immobilization. *Theriogenology* 79:558–65
59. Ashizawa K, Kawaji N, Nakamura S, Nagase D, Tatemoto H, et al. 2010. Temperature-dependent regulation of sperm motility of Ijima's copper pheasants (*Syrmaticus soemmerringii ijimae*), one of 'near threatened' species. *Anim. Reprod. Sci.* 121:181–87
60. Ashizawa K, Omura Y, Katayama S, Tatemoto H, Narumi K, Tsuzuki Y. 2009. Intracellular signal transduction pathways in the regulation of fowl sperm motility: evidence for the involvement of phosphatidylinositol 3-kinase (PI3-K) cascade. *Mol. Reprod. Dev.* 76:603–10
61. Zaniboni L, Bakst M. 2004. Localization of aquaporins in the sperm storage tubules in the turkey oviduct. *Poult. Sci.* 83:1209–12
62. Freedman S, Akuffo V, Bakst M. 2001. Evidence for the innervation of sperm storage tubules in the oviduct of the turkey (*Meleagris gallopavo*). *Reproduction* 121:809–14
63. Breque C, Surai P, Brillard JP. 2006. Antioxidant status of the lower oviduct in the chicken varies with age and dietary vitamin E supplementation. *Mol. Reprod. Dev.* 73:1045–51
64. Fitzpatrick JL, Lupold S. 2014. Sexual selection and the evolution of sperm quality. *Mol. Hum. Reprod.* 20:1180–89
65. Edward DA, Stockley P, Hosken DJ. 2014. Sexual conflict and sperm competition. *Cold Spring Harb. Perspect. Biol.* 7:a017707
66. Simmons LW. 2005. The evolution of polyandry: sperm competition, sperm selection, and offspring viability. *Annu. Rev. Ecol. Evol. Syst.* 36:125–46
67. Holt WV, Fazeli A. 2015. Do sperm possess a molecular passport? Mechanistic insights into sperm selection in the female reproductive tract. *Mol. Hum. Reprod.* 21(6):491–501
68. Hunter RHF. 2012. Components of oviduct physiology in eutherian mammals. *Biol. Rev. Camb. Philos. Soc.* 87:244–55
69. Rath D, Schuberth HJ, Coy P, Taylor U. 2008. Sperm interactions from insemination to fertilization. *Reprod. Domest. Anim.* 43:2–11
70. Guthrie HD, Welch GR. 2012. Effects of reactive oxygen species on sperm function. *Theriogenology* 78:1700–8

71. Gadea J, Gumbao D, Cánovas S, Garcia-Vázquez FA, Grullón LA, Gardón JC. 2008. Supplementation of the dilution medium after thawing with reduced glutathione improves function and the in vitro fertilizing ability of frozen-thawed bull spermatozoa. *Int. J. Androl.* 31:40–49
72. Hu JH, Tian WQ, Zhao XL, Zan LS, Wang H, et al. 2010. The cryoprotective effects of ascorbic acid supplementation on bovine semen quality. *Anim. Reprod. Sci.* 121:72–77
73. Monteiro JC, Gonçalves JS, Rodrigues JA, Lúcio CF, Silva LC, et al. 2009. Influence of ascorbic acid and glutathione antioxidants on frozen-thawed canine semen. *Reprod. Domest. Anim.* 44(Suppl. 2):359–62
74. Roy M, Gauvreau D, Bilodeau JF. 2008. Expression of superoxide dismutases in the bovine oviduct during the estrous cycle. *Theriogenology* 70:836–42
75. Lapointe J, Bilodeau JF. 2003. Antioxidant defenses are modulated in the cow oviduct during the estrous cycle. *Biol. Reprod.* 68:1157–64
76. Kobayashi M, Wada M, Hori T, Kawakami E. 2014. Superoxide dismutase activity in the oviductal and uterine fluid of the bitch and the effects of the enzyme on viability, motility and hyperactivation of canine sperm in vitro. *J. Vet. Med. Sci.* 76:741–43
77. Roy VK, Krishna A. 2013. Changes in glucose and carnitine levels and their transporters in utero-tubal junction in relation to sperm storage in the vespertilionid bat, *Scotophilus heathi*. *J. Exp. Zool. A Ecol. Genet. Physiol.* 319:517–26
78. Holleley CE, Dickman CR, Crowther MS, Oldroyd BP. 2006. Size breeds success: multiple paternity, multivariate selection and male semelparity in a small marsupial, *Antechinus stuartii*. *Mol. Ecol.* 15:3439–48
79. Naylor R, Richardson SJ, McAllan BM. 2008. Boom and bust: a review of the physiology of the marsupial genus *Antechinus*. *J. Comp. Physiol. B* 178:545–62
80. Rijsselaere T, England G, Freeman S, Maes D, Van Soom A. 2014. Current knowledge on the transport and fate of spermatozoa in the reproductive tract of the bitch. *Reprod. Domest. Anim.* 49(Suppl. 2):2–7
81. Day FT. 1942. Survival of spermatozoa in the genital tract of the mare. *J. Agric. Sci.* 32:108–11
82. Rodríguez-Martínez H, Saravia F, Wallgren M, Tienthai P, Johannisson A, et al. 2005. Boar spermatozoa in the oviduct. *Theriogenology* 63:514–35
83. England GCW, Burgess CM, Clutterbuck AL, Freeman SL. 2013. Epithelial surface changes and spermatozoa storage in the reproductive tract of the bitch. *Vet. J.* 195:185–91
84. England GC, Burgess CM, Freeman SL, Smith SC, Pacey AA. 2006. Relationship between the fertile period and sperm transport in the bitch. *Theriogenology* 66:1410–18
85. Yániz JL, Carretero T, Recreo P, Arceiz E, Santolaria P. 2014. Three-dimensional architecture of the ovine oviductal mucosa. *Anat. Histol. Embryol.* 43:331–40
86. Suarez SS. 2008. Regulation of sperm storage and movement in the mammalian oviduct. *Int. J. Dev. Biol.* 52:455–62
87. Bedford JM, Phillips DM, Mover-Lev H. 1997. Novel sperm crypts and behavior of gametes in the fallopian tube of the white-toothed shrew, *Crocidura russula monacha*. *J. Exp. Zool.* 277:262–73
88. Kadirvel G, Machado SA, Korneli C, Collins E, Miller P, et al. 2012. Porcine sperm bind to specific 6-sialylated biantennary glycans to form the oviduct reservoir. *Biol. Reprod.* 87:147
89. Talevi R, Gualtieri R. 2010. Molecules involved in sperm-oviduct adhesion and release. *Theriogenology* 73:796–801
90. Talevi R, Gualtieri R. 2001. Sulfated glycoconjugates are powerful modulators of bovine sperm adhesion and release from the oviductal epithelium in vitro. *Biol. Reprod.* 64:491–98
91. Racey PA, Uchida TA, Mori T, Avery MI, Fenton MB. 1987. Sperm-epithelium relationships in relation to the time of insemination in little brown bats (*Myotis lucifugus*). *J. Reprod. Fertil.* 80:445–54
92. Smith TT, Nothnick WB. 1997. Role of direct contact between spermatozoa and oviductal epithelial cells in maintaining rabbit sperm viability. *Biol. Reprod.* 56:83–89
93. Lloyd RE, Elliott RMA, Fazeli A, Watson PF, Holt WV. 2009. Effects of oviductal proteins, including heat shock 70 kDa protein 8, on survival of ram spermatozoa over 48 h in vitro. *Reprod. Fertil. Dev.* 21:408–18
94. Fazeli A, Elliott RM, Duncan AE, Moore A, Watson PF, Holt WV. 2003. In vitro maintenance of boar sperm viability by a soluble fraction obtained from oviductal apical plasma membrane preparations. *Reproduction* 125:509–17

95. Elliott RM, Lloyd RE, Fazeli A, Sostaric E, Georgiou AS, et al. 2009. Effects of HSPA8, an evolutionarily conserved oviductal protein, on boar and bull spermatozoa. *Reproduction* 137:191–203
96. Boilard M, Reyes-Moreno C, Lachance C, Massicotte L, Bailey JL, et al. 2004. Localization of the chaperone proteins GRP78 and HSP60 on the luminal surface of bovine oviduct epithelial cells and their association with spermatozoa. *Biol. Reprod.* 71:1879–89
97. Lachance C, Bailey JL, Leclerc P. 2007. Expression of Hsp60 and Grp78 in the human endometrium and oviduct, and their effect on sperm functions. *Hum. Reprod.* 22:2606–14
98. Lloyd RE, Fazeli A, Watson PF, Holt WV. 2012. The oviducal protein, heat-shock 70-kDa protein 8, improves the long-term survival of ram spermatozoa during storage at 17°C in a commercial extender. *Reprod. Fertil. Dev.* 24:543–49
99. Moein-Vaziri N, Phillips I, Smith S, Alminana C, Maside C, et al. 2014. Heat shock protein A8 restores sperm membrane integrity by increasing plasma membrane fluidity. *Reproduction* 147:719–32
100. Daugaard M, Rohde M, Jaattela M. 2007. The heat shock protein 70 family: highly homologous proteins with overlapping and distinct functions. *FEBS Lett.* 581:3702–10
101. Tytell M, Greenberg SG, Lasek RJ. 1986. Heat shock-like protein is transferred from glia to axon. *Brain Res.* 363:161–64
102. Dehghani-Mohammadabadi M, Salehi M, Farifteh F, Nematollahi S, Arefian E, et al. 2014. Melatonin modulates the expression of BCL-xl and improve the development of vitrified embryos obtained by IVF in mice. *J. Assist. Reprod. Genet.* 31:453–61
103. Jang HY, Ji SJ, Kim YH, Lee HY, Shin JS, et al. 2010. Antioxidative effects of astaxanthin against nitric oxide-induced oxidative stress on cell viability and gene expression in bovine oviduct epithelial cell and the developmental competence of bovine IVM/IVF embryos. *Reprod. Domest. Anim.* 45:967–74
104. Bhattacharya A, Kurochkin AV, Yip GNB, Zhang YB, Bertelsen EB, Zuiderweg ERP. 2009. Allostery in Hsp70 chaperones is transduced by subdomain rotations. *J. Mol. Biol.* 388:475–90
105. Derbigny WA, Shobe LR, Kamran JC, Toomey KS, Ofner S. 2012. Identifying a role for Toll-like receptor 3 in the innate immune response to *Chlamydia muridarum* infection in murine oviduct epithelial cells. *Infect. Immun.* 80:254–65
106. Liu JH, Marey MA, Kowsar R, Hambruch N, Shimizu T, et al. 2014. An acute-phase protein as a regulator of sperm survival in the bovine oviduct: alpha 1-acid-glycoprotein impairs neutrophil phagocytosis of sperm in vitro. *J. Reprod. Dev.* 60:342–48
107. Taylor U, Rath D, Zerbe H, Schuberth HJ. 2007. Influence of spermatozoa, seminal plasma and semen extender on the migration of porcine neutrophils in vivo and in vitro. *Reprod. Domest. Anim.* 42:71–72
108. Taylor U, Rath D, Zerbe H, Schuberth HJ. 2008. Interaction of intact porcine spermatozoa with epithelial cells and neutrophilic granulocytes during uterine passage. *Reprod. Domest. Anim.* 43:166–75
109. Taylor U, Zerbe H, Seyfert HM, Rath D, Schuberth HJ. 2008. Spermatozoa inhibit breeding-induced cytokine induction in porcine endometrial cells in vivo. *Reprod. Domest. Anim.* 43:123–24
110. Dorin JR, Barratt CL. 2014. Importance of β-defensins in sperm function. *Mol. Hum. Reprod.* 20:821–26
111. Ellington JE, Ignotz GG, Ball BA, Meyerswallen VN, Currie WB. 1993. De novo protein synthesis by bovine uterine tube (oviduct) epithelial-cells changes during co-culture with bull spermatozoa. *Biol. Reprod.* 48:851–56

112. Fazeli A, Affara NA, Hubank M, Holt WV. 2004. Sperm-induced modification of the oviductal gene expression profile after natural insemination in mice. *Biol. Reprod.* 71:60–65

112. The first in vivo demonstration using DNA microarrays in a mammal to illustrate that the arrival of spermatozoa in the female reproductive tract induces de novo expression of a selected cohort of genes.

113. Georgiou AS, Snijders AP, Sostaric E, Aflatoonian R, Vazquez JL, et al. 2007. Modulation of the oviductal environment by gametes. *J. Proteome Res.* 6:4656–66
114. Aldarmahi A, Elliott S, Russell J, Fazeli A. 2013. Effects of spermatozoa-oviductal cell coincubation time and oviductal cell age on spermatozoa-oviduct interactions. *Reprod. Fertil. Dev.* 26:358–65
115. Yeste M, Holt WV, Bonet S, Rodríguez-Gil JE, Lloyd RE. 2014. Viable and morphologically normal boar spermatozoa alter the expression of heat-shock protein genes in oviductal epithelial cells during co-culture in vitro. *Mol. Reprod. Dev.* 81:805–19
116. Dobrinski I, Suarez SS, Ball BA. 1996. Intracellular calcium concentration in equine spermatozoa attached to oviductal epithelial cells in vitro. *Biol. Reprod.* 54:783–88
117. Dobrinski I, Smith TT, Suarez SS, Ball BA. 1997. Membrane contact with oviductal epithelium modulates the intracellular calcium concentration of equine spermatozoa in vitro. *Biol. Reprod.* 56:861–69

118. Overstreet JW, Cooper GW. 1975. Reduced sperm motility in the isthmus of the rabbit oviduct. *Nat. Lett.* 258:718–19
119. Publicover SJ, Giojalas LC, Teves ME, de Oliveira GSMM, Garcia AA, et al. 2008. Ca2+ signalling in the control of motility and guidance in mammalian sperm. *Front. Biosci.* 13:5623–37
120. Tulsiani DR, Zeng HT, Abou-Haila A. 2007. Biology of sperm capacitation: evidence for multiple signalling pathways. *Soc. Reprod. Fertil. Suppl.* 63:257–72
121. Suarez SS, Marquez B, Harris TP, Schimenti JC. 2007. Different regulatory systems operate in the midpiece and principal piece of the mammalian sperm flagellum. *Soc. Reprod. Fertil. Suppl.* 65:331–34
122. Holt WV, Harrison RA. 2002. Bicarbonate stimulation of boar sperm motility via a protein kinase A-dependent pathway: Between-cell and between-ejaculate differences are not due to deficiencies in protein kinase A activation. *J. Androl.* 23:557–65
123. Coy P, Lloyd R, Romar R, Satake N, Matas C, et al. 2010. Effects of porcine pre-ovulatory oviductal fluid on boar sperm function. *Theriogenology* 74:632–42
124. Hernandez M, Lloyd R, Holt WV. 2009. Effects of the heat shock 70 kDa protein 8 (HSPA8) on boar sperm motility. In *Maternal Communication with Gametes and Embryos*, ed. A Fazeli, F Gandolf, S Ledda, p. 68. Brussels: Gemini COST Action FA0702. ISBN 978-0-9563694-0-6
125. Satake N, Elliott RMA, Watson PF, Holt WV. 2006. Sperm selection and competition in pigs may be mediated by the differential motility activation and suppression of sperm subpopulations within the oviduct. *J. Exp. Biol.* 209:1560–72
126. Rodriguez-Martinez H. 2007. Role of the oviduct in sperm capacitation. *Theriogenology* 68:S138–46
127. Killian G. 2011. Physiology and endocrinology symposium: evidence that oviduct secretions influence sperm function: a retrospective view for livestock. *J. Anim. Sci.* 89:1315–22
128. Yang X, Zhao Y, Yang X, Kan FW. 2015. Recombinant hamster oviductin is biologically active and exerts positive effects on sperm functions and sperm-oocyte binding. *PLOS ONE* 10:e0123003
129. Teijeiro JM, Dapino DG, Marini PE. 2011. Porcine oviduct sperm binding glycoprotein and its deleterious effect on sperm: A mechanism for negative selection of sperm? *Biol. Res.* 44:329–37
130. Coy P, Aviles M. 2009. What controls polyspermy in mammals, the oviduct or the oocyte? *Biol. Rev. Camb. Philos. Soc.* 85:593–605
131. Suarez SS. 1998. The oviductal sperm reservoir in mammals: mechanisms of formation. *Biol. Reprod.* 58:1105–7
132. Suarez SS, Pacey AA. 2006. Sperm transport in the female reproductive tract. *Hum. Reprod. Update* 12:23–37
133. Holt WV, Fazeli A. 2010. The oviduct as a complex mediator of mammalian sperm function and selection. *Mol. Reprod. Dev.* 77:934–43
134. Orr TJ, Zuk M. 2014. Reproductive delays in mammals: an unexplored avenue for post-copulatory sexual selection. *Biol. Rev. Camb. Philos. Soc.* 89:889–912
135. Birkhead TR. 1998. Cryptic female choice: criteria for establishing female sperm choice. *Evolution* 52:1212–18
136. Dziuk PJ. 1996. Factors that influence the proportion of offspring sired by a male following heterospermic insemination. *Anim. Reprod. Sci.* 43:65–88
137. Robl JM, Dziuk PJ. 1988. Comparison of heterospermic and homospermic inseminations as measures of male fertility. *J. Exp. Zool.* 245:97–101
138. Krishna A, Dominic CJ. 1978. Storage of spermatozoa in the female genital tract of the vespertilionid bat, *Scotophilus heathi*. *J. Reprod. Fertil.* 54:319–21
139. Singh K, Krishna A. 1996. Seasonal changes in circulating serum concentration and in vitro testicular secretion of testosterone and androstenedione in the male vespertilionid bat (*Scotophilus heathi*). *J. Exp. Zool.* 276:43–52
140. Abhilasha, Krishna A. 1997. Adiposity and androstenedione production in relation to delayed ovulation in the Indian bat, *Scotophilus heathi*. *Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol.* 116:97–101
141. Abhilasha, Krishna A. 1996. High androgen production by ovarian thecal interstitial cells: a mechanism for delayed ovulation in a tropical vespertilionid bat, *Scotophilus heathi*. *J. Reprod. Fertil.* 106:207–11
142. Roy VK, Krishna A. 2010. Evidence of androgen-dependent sperm storage in female reproductive tract of *Scotophilus heathi*. *Gen. Comp. Endocrinol.* 165:120–26

130. Experimental data showing that oviductal proteins initiate significant hardening of the zona pellucida are interpreted as showing that the oviduct is just as responsible for preventing polyspermy during fertilization as the cortical granules of the oocyte.

134. An extensive review of the relevance of sperm storage in mammals to hypotheses and predictions about its evolutionary significance; presents detailed tables showing data about sperm storage durations.

136. An excellent review of the theory and benefits of comparing the fertility of different males by the use of heterospermic insemination trials.

143. Doval J, Krishna A. 1998. Ovarian androstenedione production is enhanced by insulin during the period of delayed ovulation in a vespertilionid bat, *Scotophilus heathi*. *J. Reprod. Fertil.* 114:63–68
144. Roy VK, Krishna A. 2012. Changes in the expression of HSL and OCTN2 in the female reproductive tract of the bat, *Scotophilus heathii* in relation to sperm storage. *Acta Histochem.* 114:358–62
145. Roy VK, Krishna A. 2011. Sperm storage in the female reproductive tract of *Scotophilus heathii:* role of androgen. *Mol. Reprod. Dev.* 78:477–87
146. Chipuk JE, Moldoveanu T, Llambi F, Parsons MJ, Green DR. 2010. The BCL-2 family reunion. *Mol. Cell* 37:299–310
147. Llambi F, Green DR. 2011. Apoptosis and oncogenesis: give and take in the BCL-2 family. *Curr. Opin. Genet. Dev.* 21:12–20
148. Degterev A, Boyce M, Yuan J. 2003. A decade of caspases. *Oncogene* 22:8543–67
149. Johnston SD, Holt WV. 2001. Germplasm conservation in marsupials. In *Cryobanking the Genetic Resource: Wildlife Conservation for the Future?*, ed. PF Watson, WV Holt, pp. 203–25. London: Taylor & Francis
150. Allen CD, Burridge M, Mulhall S, Chafer ML, Nicolson VN, et al. 2008. Successful artificial insemination in the koala (*Phascolarctos cinereus*) using extended and extended-chilled semen collected by electroejaculation. *Biol. Reprod.* 78:661–66

RELATED RESOURCES

Almiñana C, Caballero I, Heath PR, Maleki-Dizaji S, Parrilla I, et al. 2014. The battle of the sexes starts in the oviduct: modulation of oviductal transcriptome by X and Y-bearing spermatozoa. *BMC Genom.* 15:293

Chang H, Suarez SS. 2012. Unexpected flagellar movement patterns and epithelial binding behavior of mouse sperm in the oviduct. *Biol. Reprod.* 86:140

Druart X, Cognie J, Baril G, Clement F, Dacheux JL, Gatti JL. 2010. In vivo imaging of in situ motility of fresh and liquid-stored ram spermatozoa in the ewe genital tract. *J. Dairy Sci.* 93:505

Kolle S, Reese S, Kummer W. 2010. New aspects of gamete transport, fertilization, and embryonic development in the oviduct gained by means of live cell imaging. *Theriogenology* 73:786–95

Matsuzaki M, Hiyama G, Mizushima S, Shiba K, Inaba K, Sasanami T. 2014. Specific mechanism of sperm storage in avian oviducts. In *Sexual Reproduction in Animals and Plants*, ed. H Sawada, N Inoue, M Iwano, pp. 23–29. New York: Springer

Wolfner MF. 2011. Precious essences: female secretions promote sperm storage in *Drosophila*. *PLOS Biol.* 9:e1001191