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# Sperm chemotaxis

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Communication between spermatozoa and egg before contact by chemotaxis appears to be prevalent throughout the animal kingdom. In non-mammalian species, sperm chemotaxis to factors secreted from the egg is well documented. In mammals, sperm chemotaxis to follicular factors *in vitro* has been established in humans and mice. The attractants of female origin in non-mammalian species are heat-stable peptides or proteins of various sizes, or other small molecules, depending on the species. Species specificity of the attractants in non-mammalian species may vary from high species specificity, through specificity to families with no specificity within a family, to absence of specificity. The mammalian sperm attractants have not been identified but they appear to be heat-stable peptides. The claim that progesterone is the attractant for human spermatozoa has failed to be substantiated, neither have claims for other mammalian sperm attractants been verified. The molecular mechanism of sperm chemotaxis is not known. Models involving modulation of the intracellular $Ca^{2+}$ concentration have been proposed for both mammalian and non-mammalian sperm chemotaxis. The physiological role of sperm chemotaxis in non-mammalian species appears to differ from that in mammals. In non-mammalian species, sperm chemotaxis strives to bring as many spermatozoa as possible to the egg. However, in mammals, the role appears to be recruitment of a selective population of capacitated ('ripe') spermatozoa to fertilize the egg.

Sperm chemotaxis is prevalent throughout the Metazoa, from marine species with external fertilization, such as sea urchins and corals, to humans (Miller, 1985; Cosson, 1990; Eisenbach and Tur-Kaspa, 1994). Although sperm chemotaxis in marine species was established and accepted from the mid-1960s, the occurrence of sperm chemotaxis in mammals was questioned until the last decade but has now been established *in vitro* beyond any doubt.

There were two primary reasons for resistance to the concept of mammalian sperm chemotaxis. First, in mammals, very large numbers of spermatozoa ($10^7$–$10^9$) are ejaculated directly into the female reproductive tract, where many may reach the egg by chance, avoiding a need for sperm chemotaxis. Second, technical difficulties in studying mammalian sperm chemotaxis prevented the acquisition of conclusive evidence. The main technical difficulty was a very low signal-to-noise ratio in the measurements, resulting from the fact that, at least in humans and mice, only a small fraction of the sperm population is chemotactically responsive at a given moment (Cohen-Dayag *et al.*, 1994; Oliveira *et al.*, 1998). This low signal-to-noise ratio taken together with the large variability between sperm samples, the fact that only about one half of the follicular fluids (commonly used as a source for female-derived attractants) are chemotactically active, the fact that many studies used attractant concentrations that were too high (for example, insufficiently-diluted follicular fluid) that yielded false results, and the fact that many studies did not examine whether the criteria for chemotaxis were fulfilled and did not distinguish between chemotaxis and other processes that may cause sperm accumulation (see Box 1), all resulted in inconsistent results and ambiguity (Eisenbach and Tur-Kaspa, in press). Chemotaxis was established only when the behaviour of mammalian sperm was analysed according to parameters that distinguish chemotaxis from chemokinesis and trapping. This short review not only presents the current state of the art of sperm chemotaxis, but also demonstrates that there is a good physiological reason for the small fraction of chemotactically responsive spermatozoa and concludes that sperm chemotaxis fulfills different goals in different species. Because of editorial limitations, the reference listing in this review is incomplete. Therefore, whenever possible, reference is made to reviews or to papers that provide access to the original literature.

## Sperm chemotaxis in non-mammalian species

Since the discovery of sperm attraction to the female gametes in ferns over a century ago (Pfeffer, 1884), the process has been established in a large variety of species, primarily due to the systematic and extensive work of R. L. Miller over the last three decades in marine invertebrates. Extensive reviews by Miller (1985), Cosson (1990) and Morisawa (1994) give detailed summaries of the observations made in each species. In general, there is no single rule that determines the existence of sperm chemotaxis in the species studied. In some species (for example, hydroids such as *Campanularia* or tunicates such as *Ciona*), the swimming direction of the spermatozoa changes abruptly towards the source of the attractant (Fig. 1a). In other species (for example, hydromedusa, ferns, or fish such as Japanese bitterlings), the approach to the attractant source is indirect and the movement is by repetitive loops of small radii (compare Fig. 1b with c and d). In others (for example, Arthropoda, in which fertilization is internal), sperm chemotaxis does not appear to occur at all. In certain species (for example, herring or the ascidian *Ciona*), activation of motility


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**Box 1.** Glossary

| | |
|---|---|
| Chemotaxis: | Modulation of the direction of movement of motile cells in response to a chemical gradient of a stimulus, resulting in approach to an attractant or retreat from a repellent. |
| Chemokinesis: | Change in the steady-state speed of a cell, governed by stimulus concentration. Chemokinesis is independent of the direction of the stimulus chemical gradient. |
| Trapping: | Accumulation of cells at a certain location as a result of reduced net vectorial speed at that location. Trapping may result from a negative effect of a stimulus on motility, from a gradient-independent change in swimming behaviour at a particular stimulus concentration, from mechanical effects such as adsorption to glass or capillary, or from any combination of these. |

precedes chemotaxis. There is no single rule for the specificity of sperm chemotaxis: in some groups (for example, hydromedusae and certain ophiuroids), high species specificity is observed; in others (for example, starfish), the specificity is at the family level and, within the family, there is crossreactivity, and in molluscs, for example, there appears to be no specificity at all (see Miller, 1985, 1997; Cosson, 1990 and references therein). In plants, a unique simple compound (for example, fucoserratene, a linear, unsaturated alkene, 1,3-trans 5-cis-octatriene) might be an attractant for various species (Maier and Müller, 1986). These differences in specificity among species may reflect the different physiological tasks that sperm chemotaxis fulfills in different species.

### *Sperm attractants*

Most sperm attractants that have been identified in animals are peptides or proteins of low molecular mass (1–20 kDa), which are heat stable and sensitive to proteases (Miller, 1985; Cosson, 1990). Exceptions to the rule are the sperm attractants of corals, which are lipid-like substances of 140–250 Da (Coll and Miller, 1992), and the attractants of ascidians *Ciona*, which are nonproteinaceous small molecules (Yoshida *et al.*, 1993). In plants such as ferns, the partially ionized form of malic acid and a large variety of unsaturated four-carbon *cis*-dicarboxylic acids are sperm attractants (Cosson, 1990). In algae, pheromones of low molecular mass are sperm attractants (Maier and Müller, 1986; Cosson, 1990). Perhaps the most investigated metazoan sperm attractants are those of sea urchins. Resact, a 14-residue peptide (see Box 2) isolated from the egg jelly layer, is not only a specific sperm attractant for the sea urchin *Arbacia punctulata* (Ward *et al.*, 1985), but also a stimulator of sperm motility and respiration, and belongs to a large family of sperm-activating peptides (Suzuki, 1995). Most other peptides of this family have not been demonstrated to be attractants, although there is some indirect evidence that suggests that the peptide speract may be a sperm attractant for the sea urchin *Strongylocentrotus purpuratus* (Cook *et al.*, 1994).

### *Physiological role*

In non-mammalian species, it appears that the physiological role of sperm chemotaxis is to bring as many spermatozoa as possible to the egg. There seems to be no sperm selection. This is evident, for example, in teleost fish such as herring, in which all the spermatozoa appear to respond to the egg factors (Yanagimachi *et al.*, 1992), and where the first spermatozoon that happens to enter the micropile in the thick coat surrounding the egg is the one that penetrates the egg (Hart, 1990).

### *Molecular mechanism*

Very little is known about the molecular mechanism of sperm chemotaxis. In sea urchins, hydroids, and one ascidian species, it is known that sperm chemotaxis requires $Ca^{2+}$ (see Yoshida *et al.*, 1994 and references therein). The intracellular $Ca^{2+}$ concentration in sea urchin spermatozoa can be modulated by the sperm-activating peptides, resact and speract. Since both peptides similarly affected the $Ca^{2+}$ concentration, it was suggested that they, and perhaps sperm-activating peptides in general, use fundamentally similar mechanisms to control the intracellular $Ca^{2+}$ concentration and, thereby, control swimming behaviour in chemotaxis (Cook *et al.*, 1994). On the basis of this possibility and the relatively well-understood mechanism of signal transduction in response to speract, Cook *et al.* (1994) proposed a model for the molecular mechanism of sperm chemotaxis in sea urchins. According to this model (Fig. 2), an increase in the attractant concentration activates the receptor, a guanylate cyclase. The consequent increase in cGMP causes the sequential opening of $K^+$ channels, hyperpolarization, and the blockage of $Ca^{2+}$ entry. The resulting decrease in the intracellular $Ca^{2+}$ concentration causes more linear swimming, and the spermatozoa continue to swim up the gradient towards the source of the attractant. Conversely, a decrease in the attractant concentration results in reduced cGMP concentrations, $K^+$ channel activity and membrane potential. The reduced membrane potential activates $Na^+$–$H^+$ exchange, increasing the intracellular pH. Consequently, adenylate cyclase is activated; the cAMP concentration is increased; and cAMP-sensitive $Ca^{2+}$ channels are activated. The result is a transient increase in the intracellular $Ca^{2+}$ concentration, producing flagellar asymmetry and reorientation of the spermatozoa until they are directed towards the egg and sense an increase in the attractant concentration. With such a detailed model for the molecular mechanism of sperm chemotaxis, it should now be possible to test its validity experimentally.

## Sperm chemotaxis in mammals

### *Indirect potential indications for the occurrence of sperm chemotaxis in mammals*

A number of observations made in laboratory and farm animals raised the possibility that a process directing spermatozoa to the egg, perhaps chemotaxis, may be involved in mammalian fertilization (Eisenbach and Ralt, 1992; Hunter, 1993). Of these observations, those that appear to be most meaningful are the following. (i) At least in mammals other than humans, a considerable fraction of the spermatozoa ejaculated into the female reproductive tract is retained with reduced motility in

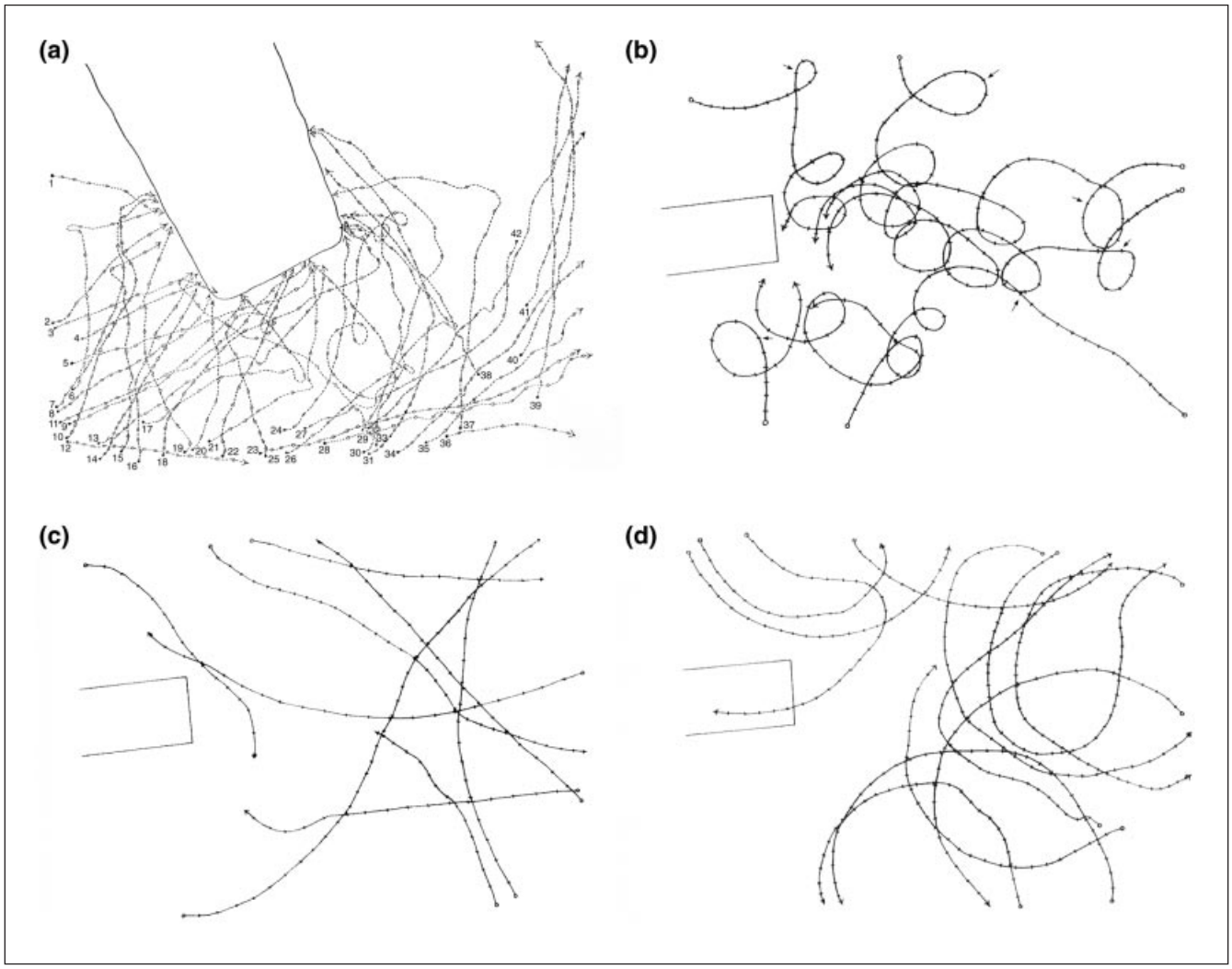


**Fig. 1.** Sperm trails in non-mammalian species. (a) Spermatozoa of the hydroid *Campanularia flexuosa* approaching the female gonangium: an example of spermatozoa whose swimming direction changes abruptly towards the attractant source. The solid circles indicate the start of each trail. The open circles are 0.45 s apart. Three types of trails can be seen: trails directed straight to the gonangium (16 trails), trails that go past the gonangium in straight line (10 trails), and trails that turn to enter or strike the gonangium (16 trails). (Reproduced with permission from Miller, 1966.) (b) Spermatozoa of the urochordate *Oikopleura dioica* approaching a pipette injecting *O. dioica* egg extract: an example of spermatozoa that approach the attractant source by indirect movement with repetitive loops. (c,d) Trajectories of *O. dioica* spermatozoa near a pipette injecting sea water as a control. (c) Shows mainly straight or curved trails, whereas (d) shows mainly curved trails. Pipette diameter is 30 mm. Each interval on the trail represents 0.08 s. (b–d, Reproduced with permission from Miller and King, 1983.)

storage sites (usually the oviductal isthmus; Fig. 3). As the spermatozoa move up the oviductal isthmus, they encounter a high mucus-containing narrow lumen which impedes their forward progression, and frequently they come into contact with the oviductal epithelium, where they bind strongly to carbohydrate moieties on glycoproteins or glycolipids on the surface of the oviductal epithelium and are consequently stored there (Suarez, 1998). When ovulation occurs, some of the spermatozoa in the sperm reservoir resume high motility and travel the distance between the storage site and the fertilization site at the oviductal ampulla within minutes (Barratt and Cooke, 1991; Overstreet and Drobnis, 1991; Hunter, 1993). Only capacitated spermatozoa, that is, spermatozoa that possess the potential to undergo the acrosome reaction (a release of proteolytic enzymes enabling sperm penetration through the egg coat) and to fertilize the egg (Yanagimachi, 1994; Jaiswal and Eisenbach, in press), are detached from the epithelium and released from the storage site (Smith and Yanagimachi, 1991; Lefebvre and Suarez, 1996). This finding suggests that there could be a signal received by the spermatozoa or the oviduct at ovulation that results in the release of capacitated spermatozoa from the storage site and their movement towards the eggs (Fig. 3). (ii) Although the number of spermatozoa moving from the uterus to the oviduct and accumulating before ovulation in the oviductal isthmus is several hundreds or thousands (depending on the species), there is seldom more than one spermatozoon in the immediate vicinity of each egg at the time of initial penetration of the egg membrane (see Hunter, 1993 and references cited

**Box 2.** Some examples of sperm attractants in non-mammalian species

| | |
|---|---|
| In corals: | A lipid-like long chain fatty alcohol $CH_3$-$(CH_2)_8$-CH=CH-CH=CH-$CH_2OH$ (Coll and Miller, 1992) |
| In sea urchins: | Resact: a 14-mer peptide with the sequence CVTGAPGCVGGGRL-$NH_2$ (Ward *et al*., 1985) |
| In starfish: | Startrak: a 13 kDa heat-stable protein (Miller and Vogt, 1996) |
| In ascidians: | SAAF: an unidentified heat-stable, proteinase-resistant small molecule (Yoshida *et al*., 1993, 1994) |
| In algae: | Low molecular weight unsaturated pheromones of cyclic or linear structure (Maier and Müller, 1986; Cosson, 1990) |
| In ferns: | Dicarboxylic acids, for example malic acid in its partially ionized form (Brokaw, 1958) |

therein). In mice, rats and pigs, the observations that a single spermatozoon is guided to each egg, and that this guidance is not random (since numerous spermatozoa do not arrive together at a single egg and spermatozoa do not arrive at other eggs) support the notion of sperm chemotaxis. If this notion is correct, there should be a mechanism that stops the signal subsequent to sperm penetration (Hunter, 1993). (iii) A number of studies have provided evidence that the products of ovulation are essential for sperm transport in the oviduct (see Harper, 1973; Ito *et al*., 1991 and references therein) by comparing superovulated with non-ovulated animals and by blocking the products of ovulation from entering the oviduct by ligation. However, these studies could not distinguish either between enhancement of sperm motility and stimulation of chemotaxis, or between the spermatozoa and the oviduct as the effectors on which the ovulatory products act.

### *Early observations interpreted as sperm chemotaxis*

Observations interpreted as sperm chemotaxis in mammals were made in the 1950s and 1960s. Moricard and Bossu (1951) noticed rat spermatozoa near dissociated follicular cells and interpreted this selective localization of spermatozoa as the result of chemotactic attraction by the oocyte. Schwartz *et al.* (1957) found that human ovarian cyst fluids, the outer liquid of egg white, and follicular fluid (from a single follicle) caused, among other effects, sperm accumulation. This accumulation was also interpreted as sperm chemotaxis (Schwartz *et al*., 1957). Dickman (1963) found that, when rat and rabbit eggs were transferred into the oviducts of previously mated rabbits, a larger number of spermatozoa collected within the rabbit eggs than on the zonae of the rat eggs. These findings were interpreted as chemotaxis of rabbit spermatozoa to the egg. However, lack of controls for other processes that might cause sperm accumulation (for example, sperm trapping, adhesion or swimming speed modulation), renders these early observations more suggestive than definitive (Eisenbach and Ralt, 1992). Conversely, Bronson and Hamada (1977) found that the

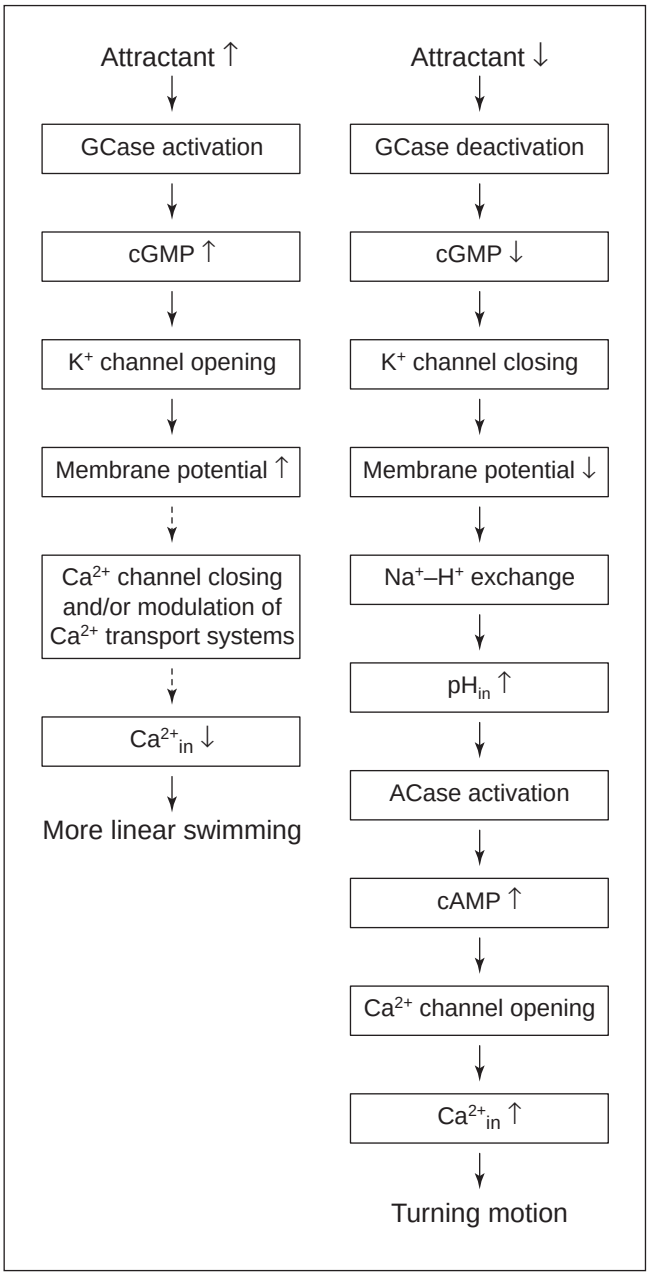


**Fig. 2.** A simplified scheme of a model, developed by Cook *et al.* (1994), for sperm chemotaxis in sea urchins. An upward or a downward arrow within a box indicates an increase or a decrease, respectively. Dashed arrows indicate processes for which no evidence is available.

cumulus oophorus secretes a substance *in vitro* that alters the pattern of mouse sperm movement. They noted that spermatozoa traversing microcapillary tubes in the environment of unfertilized eggs moved in erratic paths, owing to the repeated adherence of the sperm head to the wall of the microcapillary tube. However, when the cumulus oophorus had been removed, the spermatozoa moved in linear trajectories, as they do in the absence of eggs. Although Bronson and Hamada (1977) suggested that there was a selective trapping of mature

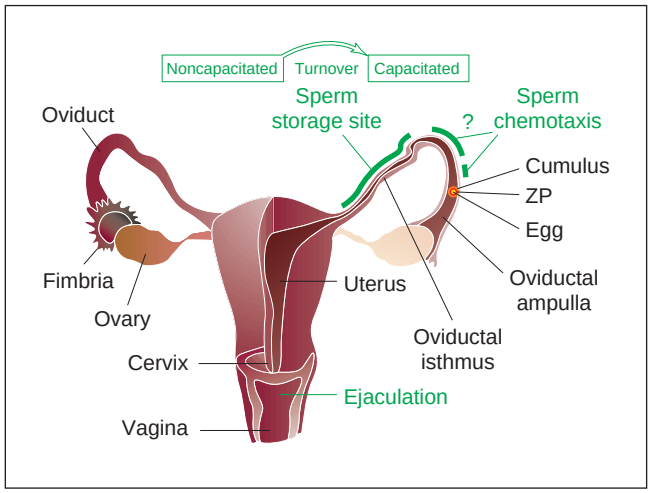


**Fig. 3.** A scheme of the genital tract in women. Spermatozoa move through the cervix and the uterus, find the opening of the oviduct and enter into it. If they arrive before ovulation, the spermatozoa are retained with reduced motility in the sperm reservoir within the oviductal isthmus. During this time there is a turnover of capacitated spermatozoa. The steady-state proportion of capacitated spermatozoa is low (usually around 10% in human spermatozoa *in vitro*). When ovulation occurs, a few capacitated spermatozoa are detached from the oviductal epithelium at the storage site, respond to attractant(s) secreted from the egg or the surrounding cumulus oophorus, and are thereby recruited to the egg. The exact location of sperm chemotaxis is not known. Only capacitated spermatozoa can penetrate the cumulus oophorus, and (according to most studies) bind to the zona pellucida (ZP) of the egg (Eisenbach, 1995). A spermatozoon bound to the zona pellucida undergoes the acrosome reaction, penetrates and fertilizes the egg. The dimensions of the egg and the cumulus oophorus surrounding it are exaggerated to make them visible.

spermatozoa by the cumulus oophorus, their observations could also be explained by sperm chemotaxis. Other examples are reviewed in Eisenbach and Ralt (1992).

### *A criterion for sperm chemotaxis*

The uncertainty about whether the phenomena described above reflect chemotaxis emphasizes the need to apply clear-cut criteria for distinguishing between chemotaxis and other processes. An important criterion for chemotaxis is the directional change of movement of spermatozoa towards the source of the attractant (Fig. 1): a unique feature of sperm chemotaxis. In the case of non-mammalian species, this criterion has been applied in most, if not all, of the published studies of sperm chemotaxis. It is regrettable that this criterion has not been applied uniformly in many studies with mammalian spermatozoa. As indicated below, many 'chemotaxis assays' have failed to distinguish between chemotaxis and other accumulation-causing processes.

### *Assays used for studying sperm chemotaxis in mammals*

The most commonly used technique for studying sperm chemotaxis in mammals is an accumulation assay in which spermatozoa sense an ascending gradient of the attractant and accumulate near or at its source. The principle is that spermatozoa from one reservoir accumulate in another reservoir that contains the attractant. The two reservoirs are connected and the attractant gradient is established by diffusion. Variations on this assay include an apparatus in which wells containing spermatozoa and the wells containing attractant are separated from each other by a thin polycarbonate membrane (Fig. 4a) or a tube (Fig. 4b), or in which a capillary containing the attractant is immersed in a well that contains a sperm suspension (Fig. 4c) (Eisenbach and Tur-Kaspa, in press). The main disadvantage of these assays is that they cannot distinguish between chemotaxis and other causes of sperm accumulation.

A similar technique that does distinguish between chemotaxis and other means of sperm accumulation is the inverted capillary assay in which spermatozoa in the well are suspended in a solution containing the presumed attractant (Fig. 4d). The capillary that is immersed into the sperm suspension contains either control buffer or the attractant. When the capillary contains buffer only, the spermatozoa sense a descending gradient of the attractant as they move from the well to the capillary (Fig. 4d). When the attractant is in both the capillary and the well, they sense no gradient at all. Thus, this assay measures the tendency of the spermatozoa to leave the attractant rather than to accumulate in it. If sperm chemotaxis is taking place, sperm accumulation in the capillary is expected to be relatively low when only the well contains the attractant; when there is no gradient and the attractant (or buffer) is everywhere, sperm accumulation in the capillary is expected to be relatively high. It is possible to distinguish between chemotaxis, speed enhancement (chemokinesis), and trapping by counting the spermatozoa accumulated in the capillaries in these settings (Ralt *et al.*, 1994) because only chemotaxis, unlike the chemokinetic and trapping effects, is dependent on the presence of a chemical gradient. Unfortunately, most investigators have not performed such assays, which could have avoided much of the confusion present in the current literature.

Another commonly used technique is a 'choice' assay in which spermatozoa choose between two wells (or two

**Fig. 4.** Various assays and designs used to study sperm accumulation and chemotaxis. (a) Accumulation assay in an apparatus consisting of two wells separated by a thin polycarbonate membrane (Gnessi *et al.*, 1985). (b) Accumulation assay in an apparatus consisting of two wells connected via a tube (Cohen-Dayag *et al.*, 1994). (c) Sperm accumulation in a capillary assay (Ralt *et al.*, 1994). (d) Inverted capillary assay (Ralt *et al.*, 1994). (e) Top view of a microscopic choice assay in sealed chamber (Makler *et al.*, 1992). (f) Choice assay in an apparatus consisting of two wells (Villanueva-Díaz *et al.*, 1992). (g) Choice assay in an apparatus consisting of three wells (Jaiswal *et al.*, in press). (h) Top view of an apparatus consisting of five wells for a choice assay (Villanueva-Díaz *et al.*, 1990; Sliwa, 1993a). (i) Schematic tracks of human spermatozoa in an attractant gradient (Ralt *et al.*, 1994).

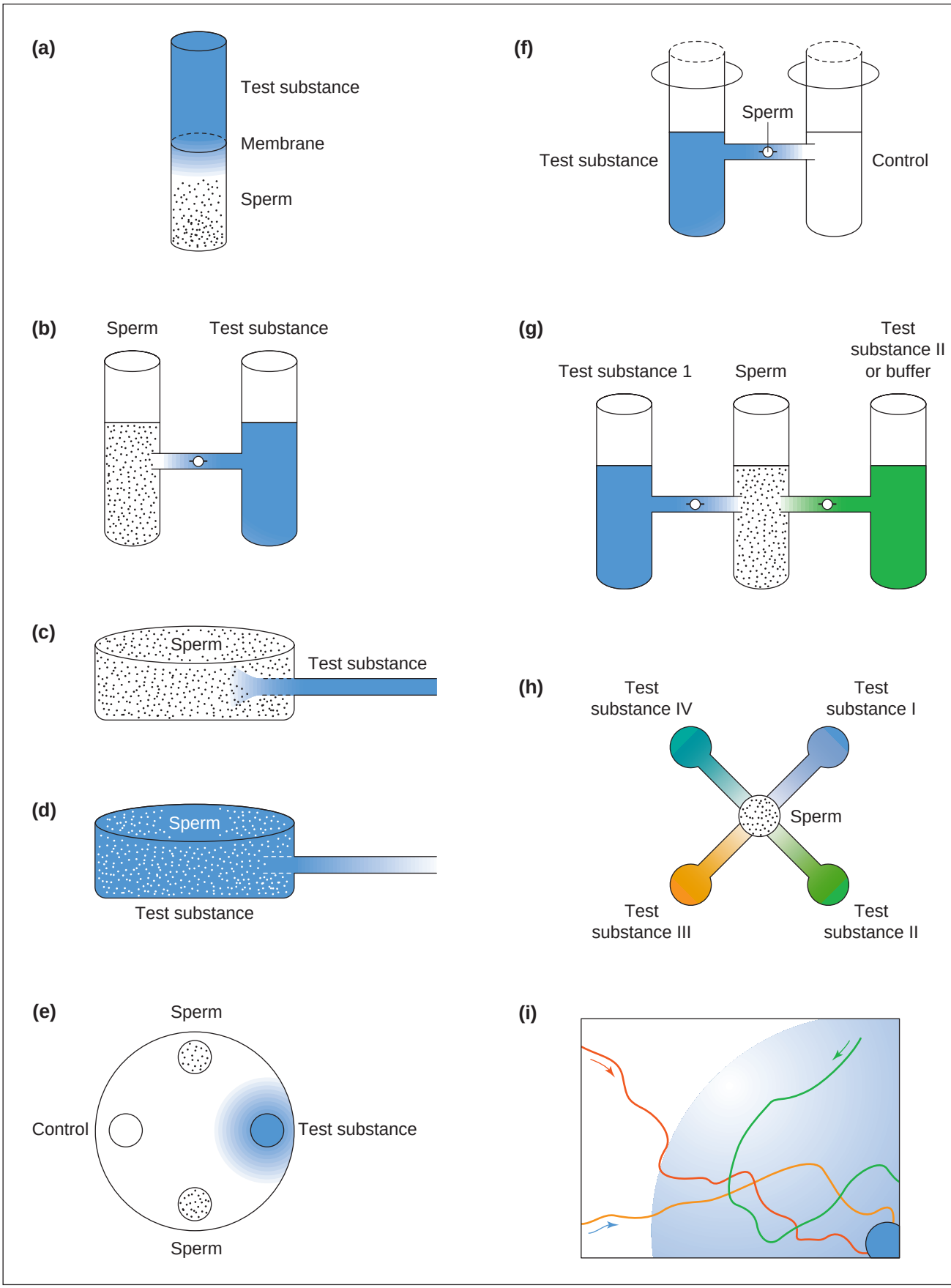
(a)
Test substance
Membrane
Sperm
(b)
Sperm
Test substance
(c)
Sperm
Test substance
(d)
Sperm
Test substance
(e)
Sperm
Control
Test substance
Sperm
(f)
Sperm
Test substance
Control
(g)
Test substance 1
Sperm
Test substance II or buffer
(h)
Test substance IV
Test substance I
Sperm
Test substance III
Test substance II
(i)

**Table 1.** Substances reported to cause sperm accumulation in mammals

| Substance | Species | Assay type* | Reference | Chemotaxis criterion fulfilled |
|---|---|---|---|---|
| Acetylcholine | Mouse | c | Sliwa, 1995 | – |
| Adrenaline | Mouse | c | Sliwa, 1994 | – |
| Atrial natriuretic peptide | Human | a,b,c | Zamir *et al.*, 1993; Anderson *et al.*, 1995 | + / –† |
| Antithrombin III | Boar | a | Lee *et al.*, 1994 | – |
| Calcitonin | Mouse | c | Sliwa, 1995 | – |
| Follicular fluid | Human | a,c | Villanueva-Díaz *et al.*, 1990, 1992 | – |
| | Human | a,b,c,d | Ralt *et al.*, 1991, 1994; Cohen-Dayag *et al.*, 1994 | + |
| | Mouse | d | Giojalas and Rovasio, 1998; Oliveira *et al.*, 1998 | + |
| Heparin | Human | c | Sliwa, 1993a | – |
| | Mouse | c | Sliwa, 1993b | – |
| Oxytocin | Mouse | c | Sliwa, 1994 | – |
| Progesterone | Human | c | Villanueva-Díaz *et al.*, 1995 | – |
| Synthetic *N*-formylated peptides | Human, bull | a | Iqbal *et al.*, 1980; Gnessi *et al.*, 1985 | –‡ |

* (a) Sperm accumulation in an ascending gradient of the test substance; (b) sperm accumulation in a descending gradient of the test substance; (c) choice assay; (d) track analysis of swimming spermatozoa.

† A distinction between chemotaxis and other processes causing accumulation has been made (Zamir *et al.*, 1993). However, a sperm track analysis has not been carried out.

‡ Chemotaxis was apparently ruled out as a cause of sperm accumulation (Miller, 1982; Makler *et al.*, 1992).

chambers): one containing the attractant and the other containing buffer as a control. A number of experimental designs have been published: a sealed chamber for microscopic measurements (Fig. 4e) and, for macroscopic measurements, apparatuses with two (Fig. 4f), three (Fig. 4g), or five (Fig. 4h) wells or chambers, connected by a tube or a groove (Eisenbach and Tur-Kaspa, in press). Such assays can distinguish between chemotaxis and chemokinesis, but cannot distinguish between chemotaxis and trapping.

An assay that addresses the criterion for chemotaxis directly is the tracing of video-recorded tracks made by spermatozoa in a gradient of an attractant (Fig. 4i) (Eisenbach and Tur-Kaspa, in press). This can be done either manually or using a computerized motion analysis system.

From this list of assays it is obvious that not every assay used for measuring sperm chemotaxis can actually distinguish chemotaxis from other causes of sperm accumulation and this experimental shortfall should be taken into account when evaluating the results of studies of mammalian sperm chemotaxis. Recent *in vitro* studies that demonstrated sperm accumulation in mammals are listed (Table 1). The table indicates the studies in which the criteria for sperm chemotaxis have been fulfilled.

### *Sperm chemotaxis to follicular fluid*

Follicular fluid contains secretions of the egg and its surrounding cells. For this reason and because of the availability of follicular fluid from women undergoing *in vitro* fertilization, many of the studies investigating sperm chemotaxis in humans were carried out with this fluid. Follicular fluid *per se* may have no physiological role after ovulation because only small quantities of it are transported into the oviduct (Hansen *et al.*, 1991). For the non-expert reader, the literature on sperm chemotaxis may appear confusing owing to the apparent lack of consensus. Of the four groups that studied chemotaxis to follicular fluid in the last decade, three groups demonstrated sperm chemotaxis (Table 1), and one group (Makler *et al.*, 1992, 1995) did not. One of the reasons for the absence of agreement is inappropriate experimental conditions to measure chemotaxis in some studies, specifically insufficient dilution of follicular fluid (for review, see Eisenbach and Tur-Kaspa, in press). When properly executed with a rigorous criterion for chemotaxis (Table 1), sperm chemotaxis to follicular fluid was observed consistently in both humans (Cohen-Dayag *et al.*, 1994; Ralt *et al.*, 1994) and mice (Giojalas and Rovasio, 1998; Oliveira *et al.*, 1998).

### *Physiological significance of chemotaxis to follicular fluid*

The first indication that sperm chemotaxis may indeed be involved in fertilization came from the observation that not all follicular fluids are active in causing sperm accumulation and that all the active fluids are from follicles containing eggs that can be fertilized (Ralt *et al.*, 1991). The first hint of the potential physiological role of sperm chemotaxis *in vivo* came from the findings that, in a given sperm population, there are chemotactic and non-chemotactic spermatozoa, and that the fraction of chemotactic spermatozoa in the total sperm population is small (2–12% in humans (Cohen-Dayag *et al.*, 1994) and approximately 10% in mice (Giojalas and Rovasio, 1998; Oliveira *et al.*, 1998); this small fraction of responsive spermatozoa may have been another reason for the lack of response found in the studies of Makler *et al.* (1992, 1995). In addition, the chemotactic responsiveness was also demonstrated to be temporary, and the responsive spermatozoa were found to change with time, that is, there is a continuous replacement of chemotactic spermatozoa within a sperm population (Cohen-Dayag *et al.*, 1994). Finally, it was demonstrated that only capacitated spermatozoa are chemotactic, that they acquire their chemotactic responsiveness as part of the capacitation process, and that they lose this responsiveness when the capacitated state is terminated

(Cohen-Dayag *et al.*, 1995). The association of chemotactic responsiveness with the capacitated state relied on the similar percentages of chemotactic and capacitated spermatozoa in a sperm population, on the occurrence of turnover of capacitated and chemotactic spermatozoa with similar kinetics, and on the fact that deliberate depletion of capacitated spermatozoa results in total loss of chemotaxis and, vice versa, depletion of chemotactic spermatozoa results in depletion of capacitated spermatozoa. This association raised the possibility that the role of human sperm chemotaxis *in vivo* is not to direct many spermatozoa to the egg, but rather to recruit a selective population of spermatozoa, that is, capacitated spermatozoa, to the egg for fertilization. A possible role of the turnover of capacitated spermatozoa may be to ensure the availability of capacitated spermatozoa for an extended period of time, despite the short lifespan of the capacitated state in any one spermatozoon (Eisenbach and Ralt, 1992; Cohen-Dayag *et al.*, 1995). It would be interesting to investigate whether turnover and chemotaxis of capacitated spermatozoa also occur in rabbits, in which ovulation is induced by copulation and, therefore, there is no obvious need for these processes.

### *Potential locations of sperm chemotaxis* in vivo

The timing and location of sperm chemotaxis *in vivo* is not known. One possibility, based on the observations that only capacitated spermatozoa appear to be released from the storage site at ovulation (Smith and Yanagimachi, 1991; Lefebvre and Suarez, 1996; and for review, see Eisenbach and Ralt, 1992; Suarez, 1998), is that chemotaxis is involved in directing the released capacitated spermatozoa towards the egg (Fig. 3). This may be significant in view of the relatively small number of spermatozoa released from the storage site (Hunter, 1993). However, because the oviduct undergoes contractile movements that appear to move fluid in a direction opposite to that of follicular fluid transport (Battalia and Yanagimachi, 1979), it is unlikely that a chemical gradient can be established over a long range (Cohen-Dayag *et al.*, 1994). Thus, the chemotaxis process may function only in close proximity to the egg. It is possible that the egg itself is the attractant source, and that the gradient is established within the cumulus oophorus and at a short range within its vicinity (Fig. 3). Only capacitated spermatozoa can penetrate the cumulus oophorus (Eisenbach, 1995), and the first spermatozoon that enters the cumulus locates the egg very effectively (Bedford and Kim, 1993; Hunter, 1993). It is possible that the cumulus cells are not homogeneous, and that only those closer to the egg secrete the attractant. Such a situation, if correct, can form an attractant gradient within the cumulus even if the egg is not the organelle that secretes the attractant. Another possibility, based on the finding that, in mice, both oviductal and follicular fluids are chemotactically active, is that there are two sequential steps of chemotaxis, each to a different attractant (Oliveira *et al.*, 1998) (Fig. 3). Determination of the cellular origin of the sperm attractant(s) may distinguish between some of these possibilities.

### *Potential attractants in follicular fluid*

The identity of the attractant(s) in follicular fluid is not known. An active fraction of follicular fluid that contains the attractant, probably a heat-stable peptide (Manor, 1994), has been identified (Ralt *et al.*, 1994). In addition, other constituents of follicular fluid, including heparin and the hormones: progesterone, atrial natriuretic peptide (ANP), adrenaline, oxytocin, calcitonin and acetylcholine, have been proposed as the attractant (Table 1).

*Progesterone.* Villanueva-Díaz *et al.* (1995) demonstrated in a choice assay that progesterone causes human sperm accumulation, that preincubation of spermatozoa with a progesterone receptor antagonist eliminates the accumulation, that dialysis of follicular fluid causes loss of this activity, that a lipid extract of follicular fluid causes sperm accumulation as does crude follicular fluid, and that heat or trypsin treatment does not affect the accumulation in follicular fluid. On the basis of these observations, Villanueva-Díaz *et al.* (1995) suggested that progesterone is the attractant in follicular fluid. However, this suggestion appears to be in conflict with earlier results that demonstrate an absence of correlation between sperm accumulation in follicular fluid and the concentration of progesterone in the fluid (Ralt *et al.*, 1991), as well as an absence of correlation between the characteristics of the active fractions of follicular fluid and those of progesterone (Manor, 1994). Jaiswal *et al.* (in press) demonstrated that progesterone does bring about sperm accumulation, but that this accumulation is due to physiological trapping and not to chemotaxis, thus resolving the apparent contradiction. Chemotaxis to progesterone was eliminated using track analysis, which demonstrated that most of the spermatozoa present near the progesterone-containing well apparently reached the well by coincidence, not by changing the direction of the swimming path. Physiological trapping was apparently caused by an acquisition of motility patterns resembling hyperactivation (a motility pattern related to capacitated spermatozoa, involving wide amplitude and marked lateral displacement of the head (Burkman, 1990)). Progesterone is known to cause sperm hyperactivation (Uhler *et al.*, 1992). Jaiswal *et al.* (in press) found that upon approaching a progesterone-containing well, a significant portion of the spermatozoa present in the accumulation zone acquired hyperactivation-like motility, resulting in very small progressive motility in spite of the vigorous motion. Consequently they remained in the vicinity of the well. In this manner, some of the spermatozoa that reach the neighbourhood of the progesterone-containing well by chance were essentially trapped there. Further evidence that progesterone is not the attractant in follicular fluid was provided by the demonstration that removal of progesterone from follicular fluid does not eliminate the chemotactic activity of the fluid but does eliminate its ability to cause hyperactivation (Jaiswal *et al.*, in press). It should be noted that the correlation, if any, between chemotaxis and hyperactivation remains unproven. It is reasonable to assume that, even though both chemotactic responsiveness and the ability to become hyperactivated are associated with capacitated spermatozoa, chemotaxis and hyperactivation do not occur simultaneously, thus avoiding mutual perturbations. Capacitated spermatozoa appear to acquire hyperactivated motility only temporarily, primarily when they encounter a proper stimulus (that is, one different from the chemotactic stimulus) such as progesterone. Thus, this motility pattern may assist spermatozoa at sites where they face mechanical

resistance, for example, when penetrating the zona pellucida (Suarez, 1996).

*Atrial natriuretic peptide.* ANP is a polypeptide hormone secreted in large quantities by the atrial portion of the heart and from a variety of other mammalian cell types. It exerts many of its actions via activation of particulate guanylate cyclase (Brenner *et al.*, 1990; Ruskoaho, 1992). ANP is present in human follicular fluids (Sundfjord *et al.*, 1989) and specific ANP receptors have been identified on human spermatozoa (Silvestroni *et al.*, 1992). Sperm chemotaxis to ANP was demonstrated by sperm accumulation in capillaries with ascending (Anderson *et al.*, 1995) and descending (Zamir *et al.*, 1993) gradients and by choice assays (Zamir *et al.*, 1993) (Table 1). It is not yet known whether ANP is involved in sperm chemotaxis *in vivo* and whether the physiological attractant for human spermatozoa is an ANP-like substance. Since chemotaxis to ANP at physiological concentrations can be observed only in the presence of a neutral endopeptidase inhibitor such as phosphoramidon, which is probably absent in follicular fluid (Zamir *et al.*, 1993), there seem to be two alternatives: either that follicular fluid contains a neutral endopeptidase inhibitor, or that ANP is not the attractant in follicular fluid. According to the latter alternative, ANP may directly affect guanylate cyclase *in vitro* in a similar manner to the physiological attractant *in vivo* (Zamir *et al.*, 1993). In support of this hypothesis, no correlation was found between the chemotactic activities of follicular fluids and their ANP content (Anderson *et al.*, 1995).

*Other hormones and heparin.* In a series of studies, Sliwa (1993a,b, 1994, 1995) demonstrated human and mouse sperm accumulation in heparin, and mouse sperm accumulation in adrenaline, oxytocin, calcitonin, and acetylcholine. Negative mouse sperm accumulation (that is, apparent repulsion) was demonstrated with glucagon and vasopressin. However, since only a single assay was used in these studies (a choice assay that did not distinguish between chemotaxis and trapping), the significance of these observations with respect to chemotaxis is not clear. Heparin has been shown to induce capacitation of bull spermatozoa and an acrosome reaction of human spermatozoa (for references, see Sliwa, 1993a), and it is possible that, like progesterone, it causes hyperactivation and trapping to occur. Because of these ancillary phenomena, unless the criteria for chemotaxis are fulfilled, positive and negative sperm accumulation should not be attributed to sperm chemotaxis.

*Antithrombin III.* Lee *et al.* (1994) demonstrated boar sperm accumulation in purified antithrombin III, a component of follicular fluid not synthesized in the follicles, although assays that distinguish chemotaxis from other processes causing accumulation have not been carried out. However, since Lee *et al.* (1994) found that antithrombin III enhances sperm motility, the sperm accumulation could be the result of chemokinesis.

### *Chemotaxis to (or away from) other substances*

Small synthetic *N*-formylated peptides, such as *N*-formyl-Met-Leu-Phe (fMLP), are attractants for neutrophils and macrophages (Schiffmann *et al.*, 1975). Such peptides bind to specific sites on human spermatozoa (Gnessi *et al.*, 1986; Ballesteros *et al.*, 1988) and cause accumulation of both human (Gnessi *et al.*, 1985) and bull (Iqbal *et al.*, 1980) spermatozoa. However, studies using the choice assay for human spermatozoa (Makler *et al.*, 1992) and track analysis of bull spermatozoa (Miller, 1982) ruled out chemotaxis as the cause of accumulation. In the case of bull spermatozoa, the accumulation was demonstrated to result from sperm adhesion to the glass surface inside the peptide-containing capillaries (Miller, 1982). These complications demonstrate the importance of carrying out assays that distinguish chemotaxis from other processes resulting in accumulation.

Not only sperm attractants have been found. Tso *et al.* (1979) demonstrated, by accumulation assays with ascending and descending chemical gradients, that *p*-nitro–phenyl–glycerol is a repellent for rat spermatozoa, in addition to being an inhibitor of sperm motility. Makler *et al.* (1995) studied hydrochloric acid, sodium hydroxide, ethanol, and glutaraldehyde as potential repellents for human spermatozoa, but found no evidence for sperm repulsion. No other studies of potential sperm repellents have been reported.

In conclusion, follicular fluid and ANP are the only substances that have been demonstrated to act as chemotactic attractants for spermatozoa by assays that differentiate between chemotaxis and other processes causing accumulation (Table 1). ANP may not be a direct physiological attractant but rather a guanylate cyclase activator. The identity of the attractant(s) in follicular fluid has yet to be revealed.

### *Molecular mechanism*

The molecular mechanism of sperm chemotaxis in mammals is obscure. If universality is assumed, the mechanism may be similar to the guanylate cyclase-mediated mechanism proposed for sperm chemotaxis in sea urchins (see above). The finding that ANP is chemotactically active (Table 1) and the suggestion that it directly affects guanylate cyclase in a manner similar to that caused by the physiological attractant (Zamir *et al.*, 1993) are in line with this possibility. However, the finding of G protein-coupled olfactory receptors in spermatozoa raises the possibility that some of these proteins are the chemotaxis receptors (Vanderhaeghen *et al.*, 1997). The observation that only a limited population of spermatozoa is stained by an antibody against a testis olfactory receptor in dogs (Walensky *et al.*, 1995) is consistent with the fact that only a small fraction of the sperm population is chemotactically responsive at a given time (Cohen-Dayag *et al.*, 1994). In dogs, the olfactory receptors were found to be localized to the midpiece of the tail of mature spermatozoa (a region rich in mitochondria), consistent with a role for these receptors in transducing chemotactic signals (Walensky *et al.*, 1995). The binding of an attractant to its receptor on the spermatozoon may thus trigger a signal transduction pathway similar to that of the olfactory system, particularly in view of the recent finding that male germ cells appear to contain all the elements of the signalling cascade present in olfactory cells (Defer *et al.*, 1998; Gautier-Courteille *et al.*, 1998).

Walensky *et al.* (1995) proposed a model for signal transduction in chemotaxis that involves modulation of adenylate cyclase or phospholipase C by the appropriate receptor-coupled G protein. As the cAMP concentration is increased, there is stimulation of respiration and motility. In parallel, the

concentration of inositol 1,4,5-triphosphate ($IP_3$) is increased, resulting in $Ca^{2+}$ release from stores in the midpiece. The resulting increased $Ca^{2+}$ concentration modulates sperm motility.

Since an increase in the intracellular $Ca^{2+}$ concentration produces flagellar beat asymmetry, and since mammalian spermatozoa possess the two types of $Ca^{2+}$ channels found in sea urchin spermatozoa, it is likely that intracellular $Ca^{2+}$ mediates the mammalian chemotactic response (see Cook *et al.* (1994) and references cited therein). This possibility is independent of whether the molecular mechanism involves guanylate cyclase or whether it involves receptor-coupled G proteins. Once the mammalian attractants are identified, the rate of progress in revealing the molecular mechanism of sperm chemotaxis is likely to increase.

## Conclusion

Sperm chemotaxis to female-originated factors appears to be involved in the fertilization of many species. In mammals, understanding the role sperm chemotaxis plays in fertilization is just beginning. Defects in sperm chemotaxis may lead to infertility and it is reasonable to suppose that, in the future, sperm chemotaxis may be exploited as a diagnostic tool for sperm quality and used as a therapeutic procedure in male infertility. In addition, the prevention of sperm chemotaxis may become an exciting new approach to contraception.

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