

SPERM THERMOTAXIS

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Abstract

Thermotaxis — movement directed by a temperature gradient — is a prevalent process, found from bacteria to human cells. In the case of mammalian sperm, thermotaxis appears to be an essential mechanism guiding spermatozoa, released from the cooler reservoir site, towards the warmer fertilization site. Only capacitated spermatozoa are thermotactically responsive. Thermotaxis appears to be a long-range guidance mechanism, additional to chemotaxis, which seems to be short-range and likely occurs at close proximity to the oocyte and within the cumulus mass. Both mechanisms probably have a similar function — to guide capacitated, ready-to-fertilize spermatozoa towards the oocyte. The temperature difference between the site of the sperm reservoir and the fertilization site is generated at ovulation by a temperature drop at the former. The molecular mechanism of sperm thermotaxis waits to be revealed.

Keywords: Thermotaxis (sperm); Guidance (sperm); Thermosensing (sperm); Fertilization; Spermatozoa (mammalian); Female genital tract.

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1. Introduction

A new life begins after the sperm cell (spermatozoon) meets the oocyte and initiates a series of processes that leads to sperm penetration, sperm-oocyte fusion, and zygote division. However, the chance of an incidental encounter between the gametes is very slim (Eisenbach and Tur-Kaspa, 1999; Hunter, 1993) due to a number of reasons. First, the number of ejaculated spermatozoa that reach the oviductal isthmus [where they become trapped and form a sperm reservoir (Suarez, 2002)] is small (Harper, 1982). Second, only a fraction of these spermatozoa [~10% in humans (Cohen-Dayag et al., 1995)] become capacitated and acquire a state of readiness for fertilizing the oocyte (Jaiswal and Eisenbach, 2002). Third, the torturous way that the spermatozoa have to make between the reservoir and the oocyte at the fertilization site is relatively long [3–5 cm in humans (Harper, 1982; Tur-Kaspa, 1992)]. Therefore, it is believed that, for reaching the oocyte, spermatozoa must be guided in the oviduct. One of the most common guidance mechanisms is chemotaxis, shown to occur in spermatozoa as well [Eisenbach (2004a) for an elaborated, recent review]. However, chemotaxis alone may not be sufficient due to peristaltic movements of the oviduct (Battalia and Yanagimachi, 1979) that may prevent the formation of a long-range chemoattractant gradient *in vivo* and restrict chemotaxis to a short distance from the oocyte (Eisenbach, 1999). Another potential guidance mechanism is thermotaxis. In this mechanism, recently discovered in mammalian spermatozoa, cells (or organisms) are guided by a temperature gradient. Here we summarize the available information on mammalian sperm thermotaxis and compare it to the knowledge of thermotaxis in other cells and organisms.

2. Thermotaxis as a sperm guidance mechanism

Earlier studies found that, at ovulation, a temperature difference exists between the sperm reservoir's site (cooler) and the fertilization site (warmer). Thus, a temperature difference of ~2°C

exists in rabbits between the isthmus and the isthmic-ampullary junction (David et al., 1972) and $\sim 0.7^{\circ}\text{C}$ difference in mated pigs between the isthmus and the ampulla (Hunter and Nichol, 1986). These findings raised the possibility that the temperature difference might serve as a cue for guiding spermatozoa to the site of fertilization (Hunter, 1998). Subsequently, Bahat *et al.* (2003) demonstrated that rabbit and human spermatozoa have the capacity to sense small temperature differences (as small as 0.5°C and, perhaps, even lower) and respond to it by thermotaxis. As in sperm chemotaxis (Cohen-Dayag et al., 1994; Cohen-Dayag et al., 1995; Fabro et al., 2002), only capacitated spermatozoa are thermotactically responsive (Bahat et al., 2003). *In vivo*, thermotaxis may be complementary to chemotaxis, each mechanism being functional in a region where the other mechanism is ineffective. One region is apparently between the isthmic sperm reservoir and the fertilization site, where a temperature gradient exists at ovulation and where long-range chemotaxis may not occur due to a mixing effect of the peristaltic movements of the oviduct, described above. Since these movements are not expected to affect the temperature gradient, it is reasonable that thermotaxis is the predominant guidance mechanism in this region. The other region is probably the immediate vicinity of the oocyte, including the viscous milieu of the cumulus cells that surround the oocyte. In this region, the opposite seems to hold: a measurable temperature gradient probably cannot be maintained, whereas a chemoattractant gradient appears to be very effective because of the relatively short distances and the viscoelastic milieu of the cumulus that resists the stirring action of the oviduct (Cohen-Dayag et al., 1994). It is, therefore, reasonable that, *in vivo*, capacitated spermatozoa are first guided by thermotaxis from the reservoir towards the warmer fertilization site. The oviductal contractions, which move liquid from the isthmus to the ampulla (Battalia and Yanagimachi, 1979), may assist the swimming spermatozoa. Then, at close proximity to the oocyte and within the cumulus mass, the guidance is likely carried out by chemotaxis (Fig. 1).

3. Temperature gradient *in vivo*

Two questions concerning the existence of a temperature gradient in the female genital tract are: How is the temperature difference within the oviduct established — is it by ovulation-dependent temperature rise at the fertilization site or by temperature decrease at the reservoir? And what is the mechanism by which the temperature difference is established between two sites, only a few centimeters apart, within the same abdominal organ? Recently, Bahat *et al.* (2005) demonstrated that the temperature difference in the rabbit's oviduct is created by a temperature drop at the reservoir. They further showed that this difference is time dependent and is built up at ovulation (10.5-11.0 h post-mating), rising from $0.8\pm 0.2^{\circ}\text{C}$ before ovulation to $1.6\pm 0.1^{\circ}\text{C}$ after ovulation (Fig. 2). This temperature difference lasts for at least 10 h after ovulation (Bahat *et al.*, 2005), more than enough for attracting the capacitated spermatozoa by thermotaxis [in the rabbit — an induced ovulator in which the timing of the capacitated state is synchronized with oocyte availability in the female genital tract — spermatozoa are capacitated during a time window of ~8 h, ranging from ~12 h to ~20 h post-mating (Giojalas *et al.*, 2004)]. Although human spermatozoa are thermotactically responsive as well (Bahat *et al.*, 2003), it is not known whether an ovulation-dependent temperature gradient exists there too. Since a temperature difference has already been detected in rabbits and pigs, it is reasonable that a similar difference is also built in the human oviduct. The finding of Cicinelli *et al.* (2004) that, in humans, the temperature in the region extending from the isthmus to the uterus decreases at ovulation, endorses this possibility.

At least three mechanisms (and any combination thereof) may account for the ovulation-dependent temperature drop at the isthmus. (i) The temperature may decrease by an endothermic hydration, as was proposed by Luck *et al.* (2001) for the lower temperature found in human (Grinsted *et al.*, 1985), pig (Hunter *et al.*, 1997) and rabbit (Grinsted *et al.*, 1980) follicles relative to their adjacent tissue. According to this possibility, an acid mucus glycoprotein (or a similar macromolecule), known to be hydrated to a large extent and to be secreted predominantly from

the isthmus (Jansen and Bajpai, 1982) in a process controlled by the level of circulating steroid hormones (Gandolfi, 1995), may cause at ovulation a temperature decrease in the isthmus. (ii) Cold blood from the ovary may cool the blood that enters the reservoir by counter-current heat exchange (David et al., 1972; Hunter and Nichol, 1986), in a manner similar to the heat transfer that occurs between the testicular veins and artery (Glad Sorensen et al., 1991). The basis of this possibility is the fact that the artery that supplies blood to the reservoir is different from the artery that supplies blood to the fertilization site, and both of them are aligned close to the ovarian vein, flowing blood in the opposite direction (Leese, 1988). (iii) Since, in women, the source of blood supply to the reservoir appears to change at ovulation from the ovarian to the uterine artery and since the blood in the latter is thought to be cooled by counter-current heat exchange with blood flowing from the vagina (Cicinelli et al., 2004), it is possible that this change reduces the temperature at the reservoir.

4. Molecular mechanism of sperm thermotaxis

In thermotaxis of bacteria and, perhaps, nematodes (see below), some of the chemotaxis receptors also serve as thermosensors (Imae, 1985; Mori, 1999; Nara et al., 1996). The situation in mammalian spermatozoa is not known. Most thermosensors of eukaryotic cells are temperature-gated ion channels, belonging to the large superfamily of ion channels termed TRP (for transient receptor potential) (Patapoutian et al., 2003). These include a heat-activated calcium channel in *Paramecium* (Imada and Oosawa, 1999) and dopaminergic neurons (Guatteo et al., 2005), and a heat-activated potassium channel in sponges (Zocchi et al., 2001). Although TRPC (one of the three families constituting the TRP superfamily) Ca²⁺ channels have been identified on mammalian spermatozoa, e.g., in mouse spermatozoa — TRPC1 and TRPC3 on the flagellum and TRPC2 and TRPC6 on the head (Jungnickel et al., 2001; Trevino et al., 2001), it has not yet been established whether a channel of this family serves as a thermosensor.

The signal-transduction pathways in bacteria, nematodes and amoeba are, at least in part, common to thermotaxis and chemotaxis. In mammals, the signaling pathway of sperm thermotaxis is still unknown, but if it shares at least some of the chemotaxis signaling pathway (which is also obscure at large), it may involve cGMP-mediated transient elevation of intracellular Ca^{2+} or a signaling cascade similar to that of the olfactory system, including membrane adenylyl cyclase-mediated transient Ca^{2+} rise (Eisenbach, 2004b; Kaupp et al., 2003; Spehr et al., 2004).

5. Sperm thermotaxis in comparison to other systems

Temperature sensing is an essential property of cells and organisms. Mammals use thermosensing to detect changes in their ambient and body temperatures. Cells and lower organisms commonly employ thermotaxis for migrating to preferable temperatures. Thermotaxis was first discovered in the unicellular slime mold *Dictyostelium discoideum* (Bonner et al., 1950; Raper, 1940) and then, among others, in *Caenorhabditis elegans* (Hedgecock and Russell, 1975), bacteria (Maeda et al., 1976), parasites like *Dirofilaria immitis* larvae (Mok et al., 1986), *Drosophila* (Sayeed and Benzer, 1996), human polymorphonuclear leukocytes (Kessler et al., 1979; Mizuno et al., 1992), human trophoblastic cells (Higazi et al., 1996), and mammalian spermatozoa (Bahat et al., 2003). In some systems [e.g., bacteria (Maeda et al., 1976), *D. discoideum* (Whitaker and Poff, 1980) and *C. elegans* (Hedgecock and Russell, 1975)], the thermotaxis process is functional in both directions, i.e., the cells can sense and respond to both positive and negative temperature gradients. In sperm thermotaxis, only positive thermotaxis (movement towards the warmer temperature) has thus far been reported (Bahat et al., 2003).

In thermotaxis, very small temperature differences can be sensed. For example, *D. discoideum* can respond to a gradient as small as $0.04^{\circ}\text{C}/\text{cm}$ (!) (Poff and Skokut, 1977). In view of (a) the measured temperature difference between the sperm reservoir in the mammalian

isthmus and the fertilization site and (b) the distance between them (Bahat et al., 2005), thermotaxis of mammalian spermatozoa is probably very sensitive, too. If, indeed, mammalian spermatozoa have a temperature sensitivity similar to that of *D. discoideum*, it would be more than enough for thermotaxis *in vivo*, where the gradient (in the case of rabbits) is at the order of 0.1°C/cm.

6. Open questions

Sperm thermotaxis, discovered only two years ago (Bahat et al., 2003), is still, at large, obscure. Major questions are: What is the threshold temperature gradient that can be sensed by spermatozoa? Is the thermotaxis process sufficiently sensitive for detecting the shallow gradient that exists in the oviduct? Can spermatozoa respond thermotactically to negative temperature gradients as well? What are the thermosensors? How do the thermosensors communicate with the sperm's flagellum? These are only a few of the questions waiting to be addressed.

Acknowledgments

M.E. is an incumbent of the Jack and Simon Djanogly Professorial Chair in Biochemistry.

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Figure legends

Fig. 1. Sperm guidance mechanisms in the mammalian female genital tract. The scheme, which is not drawn to scale, is based on the human tract.

Fig. 2. Time-dependent temperature differences between the sperm reservoir and the fertilization site within the rabbit oviduct. The squares stand for the experimental points (mean \pm SEM; 4–8 oviducts for each point). The connecting line is a theoretical sigmoidal-curve fitting ($R^2 = 0.99$).

[Taken from Bahat *et al.* (Bahat et al., 2005) with permission.]



