Thermotaxis of mammalian sperm cells: A potential navigation mechanism in the female genital tract

To the editor—One of the puzzles in mammalian fertilization is how sperm cells navigate within the female genital tract towards the egg. Sperm chemotaxis, demonstrated *in vitro* in humans and other mammals, is a navigation mechanism that, due to gradient-disturbing peristaltic movements of the oviduct, probably functions *in vivo* only over a short distance from the egg[1]. Another potential cue for sperm guidance is the ovulation-dependent temperature difference within the female genital tract[2–4]. In the rabbit, this difference amounts to approximately 2 °C between the sperm reservoir's site (the isthmus[5]) and the warmer fertilization site (the isthmic-ampullary junction) at ovulation[2]. Here we examined whether rabbit spermatozoa can sense this temperature difference and respond to it by thermotaxis.

We measured the temperatures in the rabbit's isthmus (near the uteri-isthmic junction) and the isthmic-ampullary junction at ovulation (10.5–11.0 hours post-mating), and found them to be –3.1 ± 0.4 °C and –1.5 ± 0.8 °C (mean ± s.d. of four oviducts), respectively, relative to the rectal temperature. These results confirm the published approximately 2 °C difference between the storage and fertilization sites[2] and further suggest that this difference is achieved not by an elevated temperature at the fertilization site, but rather by a reduced temperature at the sperm reservoir's site.

For measuring thermotaxis, we used a directionality-based assay, independent of the sperm's speed and pattern of movement[6]. For this purpose we modified a Zigmond chamber[7], consisting of two parallel wells separated by a partition wall, so that the temperature in each well could be accurately controlled and measured (± 0.2 °C). We put equal rabbit sperm concentrations in both wells and video-recorded the tracks of the spermatozoa swimming on top of the partition wall. Using a computerized motion analysis system, we analyzed them for thermotaxis according to three directionality-based parameters[6]: the mean net distance traveled along the temperature gradient (ΔX ¯), the percentage of cells whose net distance of swimming was towards the warmer well (cells with $\Delta X>0$), and the percentage of cells traveling a longer distance in the direction of the temperature gradient than in a gradient-less direction, perpendicular to the former (cells with $\Delta X/|\Delta Y|>1$). We either maintained a temperature difference of 2 °C between the wells, similar to the difference within the rabbit oviduct at ovulation, or, as a no-gradient control, kept both wells at the same temperature, either 37 °C or 39 °C. When we kept both wells at the same temperature, all three parameters had values expected for random movement: approximately 0 µm for ΔX ¯, approximately 50% for the percentage of cells with $\Delta X>0$, and approximately 25% for the percentage of cells with $\Delta X/|\Delta Y|>1$. However, when we kept a 2 °C difference between the wells, all these parameters were larger than the expected values for a random movement (Fig. 1*a*, right black columns, $P \le 0.003$, 0.0001 and 0.002 from top to bottom), indicating the occurrence of sperm thermotaxis from 37 °C to 39 °C. This is the first demonstration that spermatozoa can navigate according to a temperature gradient. The speed and swimming pattern were not significantly affected by the temperature difference. When we further reduced the temperature difference to 1 °C or 0.5 °C (the lowest limit of our experimental system), the thermotactic response was as strong as at 2 °C (Fig. 1*b*, black columns, $P \le 0.01$, 0.0004 and 0.0001 from top to bottom). As in sperm chemotaxis[6,8,9], only a fraction of the spermatozoa were thermotactically responsive—the capacitated spermatozoa (that is, those in a state of readiness for fertilizing the egg[10]; Fig. 1*a*). Furthermore, when we put spermatozoa in the 37 °C well only and measured the level of capacitated spermatozoa among the cells that reached the 39 °C well, we found that the level of capacitated spermatozoa there was about two-fold

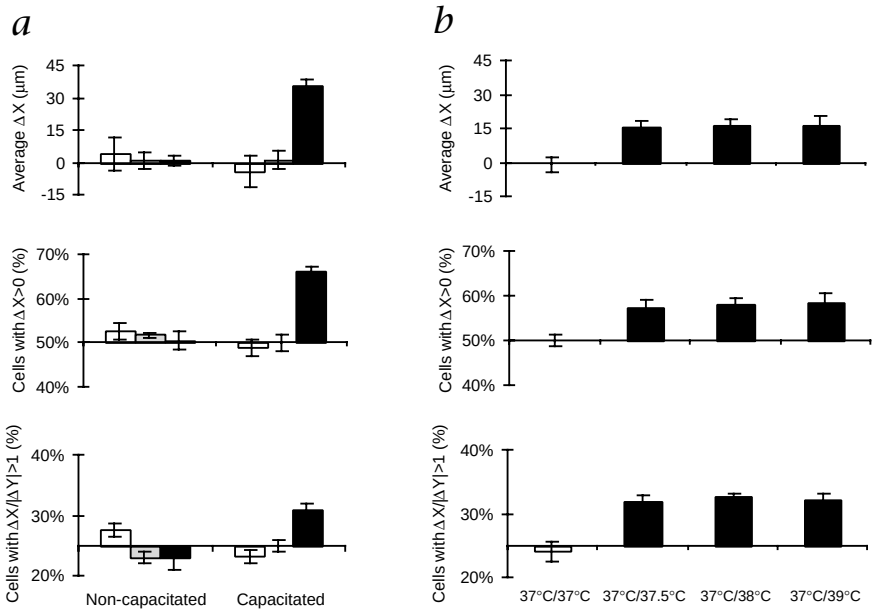

Fig. 1 Thermotactic responses of rabbit spermatozoa. The results, shown with respect to the expected values in the case of random movement, are averages of four determinations ± s.e.m. ***a***, The response of non-capacitated and capacitated spermatozoa to a temperature difference of 2 °C, studied 1 h and 14–18 h post-ejaculation, respectively. Under these conditions, the percentages of capacitated spermatozoa, identified according to their ability to undergo the acrosome reaction[6,9,10] (1,270 cells in total for each set of conditions), were 0% (± s.e.m.) and 15.7 ± 2.6%, respectively. The total numbers of cells analyzed for thermotaxis under each set of conditions were 475–688 or 743–1,194 for non-capacitated or capacitated spermatozoa, respectively. □, 37 °C/37 °C; ■, 39 °C/39 °C; ■, 37 °C/39 °C. ***b***, The response of capacitated spermatozoa to smaller temperature differences. The total numbers of cells analyzed under each set of conditions were 1,145–2,143. The experiments were approved by the Weizmann Institute Institutional Animal Care and Use Committee.

higher than in the original 37 °C well. These results indicate that, as with chemotactic responsiveness, thermotactic responsiveness is acquired during sperm capacitation.

We observed a similar thermotactic response with human spermatozoa, but the percentage of responding cells was lower (3–5% *versus* 7–17%), in line with the observations that the level of capacitated cells is lower in human spermatozoa than in rabbit spermatozoa[6,9]. These observations indicate that sperm thermotaxis may be a general phenomenon in mammals.

The findings made herein, together with the probability that sperm chemotaxis is restricted to the immediate surroundings of the egg[1], suggest that sperm thermotaxis and chemotaxis are long- and short-range mechanisms, respectively, which occur consecutively—each in a region where the other is not functional. First, capacitated spermatozoa, released from the isthmic sperm reservoir, may be guided by thermotaxis towards the warmer fertilization site. Then, at close proximity to the egg and within the cumulus mass, the guidance is likely carried out by chemotaxis.

Competing interests statement

The authors declare that they have no competing financial interests.

1. Eisenbach, M. Mammalian sperm chemotaxis and its association with capacitation. *Dev. Genet.* **25**, 87–94 (1999).
2. David, A., Vilensky, A. & Nathan, H. Temperature changes in the different parts of the rabbit's oviduct. *Int. J. Gynaec. Obstet.* **10**, 52–56 (1972).
3. Hunter, R.H.F. & Nichol, R. A preovulatory temperature gradient between the isthmus and the ampulla of pig oviducts during the phase of sperm storage. *J. Reprod. Fert.* **77**, 599–606 (1986).
4. Hunter, R.H.F. Sperm-epithelial interactions in the isthmus and ampulla of the Fallopian tubes and their ovarian control. in *Gametes: Develoment and Function* (ed. Lauria, A. *et al.*) 355–367 (Serono Symposia, Rome, 1998).
5. Suarez, S.S. Gamete transport. in *Fertilization* (ed. Hardy, D.M.) 3–28 (Academic Press, San Diego, 2002).
6. Fabro, G. *et al.* Chemotaxis of capacitated rabbit spermatozoa to follicular fluid revealed by a novel directionality-based assay. *Biol. Reprod.* **67**, 1565–1571 (2002).
7. Zigmond, S.H. Ability of polymorphonuclear leukocytes to orient in gradients of chemotactic factors. *J. Cell Biol.* **75**, 606–616 (1977).
8. Cohen-Dayag, A. *et al.* Sequential acquisition of chemotactic responsiveness by human spermatozoa. *Biol. Reprod.* **50**, 786–790 (1994).
9. Cohen-Dayag, A., Tur-Kaspa, I., Dor, J., Mashiach, S. & Eisenbach, M. Sperm capacitation in humans is transient and correlates with chemotactic responsiveness to follicular factors. *Proc. Natl. Acad. Sci. USA* **92**, 11039–11043 (1995).
10. Jaiswal, B.S. & Eisenbach, M. Capacitation. in *Fertilization* (ed. Hardy, D.M.) 57–117 (Academic Press, San Diego, 2002).

ANAT BAHAT[1], ILAN TUR-KASPA[2], ANNA GAKAMSKY[1], LAURA C. GIOJALAS[3], HAIM BREITBART[4] & MICHAEL EISENBACH[1]

[1]*Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot, Israel*

[2]*Department of Obstetrics and Gynecology, In-vitro Fertilization Unit, Barzilai Medical Center, Ben-Gurion University, Ashkelon, Israel*

[3]*Laboratory of Cell Biology, Faculty of Science, National University of Córdoba, Córdoba, and Council of Scientific and Technological Research (CONICET), Argentina*

[4]*Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel*

Correspondence should be addressed to M.E.; email: m.eisenbach@weizmann.ac.il

Is alloantigen expression by host epithelium required for acute graft-versus-host disease?

To the editor—In the June 2002 issue of *Nature Medicine*, Teshima *et al.*[1] reported that in contrast to the need for alloantigen expression on host antigen-presenting cells (APC), the presence of alloantigen on host target epithelium was not a prerequisite for initiation of acute graft-versus-host disease (GVHD). The authors' conclusions were based on studies of murine bone marrow chimeras, in which major histocompatibility complex (MHC) class I or class II antigens were expressed only on hematopoietic cells, including APCs, but not on prototypical GVHD target tissues. Recipient chimeric mice developed GVHD after myeloablative irradiation and infusion of T cell–depleted marrow, which was supplemented with $CD8^+$ or $CD4^+$ donor T cells from MHC class I– or class II–disparate donors, respectively. The authors concluded that the GVHD effector phase was mediated by inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) because neutralization of these cytokines completely prevented acute GVHD in this model, according to all parameters tested (mortality, clinical score and pathology).

At issue is whether the authors' findings, which were obtained using highly inbred strains of mice, can be generalized and applied to random-bred large animals and human patients, in whom hematopoietic stem cell transplantation is usually performed using MHC-identical donors. The available evidence would suggest not, as shown by three examples.

First, experimental evidence from the dog leukocyte antigen (DLA)–identical bone marrow transplant model indicates that it is unlikely that inflammatory cytokines alone cause clinical and pathological changes consistent with acute GVHD. When GVHD was induced in long-term recipients of DLA-identical marrow grafts by infusing lymphocytes from marrow donors sensitized against recipient minor histocompatibility antigens (mHAg), severe acute skin GVHD developed only in recipient skin but not in areas of previously placed contiguous donor skin grafts[2]. Histological evidence for GVHD in recipient skin consisted of extensive necrosis and degeneration of the basal cell layer, separation of epidermis and dermis, and infiltration with inflammatory cells; donor skin grafts did not show any gross or histologic abnormalities. Dogs with signs of skin GVHD also had functional abnormalities of the liver, indicative of liver GVHD. In this study, donor lymphocytes were sensitized by skin grafts before infusion, whereas lymphocytes causing spontaneous GVHD become sensitized to presumably the same mHAg after transplantation. Regardless of the timing of sensitization, the lymphocytes' effects on their target, the skin, should be comparable. These findings are consistent with the notion that development of skin GVHD in mHAg-mismatched transplantation involves a specific attack by cytotoxic donor lymphocytes on recipient cells expressing different mHAg, rather than only nonspecific cytokine activity.

Second, a human patient given a marrow graft from an MHC-identical sibling for treatment of severe aplastic