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REVIEW

Thermotaxis of mammalian sperm

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ABSTRACT: Sperm are guided through the female reproductive tract. A temperature difference of about 2°C exists between the storage site and fertilization site of the mammalian oviduct, leading to the hypothesis that sperm can sense and swim towards the oocyte along a rising temperature gradient, known as thermotaxis. Research over the past two decades has reported that sperm feature a sophisticated thermal detection system to detect and track ambient temperature gradients. More recently, thermotaxis is expected to be added to the microfluidic isolation method based on sperm tactic responses for sperm selection. In this article, mammalian sperm thermotaxis is discussed, explaining the underlying behavioural mechanisms and molecular basis, according to the latest research. Finally, this article explores the possible application of sperm thermotaxis in ART.

Key words: spermatozoa / thermotaxis / assisted reproductive technology / opsin / thermoTRP

Introduction

Thermotaxis, or temperature-oriented cell motility, is a universal phenomenon in biology. Lower organisms such as bacteria (Paulick *et al.*, 2017), nematodes (Kimata *et al.*, 2012), parasites (Mok *et al.*, 1986) and *Drosophilas* (Ni *et al.*, 2013) mostly exhibit a thermotactic response to suitable temperatures. In recent years, mammalian spermatozoa have also been found to have the ability to swim along positive temperature gradients *in vitro*. Together with the temperature differences measured in the oviducts of pigs and rabbits (Hunter and Nichol, 1986; Bahat *et al.*, 2003), Bahat *et al.* (2003) innovatively proposed that thermotaxis may serve as a navigation mechanism to guide the transport of mammalian sperm from the storage site (isthmus) to the warmer fertilization site (ampulla).

Resulting from research efforts over the past two decades, scientists have described multiple aspects of mammalian sperm thermotaxis. The temperature difference within the female reproductive tract (FRT) and its underlying mechanism have been explored. Temperature gradient detection and tracking methods for sperm have been described. Furthermore, the temperature range for thermotactic behaviour and the optimal temperature gradient has been researched, focusing on the properties of the sperm temperature sensors. More recently, the feasibility of applying thermotaxis to sperm selection in assisted reproduction has been explored. This article addresses these topics by synthesizing the currently available knowledge, focusing on the characteristics of the recently discovered temperature sensors.

Thermotaxis at a glance

A temperature difference of about 1°C was initially measured across the oviducts of pigs and rabbits through thermistor probes (Hunter and Nichol, 1986; Bahat *et al.*, 2003). This difference is ovulation-dependent, with the temperature difference doubling to 2°C at ovulation (Bahat *et al.*, 2005). The enlarged temperature difference reflects the vascular and lymphatic bed as well as muscle tissue activity. The temperature difference is generated by a temperature drop at the storage site. Theoretically, three mechanisms are involved: (i) an endothermic effect through acid mucus glycoprotein hydration; (ii) a counter-current heat exchange in the ovarian vein at the storage site; and (iii) an ovulation-dependent change in blood supply (Bahat *et al.*, 2005; Eisenbach and Giojalas, 2006). For obvious reasons, data on intratubal temperature differences in humans are not yet available. However, human sperm thermotaxis has been the focus of extensive research (Bahat and Eisenbach, 2010; Bahat *et al.*, 2012; Li *et al.*, 2014; De Toni *et al.*, 2016), along with that of boar (Martin-Hidalgo *et al.*, 2018; Rodriguez-Gil, 2019), rabbit (Bahat *et al.*, 2003), mouse (Perez-Cerezales *et al.*, 2015a; Hamano *et al.*, 2016; Ko *et al.*, 2018), bull (Mondal *et al.*, 2017) and stallion (Ruiz-Díaz *et al.*, 2020) sperm, which have been demonstrated to accumulate towards higher temperatures in various *in vitro* systems. Thus, thermotaxis has been recognized as a general property of mammalian sperm. Furthermore, as in the case of chemotaxis, only capacitated sperm are thermotactically responsive, accounting for about 5–10% of the total (Bahat *et al.*,

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2003; Li et al., 2014). Human sperm can respond to temperature gradients as low as 0.014°C mm^{-1} over a wide range (29–41°C) (Bahat et al., 2012). Such a wide effective temperature range and excellent sensitivity suggest that sperm cells have a thermal detection system comprising many sensors. The sensors detect different temperature ranges and overlap to some extent. The optimal temperature gradient for mouse sperm was determined to be 0.154°C mm^{-1} in a microfluidic chip when the temperature difference was 2°C, whereas the optimal temperature gradient for human sperm was further narrowed to about 0.07–0.15°C mm^{-1} (Yan et al., 2021).

Thermotaxis has been proposed as one of the physiological mammalian sperm navigation mechanisms (Bahat et al., 2003; Perez-Cerezales et al., 2015b) (Fig. 1A). Sperm movement from the isthmus to the ampulla towards the oocyte covers a considerable distance relative to its body length, so appropriate guidance is required to locate the oocyte (Suarez and Pacey, 2006). During this adventure, various biochemical factors, such as compounds (Brenker et al., 2012) and cell interactions (Suarez, 2016; Li et al., 2022) and physical information, such as temperature (Bahat et al., 2003), fluid (Kantsler et al., 2014; Zaferani et al., 2021a) and the tube wall (Zaferani et al., 2021b; Li et al., 2022), in the FRT provide directional cues for sperm guidance. Chemoattractants in the follicular fluid, such as progesterone secreted by cumulus cells, may facilitate sperm mobilization towards the oocyte, a process known as chemotaxis (Sun et al., 2005; Teves et al., 2006). However, due to the perturbations in oviductal peristalsis and fluid flow, a gradient of chemoattractants may only be effective when the sperm approaches the oocyte (Cohen-Dayag et al., 1994; Hino and Yanagimachi, 2019). On the other hand, thermotaxis can function over longer distances due to the stability of temperature gradients and the sperm's sensitivity to minimal temperature differences (Bahat et al., 2003). In addition, sperm also exhibit counter-current swimming, which seems to be a completely passive fluid-dependent process called rheotaxis (Miki and Clapham, 2013; Schiffer et al., 2020). A popular view is that long-range temperature gradients and short-range chemoattractant gradients are sequentially involved in sperm migration guidance, with passive rheotaxis playing a decisive role (Bahat and Eisenbach, 2006; Miki and Clapham, 2013).

Behavioural mechanisms

Some groups have raised the possibility that thermotaxis could be a consequence of rheological behaviour induced by thermal convection (Miki and Clapham, 2013) or metabolic changes promoted by elevated temperature (Chan et al., 1998; Miller et al., 2001; Marin-Briggiler et al., 2002). However, these possibilities were demonstrated to be incorrect (Bahat et al., 2012; Perez-Cerezales et al., 2015b). To explore thermotaxis in the FRT environment, the underlying behavioural mechanisms need to be revealed, confirming whether temperature gradients confer the ability to change the direction of sperm movement (Perez-Cerezales et al., 2015b). In chemotaxis, the capacitated sperm reorient in the chemoattractant gradient by inhibiting their hyperactivation events (an asymmetric, high-amplitude flagellar beating pattern) (Armon and Eisenbach, 2011), while in rheotaxis, the sperm responds to shear flow mostly by rotating along its longitudinal axis (Zaferani et al., 2021a). However, little is known about how sperm responds to temperature differences, and the current research suggests that

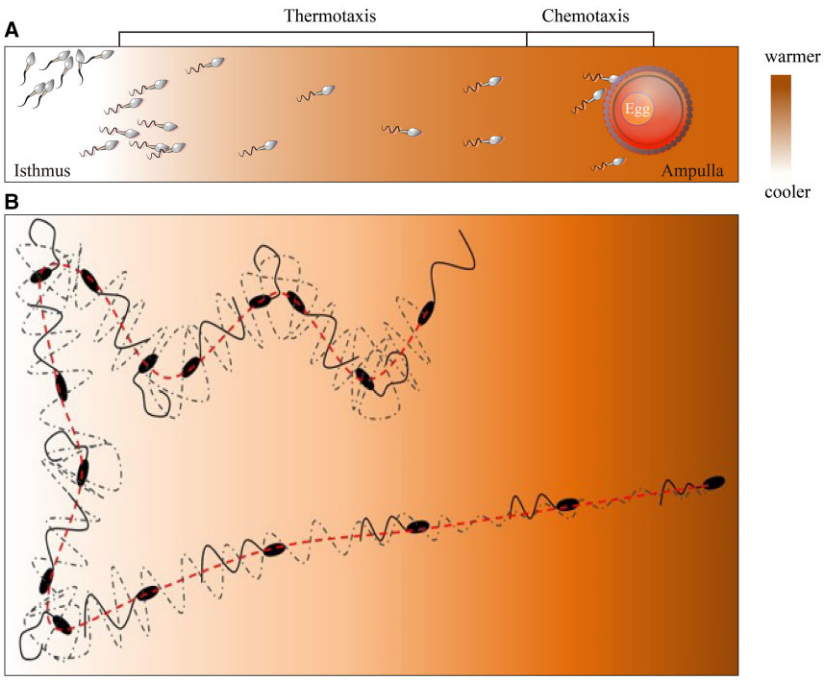

Figure 1. Thermotaxis navigation in mammalian sperm. (**A**) Sperm use thermotaxis to swim from the isthmus to the warmer ampulla, and chemotaxis plays a role near the oocyte. (**B**) A behavioural model of thermotaxis. The black dashed line represents the trajectory of the sperm head, and the red dashed line represents its average path. Part B is reprinted with permission from Boryshpolets et al. (2015).

hyperactivation is involved. Boryshpolets *et al.* (2015) observed that rapid positive temperature changes (from 31°C to 37°C) affect human sperm behaviour in two ways: warming increases the linear velocity and cooling increases the flagellar wave amplitude. The latter is essentially an increase in hyperactivation and turning frequency. In other words, the speed increases while the frequency of hyperactivation and turn decreases along the positive temperature gradient. Furthermore, similar to bacterial behaviour (Macnab and Koshland, 1972), one possible motility model for swimming sperm is that they turn and hyperactivate more frequently at cooler temperatures, whereas their linear velocity intensifies at higher temperatures (Boryshpolets *et al.*, 2015). This effect accumulates and persists, eventually resulting in linear swimming along a positive temperature gradient (Fig. 1B). The linear sperm movement along a vertical isotherm was clearly observed in a microfluidic chip, confirming that the change in velocity and direction is a result of temperature differences (Yan *et al.*, 2021). It is worth mentioning that mammalian sperm chemotaxis also involves hyperactivation (Armon and Eisenbach, 2011). Considering that both thermotaxis and chemotaxis are activated during capacitation, inhibition of hyperactivation by temperature and chemoattractant gradients may be a general directional means for capacitated sperm.

Molecular basis

Since its introduction in 2003, the molecular basis for thermotaxis and its remarkable temperature sensitivity has been a subject of research (Bahat *et al.*, 2003). It is well-known that sperm navigation in the FRT is regulated by a series of calcium-dependent events, including motility hyperactivation, capacitation, acrosome reaction and chemotaxis (Jimenez-Gonzalez *et al.*, 2006). Temperature information may likewise be coded into changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), which in turn activates the sAC/cAMP/PKA pathway to phosphorylate proteins. This is crucial for sperm motility (Stock *et al.*, 2013; Wachten *et al.*, 2017). Temperature-induced stimulation of sperm motility requires calcium signals to be sent to the flagellum (Martin-Hidalgo *et al.*, 2018). Therefore, calcium signalling was considered in the molecular mechanisms of sperm thermotaxis. Previous studies of human sperm thermotaxis had identified an obvious calcium pathway, in that temperature changes were described to activate phospholipase C (PLC), leading to hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) to diacylglycerol (DAG) and inositol trisphosphate (IP3). IP3 then binds to the inositol 1,4,5-trisphosphate receptor (IP3R) on the calcium pool to release Ca^{2+}, which regulates the flagellar beating waveform to control the swimming path (Bahat and Eisenbach, 2010). Calcium signalling has thus been postulated as the molecular basis of sperm thermotaxis. Based on the current expression studies, combined with applied electrophysiological, genetic and pharmacological approaches, it is possible to design a profiling model of sperm thermotaxis, which is that thermosensory signalling cascades initiated by temperature sensors control thermotactic behaviour in a calcium concentration-regulated manner.

Temperature sensors

Two families of membrane proteins are known to be responsible for sensory transduction in eukaryotes: G protein-coupled receptors (GPCRs) and ion channels (Cygankiewicz *et al.*, 2014; Dalesio *et al.*, 2018). Respectively, current studies are investigating the possibility of opsins and thermosensitive transient receptor potential (thermoTRP) channels as thermotaxis sensors (Fig. 2).

ThermoTRP

ThermoTRP is a highly temperature-sensitive subset of the non-selective cation channel TRP superfamily, responsible for ambient temperature detection and maintenance of body thermal homeostasis (Patapoutian *et al.*, 2003; Dhaka *et al.*, 2006; Vandewauw *et al.*, 2018). Once regarded as the main, or even the only, molecular thermometer of an organism, thermoTRP possesses temperature-sensing properties sufficient to cover the physiological range (Cao *et al.*, 2013; Kashio, 2021). Heat-activated transient receptor potential vanilloid (TRPV)1–4, cold-activated transient receptor potential cation channel subfamily M member 8 (TRPM8) and transient receptor potential cation channel subfamily A member 1 (TRPA1) are classified as conventional thermoTRPs. Several others, such as TRPM2–5 and transient receptor potential channel 5 (TRPC5), also sense temperature (Vay *et al.*, 2012). Thermal stimulation induces gating after global conformational changes of thermoTRPs to regulate sperm function with electrical signals (Chowdhury *et al.*, 2014). Among them, TRPV1 (Maccarrone *et al.*, 2005; Francavilla *et al.*, 2009; Gervasi *et al.*, 2011), TRPV4 (Hamano *et al.*, 2016; Kumar *et al.*, 2016; Mundt *et al.*, 2018) and TRPM8 (De Blas *et al.*, 2009; Martínez-López *et al.*, 2011; Majhi *et al.*, 2015) are endogenously expressed in vertebrate sperm cells and are currently strong candidates for sperm temperature receptors. TRPV1 and TRPM8 protect germ cells from heat stress and cold shock, respectively, and TRPV4 is a necessary membrane depolarization channel for sperm hyperactivation (Mizrak and van Dissel-Emiliani, 2008; Borowiec *et al.*, 2016; Mundt *et al.*, 2018). The remaining thermoTRPs, although also present in sperm, have not been tested in thermotropic processes (Castellano *et al.*, 2003; Majhi *et al.*, 2020). In 2006, Hamano *et al.* (2016) proposed that TRPV4 is involved in sperm thermotaxis, as the proportion of spermatozoa in spermatozoa with *Trpv4* knockout and following ruthenium red (TRPV antagonist) treatment was significantly reduced. In the same year, De Toni *et al.* (2016) observed that migration towards a temperature gradient enabled the selection of sperm cells characterized by high TRPV1 expression, whereas pre-incubation with a TRPV1 antagonist inhibited thermotaxis in a manner that severely reduced calcium concentrations. Given the complex regulation of TRPV1/TRPV4 in other aspects of sperm function such as cell migration, capacitation and acrosome responses, caution is still required in identifying TRPV1/TRPV4 as thermotaxis temperature sensors (Waning *et al.*, 2007; Xiao and Chen, 2022). Interestingly, there is now a consensus that thermotaxis is not regulated by a single receptor, as neither antagonism nor knockout of either putative sensor completely eliminates sperm thermotaxis.

Opsins

Another group proposed as temperature sensors for sperm thermotaxis are the GPCR opsins, inspired by the surprising breakthrough of rhodopsin in temperature discrimination in *Drosophila* (Minke and Peters, 2011). The dogma in the past held that opsins were restricted to light-exposed tissues (eyes and skin), however, expression of opsins was detected in other organs and exhibited light-independent effects (Leung and Montell, 2017; Moraes *et al.*, 2021). The ability of *Drosophila* to select the optimum temperature (18°C) within the comfort temperature range (18–

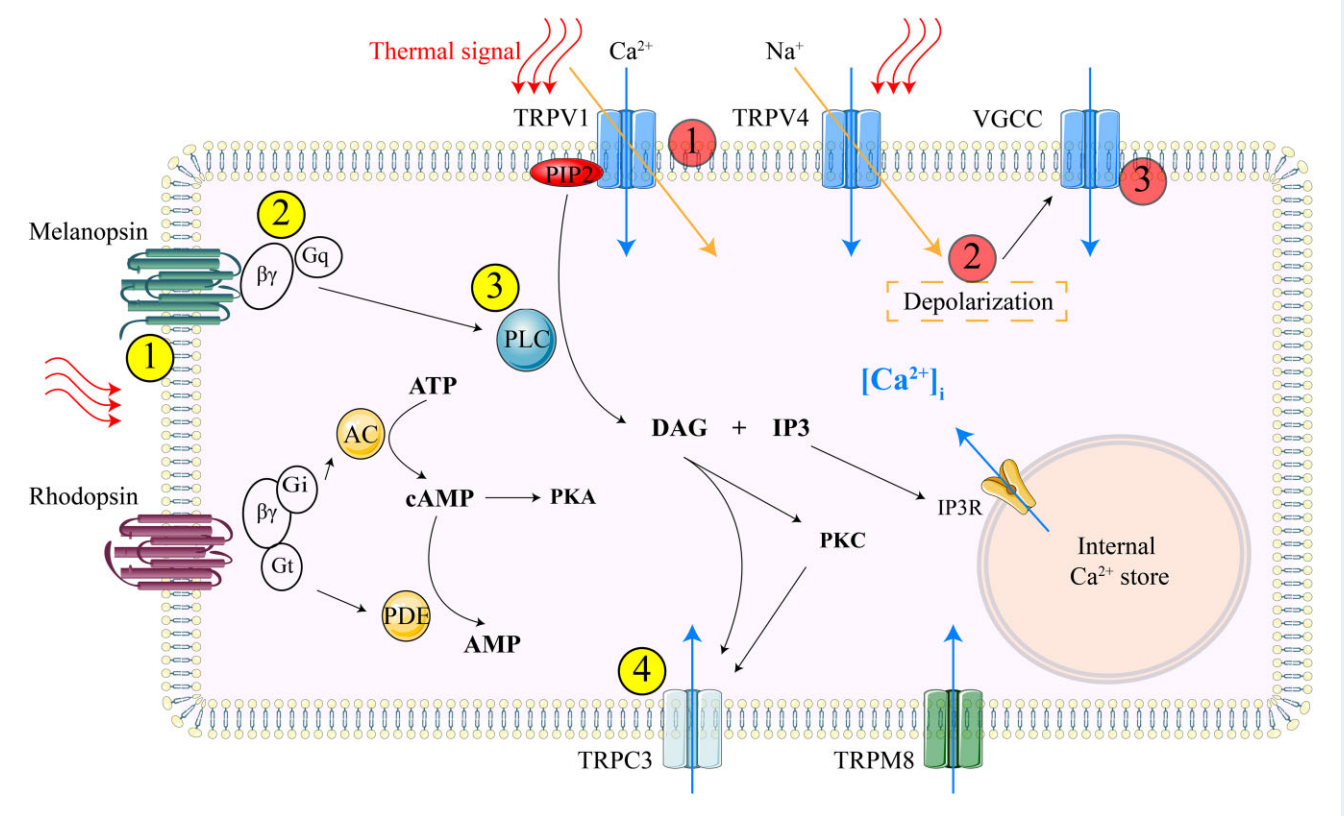

Figure 2. A molecular model of mammalian sperm thermotaxis. ThermoTRPs and opsins initiate a PLC signalling cascade that controls thermotaxis in a calcium-regulated manner. Blue and yellow arrows indicate sodium and calcium currents, and red arrows indicate thermal signals. The red numbers indicate thermoTRPs thermotransduction steps: (1) TRPV1/TRPV4 are activated by thermal signal or hydrolysis of PIP2; (2) membrane depolarization; and (3) calcium channel opening. Yellow numbers indicate the opsins thermotransduction steps: (1) opsins receive thermal signal; (2) G protein activation; (3) PLC activation; (4) downstream TRP channels opening. See text for details. thermoTRPs, thermosensitive transient receptor potential; PLC, phospholipase C; VGCC, voltage-gated calcium channel; IP3R, inositol 1,4,5-trisphosphate receptor; $[Ca^{2+}]i$, intracellular Ca^{2+} concentration; PIP2, phosphatidylinositol 4,5-bisphosphate; DAG, diacylglycerol; PDE, phosphodiesterase; PKA, protein kinase A; PKC, protein kinase C.

24°C), and the change in thermal preference exhibited by third instar larvae, result from the activation of TRP channels by an enzymatic cascade initiated by rhodopsin (Shen *et al.*, 2011; Sokabe *et al.*, 2016). Rhodopsin and various opsins (melanopsin, encephalopsin and neuropsin) were recently discovered in mammalian spermatozoa by qRT-PCR, western blotting and immunocytochemistry (Perez-Cerezales *et al.*, 2015a). Rhodopsin is mainly located in the head of human sperm and the tail of mouse sperm. To investigate whether opsins act as temperature sensors for sperm, Perez-Cerezales *et al.* (2015a) first demonstrated the involvement of GPCRs in thermotaxis, with a temperature gradient-dependent accumulation in sperm being inhibited by m119k, a G protein inhibitor. Moreover, the use of hydroxylamine, a nucleophile that disrupts opsin function, resulted in an inhibition of thermotaxis. Another study observed enrichment of rhodopsin in a subset of spermatozoa selected by thermotaxis, supporting opsins as markers of thermotaxis (Pérez-Cerezales *et al.*, 2018). It was recently recorded that rhodopsin and melanopsin co-localize in mammalian sperm cells (Roy *et al.*, 2020). The thermotactic activity of sperm in mice with knockout of either rhodopsin or melanopsin was reduced by 70% and 50%, respectively (Perez-Cerezales *et al.*, 2015a; Roy *et al.*, 2020). This finding is more pronounced than the expected effect of knocking out a single protein. A plausible explanation is that, as in the retina, opsins are organized in sperm cells in paracrystalline arrays of dimers (Fotiadis *et al.*, 2003; Perez-Cerezales *et al.*, 2015a). This structure is so delicately adapted to construct thermal detection of sperm that removal of individual proteins can lead to disruption of the array system. Moreover, the cooperation of the two opsins is recapitulated in *Drosophila*, and *Drosophila* expressing melanopsin can reverse the Rh1 (rhodopsin) knockout defect in thermal sensory recognition (Shen *et al.*, 2011). However, rhodopsin and melanopsin appear to be involved in two distinct thermotaxis mechanisms, corresponding to the transducin/cyclic nucleotides and PLC pathways, respectively (see next subsection) (Roy *et al.*, 2020). The study demonstrates that the extraordinary thermal recognition of sperm relies on multiple opsins, potentially initiating G protein-coupled signalling cascades similar to that on visual cells. These signalling cascades have an amplifying effect, granting sperm cells the ability to sense tiny temperature differences.

Thermosensory signalling cascades

Sperm possess far sharper temperature sensations than *Drosophila*, responding to very small differences (<0.0006°C) between the head and tail of the cell (Bahat *et al.*, 2012). This ability, in addition to the

requirement that temperature sensors have a large molecular composition, means that thermosensory signalling cascades may have to amplify small temperature differences to the optimum. Such cascades are evident in vision as well as in the thermotaxis of invertebrates, and mammalian sperm may be no exception (Fu and Yau, 2007; Kwon *et al.*, 2008).

In one case, PLC is activated by opsin-coupled G proteins, and TRPs act as downstream transduction channels. In melanopsin knockout mouse sperm, application of the general phosphodiesterase (PDE) inhibitors caffeine and 3-isobutyl-1-methylxanthine (IBMX) and the specific PDE5/6 inhibitor sildenafil both partially inhibited thermotaxis (Perez-Cerezales *et al.*, 2015a; Roy *et al.*, 2020). It has also been indicated that rhodopsin expression leads to changes in the intracellular cyclic nucleotide content of sperm through the G_t/PDE pathway, with effects that closely match the behavioural responses of vertebrate rods and cones to light (Fu and Yau, 2007). Notably, rhodopsin in *Drosophila* senses temperature differences via G_q/PLC rather than G_t/PDE, but rhodopsin in sperm does not appear to be involved in the PLC cascade (Roy *et al.*, 2020). Instead, mammalian sperm melanopsins are functionally similar to invertebrate (*Drosophila*) opsins, activating downstream TRP channels through a pathway coupled to G_q/PLC and hydrolysis of PIP2 (Roy *et al.*, 2020). These TRPs are most likely TRPC family members represented by TRPC3, as both the TRPC inhibitor SKF96365 and the TRPC3 inhibitor Pyr3 reduce the amount of sperm accumulating in the warmer compartment (Panda *et al.*, 2005; Perez-Cerezales *et al.*, 2015a). In addition, opsin cascade downstream signalling appear to modulate many important events associated with thermotaxis, including the control of thermoTRP thermosensitivity (Kaneko and Szallasi, 2014), capacitation (Li *et al.*, 2021) and hyperactivation (Fujinoki, 2013), through DAG/protein kinase C (PKC) and cAMP/protein kinase A (PKA) pathway-dependent phosphorylation rather than dephosphorylation.

Alternatively, thermoTRP acts as the primary temperature sensor, mediated by PLC activation and the triggering of action potential. Thermal activation of thermoTRP leads to membrane depolarization and Ca^{2+} influx. It has been demonstrated in human sperm that temperature-sensitive TRPV4 mediates Na^{+} influx to induce sperm membrane depolarization when exposed to the warm FRT, which is necessary to facilitate gating of ion channels (Mundt *et al.*, 2018). TRPV1 also acts as a depolarizing channel and opens voltage-gated calcium channels (VGCCs), because activation of TRPV1 causes a transient rise in sperm $[Ca^{2+}]_i$, an effect that is severely inhibited in the absence of extracellular sodium or the presence of the VGCC inhibitor verapamil (Bernabo *et al.*, 2010; De Toni *et al.*, 2016). Furthermore, a temperature-dependent $[Ca^{2+}]_i$ increase mediated by TRPM8 in the 21∼23°C range was directly detected in sperm (De Blas *et al.*, 2009). The above results imply that the release of internal Ca^{2+} stores and/or subsequent opening of new calcium channels (VGCCs or store-operated calcium channels (SOCCs)) are the main consequences of thermoTRP sensing of thermal stimuli. Indeed, the extracellular calcium chelator EGTA and the intracellular calcium chelator BAPTA abolish thermotaxis of bull sperm (Mondal *et al.*, 2017). Coincidentally, the most likely additional candidate calcium channel is the non-temperature-sensitive TRPC3. TRPC3 is not only a SOCC but also activated by DAG and Ca^{2+} downstream of PLC (Wang *et al.*, 2020). There may be sequential activation events between opsins and thermoTRPs, and the complex crosstalk between the two systems on thermotaxis remains to be further investigated.

Thermotaxis-driven microfluidics in ART

Sperm entering the FRT are subjected to rigorous selection, allowing only the best candidate from the millions of ejaculated gametes to complete fertilization (Suarez and Pacey, 2006). However, the harsh elimination mechanisms lead to infertility issues in men with asthenozoospermia. Infertility is estimated to affect nearly 10% of men worldwide, with abnormal semen parameters being the leading cause of male infertility (Barratt *et al.*, 2017). Embryologists use ART to select the fertile and healthy sperm subpopulations to overcome the barriers of FRT and fertilize an oocyte. IVF and IUI retain the cumulus and zona pellucida selection, while ICSI directly bypasses all natural selection processes. Therefore, to reduce the genetic risk of paternal genomic defects, sperm selection from semen samples is critical for ART. The microfluidic lab-on-a-chip device developed in the past fifteen years can establish a laminar flow field that strictly simulates sperm migration in the FRT and avoids the generation of reactive oxygen species and DNA fragmentation in the centrifugation step of traditional sorting techniques (Nosrati *et al.*, 2017).

Applying thermotaxis to ART is a relatively new idea that is recently being tested on microfluidic chips (Li *et al.*, 2014; Pérez-Cerezales *et al.*, 2018). The basic principle of thermotaxis-driven microfluidics is to place sperm in a microchannel that allows free swimming and to use a microheater to apply a lateral temperature gradient in the channel, thereby selecting and trapping thermotactic sperm in specific branches (Li *et al.*, 2014; Karbalaei and Cho, 2018; Ko *et al.*, 2018; Yan *et al.*, 2021). Li *et al.* (2014) first designed a microfluidic device utilizing a gas-liquid interface valve to isolate branches. Migration of human spermatozoa was demonstrated in four preset temperature gradients: 34.0–35.3°C, 35.0–36.3°C, 36.0–37.3°C and 37.0–38.3°C. Despite the on-chip device validating sperm thermotaxis, it was unable to replicate the complex natural selection of FRT. As demonstrated in microfluidics, sperm sorting platforms need to simultaneously combine the molecular characteristics of fertile sperm and their response to external stimuli. In terms of sperm strategy responses, temperature gradients can be used as a new parameter to enhance microfluidic systems along with chemotaxis and rheotaxis. Indeed, fully integrated biomimetic microfluidic devices have recently been developed, enabling the simultaneous assessment and quantification of chemotaxis and thermotaxis (Ko *et al.*, 2018; Yan *et al.*, 2021). The microfluidic chip combining thermotaxis and chemotaxis collected more sperm than when thermotaxis or chemotaxis was applied alone (Ko *et al.*, 2018). It is worth mentioning that no statistical association was observed between thermotaxis and chemotaxis, which may be explained by the fact that different mechanisms drive the two strategy responses.

Sperm selection by thermotaxis contributes to improved ART outcomes. Studies have shown that mouse, human and stallion sperm subpopulations selected by thermotaxis have higher DNA integrity and looser chromatin compared to unselected sperm (Pérez-Cerezales *et al.*, 2018; Ruiz-Díaz *et al.*, 2020). In mice, the use of thermotactically selected sperm significantly increased blastocyst production rates and embryo quality for ICSI (Pérez-Cerezales *et al.*, 2018). However, the actual application of thermotaxis to improve human ART requires support from more extensive clinical studies.

Conclusions

This article reviews all the current knowledge since the introduction of mammalian sperm thermotaxis, reviews aspects of thermotaxis navigation and sperm temperature sensing mechanisms based on temporal cues, and highlights their potential for use in clinical procedures. Although two other guiding mechanisms, chemotaxis and rheotaxis, are well described, many aspects of thermotaxis remain unknown. Sperm cell function is temperature-dependent, creating difficulties distinguishing between thermotaxis and ambient temperature effects. Furthermore, the molecular mechanisms of thermotaxis are complex and appear to result from an extensive intersection of intracellular signalling pathways. It is now known that at least two classes of opsins and thermoTRPs make up the sperm thermal detection system. However, establishing thermoTRP members as thermotaxis temperature sensors requires further research. The role of ion channels in temperature sensing and sperm cell migration remains unclear. On the other hand, considering that thermotaxis is limited to capacitated sperm, it would be very interesting to investigate the mechanism behind the acquisition of thermotaxis.

Data availability

No new data were generated or analysed in support of this research.

Authors' roles

W.X., M.Y. and Y.Y.: conceptualization and initial draft. X.L.: revision. Y.C.: design and supervision, critical revision and suggestions. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare to have no conflicts of interest.

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