Sperm guidance in mammals — an unpaved road to the egg

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Abstract | Contrary to the prevalent view, there seems to be no competition in the mammalian female genital tract among large numbers of sperm cells that are racing towards the egg. Instead, small numbers of the ejaculated sperm cells enter the Fallopian tube, and these few must be guided to make the remaining long, obstructed way to the egg. Here, we review the mechanisms by which mammalian sperm cells are guided to the egg.

Capacitation
A ripening process that spermatozoa must undergo in order to penetrate the female's egg and fertilize it.

Acrosome reaction
The release of proteolytic enzymes from the top part of the sperm's head, known as the acrosome, which enables sperm penetration through the egg coat.

Chemotaxis
The movement of cells in the direction of a chemoattractant gradient.

Chemoattractant
A factor (a peptide or any other chemical) that attracts specific cells by chemotaxis.

Thermotaxis
The movement of cells that is directed according to a temperature gradient.

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For fertilization to occur in mammals, ejaculated spermatozoa must reach the egg, which, following ovulation, has moved from the ovary into the Fallopian tube. Until not too long ago, the common belief was that, in mammals, following ejaculation into the female genital tract, large numbers of spermatozoa 'race' towards the egg and compete to fertilize it. This dogma, as well as conflicting results in the literature, instilled the idea that the guidance of sperm to the egg was superfluous in mammals. The 'competitive-race model' dismantled when it became clear that, in fact, few of the ejaculated spermatozoa (in humans, only ~1 of every million spermatozoa) succeed in entering the Fallopian tubes[1–3]. Furthermore, the number of spermatozoa that can fertilize the egg is even smaller. Spermatozoa must undergo a process of ripening, known as capacitation, and only capacitated spermatozoa can penetrate the cumulus layer that surrounds the egg, bind to the sperm receptor on the egg coat, and undergo the acrosome reaction that enables sperm penetration through the egg coat and then fusion with the egg (see REF. 4 for a review).

The percentage of capacitated spermatozoa is low (~10% in humans)[4–6] and, therefore, the number of spermatozoa that can reach and fertilize the egg is small. The chances that such low numbers of spermatozoa will successfully reach the egg by coincidence, without a guidance mechanism, are very slim. This realization has fuelled an interest in the study of sperm guidance in mammals, and here we summarize the state of the art in this young and rapidly changing field, comment on new findings and discuss the direction in which this research will probably go.

Mechanisms of mammalian sperm guidance

Two active mechanisms of sperm guidance have been shown in mammals: chemotaxis, which is the movement of cells up a concentration gradient of chemoattractant (see REF. 7 for an extensive review), and thermotaxis[8] — the directed movement of cells along a temperature gradient.

Chemotaxis was discovered as a form of sperm guidance in the mid-1960s in marine species[9,10], and has only been recognized in amphibians and mammals in the past 15 years[7,11]. The initial resistance to the notion of sperm chemotaxis in mammals was compounded by the inconsistent conclusions drawn from initial studies (see REFS 3,11 for reviews). One of the main reasons for the apparent inconsistency was the very low signal-to-noise ratio that was obtained in chemotaxis assays with mammalian spermatozoa. This factor, combined with suboptimal experimental conditions and a failure to distinguish between true chemotaxis and other causes of sperm accumulation (reviewed in REF. 3), resulted in conflicting results and ambiguity. Today, we know that the low signal-to-noise ratio is caused by the fact that, in mammals (unlike sperm chemotaxis in marine species[9]), only the small fraction of capacitated spermatozoa are chemotactically responsive[5,12–14]. This realization, combined with the development of assays that allow chemotaxis to be distinguished from other processes[7] (BOX 1), has established that chemotaxis is indeed a mammalian sperm-guidance mechanism.

The first experiments in mammals showed that human sperm accumulated in the follicular fluid[15,16], and that there was a remarkable correlation between this *in vitro* accumulation and egg fertilization[16]. Subsequent experiments confirmed that this accumulation was indeed the consequence of chemotaxis[17]. Sperm chemotaxis was later also demonstrated in frogs[18], mice[14,19] and rabbits[13]. In addition, the accumulation of sperm in follicular fluid (but without substantiating that it truly reflects chemotaxis) was demonstrated in horses[20] and pigs[21]. So, sperm chemotaxis seems to be a widespread mechanism that guides spermatozoa to the egg, both when fertilization is external (as in marine species) or internal.

Box 1 | **The measurement of sperm chemotaxis**

In sperm chemotaxis assays, it is essential to apply well-defined criteria that distinguish between chemotaxis and other processes that might cause sperm accumulation. There are two main criteria: the directional change of sperm movement towards the chemoattractant source — a unique feature of chemotaxis, which, unfortunately, is fulfilled in only the first two assays that are commonly used to measure sperm chemotaxis (see below), and a peak-like dependence (rather than a saturation-curve dependence) of the measured response on the chemoattractant concentration. The latter criterion applies to all chemotaxis assays and it relies on the fact that when a certain chemoattractant concentration saturates its cognate receptor, the cell cannot sense any further increases in the chemoattractant concentration and, as a result, the chemotactic response drops.

The assays listed below have been used to determine sperm chemotaxis. In all of them, a comparison is made to a no-gradient situation as a control.

Directionality assays
The video-recorded movement tracks of spermatozoa in a chemoattractant gradient are analysed to determine which tracks involve directional changes towards the source of the chemoattractant. The gradient can be two-dimensional (when the source is point-like)[17,35,36,49] or linear (when the source is linear)[13].

Descending chemoattractant gradient assays
Spermatozoa are suspended in a solution containing a chemoattractant and their accumulation at the bottom of a chemoattractant gradient is compared with their accumulation at the same location when the chemoattractant concentration is constant throughout the measuring unit (a no-gradient control)[11,17]. Chemotactically responsive spermatozoa (unlike chemokinetically responsive spermatozoa that respond to the chemoattractant by only increasing their speed of movement) are expected to accumulate to a lesser extent in the former setup. Trapped spermatozoa would not distinguish between the presence and absence of a gradient. Therefore, in most cases, this assay allows a distinction between chemotaxis, chemokinesis and trapping.

Sperm accumulation in an ascending chemoattractant gradient
The number of spermatozoa that sense an ascending chemoattractant gradient and accumulate near or at its source is counted. Because sperm accumulation can also be caused by trapping or changes in swimming speed, this method cannot distinguish between chemotaxis and other causes of sperm accumulation[11].

Choice assay
Spermatozoa 'choose' between a chemoattractant-containing and a chemoattractant-free well. Such assays can distinguish between chemotaxis and chemokinesis. However, because a higher sperm concentration near the chemoattractant-containing well can be also caused by trapping, these assays cannot distinguish between chemotaxis and trapping[11,17].

Follicular fluid
A fluid consisting of sex steroid hormones, plasma proteins, mucopolysaccharides and electrolytes that surrounds the ovum in the vesicular ovarian follicle (Graafian follicle).

Oviduct
A tube between the ovary and the uterus, through which the egg is transported from the former to the latter and in which fertilization occurs. It consists of two parts: the isthmus — a narrow part that is closer to the uterus — and the ampulla — a wider part that is closer to the ovary.

Cumulus cells
The cells that form dense layers surrounding a mature egg.

In thermotaxis, spermatozoa are directed towards a higher temperature and this mechanism of sperm guidance has so far been demonstrated in only two species: humans and rabbits[8]. Chemotaxis and thermotaxis seem to have a similar function — to guide capacitated, 'ready-to-fertilize' spermatozoa towards the egg that resides at the fertilization site. Another potential passive guidance mechanism might be the drag of spermatozoa by muscle contractions in the female genital tract[22]. The existence of two or more sperm-guidance mechanisms implies that sperm guidance is essential for fertilization.

Mammalian sperm guidance in the female genital tract. In mammals, the semen is ejaculated either into the vagina (in primates, ruminants and rabbits) or into the uterus (in rodents, pigs and horses)[1]. From there, spermatozoa of the former group use a combination of active swimming and passive drag through muscle contraction to reach the storage site in the isthmus of the oviduct (FIG. 1; BOX 2). *In vitro* experiments imply that, of the few hundreds spermatozoa that reach the storage site, only some become capacitated[5]. These few spermatozoa have to negotiate the long (2–6 cm in humans[1,23] and ~10 cm in rabbits[24]) obstructed road that separates them from the egg at the fertilization site[25]. *In vitro* findings indicate that capacitated spermatozoa are guided from the storage site to the egg primarily by a combination of chemotaxis[5,26] and thermotaxis[8], assisted perhaps by oviductal contractions[27].

It would seem logical that the capacitated spermatozoa at the storage site use an ovulation-dependent temperature gradient between this site and the fertilization site[24,28,29] as a 'road sign' to guide them to the site of fertilization by thermotaxis. Additionally, the finding that, at least in mice, oviductal fluid contains one or more chemoattractants[14] raises the possibility that different chemoattractants might be secreted along the oviduct to promote sequential, short-range chemotactic processes towards these chemoattractants. As the spermatozoa approach the vicinity of the fertilization site, they probably sense a chemoattractant gradient that originates at the cumulus cells[26] (see below), which guides them to the egg–cumulus complex. However, the arrival at this complex does not guarantee entry into the egg itself[30], because the egg is covered with a dense matrix that is composed of hundreds of cumulus cells (FIG. 1). It is thought that a chemoattractant gradient that originates at the egg is established within the cumulus matrix, which guides the spermatozoa to the egg[26]. Indeed, the first few spermatozoa that enter the cumulus are successful in finding the egg[31].

Mammalian sperm chemotaxis

Physiology of sperm chemotaxis. The encounter between the gametes of marine species, in which fertilization is external, occurs in a turbulent, aqueous environment that usually contains eggs and spermatozoa from various species. Therefore, the aim of the long-recognized process of sperm chemotaxis in these animals is suggested to be not only to recruit as many spermatozoa as possible to the eggs, but also to prevent cross-species fertilization. Accordingly, the main physiological characteristics of sperm chemotaxis in marine species include: first, the responsiveness of a large fraction of the sperm population and, second, in most genera, a high species specificity[9,10] (that is, a chemoattractant for one marine species is usually not recognized by the spermatozoa of another marine species). By contrast, in mammals, semen is placed inside the female genital tract, and sperm competition, if it exists, is limited to semen from different individuals of the same species[32]. It is therefore not surprising that species specificity was not detected in experiments that compared the chemotactic responsiveness of spermatozoa of a few mammalian species to follicular fluids or egg-conditioned media[33].

An intriguing question is how large the fraction of chemotactically responsive spermatozoa in mammals is. On the one hand, several studies concluded that, in human and rabbit spermatozoa, only capacitated spermatozoa — which constitute a small fraction of the sperm population — are chemotactically responsive[5,6,13,34]. This conclusion was based on the similarity between capacitated and chemotactically responsive spermatozoa in terms of the

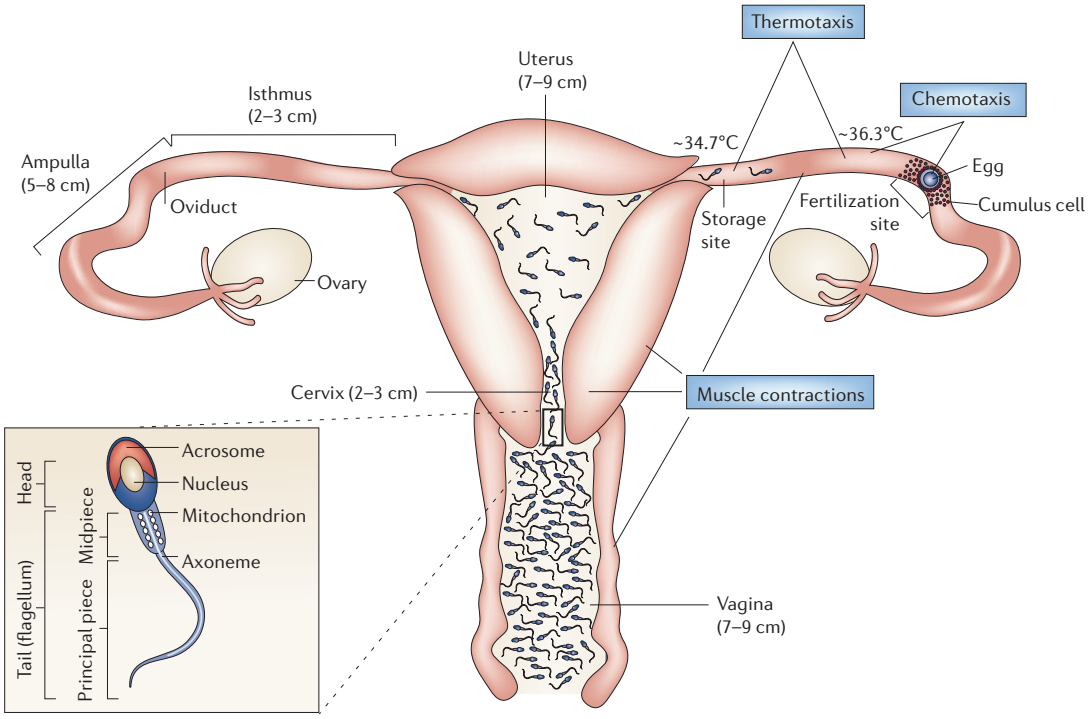

Figure 1 | **The mammalian female genital tract and possible guidance mechanisms.** The diagram, which is not drawn to scale, and the dimensions shown are derived from studies in humans[1], whereas the temperature values shown are those measured in rabbits[24] (there are no published measurements in humans). The dimensions in parentheses indicate the length of the respective organ. The spermatozoa in the vagina use both active swimming and passive drag by female genital-tract muscular contraction to reach the storage site in the oviduct. Once here, a small fraction of spermatozoa undergoes ripening, or capacitation, which enables them to fertilize the egg at the fertilization site. Capacitated spermatozoa are guided from the storage site to the egg by a combination of chemotaxis, thermotaxis and, perhaps, oviductal contractions. An ovulation-dependent temperature gradient between the storage site and the fertilization site provides the thermotactic stimulus. Chemoattractants are present in the oviductal fluid and are also secreted by the egg and the surrounding cumulus cells — providing the chemotactic stimulus that guides the spermatozoa to the egg–cumulus complex. The insert shows a human spermatozoon, which comprises the head (with the acrosome and the nucleus) and the tail or the flagellum, consisting of the midpiece (where mitochondria are located around the axoneme) and the principal piece.

size of the fraction in the sperm population, and on the observation that their lifespans are similarly short (see below) and that they similarly undergo, and with similar kinetics, continuous replacement (thereby ensuring the continuous availability of capacitated and chemotactically responsive spermatozoa in spite of their short lifespan). In addition, this conclusion was also based on the fact that the deliberate depletion of capacitated spermatozoa results in a total loss of chemotaxis and, *vice versa*, the depletion of chemotactic spermatozoa results in the depletion of capacitated spermatozoa (see REF. 34 for a review). A similar fractional chemotactic responsiveness was observed in mouse spermatozoa[14,19].

On the other hand, Spehr *et al.*[35] showed that ~90% of the motile cells in human spermatozoa swim up the gradient of a chemoattractant known as bourgeonal. The same group noticed, however, that only about one third of the cells responded to bourgeonal with a rise in the intracellular level of Ca^{2+} — a response thought to occur in every cell that responds chemotactically[36]. The reason for the much higher fraction of responsive cells in the chemotaxis assays of Spehr *et al.*[35,36] (compared with other published chemotaxis assays and compared with the fraction of cells exhibiting Ca^{2+} elevation in their own experiments[36]) awaits clarification. The discrepancy is not due to the chemoattractant used, because it was noted that the chemotactic response of human spermatozoa to bourgeonal was as fractional and small as the response to other chemoattractants (M.E., unpublished data).

The capacitated and chemotactic responsive state is neither static nor prolonged. Rather, it is transient and short (1–4 hours in humans, as measured *in vitro*), and occurs only once in the sperm's lifetime[5,12] (3–5 days in humans[37,38]). Nevertheless, as different spermatozoa become capacitated at different times, this continuous replacement of capacitated cells in the sperm population ensures that capacitated spermatozoa are present in the female genital tract for extended periods[5]. This is essential in species that ovulate periodically (for example, humans), in whom this extended, continuous replacement probably ensures the availability of capacitated and chemotactic competent cells throughout the lifespan of spermatozoa in the female genital tract[5,6]. The extended availability of capacitated, chemotactic spermatozoa seems to be less

Axoneme
An axial filament complex at the centre of the sperm tail.

Hydrozoa
A class of radially symmetrical marine or freshwater invertebrates of the phylum Cnidaria, with one end of the body bearing the mouth and tentacles. This class includes polyps and medusa.

Hydromedusa
A hydrozoan in the medusoid stage of its life cycle.

Ascidian
A marine invertebrate animal that has a transparent sac-shaped body with openings through which water passes; also known as sea squirt.

Chemokinesis
The speed enhancement of actively moving cells in response to a stimulus.

essential in species with induced ovulation (for example, rabbits), in which ovulation is induced by mating. In such species, capacitation seems to be synchronized with ovulation[6]. It therefore seems that the timing of the availability of capacitated, chemotactic spermatozoa is programmed in mammals according to the time at which an ovulated egg is available in the female genital tract[6].

Behavioural mechanisms of chemotaxis. Chemotaxis is characterized by directional changes in the movement towards the source of the chemoattractant. Therefore, the most direct assays for the measurement of sperm chemotaxis are based on the direction of sperm movement[7,11]. The behaviour of spermatozoa in response to a chemoattractant depends on their variable swimming patterns. In the most well-studied spermatozoa of marine and freshwater species[9,39], the swimming patterns and the changes that occur in response to a chemoattractant can be classified into two categories (FIG. 2). The first category includes spermatozoa that swim in almost straight or curved lines but, when exposed to a chemoattractant gradient, there is an abrupt change in the direction of swimming towards the chemoattractant source. Spermatozoa of hydrozoa (for example, *Campanularia flexuosa* and *Gonionemus vertens*) belong to this category[9]. The second category includes spermatozoa that swim in circles but, when exposed to a chemoattractant gradient, the circles become biased towards the chemoattractant source, resulting in a spiral path towards the gradient. The spermatozoa of hydromedusa, ascidian, sea urchin and starfish belong to this category[9,40,41]. A detailed model of the intracellular molecular events that mediate the formation of this spiral path has recently been proposed by Böhmer *et al.*[41] Unique features of this model are that it does not require the ability of sperm to sense a descending chemoattractant gradient, and that the spermatozoa sample the environment intermittently rather than continuously.

Mammalian spermatozoa usually swim in either a straight or a curved line, depending on the pattern of flagellar movement[42]. However, little is known about the behavioural response of these spermatozoa in a chemoattractant gradient. What is known is that when human spermatozoa are swimming in an ascending chemoattractant gradient, they beat their flagella symmetrically and reach the chemoattractant source by maintaining the same swimming direction[35]. Concomitantly, their swimming speed increases (this phenomenon is known as chemokinesis)[17] as a result of increased flagellar beat frequency[35]. Conversely, when human spermatozoa are swimming away from the chemoattractant source bourgeonal, they abruptly turn around as a result of an asymmetrical flagellar beat and then swim towards the source[35].

This response to a descending chemoattractant gradient apparently differs from the presumed inability of sea-urchin spermatozoa to sense a descending gradient of their chemoattractant, resact, owing to the irreversible resact–sperm binding[41,43]. This inability might also occur in human spermatozoa with the chemoattractant progesterone (dissociation constant $K_d \approx 6 \times 10^{-10}$ M; REF. 44). Therefore, it is reasonable to postulate that there are multiple mechanisms by which spermatozoa respond to a chemoattractant gradient, depending on the species and the chemoattractant.

Another important question is how spermatozoa sense the chemoattractant gradient. Do they compare the chemoattractant concentrations at different locations on the cell membrane (the detection of a spatial gradient), or do they compare the chemoattractant concentrations at different time points (the detection of a temporal gradient), as do bacteria[45]? This question has not been addressed in mammals. However, the finding that a sudden temporal increase in the concentration of the chemoattractant

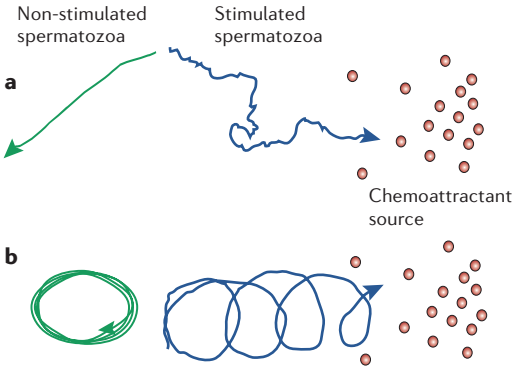

Figure 2 | **Two types of sperm response to chemoattractants.** The green and blue lines represent tracks made by swimming spermatozoa. **a** | Non-stimulated spermatozoa swim either in almost straight or in curved lines. In the presence of a chemoattractant gradient, the direction of the swimming movements changes abruptly towards the chemoattractant source. The drawn tracks are based on the swimming of human spermatozoa as reported in REFS 17,49. **b** | Non-stimulated spermatozoa swim in concentric circles that, when in a chemoattractant gradient, change into loops towards the chemoattractant source. The drawn tracks are based on the swimming of ascidian and sea-urchin spermatozoa as reported in REF. 40 and REFS 43,66, respectively.

Box 2 | **Sperm transport in the female genital tract**

In mammals that ejaculate semen into the vagina, sperm transport through the cervix depends on sperm motility as well as on the muscular activity of the vagina, cervix and uterus[90]. In the uterus, the spermatozoa are passively driven towards the small opening of the oviduct (Fallopian tube) by waves of uterine smooth muscle contractions[22,90,91]. This small opening at the uterotubal junction provides an additional barrier to sperm entry into the oviduct, and successful entry seems to require active sperm motility[91]. The number of spermatozoa that enter the oviduct is remarkably constant[90] (in humans, only ~10% of the spermatozoa that were present in the uterus[3]). The oviduct has two regions, the isthmus and the ampulla (FIG. 1), each of which has different anatomical and physiological characteristics. The spermatozoa that enter the isthmus bind strongly to the oviductal epithelium and become trapped, forming a reservoir[91] (FIG. 1). It is thought that, while residing in this storage site, the spermatozoa undergo capacitation[4,91], although asynchronously[5,34]. Owing to their lower affinity for the epithelium, spermatozoa that become capacitated are released from the storage site. The few spermatozoa that are released probably require guidance to reach the ovulated egg successfully[3], which, depending on the species, either resides in the isthmic–ampullary junction or in the ampulla of the oviduct[1] (FIG. 1).

Table 1 | **Confirmed and putative chemoattractants for mammalian spermatozoa**

Substance	Female source	Species	Reference
Confirmed substances			
Atrial natriuretic peptide	FF	Human	59,60
Bourgeonal	Unknown	Human	36
Lyral	Unknown	Mouse	51
Peptides (<1.3 and ~13 kDa)	FF	Human	92
Progesterone*	CO, FF, OF	Human, rabbit	50
RANTES	FF	Human	54
Putative chemoattractants**			
Acetylcholine	FF	Mouse	93
Adrenaline	FF	Mouse	94
Antithrombin III	FF	Pig	95
Calcitonin	FF	Mouse	93
β-Endorphin	FF	Mouse	96
Heparin	FF	Human, mouse	97,98
Hyaluronic acid	CO	Human	99
Oxytocin	FF	Mouse	94
Peptide (8.6 kDa)	FF	Pig	21
Substance P	FF	Mouse	96

*Of the currently identified chemoattractants, only progesterone has been shown to be present near the egg in an aspirated human egg–cumulus complex[50]. **Substances that have been reported to cause sperm accumulation but have not been confirmed as chemoattractants. CO, cumulus oophorus; FF, follicular fluid; OF, oviductal fluid.

resact (achieved by the photorelease from caged resact — a chemotactically inactive analogue of resact that releases resact in response to a short pulse of light) causes a behavioural response of sea-urchin spermatozoa in the absence of a spatial gradient[41,43] indicates that these spermatozoa sense a temporal gradient.

Chemoattractants and their cellular origin

Over the years, many substances have been claimed to be chemoattractants for mammalian spermatozoa[11] (TABLE 1). However, because sperm accumulation at the optimal chemoattractant concentration — which is one of the characteristics of sperm chemotaxis — can be caused by other processes such as chemokinesis and sperm trapping[7,11], a clear-cut criterion for distinguishing between these processes (BOX 1) should be used in assays that identify putative chemoattractants. So far, only a few putative chemoattractants satisfy the criterion discussed in BOX 1, and the discussion below is therefore restricted to these chemoattractants. It should be noted that the physiological significance of most of these substances is still unclear.

Sources of chemotactic activity in mammals. One of the first physiological sources to be investigated for chemotactic activity was the mammalian follicular fluid[7,11], which contains the pre-ovulatory secretions of the egg and its surrounding cells. Chemotaxis towards the follicular fluid, which has so far been shown for human[17], mouse[14] and rabbit[13] spermatozoa, seems to be highly correlated with the chance of fertilizing an egg, at least in humans[16]. However, it is unlikely that chemotaxis to the follicular fluid also occurs *in vivo*[26], because the chemoattractant gradient in the oviduct is anticipated to be maintained for as long as the egg survives in the female genital tract (~24 h post-ovulation in humans[1]). This would require a continuous supply of chemoattractant, whereas the follicular fluid is only released as a single event at ovulation. Therefore, the physiological significance of the results obtained with follicular fluid is restricted to the implication that the egg and/or its surrounding cells secrete(s) a chemoattractant(s) within the follicle prior to ovulation.

So, does the mammalian female egg–cumulus complex secrete sperm chemoattractants subsequent to ovulation? This question has recently been answered by the findings that media conditioned either with individual, mature human eggs or with the surrounding cumulus cells are chemotactically active[26]. This indicates that sperm chemoattractants are secreted, not only prior to ovulation within the follicle, but also after egg maturation outside the follicle, and that there are two chemoattractant sources: the mature egg and the surrounding cumulus cells.

Identification of mammalian chemoattractants. The identity of the chemoattractant that is secreted from the egg is not yet known. One chemoattractant that is secreted by the cumulus cells is probably progesterone. The identification of this steroid as a sperm chemoattractant was not straightforward. Initially, it was shown that nM to mM concentrations of progesterone caused the accumulation of human sperm, and that this could be inhibited by a progesterone receptor antagonist[46–48]. This and other findings led to the suggestion that progesterone

Photorelease
The rapid release of a compound from its caged (protected) analogue by a short pulse of light.

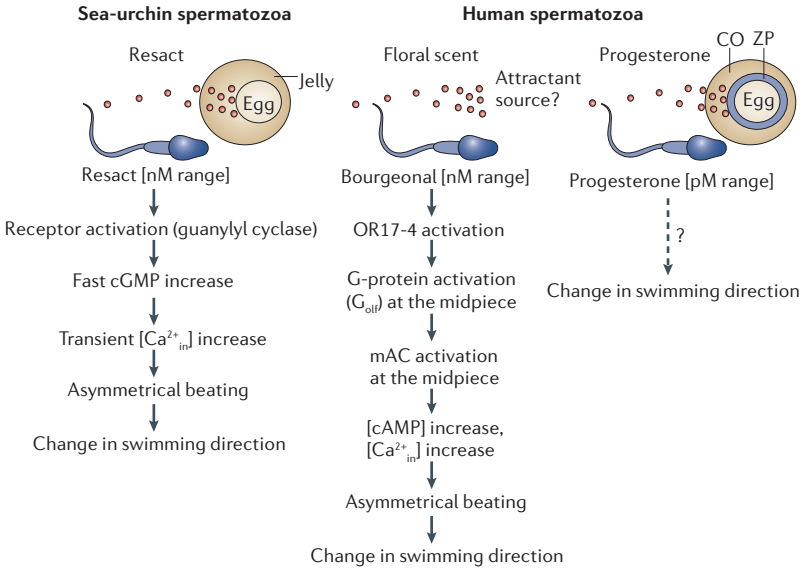

Figure 3 | **Models for the molecular mechanisms of sperm chemotaxis in marine species and mammals.** In the absence of sufficient molecular information, the models of the molecular mechanisms of sperm chemotaxis are inevitably oversimplified. In the case of spermatozoa of the sea urchin *Arbacia punctulata*, resact — a chemoattractant that is produced by the egg jelly — binds to its specific surface receptor (probably located on the flagellum), a guanylyl cyclase, and activates it. The resulting elevation of the level of cyclic GMP (cGMP) leads to a transient increase in the intracellular concentration of Ca^{2+} (Ca^{2+}_{in}). This, in turn, causes the asymmetrical beating of the flagellum and, consequently, a change in the swimming direction. In the case of human spermatozoa, the chemoattractant bourgeonal (or its physiological analogue) activates the odorant receptor OR17-4, which is bound to a G protein (G_{olf}) that is located at the midpiece of the flagellum. As a consequence, a membrane adenylate cyclase (mAC) is activated at the midpiece and the levels of cyclic AMP (cAMP) and Ca^{2+}_{in} rise. As before, these events induce the asymmetrical beating of the flagellum and, consequently, a change in the swimming direction. In the case of the chemoattractant progesterone, which is produced by the cumulus cells, the signal-transduction pathway that is triggered by its binding to the progesterone receptor is not known. The location of the progesterone chemotaxis receptor in the spermatozoon is also unknown. CO, cumulus oophorus; ZP, zona pellucida.

was the chemoattractant in follicular fluid[46]. However, as discussed in BOX 1, the occurrence of sperm chemotaxis cannot be concluded on the basis of sperm accumulation alone[11], and therefore these results were inconclusive. Indeed, it was shown that progesterone is almost inactive as a chemoattractant when used at the same concentration as that found in chemotactically active follicular fluid, and the accumulation of human spermatozoa caused by this steroid hormone was shown to be mainly owing to sperm trapping, resulting from progesterone-stimulated hyperactivation[49]. However, progesterone was recently shown to be chemotactically active at concentrations as low as 10^{-11}–10^{-10} M for both human and rabbit spermatozoa, and evidence for a progesterone gradient along the cumulus cell mass has been provided[50]. (The reason why spermatozoa chemotactically respond to progesterone at a concentration of 10^{-11}–10^{-10} M, but not at much higher concentrations[16,49], is that, in chemotaxis, the response drops at too high chemoattractant concentrations; see BOX 1.) It is not yet known whether the cumulus cells also secrete chemoattractants other than progesterone.

These findings have bearings on the issue of species specificity. A single chemoattractant that is common to various species would account for the lack of specificity in the chemotactic response of mammalian spermatozoa. Therefore, the secretion of progesterone by the cumulus cells could explain the lack of species specificity reported for follicular fluids and conditioned media of several mammals[33]. It remains to be seen whether the chemoattractant that is secreted from the egg (which is probably a different chemoattractant[26]) is species specific or common.

Recently, two odorants — bourgeonal (at concentrations ≥10^{-8} M)[36] and lyral (at concentrations in the mM range)[51] — were found to be chemoattractants for human and mouse spermatozoa, respectively. As these chemoattractants are probably not secreted in the female genital tract, it is likely that they are not the physiological sperm chemoattractants and that their cognate receptors (discussed below) identify other, structurally similar chemoattractants (not yet known).

The 8-kDa chemokine RANTES (regulated on activation, normally T-cell expressed and secreted chemokine), a potent chemoattractant for eosinophils, monocytes and T lymphocytes[52,53], was shown to be a chemoattractant for human spermatozoa. This chemokine is known to be present in follicular fluid, and the mRNA for its receptor has been found in human spermatozoa[54]. RANTES is produced by granulosa cells within the follicle in the ovaries, prior to ovulation, and its production is upregulated in some diseases that are associated with infertility[54]. It is not known whether RANTES is also secreted in the female genital tract after ovulation. If RANTES is a physiologically relevant chemoattractant for human spermatozoa, it is an example of a substance that serves as a chemoattractant for different cell types.

Atrial natriuretic peptide (ANP) is a polypeptide hormone that is secreted from the atrial portion of the heart and from other mammalian cell types, and that activates particulate guanylyl cyclase[55,56]. ANP is present in human follicular fluid[57] and ANP-specific receptors have been identified on human spermatozoa[58]. Although sperm chemotaxis to ANP was demonstrated (but only in the presence of a neutral endopeptidase inhibitor, such as phosphoramidon, to avoid the inactivation of ANP by the residual neutral endopeptidase-containing seminal fluid)[59,60], it is not known whether ANP is involved in sperm chemotaxis *in vivo*.

In the amphibian *Xenopus laevis*[18], allurin is a 21-kDa sperm chemoattractant. We mention it in the context of mammalian sperm chemoattractants because it shares homology with mammalian sperm-binding proteins[61]. This chemoattractant is expressed and secreted from the *X. laevis* oviduct in a hormone-regulated manner. It is thought that at ovulation, as the egg progresses down the oviduct, allurin is applied onto the jelly layer that surrounds the *X. laevis* egg[62]. Again, whether or not the mammalian oviduct contains allurin-like chemoattractants is an open question.

Why are there so many chemoattractants? There are several possible answers to this question. First, spermatozoa might sense different chemoattractants; that

Hyperactivation
A motility pattern that is characterized by increased velocity, decreased linearity, increased amplitude of lateral head displacement, and flagellar whiplash movement.

Granulosa cells
The cells that form layers surrounding the oocyte within the follicle.

is, they might undergo multistep chemotaxis as they travel through the female genital tract. Each step might sequentially guide the spermatozoa to the next chemoattractant source. This implies that sperm guidance might be much more elaborate than originally thought, and that chemotaxis extends over a much longer distance. Second, perhaps each spermatozoon combines the information received from the different chemotaxis receptors and 'calculates' its location according to the relative concentrations of the sensed chemoattractants. Third, it is possible that different chemoattractants elicit different behavioural sperm responses. Fourth, because fertilization is so crucial, the multiplicity of chemoattractants might serve as a 'back-up'. Finally, different spermatozoa might respond to different chemoattractants, resulting in sperm selection. This possibility is consistent with the suggestion (based on the finding that human leukocyte antigen-linked olfactory receptor genes are transcribed in the testis) that testis-expressed human leukocyte antigen and olfactory receptor proteins are functionally connected and serve in the selection of spermatozoa, enabling them to distinguish 'self' from 'non-self'[63]. It is also possible that chemoattractant-specific sperm selection, if it exists in mammals, might be involved in sperm competition or might enable the female to choose sperm. We would like to emphasize that all these possibilities are speculative and there is currently no hard evidence in support of any of them.

Molecular mechanisms of sperm chemotaxis

The molecular mechanisms of sperm chemotaxis are poorly understood. The few known pieces of the puzzle were derived from studies in marine invertebrate spermatozoa, in which sperm chemoattractants are well known and in which (unlike mammals) most of the spermatozoa are chemotactically responsive and, therefore, sperm chemotaxis is easily measurable[9,10]. Models of the molecular mechanism of sperm chemotaxis in such species have been published (for reviews, see REFS 64,65). However, recent studies, carried out primarily in the sea urchin *Arbacia punctulata*[43,66] and the starfish *Asterias amurensis*[67], do not substantiate the published models. These studies led to the proposal of another model, in which the chemotaxis receptor, a guanylyl cyclase[68] located on the flagellum[67], is activated in response to chemoattractant binding[69] (resact in *A. punctulata*[70] and asterosap in *A. amurensis*[67]). Consequently, the concentration of cyclic GMP rises rapidly and transiently[43,67], and this transient rise is thought to result in a spike of the intracellular concentration of Ca^{2+} (Ca^{2+}_{in})[43,67,71,72]. The Ca^{2+}_{in} elevation is known to bring about increased flagellar asymmetry and reduced linearity of swimming[71,73]. So, depending on the timing of the Ca^{2+}_{in} spikes, sperm swimming is altered to approach the chemoattractant[41].

The available information about the molecular mechanisms of sperm chemotaxis in mammals is limited and mainly includes the identity of some chemotaxis receptors and chemoattractant-induced Ca^{2+}_{in} changes. The finding of G-protein-coupled olfactory receptors in mammalian sperm[74–82], and their localization to the midpiece of the tail of mature spermatozoa[35,78], raised the possibility that some of these proteins might be chemotaxis receptors[74,77]. Recently, two distinct olfactory receptors — OR17-4 (also known as OR1D2) on the flagella of human spermatozoa[36] and OR23 in mouse round spermatids (exact location not yet determined)[51] — were identified and their respective ligands, bourgeonal and lyral, were found to be sperm chemoattractants.

The stimulation of human spermatozoa with bourgeonal results in a transient rise of Ca^{2+}_{in}[36], originating at the flagellar midpiece and propagating to the sperm head[35]. The finding of membrane-associated adenylyl cyclase isoforms on the sperm flagellum and the observation that a specific antagonist of this enzyme (SQ22536 at a concentration of 5 mM) inhibited the chemotactic response to bourgeonal, raised the possibility that the chemotactic response to bourgeonal might be mediated by membrane adenylyl cyclase[35]. (To substantiate this possibility, experiments demonstrating that bourgeonal indeed causes cAMP elevation, and that such elevation increases Ca^{2+}_{in}, should be carried out with human spermatozoa.) It therefore seems possible that the chemotactic stimulation of OR17-4 triggers a signalling pathway similar to that of the olfactory system[35] (FIG. 3). It should be pointed out that many of the olfactory receptors are expressed in the testes; therefore, it is quite possible that many of them are simultaneously present in sperm. If this is found to be the case, it will be in line with the multiplicity of chemoattractants, as discussed above.

The finding that progesterone at concentrations in the pM range is a chemoattractant for human and rabbit spermatozoa[50] indicates that at least one of the two progesterone receptors, which were identified on the cell surface of mammalian spermatozoa[44,83,84] (and that are distinct from the nuclear progesterone receptor of somatic cells), is a chemotaxis receptor. These progesterone receptors bind progesterone with a K_d of 6×10^{-10} and 3×10^{-5} M, respectively[44]. They have not yet been cloned, isolated or sequenced. One of the progesterone receptors is located at the head of the spermatozoon, but it is not known whether this is the chemotaxis receptor or whether the progesterone chemotaxis receptor is a different protein located on the flagellum, similar to OR17-4.

The signal-transduction pathway that takes place during chemotaxis to progesterone has not been elucidated. Recently, however, Harper *et al.*[85] showed that a concentration gradient of progesterone, starting at less than 10^{-8} M, stimulates Ca^{2+}_{in} oscillations at the junction between the caudal head and the midpiece of the spermatozoon. These Ca^{2+} oscillations, which are reminiscent of the faster oscillatory spikes observed in the sea urchin *A. punctulata* on stimulation with the chemoattractant resact[41], are synchronous with flagellar activity, indicating that they modulate the flagellar beat[85]. Other progesterone-triggered molecular events have been identified in mammalian spermatozoa (for example, cell membrane depolarization, increased intracellular pH, elevated cAMP concentration, and the activation of phospholipase A, protein kinase C and other kinases). However,

Olfactory receptor
An integral membrane protein that is associated with a G-protein and is involved in effecting the sense of smell.

Spermatid
An immature gamete that develops into a spermatozoon.

Nuclear progesterone receptor
A progesterone-inducible transcription factor that is located intracellularly.

because progesterone stimulates a number of other sperm functions, such as hyperactivation, capacitation and acrosome reaction, most of these molecular events were associated with these functions (see REF. 86 for a review). It remains to be seen whether some of these intracellular events are also part of the progesterone-induced chemotactic signalling pathway.

Another possibility is that, as in sea-urchin and starfish spermatozoa, one of the chemotaxis receptors on mammalian spermatozoa is guanylyl cyclase. However, the only indirect evidence for this is the finding that human spermatozoa respond chemotactically to ANP, which is an activator of membrane guanylyl cyclase[59]. It is possible that mammalian spermatozoa possess several signal-transduction pathways that might be complementary, or that different chemoattractants might trigger different signal-transduction systems.

Mammalian sperm thermotaxis

The fertilization site in the oviduct has been shown to be 1–2°C warmer than the storage site for spermatozoa in rabbits[28] and in pigs[29] (FIG. 1). However, data for the temperature in the human fallopian tube are not available. A recent study in rabbits showed that this temperature difference is time dependent and that the difference increases from 0.8 ± 0.2°C before ovulation to 1.6 ± 0.1°C after ovulation[24]. This change is due to a temperature decrease at the storage site rather than a temperature increase at the fertilization site. Three mechanisms have been proposed for the ovulation-dependent temperature decrease at the storage site[24]: first, the hormone-controlled localized release of acid mucus glycoprotein — a macromolecule that undergoes extensive endothermic hydration — at the storage site[87]; second, blood from the nearby ovarian vein cools the blood that enters the storage site by counter-current heat exchange[28,29,88]; and third, an ovulation-dependent change in the source of blood supply to the storage site — the warmer ovarian artery prior to ovulation and the cooler uterine artery subsequent to ovulation[89]. It is possible that these mechanisms function in concert to reduce the temperature at the storage site efficiently.

Bahat *et al.*[8] showed that rabbit and human spermatozoa can sense a temperature difference and respond to it by thermotaxis; that is, by swimming from the cooler to the warmer temperature. The thermotactic response is as strong at a temperature difference of 0.5°C as at a difference of 2°C, which indicates that spermatozoa can sense small temperature differences. However, because the distance between the storage and fertilization sites is much larger than the distance employed in the *in vitro* thermotaxis assay[8], the temperature gradient that is sensed by the spermatozoa *in vivo* is probably shallower. It is not yet known whether spermatozoa can sense as shallow a temperature gradient as the one that exists within the oviduct. As in the case of mammalian chemotaxis, only capacitated spermatozoa are thermotactically responsive[8]. The molecular mechanism of sperm thermotaxis is not yet known.

Conclusions and perspectives

The research field of mammalian sperm guidance was non-existent a little more than a decade ago. In recent years, the concept of mammalian sperm guidance has been revolutionized in the sense that, unlike the prevailing dogma, it became clear that mammalian spermatozoa have the ability to be actively guided to the egg. Two of the mechanisms by which this guidance is achieved have been revealed: chemotaxis and thermotaxis. However, many important questions are still unresolved. For example, what are the identities of all the physiological chemoattractants? Are the egg and the cumulus cells the only physiologically relevant sources of chemoattractants *in vivo*? Are there sequential processes of chemotaxis along the oviduct? Are there several different chemoattractant-specific, behavioural response mechanisms in each mammalian species? And if so, what purpose does this multiplicity of mechanisms serve? What are the signal-transduction pathways that are involved in sperm chemotaxis in response to the various chemoattractants, and do these pathways function in concert or individually under different conditions? Does thermotaxis have a unique signal-transduction pathway or does it converge on one of the chemotaxis pathways? What is the identity of the thermosensor for thermotaxis? Furthermore, all the conclusions about the function and location of sperm chemotaxis and thermotaxis have been reached on the basis of *in vitro* experiments only and they, therefore, await verification *in vivo*.

Obtaining answers to questions like these will not only increase our understanding of mammalian fertilization but might also allow obvious clinical applications. For example, both chemotaxis and thermotaxis can potentially be used in clinical procedures to obtain sperm populations that are enriched with capacitated spermatozoa *in vitro*. They can also be exploited as a diagnostic tool to assess sperm quality. In addition, these processes can potentially be used, in the long run, as a means of contraception by interfering with the normal process of fertilization.

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Competing interests statement
The authors declare no competing financial interests.

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