Introduction

Within the female genital tract mammalian spermatozoa are known to undergo a process of maturation, termed capacitation, which confers on them a number of capabilities: the ability to be released from the sperm storage site in the isthmic part of the Fallopian tube, to be guided by thermotaxis and chemotaxis (thought to serve as long-range and short-range guidance mechanisms, respectively), to penetrate the cumulus layers surrounding the oocyte, to bind to the oocyte, and to undergo the acrosome reaction that enables oocyte penetration [1,2]. Two important facts about capacitation are that the fraction of capacitated cells is small at any given moment and that the important facts about capacitation are that the fraction of capacitated cells is small at any given moment and that the number of spermatozoa that reach the oocyte is significantly lower than in proper controls. This suggests that upon sensing an increase in the chemoattractant concentration capacitated cells repress their hyperactivation events and thus maintain their course of swimming toward the chemoattractant. Furthermore, in response to a temporal concentration jump achieved by photorelease of the chemoattractant progesterone from its caged form, the responsive cells exhibited a delayed turn, often accompanied by hyperactivation events or an even more intense response in the form of flagellar arrest. This study suggests that the function of hyperactivation is to cause a rather sharp turn during the chemoattractant response of capacitated cells so as to assist them to reorient according to the chemoattractant gradient. On the basis of these results a model for the behavior of spermatozoa responding to a spatial chemoattractant gradient is proposed.

Abstract

When mammalian spermatozoa become capacitated they acquire, among other activities, chemotactic responsiveness and the ability to exhibit occasional events of hyperactivated motility—a vigorous motility type with large amplitudes of head displacement. Although a number of roles have been proposed for this type of motility, its function is still obscure. Here we provide evidence suggesting that hyperactivation is part of the chemotactic response. By analyzing tracks of spermatozoa swimming in a spatial chemoattractant gradient we demonstrate that, in such a gradient, the level of hyperactivation events is significantly lower than in proper controls. This suggests that upon sensing an increase in the chemoattractant concentration capacitated cells repress their hyperactivation events and thus maintain their course of swimming toward the chemoattractant. Furthermore, in response to a temporal concentration jump achieved by photorelease of the chemoattractant progesterone from its caged form, the responsive cells exhibited a delayed turn, often accompanied by hyperactivation events or an even more intense response in the form of flagellar arrest. This study suggests that the function of hyperactivation is to cause a rather sharp turn during the chemoattractant response of capacitated cells so as to assist them to reorient according to the chemoattractant gradient. On the basis of these results a model for the behavior of spermatozoa responding to a spatial chemoattractant gradient is proposed.
Results

A direct way to determine the involvement of hyperactivation in the chemotactic response would be specific inhibition of hyperactivation and examining its effect on chemotaxis. But, regretfully, such specific inhibitors are currently unknown. Moreover, even inhibition of molecular processes known to be involved in hyperactivation (e.g., Ca\(^{2+}\) elevation [5]) is impossible because these processes are also involved in chemotaxis (e.g., [2,23,26]). An exception could have been inhibition of the CatSper channel. However, the recently suggested possibility that this channel (or a protein closely associated with it) is the receptor for the chemoattractant progesterone [14,15] excludes the inhibition of CatSper as a means of distinction between hyperactivation and chemotaxis.

In the absence of a suitable inhibitor, we employed an indirect approach. We anticipated that if, indeed, hyperactivation is part of the chemotactic response and its role is to change the direction of swimming, the fraction of hyperactivated spermatozoa (reflecting the number of hyperactivation events because the identification of a cell as hyperactivated is based on the average behavior along the whole track) would be lower in a chemoattractant gradient, where capacitated spermatozoa would maintain their course of swimming once they find the right direction (up the gradient). However,

Figure 1. A comparison between the fractions of hyperactivated cells in chemotactically responsive and non-responsive spermatozoa. A: Sperm populations chemotactically responsive to progesterone (0.01–10 nM, depending on the sperm sample). The data shown are the mean±SEM of 20 or 40 runs (for the zero-gradient control or the positive gradient, respectively). B: Chemotactically non-responsive sperm populations in concentration gradients of progesterone similar to those in A. The definition of non-responsive population is explained in Materials and Methods. The data shown are the mean±SEM of 14 or 17 runs (for the zero-gradient control or the positive gradient, respectively). C: Sperm populations chemotactically responsive to progesterone (0.01–1 nM, depending on the sperm sample) in the presence of 5 mM procaine. The no-gradient control, as well, included procaine. The data shown are the mean±SEM of 6–7 runs. D: Chemotactically non-responsive sperm populations in concentration gradients of progesterone similar to those in C in the presence of 5 mM procaine. The no-gradient control included procaine. The data shown are the mean±SEM of 7–8 runs. In all cases, the no-gradient controls contained a gradient of DMSO (the solvent of progesterone) instead of a gradient of progesterone. An asterisk indicates a statistically significant difference from the control \([P < 0.05\) (one-tailed) according to Mann-Whitney test in (A) and Student’s t-test in (C)].

doi:10.1371/journal.pone.0028359.g001
The fact that a reduced number of hyperactivation events was only observed in experimental runs in which a chemotactic response was detected suggests that hyperactivation is part of the chemotactic response and that its role is to change the swimming direction. (However, not every turn must be the outcome of a hyperactivation event. Just like in bacterial swimming and chemotaxis [31], spontaneous turns or turns in response to suble stimulation may well be gradual and moderate.) In other words, chemotactically responsive human spermatozoa in a spatial chemoattractant gradient seem to swim more linearly, exhibiting fewer hyperactivation events.

Wishing to examine this conclusion at the single-cell level, we carried out flash experiments in which progesterone was photorelease in a sperm suspension. Generally speaking, the responses were similar to those observed earlier with cAMP or cGMP photorelease [22], though the fraction of cells responding to progesterone was lower (37%) (see Movie S1 in Supplementary Information for a typical example of the sperm response to photorelease of progesterone; see Figure 3 for the tracks made by the spermatozoa in this movie). The common denominator of all the responsive cells was a delayed turn, the length of the delay being 2.2 ± 0.3 s (±SEM) on average. The differences between the responses were in the intensity of the response. Thus, of 75 sperm tracks analyzed, 10 cells (13%) responded to the flash with a delayed hyperactivation event resulting in a change of the swimming direction (Figure 4 as an example; cells #2 and #6 in Figure 3). In two of these cells the hyperactivation was immediately followed by an arrest episode (e.g., cell #2 in Figure 3). Additional 14 cells (19%) responded to the flash with a delayed flagellar arrest (Figure 5 as an example; cells #1, #7 and #9 in Figure 3; the arrest, 2–10 s in length, probably reflects a very intensive response). Whenever we could measure, the arrest was followed by swimming in a new direction (10 cells; in the other 4 cells the track recording ended before the resumption of the swimming). Noteworthy, some of the cells responded by more than one hyperactivation or arrest events (e.g., cells #2 and #6, respectively, in Figure 3). Other 4 cells (5%) responded with a delayed change in their swimming direction without a hyperactivation event (e.g., cell #4 in Figure 3 for a sharp turn). The rest of the cells (63%; cells #3, #5 and #8 in Figure 3) did not exhibit a detectable change in the swimming direction. The observed link between hyperactivation and changes in swimming track following the photorelease of progesterone endorses the notion of hyperactivation being part of the chemotactic response to chemoattracts (see Discussion).

Discussion

The results of this study suggest that the rather erratic motion, known as sperm hyperactivation, is part of the chemotactic response of human spermatozoa. This conclusion is based on the reduction of hyperactivation events in chemotactically responding sperm populations in a spatial chemoattractant gradient (Figure 1), on the observation that, in response to a temporal concentration jump of a chemoattractant, turns often involve hyperactivation events (Figure 3), and on the published observations that both sperm hyperactivation and taxis are restricted to capacitated cells [3,20,21]. This conclusion is further endorsed by the recent findings that the CatSper channel (or a protein closely associated with it), well known to be involved in hyperactivation [10], is the receptor for the chemoattractant progesterone [14,15]. Below we discuss the implications of this study to sperm behavior in a spatial chemoattractant gradient and we address a number of apparent difficulties and questions.
Sperm response to a temporal chemoattractant gradient

Even though the photorelease of progesterone triggered a variety of different responses, they all seemed to share a common denominator: a delayed change in the swimming direction (Figures 3–5). It seems reasonable that the differences between the responses were more a matter of intensity than a matter of distinct response types. Thus, different cells may be differently sensitive to changes in progesterone concentration (e.g., due to differences in the number of receptor molecules in the membrane). These differences in sensitivity may be reflected in different responses to the extremely strong stimulus: from a delicate response (e.g., subtle turning), through a strong response (e.g., a series of hyperactivation events), to a very intense response (e.g., flagellar arrest in hypersensitive cells).

It is important to emphasize that in the progesterone photorelease experiments, as in any assay that measures the response of cells to a temporal chemoattractant gradient, the stimuli were orders of magnitude stronger (concentration-wise) than those encountered in a spatial gradient. This is a necessity due to differences in the detection threshold. A single small concentration change of the stimulant in a temporal assay may be undetectable whereas the same repeated change in a spatial gradient may be detectable due to the integration of the response over time [31]. In this study progesterone was photoreleased from its caged compound within ~60 ms, reaching a concentration of 50 nM. This means that the photorelease created a temporal gradient of ~1 µM/s. This gradient is 2–3 orders of magnitude steeper than the gradient in chemotaxis assays in vitro or the gradient established by the release of progesterone from cumulus cells, because progesterone is sensed at 0.1 pM ([32] and Figure 2) by spermatozoa that swim at an average speed of 70 µm/s, meaning a gradient at the order of 7 nM/s.

Comparison between the sperm responses to photorelease of progesterone and cyclic nucleotides

The responses to photorelease of progesterone and their diversity are very similar to those observed in response to the intracellular photorelease of cyclic nucleotides [22]. There is, however, one major difference. In the case of the response to intracellular cAMP and cGMP, the large majority of the cells responded [22], whereas here, in response to progesterone, only about one third of the cells responded. This difference likely has two causes. First, the concentrations of cyclic nucleotides were orders of magnitude higher than those of progesterone. Second, one of the factors that probably determines whether or not a spermatozoon would respond to a chemoattractant is its sensitivity to the chemoattractant [22], e.g., the relative number of chemoreceptor molecules exposed to the medium. The response to cAMP or cGMP inside the cell is a post-chemoreceptor step; it is, therefore, independent of the chemoreceptors level and it is non-restricted by it.
Apparent difficulties and questions

Aren’t the responses to temporal and spatial stimulations of progesterone conflicting? The response to progesterone stimulation in a temporal gradient (progesterone photorelease) was increased hyperactivation (Figure 3), whereas the response to progesterone in a spatial gradient was decreased hyperactivation (Figure 1). These observations are not conflicting, as they may seem. The difference between the observations is due to fact that the response in the temporal gradient consists of two phases: a delay followed by a hyperactivation/turn event(s) (Figure 3). Therefore, the continuous stimulation in the spatial gradient results in observing the first phase only (see below), meaning a reduced level of hyperactivation.

How could the fraction of cells responding to progesterone be higher than the fraction of capacitated cells? It is well established that only capacitated cells are chemotactically responsive [3,33,34]. Nevertheless, the fraction of cells responding to progesterone in the photorelease experiments was higher than the fraction of capacitated cells. This apparent conflict is resolved by the difference between temporal and spatial gradients. Thus, in a spatial chemoattractant gradient, where the stimuli are always mild, only capacitated spermatozoa respond [3]. However, in response to a very strong stimulation as in the case of a concentration jump of photoreleased progesterone, even non-capacitated cells, which are presumably less sensitive to the chemoattractant [22], can respond. This possibility is well in line with the recent observation that in a capacitated sperm population the CatSper channel is more sensitive to activation by progesterone [14,15] (possibly reflecting the higher number of exposed receptor molecules in capacitated cells).

Are sperm hyperactivation and bacterial tumbling similar phenomena? Even though both hyperactivation and tumbling appear to serve the same function, i.e., abrupt change in the swimming direction, they are not the same. Tumbling, unlike hyperactivation, occurs in response to negative stimulation (increased concentration of a chemorepellent or decreased concentration of a chemoattractant). Hence, in bacteria like E. coli, photorelease of a repellent results in instantaneous tumbling whereas photorelease of a chemoattractant results in swimming linearization [35]. In contrast, in sperm cells, hyperactivation occurs in response to chemoattractant stimulation and it is preceded by a delay. Thus, both phenomena are mechanistically different but they apparently serve similar functions.

How procaine does not interfere with the chemotactic response? The conclusion that hyperactivation is part of the chemotactic response raises the question of how procaine, shown to elevate the hyperactivation level [29,30] (Figure 1), does not interfere with this response (Figure 2). Two reasons may account for this. First, procaine caused partial elevation of hyperactivation, from 6–7% to 16–17% (Figure 1). This leaves sufficient room both up and down for chemotaxis-dependent modulation of hyperactivation events. In analogy, it is well known that the tumbling levels of different bacterial populations may differ from each other and, yet, the populations may be chemotactically responsive to a similar extent. Second, the chemotactic response is carried out by capacitated cells only [3,33,34] whereas the procaine effect is not selective [36]. Therefore, in the presence of procaine the fraction of capacitated cells (i.e., the fraction of the chemotactically responsive cells) may be as high as in the absence of procaine.

Figure 4. A representative swimming response, involving a hyperactivation event and a turn, to photoreleased progesterone. A: The trajectory made by the cell, monitored at 30 frames/s. The arrow indicates the direction of swimming. B–D: Kinematic parameters calculated from the trajectory. The calculations were done as described in Materials and Methods. The pink color indicates the time of the flash; the green color highlights the turn segment. doi:10.1371/journal.pone.0028359.g004
Implications to sperm behavior in a spatial chemoattractant gradient

Earlier, the response of human spermatozoa to a concentration jump in the intracellular concentration of cyclic nucleotides combined with the response to manual addition of chemoattractants led us to propose a model for the behavior of human spermatozoa in a spatial chemoattractant gradient [22]. The results of the current study and the similarity between the responses of the cells to photorelease of progesterone and cyclic nucleotides not only endorse our proposed model but they also expand it to include hyperactivation (Figure 6). According to the expanded model, when a capacitated spermatozoon swims up a concentration gradient, it is continuously stimulated, resulting in suppression of turns and hyperactivation events. The observed reduced level of hyperactivation during a response to a spatial chemoattractant gradient (Figure 1) is fully consistent with the model. When the spermatozoon swims down the gradient or when it senses no change in the chemoattractant concentration it exhibits turns and hyperactivation episodes to modify its direction of swimming. In the case of sensing no change, the cell adapts to the constant chemoattractant concentration and restores its non-stimulated swimming mode, consisting of rather straight swimming with occasional hyperactivation events and turns.

Spermatozoa exhibit two phases of response to the chemoattractant, a delay and a turn (or series of turns; Figures 3–5). According to the model, the first phase is dominant in an upward chemoattractant gradient and the second phase is dominant in a downward gradient or when the upward gradient is not sensed anymore. Two mechanisms may account for the two-phase response. One is that the two phases are independent of each other; the first phase occurs when the chemoattractant concentration increases, and the second phase occurs when no change in the concentration is sensed or when the chemoattractant concentration decreases. The other possibility is that both phases are linked, being an integral part of the very same response. The available data cannot distinguish between these possibilities.

Materials and Methods

Ethics statement

The study was approved by the Bioethics and Embryonic Stem Cell Research Oversight Committee of the Weizmann Institute of Science. Informed consent was obtained in writing from each sperm donor.

Spermatozoa

Human semen samples were obtained from ten healthy donors after 3 days of sexual abstinence. Semen samples with normal sperm density, motility and morphology (according to WHO guidelines [37]) were allowed to liquefy for 30–60 min at room temperature. Human spermatozoa were separated from the seminal plasma by the migration–sedimentation technique [38] using the commercially available Modified HTF medium (Irvine Scientific, Santa Ana, CA, USA) supplemented with 0.3% human serum albumin (HSA, Irvine Scientific, Santa Ana, CA, USA). Following this procedure, the sperm concentration was adjusted to $4 \times 10^7$ cells/ml in HTF medium containing 0.3% HSA and 3.5% polyvinylpyrrolidone (PVP 25 K, Fluka, Buchs, Switzerland). PVP
was used for two reasons. One was to elevate the viscosity of the medium, making it closer to the physiological environment in vivo [39,40]. The second reason was that PVP is a hydrophilic carrier [41], thus preventing the adsorption of the hydrophilic chemoattractants to the chemotaxis chamber. The sperm suspensions were incubated under an atmosphere of 5% CO₂ at 37°C for an additional 1 h (in total, 2 h together with the separation procedure) to obtain capacitated spermatozoa [3].

Chemotaxis assays

Chemotaxis assays were performed at room temperature in a disposable µ-slide chemotaxis, consisting of two reservoirs (40 µl each) connected by a thin slit (2×1×0.07 mm; Ibidi GmbH, Munich, Germany). Sperm suspensions and chemoattractant solutions were adjusted to room temperature prior to the experiment. When used, procaine (Sigma-Aldrich, Steinheim, Germany) was added just prior to chamber loading. Next both reservoirs and the slit were filled with spermatozoa, and only then the chemoattractant was applied within a sperm suspension (in order to avoid dilution of the cell concentration) to one of the reservoirs. In control experiments, cell suspension containing the solvent of the chemoattractant was applied instead. After loading, the slides were incubated at room temperature for 20 min to allow the establishment of a chemoattractant concentration gradient (Figure S1 in Supplementary Information). The swimming of spermatozoa in the observation area (part of the slit) was video-recorded for 4 min at two different fields (changed every 40 s) using Nikon Eclipse Ti microscope at 10× magnification. The cell tracks (10–450 µm long) were subsequently analyzed with homemade software.

Chemotaxis assessment and statistical analysis

Chemotaxis was evaluated as described [42] on the basis of distribution of the instantaneous directionality angles (θinst, the angle between the vector of the cell frame-to-frame displacement and the gradient direction; the video frequency was 25 frames/s). For each treatment the total number of angles in the gradient direction (N+) and in the opposite direction (N−) were summed from a number of experiments. A combined odds parameter was calculated as the ratio between these sums (combined Odds = ΣN+/ΣN−). The odds parameter yields values close to 1 when the swimming is random; it is >1 when the swimming is biased in the gradient direction. The intensity of the chemotactic response was reflected in the combined odds ratio (O.R.) parameter (combined O.R. = combined Oddsresp/combined Oddscontrol). The statistical significance of the response was estimated by adjusting the distribution of combined O.R. values using bootstrapping algorithm that sub-samples all the angles corresponding to a random selection of control tracks, as described in Armon et al. (submitted).

Analysis of hyperactivation

All recorded sperm tracks of chemotaxis assays were re-analyzed by the computerized motion analysis system to determine the fraction of hyperactivated spermatozoa in them. Those runs that yielded a positive response (O.R. >1.02) were defined as the responsive population. Chemotaxis assays of human spermatozoa are characterized by a very low signal-to-noise ratio — an outcome of the small fraction of responsive cells [43,44,45], i.e., of capacitated cells [2,3]. Consequently, in some runs of the assay (any type of a chemotaxis assay) the spermatozoa are similarly distributed in all directions and chemotactic activity is not detected, even though the same chemoattractant concentration is used. These runs were considered as chemotactically non-responsive. Among the non-responsive runs only those having the same concentration of progesterone (or one order of magnitude higher) as the responsive runs of the same experiment were taken into account. The determination of whether a cell is hyperactivated or not was done on the basis of its curvilinear velocity (VCL; mean velocity of the sperm head along its actual, sampled path [46,47]) and fractal dimension (FD; an indicator of the sperm trajectory regularity [describing its space-filling properties] [48]. Cells having VCL >70 µm/s and FD >1.7 were defined as hyperactive. This definition is based on the study by Mortimer et al. [48], which determined the value of FD >1.3 as representing hyperactivated tracks, irrespectively of the sampling frequency. To restrict our analysis to fully hyperactivated cells (not transitional), we elevated the threshold to FD >1.7. VCL was used as an...
additional parameter, in accordance with Mortimer et al. [48]. The percentage of hyperactivated cells was calculated out of the motile population only. It should be noted that because the VCL and FD values are averaged over the whole track, a cell that exhibits isolated brief hyperactivation events would likely not be considered as hyperactivated by the motion analysis software. The statistical analysis was carried out using InStat 3 software (Graph Pad Software, San Diego, CA, USA).

Flash-photolysis
Sperm cells were mixed with 500 nM caged progesterone [49] and placed into an observation chamber (50 µm depth). Motility of sperm cells was recorded with an inverted microscope (IX71; Olympus, Hamburg, Germany) equipped with a 20x magnification objective (UPLSAP; NA 0.5; Olympus). Experiments were carried out in a 37°C incubator (Life Imaging Services, Basel, Switzerland). Photolysis of caged progesterone was achieved using a 100 W Mercury lamp (U-RFL-T; Olympus). Light was filtered through a band-pass filter (H 350/50; AHF analysentechnik, Tubingen, Germany). The irradiation time (~60 ms) was controlled by a mechanical shutter (VS25; Uniblitz, Vincent Associates, Rochester, USA). Under the experimental conditions used, the energy density at the focal plane was 619 mJ/cm² and ~10% of the total caged progesterone was expected to be released per flash. To produce sharp images of swimming sperm, stroboscopic illumination was achieved using a white LED (K2 star; Luxeon) and a custom-made housing.

The flash-photorelease of progesterone, monitored at 30 frames/s. The flash for progesterone photorelease was at 5 s.

Analysis of the sperm response to progesterone photorelease
The analysis of sperm behavior was carried out by homemade software, which provides track coordinates and commonly used kinematic parameters of individual cells, calculated as a moving average with a 9 frames window. These kinematic parameters include the straight-line velocity (VSL; defined as the time-average velocity of the sperm head along a straight-line from its first position to its last position), VCL, the linearity of swimming path (LIN; the ratio VSL/VCL), and the amplitude of lateral head displacement (ALH; the amplitude of the variations of the actual sperm-head trajectory around its average path) [46,47]. The ALH was calculated as twice the local maximal distance from the true track coordinates to their corresponding averaged coordinates [47].

Supporting Information
Figure S1 Gradient establishment in the µ-slit chemotaxis chamber. Rhodamine B was used instead of chemotactant and the fluorescence intensity measured as a function of the distance from the chemotactant reservoir. The region shown is the recording area, which is only a part of the whole observation area. (TIF)

Movie S1 Sperm response to photorelease of progesterone.
Spermatozoa showing different types of responses to photorelease of progesterone, monitored at 30 frames/s. The flash for progesterone photorelease was at 5 s. (MOV)

Acknowledgments
We are indebted to Dr. Luis Alvarez in the lab of Prof. Dr. U. Benjamin Kaupp in CAESAR, Bonn, Germany, for so willingly carrying out the progesterone photorelease experiments for us. We thank both Dr. Alvarez and Dr. Kaupp for critical reading of the manuscript, Dr. Anna Gakamsky for her assistance in writing the various analysis programs used in the photorelease experiments, Dr. Hillary Voet for her assistance with the statistical analysis of the chemotaxis experiments, and Karin Berkovsky for technical assistance. M.E. is an incumbent of the Jack and Simon Djinogly Professorial Chair in Biochemistry.

Author Contributions
Conceived and designed the experiments: LA ME. Performed the experiments: LA ME. Analyzed the data: LA. Contributed reagents/materials/analysis tools: LA. Wrote the paper: LA ME.

References
1. Joswall BS, Eisenbach M (2002) Capacitation. In: Hardy DM, ed. Fertilization. San Diego: Academic Press, pp 57–117.
2. Eisenbach M, Giojalas GC (2001) Sperm guidance in mammals - an unpaved road to the egg. Nature Rev Mol Cell Biol 2: 276–285.
3. Cohen-Dayag A, Tur-Kaspa I, Dor J, Meshiach S, Eisenbach M (1995) Sperm capacitation in humans is transient and correlates with chemotaxic responsiveness to follicular factors. Proc Natl Acad Sci USA 92: 11039–11043.
4. Suarez SS, Ho HC (2003) Hyperactivated Motility in Sperm. Reprod Domest Anim 38: 119–124.
5. Suarez SS (2000) Control of hyperactivation in sperm. Hum Reprod Update 14: 647–657.
6. Ho HC, Suarez SS (2001) An insitol 1,4,5-triphosphate receptor-gated intracellular Ca2+ store is involved in regulating sperm hyperactivated motility. Biol Reprod 65: 1606–1615.
7. Ren DJ, Navarro B, Perez G, Jackson AC, Hu SF, et al. (2001) A sperm ion channel required for sperm motility and male fertility. Nature 413: 603–609.
8. Quill TA, Ren DJ, Clapham DE, Garbers DJ, et al. (2003) A voltage-gated ion channel expressed specifically in spermatozoa. Proc Natl Acad Sci USA 98: 12527–12531.
9. Lobhly A, Pierron V, Reynolds L, Allen L, Michalovich D (2003) Identification of human and mouse CatSper3 and CatSper4 genes: characterization of a common interaction domain and evidence for expression in testis. Reprod Biol Endocrinol 1: 53.
10. Qi H, Moran MM, Navarro B, Chong JA, Krapivinsky G, et al. (2007) All four CatSper ion channel proteins are required for male fertility and sperm cell hyperactivated motility. Proc Natl Acad Sci USA 104: 1219–1223.
11. Avidan N, Tamary H, Dgany O, Cattan D, Pariente A, et al. (2003) CATSPER2, a human autosomal nonsyndromic male infertility gene. Eur J Hum Genet 11: 497–502.
12. Nikpoor P, Mowla SJ, Movahedini M, Zaree SA, Tiruhi T (2004) CatSper gene expression in postnatal development of mouse testis and in subfertile men with deficient sperm motility. Hum Reprod 19: 124–128.
13. Li HG, Ding XF, Liao AH, Kong XB, Xiong CL (2007) Expression of CatSper family transcripts in the mouse testis during post-natal development and male eejaculated spermatozoa relationship to sperm motility. Mol Hum Reprod 13: 299–306.
14. Strunker T, Goodwin N, Brenker C, Kashikar ND, Weyand I, et al. (2011) The CatSper channel mediates progesterone-induced Ca2+ influx in human sperm. Nature 471: 382–386.
15. Lishto PV, Botchkina IL, Kirichok Y (2011) Progesterone activates the principal Ca2+ channel of human sperm. Nature 471: 387–391.
16. Pacey AA, Davies N, Warren MA, Barratt CLR, Cooke ID (1995) Hyperactivation may assist human spermatozoa to detach from intimate association with the endosalpinx. Hum Reprod 10: 2603–2609.
17. Suarez SS (2002) Gamete transport. In: Hardy DM, ed. Fertilization. San Diego: Academic Press. pp 3–28.
18. Suarez SS, Dai XB (1992) Hyperactivation enhances mouse sperm capacity for penetrating viscoelastic media. Biol Reprod 46: 686-691.
19. Stauss CR, Votta TJ, Suarez SS (1995) Sperm motility hyperactivation facilitates spermatozoa to a concentration jump of chemoattractants or intracellular cyclic nucleotides. Biol Reprod 53: 1280–1285.
20. Burkman LJ (1984) Characterization of hyperactivated motility by human spermatozoa during capacitation: comparison of fertile and oligozoospermic sperm populations. Arch Androl 13: 153–165.
21. Bahat A, Tur-Kaspa I, Gakamsky A, Giojalas LC, Breithart H, et al. (2003) Thermotaxis of mammalian sperm cells: A potential navigation mechanism in the female genital tract. Nature Med 9: 149–150.
22. Gakamsky A, Armon I, Eisenbach M (2009) Behavioral response of human spermatozoa to a concentration jump of chemoattractants or intracellular cyclic nucleotides. Hum Reprod 24: 1152–1163.
23. Teves ME, Guidobaldi HA, Unates DR, Sanchez R, Miska W, et al. (2009) Molecular mechanism for human sperm chemotaxis mediated by progesterone. PLoS One 4: e8211.

24. Chang H, Suarez SS (2011) Two Distinct Ca\textsuperscript{2+} Signaling Pathways Modulate Sperm Flagellar Beating Patterns in Mice. Biol Reprod 85: 296–305.

25. Macnab RM, Koslund DE (1972) The gradient-sensing mechanism in bacterial chemotaxis. Proc Natl Acad Sci USA 69: 2509–2512.

26. Oren-Benaroya R, Orvieto R, Gakamsky A, Pinchasov M, Eisenbach M (2008) The sperm chemotactant secreted from human cumulus cells is progesterone. Hum Reprod 23: 2339–2345.

27. Robertson L, Wolf DP, Tash JS (1988) Temporal changes in motility parameters related to acrosomal status: Identification and characterization of populations of hyperactivated human sperm. Biol Reprod 39: 797–803.

28. Uhler ML, Leung A, Chan SYW, Wang C (1992) Direct effects of progesterone and antiprogestrone on human sperm hyperactivated motility and acrosome reaction. Fertil Steril 58: 1190–1196.

29. Marquez B, Suarez SS (2004) Different signaling pathways in bovine sperm regulate capacitation and hyperacrosion. Biol Reprod 70: 1628–1633.

30. Marquez B, Igotz G, Suarez SS (2007) Contributions of extracellular and intracellular Ca\textsuperscript{2+} to regulation of sperm motility: Release of intracellular stores can hyperactivate CatSper1 and CatSper2 null sperm. Dev Biol 303: 214–221.

31. Block SM, Segall JE, Berg HC (1982) Impulse responses in bacterial chemotaxis. Cell 31: 215–226.

32. Teves ME, Barban F, Guidobaldi HA, Sanchez R, Miska W, et al. (2006) Progesterone at the picomolar range is a chemoattractant for mammalian spermatozoa. Fertil Steril 86: 745–749.

33. Eisenbach M (1999) Mammalian sperm chemotaxis and its association with capacitation. Dev Genet 25: 87–94.

34. Fahrer G, Rosario RA, Cavalero S, Frenkel A, Caplan SR, et al. (2002) Chemotaxis of capacitated rabbit spermatozoa to follicular fluid revealed by a novel directionality-based assay. Biol Reprod 67: 1565–1571.

35. Khan S, Castellano F, Spaeth JL, McGraw JA, Goody RS, et al. (1993) Excitatory signaling in bacteria probed by caged chemoreceptors. Biophys J 65: 2368–2382.

36. Mujica A, Neri-Bazan L, Tash JS, Uribe S (1994) Mechanism for procaine-mediated hyperactivated motility in guinea pig spermatozoa. Mol Reprod Devel 38: 283–292.

37. World Health Organization (1993) WHO Laboratory manual for the examination of human semen and semen-cervical mucus interaction. New York: Cambridge University Press.

38. Hau er R, Homonnai ZT, Paz GF, Yavetz H, Amit A, et al. (1992) Migration sedimentation technique as a predictive test for the fertilizing capacity of spermatozoa in an in vitro fertilization programme. Int J Androl 15: 486–505.

39. Janen RP (1980) Cyclic changes in the human fallopian tube tubis and their functional importance. American journal of obstetrics and gynecology 136: 292–300.

40. Hunter RHF (1994) Modulation of gamete and embryonic microenvironment by ovuduct glycoproteins. Mol Reprod Dev 39: 176–181.

41. Yonish-Rouach E, Shimizky M, Rubinstein M (1996) A method for preparing biologically active aqueous cyclosporin A solutions avoiding the use of detergents or organic solvents. J Immunol Meth 135: 147–153.

42. Gakamsky A, Schechtman E, Caplan SR, Eisenbach M (2008) Analysis of chemotaxis when the fraction of responsive cells is small - application to mammalian sperm guidance. Int J Dev Biol 52: 401–407.

43. Ralt D, Manor M, Cohen-Dayag A, Tur-Kaspa I, Makler A, et al. (1994) Chemotaxis and chemokinesis of human spermatozoa to follicular factors. Biol Reprod 50: 774–785.

44. Cohen-Dayag A, Ralt D, Tur-Kaspa I, Manor M, Makler A, et al. (1994) Sequential acquisition of chemotactic responsiveness by human spermatozoa. Biol Reprod 50: 786–790.

45. Eisenbach M (1999) Sperm chemotaxis. Rev Reprod 4: 56–66.

46. Davis RO, Siemers RJ (1995) Derivation and reliability of kinematic measures of sperm motility. Reprod Fertil Dev 7: 857–869.

47. Mortimer ST (1997) A critical review of the physiological importance and analysis of sperm movement in mammals. Hum Reprod Update 3: 403–439.

48. Mortimer ST, Swan MA, Mortimer D (1996) Fractal analysis of mammalian spermatozoa. Hum Reprod 11: 1049–1054.

49. Kilic F, Kashikar ND, Schmidt R, Alvarez L, Dai L, et al. (2009) Caged progesterone: a new tool for studying rapid nongenomic actions of progesterone. J Am Chem Soc 131: 4027–4030.