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ORIGINAL ARTICLE *Andrology*

The antidepressant Sertraline inhibits CatSper Ca^{2+} channels in human sperm

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STUDY QUESTION: Do selective serotonin reuptake inhibitor (SSRI) antidepressants affect the function of human sperm?

SUMMARY ANSWER: The SSRI antidepressant Sertraline (e.g. Zoloft) inhibits the sperm-specific Ca^{2+} channel CatSper and affects human sperm function *in vitro*.

WHAT IS KNOWN ALREADY: In human sperm, CatSper translates changes of the chemical microenvironment into changes of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and swimming behavior. CatSper is promiscuously activated by oviductal ligands, but also by synthetic chemicals that might disturb the fertilization process. It is well known that SSRIs have off-target actions on Ca^{2+}, Na^+ and K^+ channels in somatic cells. Whether SSRIs affect the activity of CatSper is, however, unknown.

STUDY DESIGN, SIZE, DURATION: We studied the action of the seven drugs belonging to the most commonly prescribed class of antidepressants, SSRIs, on resting $[Ca^{2+}]_i$ and Ca^{2+} influx via CatSper in human sperm. The SSRI Sertraline was selected for in-depth analysis of its action on steroid-, prostaglandin-, pH- and voltage-activation of human CatSper. Moreover, the action of Sertraline on sperm acrosomal exocytosis and penetration into viscous media was evaluated.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The activity of CatSper was investigated in sperm of healthy volunteers, using kinetic Ca^{2+} fluorimetry and patch-clamp recordings. Acrosomal exocytosis was investigated using *Pisum sativum* agglutinin and image cytometry. Sperm penetration in viscous media was evaluated using the Kremer test.

MAIN RESULTS AND THE ROLE OF CHANCE: Several SSRIs affected $[Ca^{2+}]_i$ and attenuated ligand-induced Ca^{2+} influx via CatSper. In particular, the SSRI Sertraline almost completely suppressed Ca^{2+} influx via CatSper. Remarkably, the drug was about four-fold more potent to suppress prostaglandin- versus steroid-induced Ca^{2+} influx. Sertraline also suppressed alkaline- and voltage-activation of CatSper, indicating that the drug directly inhibits the channel. Finally, Sertraline impaired ligand-induced acrosome reaction and sperm penetration into viscous media.

LIMITATIONS, REASONS FOR CAUTION: This is an *in vitro* study. Future studies have to assess the physiological relevance *in vivo*.

WIDER IMPLICATIONS OF THE FINDINGS: The off-target action of Sertraline on CatSper in human sperm might impair the fertilization process. In a research setting, Sertraline may be used to selectively inhibit prostaglandin-induced Ca^{2+} influx.

STUDY FUNDING/COMPETING INTEREST(S): This work was supported by the Swiss Centre for Applied Human Toxicology (SCAHT), the Département de l'Instruction Publique of the State of Geneva, the German Research Foundation (CRU326), the Interdisciplinary Center for Clinical Research, Münster (IZKF; Str/014/21), the Innovation Fund Denmark (grant numbers 14-2013-4) and the EDMaRC research grant from the Kirsten and Freddy Johansen's Foundation. The authors declare that no conflict of interest could be perceived as prejudicing the impartiality of the research reported.

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Key word: human sperm / antidepressants / SSRI / calcium signaling / CatSper / acrosomal exocytosis / sperm motility

Introduction

Infertility affects 10–15% of couples worldwide (Barratt *et al.*, 2017). Causes can be of male or female origin or due to a combination of both; however, in 15–20% of the cases, infertility remains idiopathic (Nieschlag, 2010; Barratt *et al.*, 2017; Cunningham, 2017). Among the etiological factors involved in infertility, adverse effects of common medications are often neglected in the clinical setting and rather understudied (Jarow *et al.*, 2010; Samplaski and Nangia, 2015; Semet *et al.*, 2017). It is however well known that a plethora of synthetic exogenous compounds affects the function of human sperm *in vitro* (Gore *et al.*, 2015). Several studies have revealed that diverse endocrine disrupting chemicals (EDCs) activate the sperm-specific Ca^{2+} channel CatSper (Tavares *et al.*, 2013; Schiffer *et al.*, 2014; Rehfeld *et al.*, 2016; Brenker *et al.*, 2018; Majzoub *et al.*, 2018; Yuan *et al.*, 2020) that controls the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and, thereby, sperm function (reviewed by Kaupp and Strünker, 2017; Rahban and Nef, 2020; Wang *et al.*, 2021). Physiological stimuli that activate CatSper are depolarization of the membrane potential (V_m), alkalization of the intracellular pH (pH_i) as well as steroids and prostaglandins released in the oviduct (Kirichok and Lishko, 2011; Strünker *et al.*, 2011). Activation of CatSper by steroids or prostaglandins has been shown to be implicated in critical sperm functions like capacitation (Sumigama *et al.*, 2015), chemotaxis (Eisenbach and Giojalas, 2006; Publicover *et al.*, 2008), hyperactivation (Alasmari *et al.*, 2013; Williams *et al.*, 2015), penetration into viscous media (Williams *et al.*, 2015; Rennhack *et al.*, 2018; Luo *et al.*, 2019) and acrosomal exocytosis (Tamburrino *et al.*, 2014; Luo *et al.*, 2019). This suggests that exogenous compounds interfering with the activity of CatSper can impair the ability of sperm to reach and fertilize the egg.

Selective serotonin reuptake inhibitors (SSRIs) are the most widely prescribed antidepressants in the USA and in Europe (Preskorn, 2004; Dawson *et al.*, 2016). Rates of depressive symptoms are twice as high among infertile couples, and almost 11% of women undergoing IVF are taking SSRIs during the procedure (Dawson *et al.*, 2016; Sylvester *et al.*, 2019). Treatment with SSRIs is usually prescribed for several months and can last up to years, or even a lifetime. Sperm might be exposed to SSRIs in the male reproductive tract, during their journey through the female genital tract, and/or during the fertilization process. SSRIs primarily target serotonin transporters, but off-target actions on voltage-gated Ca^{2+}, Na^+ and K^+ channels in somatic cells have been described (Choi *et al.*, 1999; Hahn *et al.*, 1999; Lory *et al.*, 2006; Lee *et al.*, 2012, 2016; Kim *et al.*, 2017). Whether SSRIs also affect CatSper is, however, unknown. Here, using kinetic Ca^{2+} fluorimetry and patch-clamp recordings, we studied the action of SSRIs on human sperm. We show that several SSRIs affect $[Ca^{2+}]_i$ and/or suppress progesterone- and prostaglandin-evoked Ca^{2+} influx, indicating that SSRIs affect the activity of CatSper. In particular, the SSRI Sertraline inhibits in a concentration-dependent fashion ligand-, pH_i-, and voltage-activation of human CatSper. Moreover, Sertraline attenuated progesterone- and prostaglandin E1-evoked acrosomal exocytosis and sperm penetration into viscous media. Altogether, we conclude that inhibition of CatSper by antidepressant treatment with SSRIs might impair human fertilization *in vivo*.

Materials and methods

Reagents

SSRIs Dapoxetine (CAS # 129938-20-1), Escitalopram (CAS # 219861-08-2), Fluoxetine (CAS # 56296-78-7), Fluvoxamine (CAS # 61718-82-9), Sertraline (CAS # 79559-97-0), Citalopram (CAS # 59729-32-7) and Paroxetine (CAS # 110429-35-1) were purchased from Sigma-Aldrich (Buchs, Switzerland). The drugs were dissolved at a stock concentration of 20 mM in Dimethyl sulfoxide (DMSO) manufactured by PanReac AppliChem and purchased from AxonLab AG (Baden, Switzerland). Steroids, prostaglandins, Pluronic F127 and NH_4Cl were purchased from Sigma-Aldrich (Buchs, Switzerland). The fluorescent Ca^{2+} indicator Fluo-4-AM was purchased from Invitrogen (CA, USA) and Human Serum Albumin (HSA) was obtained from Polygon Diagnostics (Lucerne, Switzerland) or Irvine Scientific (Tilburg, Netherlands). For the assessment of acrosomal exocytosis and sperm motility, fluorescein isothiocyanate-conjugated *Pisum sativum* agglutinin (FITC-PSA) and 4000 cP methylcellulose (MC) were purchased from Sigma-Aldrich, MO, USA. Propidium Iodide (PI) and Hoechst-33342 (H342) were purchased from ChemoMetec A/S, Allerød, Denmark.

Semen sample preparation

Semen samples were obtained from volunteers with prior written consent, under approval from the ethical committees of the medical association Westfalen-Lippe, the medical faculty of the University of Münster (4INie), and the Capital Region of Denmark (H-16036581 and H-19089581). The study was performed in agreement with the standards set by the Declaration of Helsinki. Semen samples were produced by masturbation and ejaculated into plastic containers. Motile sperm were prepared by a swim-up procedure as previously described (Strünker *et al.*, 2011) in human tubular fluid (HTF) medium containing (in mM): 97.8 NaCl, 4.69 KCl, 0.2 $MgSO_4$, 0.37 KH_2PO_4, 2.04 $CaCl_2$, 0.33 Na-pyruvate, 21.4 lactic acid, 2.78 glucose, 21 HEPES and 4 $NaHCO_3$, pH adjusted between 7.3 and 7.4 with NaOH. HSA was added at a final concentration of 3 mg/ml. For the assessment of acrosomal exocytosis and sperm motility in viscous media, sperm were capacitated for at least 3 h at 37°C in a capacitating medium containing (in mM): 72.8 NaCl, 4.69 KCl, 0.2 $MgSO_4$, 0.37 KH_2PO_4, 2.04 $CaCl_2$, 0.33 Na-pyruvate, 21.4 lactic acid, 2.78 glucose, 21 HEPES and 25 $NaHCO_3$, pH adjusted between pH 7.3 and 7.4 with NaOH. HSA (3 mg/ml) was added to the capacitating medium.

Measurements of changes in $[Ca^{2+}]_i$

Sperm were incubated with the fluorescent calcium indicator Fluo-4-AM at a final concentration of 5 µM in the presence of Pluronic F127 (0.05% w/v) for 45 min at 37°C. After incubation, excess dye was

removed by centrifugation (700×g, 5 min, Room Temperature). The sperm pellet was resuspended in HTF to a density of 5 × 10^6 sperm/ml. A volume of 50 µl was filled into the wells of 384 multiwell plates. Fluorescence was measured in a fluorescent plate reader (FLUOstar Omega, BMG Labtech, Germany) at 30°C with an excitation wavelength of 480 nm and an emission wavelength of 520 nm with bottom optics. Fluorescence was recorded before and after the application of 25 µl (1:3 dilution) of buffer, ligands or SSRIs with an electronic multichannel pipette yielding technical duplicates for each condition within each experiment. Changes in Fluo-4 fluorescence are depicted as $\Delta F/F_0$ (%), that is, the change in fluorescence (ΔF) relative to the mean basal fluorescence (F_0) before application of buffer or stimuli (25 µl) in order to correct for intra- and inter-experimental as well as drug-induced variations in basal fluorescence among individual wells. Sperm were incubated with SSRIs for at least 5 min prior to the addition of prostaglandins, steroids, or NH_4Cl.

Patch-clamp recordings

Patch-clamp recordings from human sperm were performed in the whole-cell configuration, as previously described (Strünker *et al.*, 2011). The standard extracellular solution (HS) contained (in mM): 135 NaCl, 5 KCl, 1 $MgSO_4$, 2 $CaCl_2$, 5 glucose, 1 Na-pyruvate, 10 lactic acid and 20 HEPES, pH adjusted to 7.4 with NaOH. The sodium-based divalent-free solution (NaDVF) contained (in mM): 140 NaCl, 40 HEPES, 1 EGTA, pH adjusted to 7.4 with NaOH; the pipette solution contained (in mM): 130 Cs-aspartate, 50 HEPES, 5 EGTA, 5 CsCl, pH adjusted to 7.3 with CsOH.

Analysis of acrosomal exocytosis

Acrosome exocytosis was evaluated using an image cytometer as previously described (Egeberg Palme *et al.*, 2018). Briefly, suspensions of capacitated sperm (1 × 10^7 sperm/ml) were divided into equal parts and mixed with a staining solution containing: 5 µg/ml FITC-PSA, 0.5 µg/ml PI and 10 µg/ml H342 in HTF (final concentrations). Afterward, progesterone (5 µM), PGE1 (5 µM) or Sertraline (10 µM) were added to the parts. To study how Sertraline affects the action of progesterone and PGE1, the stained sperm were incubated for 5 minutes with Sertraline before progesterone or PGE1 was added. DMSO (0.2%) served as the negative control. Samples were then thoroughly mixed and incubated at 37°C on a gentle mixing heating plate for 30 min. After incubation, a 50 µl aliquot was mixed with 100 µl of an immobilizing solution containing 0.6 M $NaHCO_3$ and 0.37% (v/v) formaldehyde in distilled water. This mixture was immediately loaded into a two-chamber NC-Slide A2™ (ChemoMetec, Allerød, Denmark), which was analyzed by image cytometry using a NucleoCounter® NC-3000™ (ChemoMetec). Only live acrosome-reacted sperm cells were taken into account (FITC-PSA positive but PI negative).

Assessment of sperm motility

Sperm motility was assessed using the Kremer test. Sperm were evaluated for their ability to penetrate a glass capillary filled with a viscous medium containing 1% (w/v) MC (4000 centipoises) and 0.3% HSA, equilibrated overnight at 4°C, in HTF (MC-HTF). Progesterone (5 µM), PGE1 (5 µM), Sertraline (10 µM), progesterone + Sertraline, PGE1 + Sertraline or DMSO (control) was added to the MC-HTF that was then filled into flattened glass capillary tubes (0.2 × 4.0 × 50 mm, CM scientific, UK) and sealed on one end with wax (Vitrex, UK). The open ends of the tubes were then submerged in a tube with 1.5–3 × 10^6/ml capacitated sperm in presence of Sertraline, DMSO, or with Sertraline followed by application of the ligands. Sperm penetration was assessed after 60 min of incubation at 37°C by counting sperm at 1 cm using a phase-contrast microscope at a 200× magnification.

Statistical analysis

Data are shown as mean ± SD with 'n' referring to the number of independent experiments performed using sperm samples from ≥3 different donors. Statistical analysis and fitting of dose–response relations were performed using GraphPad Prism 8 (Prism, La Jolla, USA). Half-maximal inhibitory concentrations (IC_{50}) were derived by nonlinear regression analysis, using a four-parameter fit. Statistical significance between control and stimulus/inhibitor-treated conditions was either evaluated using one-way ANOVA, followed by Dunnett's test and Siddak's when comparing each of the different conditions to a single control and for multiple comparisons, respectively. A paired *t*-test was used to analyze data in Figure 6B. A *P*-value <0.05 was considered significant. The data and statistical analysis were performed according to the recommendations on experimental design and analysis in pharmacology (Curtis *et al.*, 2018).

Results

SSRIs affect the intracellular Ca^{2+} concentration in human sperm

We studied the action of the SSRIs Dapoxetine, Escitalopram, Fluoxetine, Fluvoxamine, Sertraline, Citalopram and Paroxetine on $[Ca^{2+}]_i$ in non-capacitated human sperm using a fluorescence plate reader (Strünker *et al.*, 2011; Schiffer *et al.*, 2014) (Fig. 1A). Sperm were loaded with a fluorescent Ca^{2+} indicator and $[Ca^{2+}]_i$ was monitored before and after application of SSRIs (10 µM). Progesterone (2 µM) and buffer were applied in parallel as a positive and negative control, respectively. Progesterone-activation of CatSper evoked a prototypical Ca^{2+} response, whereas application of buffer evoked only a small mixing artifact (Fig. 1B). Escitalopram and Citalopram did not affect $[Ca^{2+}]_i$, whereas Dapoxetine, Fluoxetine, Fluvoxamine and Paroxetine evoked a small, transient Ca^{2+} signal (Fig. 1B and C). Sertraline, however, decreased $[Ca^{2+}]_i$. Of note, except for Sertraline, the SSRIs did not affect the intracellular pH, indicating that the transient Ca^{2+} increase does not rest on alkaline-induced Ca^{2+} influx via CatSper (Supplementary Fig. S1). Sertraline slightly increased pH_i, which is however unlikely to account for the $[Ca^{2+}]_i$ decrease evoked by the drug. The mechanism underlying the slight pH_i increase is unclear.

SSRIs suppress steroid- and prostaglandin-evoked Ca^{2+} influx in human sperm

We next studied the action of the SSRIs on progesterone- and prostaglandin E1 (PGE1)-evoked Ca^{2+} influx via CatSper. Dapoxetine, Fluoxetine, Sertraline and Paroxetine, but not Escitalopram, Fluvoxamine and Citalopram suppressed the ligand-evoked Ca^{2+} influx (Fig. 2A and B). The SSRIs seemed to suppress the Ca^{2+} influx evoked

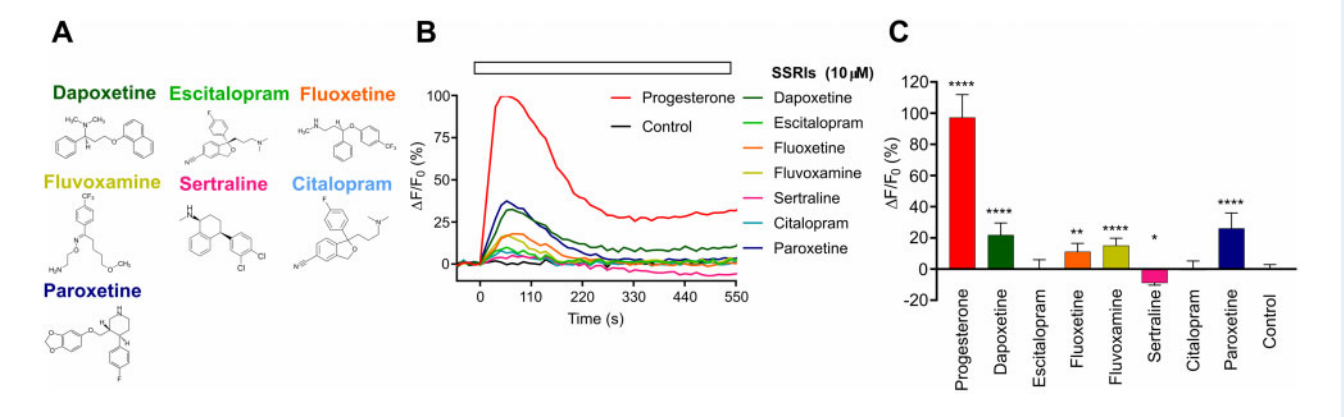

Figure 1. The action of selective serotonin reuptake inhibitors (SSRIs) on the intracellular Ca^{2+} concentration in human sperm. (**A**) Generic names and chemical structures of SSRIs. (**B**) Representative Ca^{2+} signals evoked by application of buffer (control), progesterone (2 µM) and SSRIs (10 µM). $[Ca^{2+}]_i$ was monitored using a fluorescence plate reader. Sperm were loaded with the fluorescent Ca^{2+} indicator Fluo-4-AM. $\Delta F/F_0$ (%) designates the percent change in fluorescence (ΔF) with respect to the mean basal fluorescence (F_0) before application and subsequent continuous presence (indicated by the white bar on top) of SSRIs or progesterone (2 µM) at $t = 0$. (**C**) Mean (±SD) maximal amplitude of Ca^{2+} evoked by buffer (control), progesterone and SSRIs (n = 12). *$P < 0.05$; **$P < 0.001$; ****$P < 0.00001$ versus control.

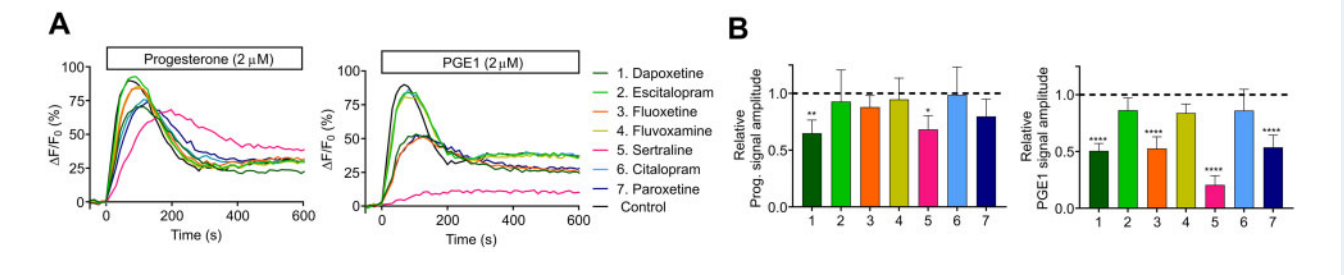

Figure 2. The action of selective serotonin reuptake inhibitors (SSRIs) on progesterone- and prostaglandin E1-evoked Ca^{2+} influx in human sperm. Representative Ca^{2+} signals in human sperm evoked by progesterone (**A**) or PGE1 (**B**) in the absence (control) and presence of SSRIs (10 µM); sperm were incubated for ≥5 min with SSRIs prior to stimulation with progesterone or PGE1. (**C and D**)**:** Mean (±SD) maximal signal amplitude evoked by progesterone (**C**) and PGE1 (**D**) in the presence of SSRIs relative to that evoked in their absence (control, set to 1) (n = 6); *$P < 0.05$; **$P < 0.001$; ****$P < 0.00001$ versus control.

by PGE1 more potently and/or efficaciously compared to that evoked by progesterone. Particularly, at 10 µM, Sertraline inhibited the PGE1 and progesterone response by 80 ± 8% and 31 ± 12%, respectively (Fig. 2D; n = 6). Altogether, we conclude that SSRIs have a complex action in human sperm: at 10 µM; some SSRIs evoke Ca^{2+} signals on their own, whereas others, such as Sertraline, decrease $[Ca^{2+}]_i$. Moreover, some, but not all, SSRIs also inhibit Ca^{2+} influx via CatSper. We decided to investigate the action of Sertraline on human CatSper in more detail.

The SSRI sertraline directly inhibits human CatSper

At first, we analyzed the action of Sertraline alone on $[Ca^{2+}]_i$ over a broad range of concentrations (Fig. 3A and B). At >0.3 µM, Sertraline caused a small, transient Ca^{2+} increase. $[Ca^{2+}]_i$ peaked and returned to basal levels within about 200 s. The signal amplitude grew with increasing concentrations, saturated at about 3 µM, and decreased again. Only at 10 µM, Sertraline caused a small sustained $[Ca^{2+}]_i$ decrease. The mechanism(s) underlying the complex, yet, small Sertraline-evoked changes in $[Ca^{2+}]_i$ are unclear but are reminiscent of the $[Ca^{2+}]_i$ changes evoked by other drugs that inhibit CatSper, such as RU1968 (Rennhack *et al.*, 2018) or H89 (Wang *et al.*, 2020). Next, we studied prostaglandin- and steroid-evoked Ca^{2+} influx in sperm incubated with different concentrations of Sertraline (Fig. 3C–H). Of note, to correct for sertraline-induced differences in the baseline prior to the application of the hormones (Fig. 3A and C), we depicted and analyzed the prostaglandin- or steroid-evoked increases in fluorescence relative to the baseline right before their application (compare Fig. 3C and D). Sertraline slowed down and almost completely suppressed the Ca^{2+} signals evoked by PGE1 and

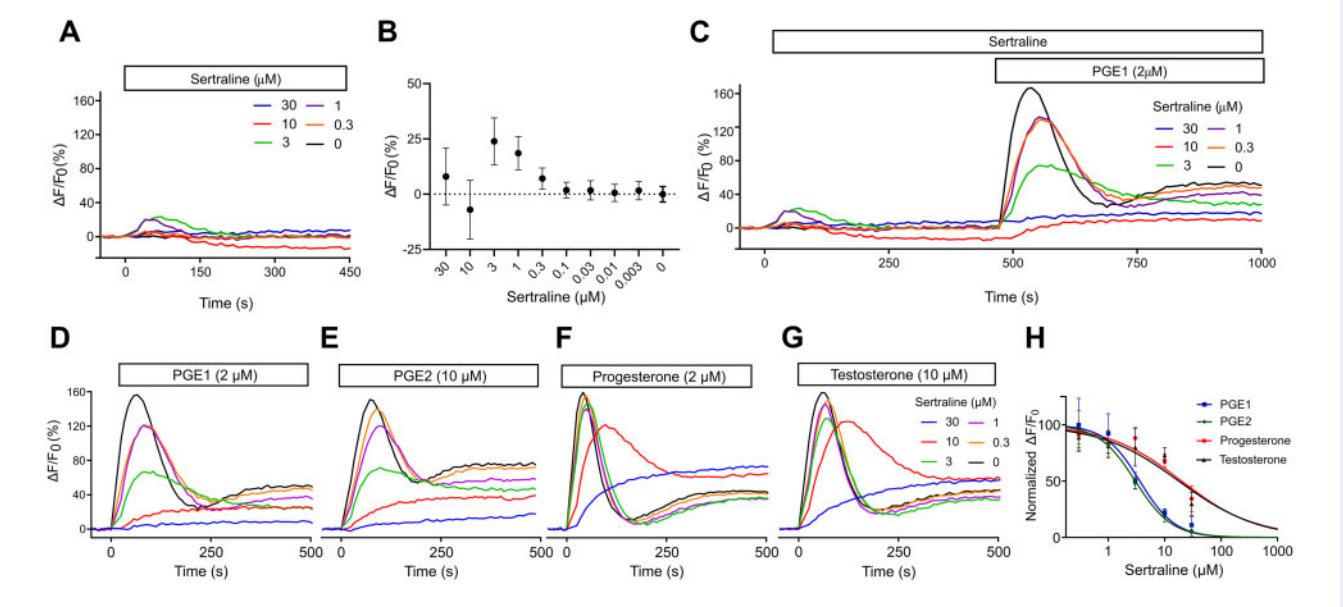

Figure 3. Inhibition of prostaglandin- and- steroid-evoked Ca^{2+} signals by Sertraline. (**A**) Representative Ca^{2+} signals in human sperm evoked by increasing concentrations of Sertraline. (**B**) Mean (±SD) maximal signal amplitudes evoked by Sertraline (n = 20). (**C**) Representative Ca^{2+} signals evoked in human sperm by Sertraline as shown in (**A**) and by a subsequent stimulation with PGE1. (**D–G**) Representative Ca^{2+} signals evoked by PGE1 (**D**) PGE2 (**E**), progesterone (**F**), and testosterone (**G**) in the absence (control, 0 μM) and presence of different concentrations of Sertraline; (**D**) shows the PGE1-evoked signals from (**C**), yet, normalized to the mean basal fluorescence right before application of PGE1. The PGE2-, progesterone- and testosterone-evoked signals were processed and analyzed similarly. (**H**) Dose–response relation for the mean (±SD) maximal signal amplitudes within the first 200 s after stimulation (n = 6). The amplitudes were normalized to that evoked in the absence of Sertraline (set to 100); the dose–response curves were fitted restraining the top and bottom values to 100 and 0, respectively. IC_{50} value (± standard error of the fit): 4.62 ± 1.18 μM for PGE1, 5.61 ± 1.27 μM for PGE2, 16.19 ± 1.16 μM for progesterone and 19.61 ± 1.10 μM for testosterone.

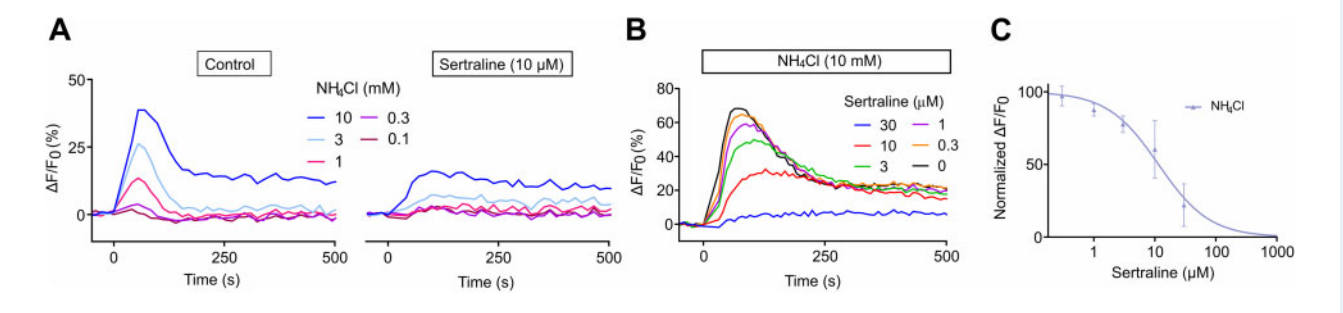

Figure 4. Inhibition of alkaline-evoked Ca^{2+} signals by Sertraline. (**A**) Representative Ca^{2+} signals in sperm incubated for 10 min in the absence (control, left panel) and presence (right panel) of Sertraline (10 μM) evoked by stimulation with different concentrations of NH_4Cl. (**B**) Ca^{2+} signals evoked by NH_4Cl in the absence (0 μM) and presence of different concentrations of Sertraline. (**C**) Dose–response relation for the mean (±SD) maximal signal amplitudes within the first 200 s after stimulation (n = 4). The amplitudes were normalized to that evoked in the absence of Sertraline (set to 100). The dose–response curve was fitted restraining the top and bottom values to 100 and 0, respectively. IC_{50} (±standard error of the fit): 11.5 ± 1.05 μM.

PGE2 with a half-maximal inhibitory concentration (IC_{50}) of 4.62 ± 1.18 μM and 5.61 ± 1.27 μM (n = 6), respectively (Fig. 3C–E and H). The drug also slowed down and attenuated the Ca^{2+} responses evoked by progesterone and testosterone (Fig. 3F–H). However, Sertraline inhibited the steroid responses only at concentrations ≥10 μM; at 30 μM, Sertraline attenuated the first transient signal phase by 72 ± 7% (n = 6). Fitting the dose–response relation yielded an IC_{50} of 16.19 ± 1.16 μM and 19.61 ± 1.10 μM for the inhibition of the progesterone and testosterone response, respectively (Fig. 3H). Sertraline thus inhibits

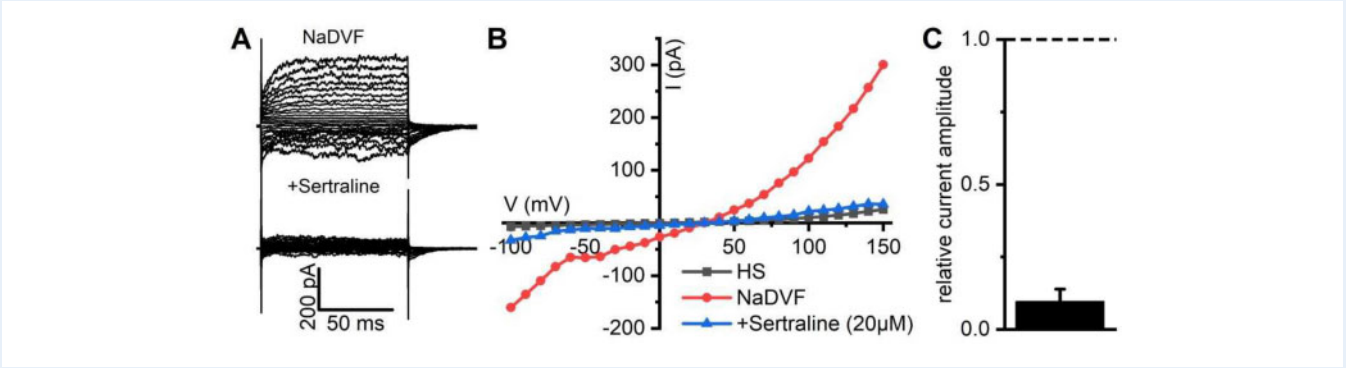

Figure 5. Inhibition of CatSper-mediated membrane currents by Sertraline. (**A**) Representative whole-cell CatSper currents recorded from a human sperm cell at pH_i 7.3 in Na^+-based divalent-free extracellular solution (NaDVF) and during perfusion with NaDVF containing Sertraline (20 μM). Currents were evoked by stepping the membrane voltage from −100 to +150 mV (in steps of 10 mV) from a holding potential of 0 mV. (**B**) Steady-state current-voltage relationships from (A), including leak currents recorded as a control in extracellular solution containing Mg^{2+} and Ca^{2+} (HS) (**C**) Mean (± SD) amplitude of CatSper currents at + 100 mV in the presence of Sertraline relative to that in its absence (set to 1) (n=3).

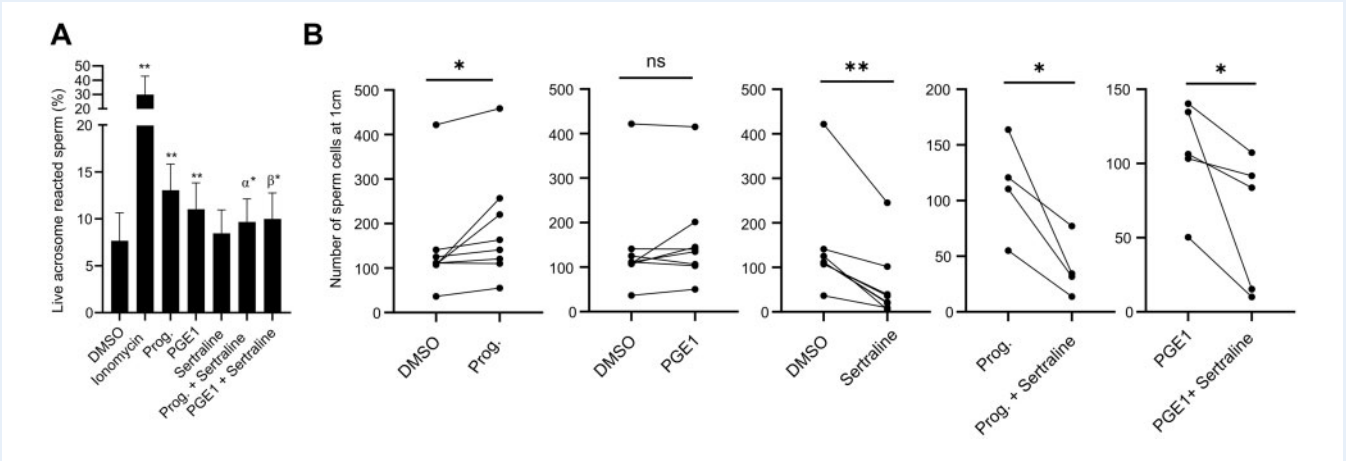

Figure 6. Action of Sertraline on progesterone- and prostaglandin E1-induced acrosomal exocytosis and on sperm penetration into viscous media. (**A**) Percentage (±SD) of live acrosome-reacted human sperm in the absence (DMSO, spontaneous acrosome reaction) and presence of progesterone (10 μM), PGE1 (5 μM), Sertraline (10 μM), ionomycin (10 μM), progesterone + Sertraline and PGE1 + Sertraline ($n \geq 7$). (**B**) Paired plots of the number of sperm at a penetration distance of 1 cm in the absence (DMSO) and presence of progesterone (10 μM), PGE1 (5 μM) or Sertraline (10 μM); paired plots of sperm number in the presence of progesterone/PGE1 and Sertraline + progesterone/PGE1 ($n \geq 5$). *$P < 0.05$, **$P < 0.001$, versus control, α * < 0.05, significantly different from progesterone, *β < 0.05, significantly different from PGE1.

prostaglandin-evoked Ca^{2+} influx via CatSper with about four-fold higher potency than the steroid-induced influx. The inhibitory action of the drug was similar in capacitated sperm (Supplementary Fig. S2). Next, we studied whether Sertraline also inhibits CatSper-mediated Ca^{2+} influx evoked by intracellular alkalization, using NH_4Cl. Sertraline slowed down and almost completely suppressed Ca^{2+} signals evoked by ≤10 mM NH_4Cl (Fig. 4A and B). The IC_{50} value for the inhibition of signals evoked by 10 mM NH_4Cl was 11.5 ± 1.13 μM (n = 4) (Fig. 4C). Finally, we studied whether Sertraline inhibits CatSper-mediated membrane currents recorded in human sperm by whole-cell patch clamping (Fig. 5). In an extracellular solution containing Ca^{2+} and Mg^{2+} (HS solution), stepping the membrane voltage from −100 mV to +150 mV with increments of 10 mV from a holding potential of 0 mV evoked only minuscule currents (Fig. 5B, HS). Upon superfusion with Na^+-based divalent-free solution (NaDVF solution), the prototypical monovalent CatSper currents were recorded (Fig. 5A and B; NaDVF). Sertraline (20 μM) almost completely suppressed currents carried by CatSper (Fig. 5A–C). Moreover, Sertraline also suppressed CatSper currents evoked in the presence of

progesterone or PGE1 (Supplementary Fig. S3). In summary, Sertraline inhibits ligand-, alkaline-, and voltage-activation of CatSper, indicating that the drug directly blocks CatSper.

Sertraline attenuates progesterone- and PGE1-induced acrosomal exocytosis and reduces the penetration of human sperm into viscous media

Using an image cytometer-based assay (Egeberg Palme *et al.*, 2018), we studied the action of Sertraline on progesterone- and prostaglandin-evoked acrosomal exocytosis in human sperm. Progesterone and PGE1 increased the fraction of acrosome reacted sperm by 1.70 ± 0.93- and 1.44 ± 0.93-fold ($n \geq 7$), respectively (Fig. 6A). Sertraline alone did not induce acrosomal exocytosis, but strongly attenuated acrosomal exocytosis evoked by progesterone and PGE1 (Fig. 6A).

Moreover, using a modified Kremer's test, we assessed the penetration of sperm into viscous media. Progesterone increased the number of sperm at a penetration distance of 1 cm by 1.50 ± 0.52 fold (Fig. 6B). PGE1 also slightly increased the number of penetrating sperm (Fig. 6B); the increase was, however, not statistically significant. Sertraline alone decreased the number of penetrating sperm by 0.33 ± 0.22-fold and suppressed the action of progesterone and PGE1 (Fig. 6B). We wondered whether Sertraline affects the basal motility parameters of human sperm, which might explain the drug's action in Kremer's test. To this end, the swimming behavior of sperm before and after application of Sertraline (10 µM) was analyzed, using standard computer-assisted sperm analysis. Incubation of sperm for 15 or 60 min with Sertraline did neither affect the fraction of motile sperm nor their kinematics (Supplementary Fig. S4); the drug seemed to slightly decrease some parameters such as spontaneous hyperactivation, but this decrease was not statistically significant. Thus, the suppression of viscous-media penetration by Sertraline is rather not due to impaired basal motility. Of note, the currently most specific and best-characterized CatSper-inhibitor RU1968 has a similar action in the Kremer test (see Rennhack *et al.*, 2018). Altogether, we conclude that inhibition of CatSper by Sertraline affects viscous-media penetration and acrosomal exocytosis of human sperm.

Discussion

Female ligands released into the genital tract assist the sperm to locate and fertilize the egg by controlling the activity of CatSper (Schaefer *et al.*, 1998; Harper *et al.*, 2004; Oren-Benaroya *et al.*, 2008; Publicover *et al.*, 2008; Baldi *et al.*, 2009; Kilic *et al.*, 2009; Alasmari *et al.*, 2013; Schiffer *et al.*, 2014; Tamburrino *et al.*, 2014, 2015; Rennhack *et al.*, 2018; Rehfeld, 2020). This renders CatSper a central signaling node required for sperm function and fertilization (Kaupp and Strünker, 2017; Rahban and Nef, 2020; Wang *et al.*, 2021). Mutations or deletions of *CATSPER* genes leading to loss of CatSper function are associated with male infertility (Avidan *et al.*, 2003; Avenarius *et al.*, 2009; Hildebrand *et al.*, 2010; Smith *et al.*, 2013; Jaiswal *et al.*, 2014; Williams *et al.*, 2015; Brown *et al.*, 2018; Luo *et al.*, 2019; Schiffer *et al.*, 2020). In the past decade, a series of studies, including our own, revealed that CatSper is affected by synthetic chemicals including EDCs, odorants as well as diverse compounds used to manipulate enzymes, receptors, and ion channels (Lishko *et al.*, 2011; Strünker *et al.*, 2011; Brenker *et al.*, 2012; Tavares *et al.*, 2013; Schiffer *et al.*, 2014; Rehfeld *et al.*, 2016, 2017; Brenker *et al.*, 2018; Rennhack *et al.*, 2018; McBrinn *et al.*, 2019; Wang *et al.*, 2020; Zhang *et al.*, 2020). The chemicals act as full or partial CatSper agonists, inhibitors, or feature rather a dual agonistic and inhibitory action at low and high concentrations, respectively. This demonstrates that the pharmacology of CatSper is highly complex, involving several so far unknown activator- and inhibitor-binding sites, which might be allosterically coupled. It seems that some drugs can bind to more than one of these binding sites at the same time, leading to complex pharmacological effects on CatSper and, thereby, $[Ca^{2+}]_i$ (see, e.g., Rennhacket *al.*, 2018; Wang *et al.*, 2020).

We show that the list of synthetic, non-physiological CatSper modulators also includes common drugs like SSRIs. These drugs have been on the market for decades and their toxicity profile, as well as their therapeutic window, is well known. Although there is no doubt about the safety of these drugs, our findings suggest that Sertraline and perhaps other SSRIs might have side effects that have thus far been unexplored. The drug's action on CatSper impairs sperm function and might thereby iatrogenically disturb the fertilization process *in vivo*, lowering the fecundity of males, females, or both. Among other factors, such previously unexplored side effects of commonly used drugs might be involved in the adverse trends in human reproduction and increasing demand for assisted reproduction (Skakkebaek *et al.*, 2016). Supporting this notion, previous studies suggested that SSRIs reduce male fertility by affecting semen quality (reviewed by Norr *et al.*, 2016; Sylvester *et al.*, 2019; Beeder and Samplaski, 2020). Treatment of rodents with SSRIs reduced the sperm count, sperm motility, testicular weight, length of seminiferous tubules, and, thereby, male fertility (Bataineh and Daradka, 2007; Attia, and Bakheet, 2013; Monteiro Filho *et al.*, 2014; Galal *et al.*, 2016; Lyons *et al.*, 2016; Atli *et al.*, 2017). In men, intake of SSRIs was associated with reduced sperm concentration, a higher number of morphologically abnormal sperm, and an increase in DNA fragmentation. In most of these studies, recovery of normal semen parameters was observed upon cessation of SSRI intake (Kumar et al., 2006; Tanrikut and Schlegel, 2007; Safarinejad, 2008; Tanrikut *et al.*, 2010; Koyuncu *et al.*, 2011; Relwani *et al.*, 2011; Akasheh *et al.*, 2014; Elnazer and Baldwin, 2014; Beeder and Samplaski, 2020). Whether the intake of SSRIs affects female fertility and/or IVF success is unclear and, in fact, a much-debated question (Klock *et al.*, 2004; Friedman *et al.*, 2009; Domar *et al.*, 2013; Casilla-Lennon *et al.*, 2016; Evans-Hoeker *et al.*, 2018; Sylvester *et al.*, 2019).

An important question concerns whether the results from our *in vitro* study are indeed of pharmacological relevance *in vivo*. Sertraline inhibits CatSper at concentration ≥0.3 µM and, thus, at pharmacologically relevant concentrations reached in body fluids: on average, peak plasma concentrations of ~400 nM were determined after oral administration of 200 mg Sertraline (DeVane, 1999; DeVane *et al.*, 2002; Hiemke *et al.*, 2011). The tissue concentration of the drug, e.g., in the lung, heart, and brain, can be more than 20-fold higher than in plasma and might, thus, reach several micromolar (DeVane, 1999; DeVane *et al.*, 2002; Reis *et al.*, 2007; Hiemke *et al.*, 2011; Lewis *et al.*, 2013; Nedahl *et al.*, 2018). To experimentally assess whether Sertraline and other SSRIs might indeed disturb sperm function and fertilization *in vivo*, quantitative data regarding their concentration in reproductive

fluids are required. To our knowledge, the concentration of Sertraline and other SSRIs in seminal, oviductal and/or follicular fluid is unknown. Most importantly, we need to study the action of the drugs on sperm under conditions that experimentally mimic the complex chemical, topographical and hydrodynamic landscapes of the female genital tract—a challenging task that has not yet been accomplished (Suarez and Pacey, 2006; Suarez, 2008; Kirkman-Brown and Smith, 2011; Miki and Clapham, 2013). In addition, animal models, such as primates, might be used to assess whether and how the action of the drugs on sperm might affect the fecundity of males and/or females.

The molecular mechanism underlying the inhibition of CatSper by Sertraline remains to be elucidated. CatSper activation by alkaline pH_i and depolarization of the membrane potential does not involve a ligand-binding site. This indicates that Sertraline directly binds to an inhibitory binding site on the CatSper-channel complex. The finding that the SSRI suppresses more potently prostaglandin- versus steroid-induced Ca^{2+} influx via CatSper might reflect the different mechanisms of action of these molecules. It has been proposed that steroids activate human CatSper via the receptor alpha/beta hydrolase domain-containing protein 2 (ABHD2) (Miller *et al.*, 2016). The mechanism of CatSper activation by prostaglandins is, however, unknown, but does not involve ABHD2 or the classical G-protein coupled prostaglandin receptors (Schaefer *et al.*, 1998; Lishko *et al.*, 2011; Strünker *et al.*, 2011; Brenker *et al.*, 2012; Miller *et al.*, 2016). The specific role and interplay of steroids and prostaglandins, which activate CatSper highly synergistically (Brenker *et al.*, 2018), during fertilization, has not yet been fully established (Baldi *et al.*, 2009). In this regard, Sertraline with its higher potency to inhibit prostaglandin versus steroid responses might serve as a tool in future studies aimed at deciphering the mechanism of prostaglandin and steroid control of Ca^{2+} signaling in human sperm.

Previous studies identified several drugs that inhibit human CatSper (Lishko *et al.*, 2011; Strünker *et al.*, 2011; Brenker *et al.*, 2012; Rennhack *et al.*, 2018), and at the same time the sperm-specific K^+ channel Slo3 (Navarro *et al.*, 2007; Carlson *et al.*, 2009; Brenker *et al.*, 2014; Mansell *et al.*, 2014), the principal K^+ channel in mouse (Santi *et al.*, 2010; Zeng *et al.*, 2011) and human sperm (Brenker *et al.*, 2014). Sertraline also inhibits human Slo3 (Supplementary Fig. S5), indicating that the off-target action of SSRIs in human sperm involves not only CatSper but also Slo3, which is thought to indirectly control the activity of CatSper (Kaupp and Strünker, 2017).

The results presented here on SSRIs are the first example of the potential impact of commonly used drugs on sperm function. Systematic studies assessing the action of other types of pharmaceuticals on CatSper and human sperm are required to evaluate their potential adverse effects on fertilization. Such an approach might also identify tools to study sperm physiology or lead structures to develop male contraceptives.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

The data underlying this article are available in the article and in its online supplementary material.

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Authors' roles

R.R., T.S. and S.N. designed and coordinated the study. R.R., A.R., C.B., T.W. and D.L.E. performed experiments; R.R., A.R., C.S., C.B., D.L.E., K.A., N.E.S., T.S. and S.N. designed experiments, analyzed and/or interpreted the data, and revised the manuscript critically for important intellectual content, R.R., C.S., T.S. and S.N. wrote the manuscript. All authors approved the manuscript.

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Conflict of interest

The authors declare that no conflict of interest could be perceived as prejudicing the impartiality of the research reported.

References

Akasheh G, Sirati L, Noshad Kamran AR, Sepehrmanesh Z. Comparison of the effect of sertraline with behavioral therapy on semen parameters in men with primary premature ejaculation. *Urology* 2014;**83**:800–804.

Alasmari W, Costello S, Correia J, Oxenham SK, Morris J, Fernandes L, Ramalho-Santos J, Kirkman-Brown J, Michelangeli F, Publicover S *et al.* Ca^{2+} signals generated by CatSper and Ca^{2+} stores regulate different behaviors in human sperm. *J Biol Chem* 2013;**288**:6248–6258.

Atli O, Baysal M, Aydogan-Kilic G, Kilic V, Ucarcan S, Karaduman B, Ilgin S. Sertraline-induced reproductive toxicity in male rats: evaluation of possible underlying mechanisms. *Asian J Androl* 2017;**19**:672–679.

Attia SM, Bakheet SA. Citalopram at the recommended human doses after long-term treatment is genotoxic for male germ cell. *Food Chem Toxicol* 2013;**53**:281–285.

Avenarius MR, Hildebrand MS, Zhang Y, Meyer NC, Smith LLH, Kahrizi K, Najmabadi H, Smith RJH. Human male infertility caused by mutations in the CATSPER1 channel protein. *Am J Hum Genet* 2009;**84**:505–510.

Avidan N, Tamary H, Dgany O, Cattan D, Pariente A, Thulliez M, Borot N, Moati L, Barthelme A, Shalmon L *et al.* CATSPER2, a

human autosomal nonsyndromic male infertility gene. *Eur J Hum Genet* 2003;**11**:497–502.

Baldi E, Luconi M, Muratori M, Marchiani S, Tamburrino L, Forti G. Nongenomic activation of spermatozoa by steroid hormones: facts and fictions. *Mol Cell Endocrinol* 2009;**308**:39–46.

Barratt CLR, Bjorndahl L, De Jonge CJ, Lamb DJ, Osorio Martini F, McLachlan R, Oates RD, van der Poel S, St John B, Sigman M *et al.* The diagnosis of male infertility: an analysis of the evidence to support the development of global WHO guidance-challenges and future research opportunities. *Hum Reprod Update* 2017;**23**: 660–680.

Bataineh HN, Daradka T. Effects of long-term use of fluoxetine on fertility parameters in adult male rats. *Neuro Endocrinol Lett* 2007; **28**:321–325.

Beeder LA, Samplaski MK. Effect of antidepressant medications on semen parameters and male fertility. *Int J Urol* 2020;**27**:39–46.

Brenker C, Goodwin N, Weyand I, Kashikar ND, Naruse M, Krähling M, Müller A, Kaupp UB, Strünker T. The CatSper channel: a polymodal chemosensor in human sperm. *EMBO J* 2012;**31**: 1654–1665.

Brenker C, Rehfeld A, Schiffer C, Kierzek M, Kaupp UB, Skakkebæk NE, Strünker T. Synergistic activation of CatSper Ca^{2+} channels in human sperm by oviductal ligands and endocrine disrupting chemicals. *Hum Reprod* 2018;**33**:1915–1923.

Brenker C, Zhou Y, Müller A, Echeverry FA, Trötschel C, Poetsch A, Xia X-M, Bönigk W, Lingle CJ, Kaupp B *et al.* The Ca^{2+}-activated K+ current of human sperm is mediated by Slo3. *Elife* 2014; **3**:1438–1438.

Brown SG, Miller MR, Lishko PV, Lester DH, Publicover SJ, Barratt CLR, Martins S, Silva D. Homozygous in-frame deletion in CATSPERE in a man producing spermatozoa with loss of CatSper function and compromised fertilizing capacity. *Hum Reprod* 2018; **33**:1812–1816.

Carlson AE, Burnett LA, del Camino D, Quill TA, Hille B, Chong JA, Moran MM, Babcock DF. Pharmacological targeting of native CatSper channels reveals a required role in maintenance of sperm hyperactivation. *PLoS One* 2009;**4**:e6844.

Casilla-Lennon MM, Meltzer-Brody S, Steiner AZ. The effect of antidepressants on fertility. *Am J Obstet Gynecol* 2016;**215**:314.e1–15.

Choi J-S, Hahn SJ, Rhie D-J, Yoon S-H, Jo Y-h, Kim MS. Mechanism of fluoxetine block of cloned voltage-activated potassium channel Kv1.3. *J Pharmacol Exp Ther* 1999;**291**:1–6.

Cunningham J. Infertility: a primer for primary care providers. *JAAPA* 2017;**30**:19–25.

Curtis MJ, Ashton JC, Moon LDF, Ahluwalia A. Clarification of the basis for the selection of requirements for publication in the British Journal of Pharmacology. *Br J Pharmacol* 2018;**175**:3633–3635.

Dawson AL, Ailes EC, Gilboa SM, Simeone RM, Lind JN, Farr SL, Broussard CS, Reefhuis J, Carrino G, Biermann J *et al.* Antidepressant prescription claims among reproductive-aged women with private employer-sponsored insurance—United States 2008–2013. *MMWR Morb Mortal Wkly Rep* 2016;**65**:41–46.

DeVane CL. Metabolism and pharmacokinetics of selective serotonin reuptake inhibitors. *Cell Mol Neurobiol* 1999;**19**:443–466.

DeVane CL, Liston HL, Markowitz JS. Clinical pharmacokinetics of Sertraline. *Clin Pharmacokinet* 2002;**41**:1247–1266.

Domar AD, Moragianni VA, Ryley DA, Urato AC. The risks of selective serotonin reuptake inhibitor use in infertile women: a review of the impact on fertility, pregnancy, neonatal health and beyond. *Hum Reprod* 2013;**28**:160–171.

Egeberg Palme DL, Rehfeld A, Bang AK, Nikolova KA, Kjærulff S, Petersen MR, Jeppesen JV, Glensbjerg M, Juul A, Skakkebæk NE *et al.* Viable acrosome-intact human spermatozoa in the ejaculate as a marker of semen quality and fertility status. *Hum Reprod* 2018; **33**:361–371.

Eisenbach M, Giojalas LC. Sperm guidance in mammals—an unpaved road to the egg. *Nat Rev Mol Cell Biol* 2006;**7**:276–285.

Elnazer HY, Baldwin DS. Treatment with citalopram, but not with agomelatine, adversely affects sperm parameters: a case report and translational review. *Acta Neuropsychiatr* 2014;**26**:125–129.

Evans-Hoeker EA, Eisenberg E, Diamond MP, Legro RS, Alvero R, Coutifaris C, Casson PR, Christman GM, Hansen KR, Zhang H *et al.* Major depression, antidepressant use, and male and female fertility. *Fertil Steril* 2018;**109**:879–887.

Friedman BE, Rogers JL, Shahine LK, Westphal LM, Lathi RB. of selective serotonin reuptake inhibitors on *in vitro* fertilization outcome. *Fertil Steril* 2009;**92**:1312–1314.

Galal AAA, Alam RTM, Abd El-Aziz RM. Adverse effects of long-term administration of fluvoxamine on haematology, blood biochemistry and fertility in male albino rats: a possible effect of cessation. *Andrologia* 2016;**48**:914–1010.

Gore AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, Toppari J, Zoeller RT. Executive summary to EDC-2: the Endocrine Society's Second Scientific statement on endocrine-disrupting chemicals. *Endocr Rev* 2015;**36**:593–602.

Hahn SJ, Choi J-S, Rhie D-J, Oh C-S, Jo Y-H, Kim M-S. Inhibition by fluoxetine of voltage-activated ion channels in rat PC12 cells. *Eur J Pharmacol* 1999;**367**:113–118.

Harper CV, Barratt CLR, Publicover SJ. Stimulation of human spermatozoa with progesterone gradients to simulate approach to the oocyte. Induction of $[Ca^{2+}]_i$ oscillations and cyclical transitions in flagellar beating. *J Biol Chem* 2004;**279**:46315–46325.

Hiemke C, Baumann P, Bergemann N, Conca A, Dietmaier O, Egberts K, Fric M, Gerlach M, Greiner C, Gründer G *et al.* AGNP consensus guidelines for therapeutic drug monitoring in psychiatry: update 2011. *Pharmacopsychiatry* 2011;**44**:195–235.

Hildebrand MS, Avenarius MR, Fellous M, Zhang Y, Meyer NC, Auer J, Serres C, Kahrizi K, Najmabadi H, Beckmann JS *et al.* Genetic male infertility and mutation of CATSPER ion channels. *Eur J Hum Genet* 2010;**18**:1178–1184.

Jaiswal D, Singh V, Dwivedi US, Trivedi S, Singh K. Chromosome microarray analysis: a case report of infertile brothers with CATSPER gene deletion. *Gene* 2014;**542**:263–265.

Jarow J, Sigman M, Kolettis PN, Lipshultz LR, McClure RD, Nangia AK, Naughton CK, Prins GS, Sandlow JI, Schlegel PN *et al. The Optimal Evaluation of the Infertile Male: AUA Best Practice Statement.* American Urological Association Education and Research, Inc, 2010;1–39. https://www.auanet.org/guidelines/archived-documents/male-infertility-optimal-evaluation-best-practice-statement.

Kaupp UB, Strünker T. Signaling in sperm: more different than similar. *Trends Cell Biol* 2017;**27**:101–109.

Kilic F, Kashikar ND, Schmidt R, Alvarez L, Dai L, Weyand I, Wiesner B, Goodwin N, Hagen V, Kaupp UB. Caged

progesterone: a new tool for studying rapid nongenomic actions of progesterone. *J Am Chem Soc* 2009;**131**:4027–4030.

Kim HS, Li H, Kim HW, Shin SE, Seo MS, An JR, Ha K-S, Han E-T, Hong S-H, Choi I-W *et al.* Escitalopram, a selective serotonin reuptake inhibitor, inhibits voltage-dependent K+ channels in coronary arterial smooth muscle cells. *Korean J Physiol Pharmacol* 2017; **21**:415–421.

Kirichok Y, Lishko PV. Rediscovering sperm ion channels with the patch-clamp technique. *Mol Hum Reprod* 2011;**17**:478–499.

Kirkman-Brown JC, Smith DJ. Sperm motility: is viscosity fundamental to progress? *Mol Hum Reprod* 2011;**17**:539–544.

Klock SC, Sheinin S, Kazer R, Zhang X. A pilot study of the relationship between selective serotonin reuptake inhibitors and *in vitro* fertilization outcome. *Fertil Steril* 2004;**82**:968–969.

Koyuncu H, Serefoglu EC, Yencilek E, Atalay H, Akbas NB, Sarıca K. Escitalopram treatment for premature ejaculation has a negative effect on semen parameters. *Int J Impot Res* 2011;**23**:257–261.

Kumar STV, Sharma VL, Tiwari P, Singh D, Maikhuri JP, Gupta G, Singh MM. The spermicidal and antitrichomonas activities of SSRI antidepressants. *Bioorg Med Chem Lett* 2006;**16**:2509–2512.

Lee H-A, Kim K-S, Hyun S-A, Park S-G, Kim SJ. Wide spectrum of inhibitory effects of sertraline on cardiac ion channels. *Korean J Physiol Pharmacol* 2012;**16**:327–332.

Lee HM, Hahn SJ, Choi BH. Blockade of Kv1.5 channels by the antidepressant drug sertraline. *Korean J Physiol Pharmacol* 2016;**20**: 193–193.

Lewis RJ, Angier MK, Williamson KS, Johnson RD. Analysis of sertraline in postmortem fluids and tissues in 11 aviation accident victims. *J Anal Toxicol* 2013;**37**:208–216.

Lishko PV, Botchkina IL, Kirichok Y. Progesterone activates the principal Ca^{2+} channel of human sperm. *Nature* 2011;**471**:387–391.

Lory P, Traboulsie A, Chemin J, Kupfer E. T-type calcium channels are inhibited by fluoxetine and its metabolite norfluoxetine. *Mol Pharmacol* 2006;**69**:1963–1968.

Luo T, Chen H-Y, Zou Q-X, Wang T, Cheng Y-M, Wang H-F, Wang F, Jin Z-L, Chen Y, Weng S-Q *et al.* A novel copy number variation in CATSPER2 causes idiopathic male infertility with normal semen parameters. *Hum Reprod* 2019;**34**:414–423.

Lyons DJ, Ammari R, Hellysaz A, Broberger C. Serotonin and antidepressant SSRIs inhibit rat neuroendocrine dopamine neurons: parallel actions in the lactotrophic axis. *J Neurosci* 2016;**36**: 7392–7406.

Majzoub A, Al Said S, Al Rumaihi K, El Ansari W, Alattar A. Geographical differences in semen characteristics of 13 892 infertile men. *Arab J Urol* 2018;**16**:3–9.

Mansell SA, Publicover SJ, Barratt CL, Wilson SM. Patch clamp studies of human sperm under physiological ionic conditions reveal three functionally and pharmacologically distinct cation channels. *Mol Hum Reprod* 2014;**20**:392–408.

McBrinn RC, Fraser J, Hope AG, Gray DW, Barratt CLR, Martins da Silva SJ, Brown SG. Novel pharmacological actions of trequinsin hydrochloride improve human sperm cell motility and function. *Br J Pharmacol* 2019;**176**:4521–4536.

Miki K, Clapham DE. Rheotaxis guides mammalian sperm. *Curr Biol* 2013;**23**:443–452.

Miller MR, Mannowetz N, Iavarone AT, Safavi R, Gracheva EO, Smith JF, Hill RZ, Bautista DM, Kirichok Y, Lishko PV. Unconventional endocannabinoid signaling governs sperm activation via the sex hormone progesterone. *Science* 2016;**352**: 555–559.

Monteiro Filho WO, de Torres SM, Amorim MJAAL, Andrade AJM, de Morais RN, Tenorio BM, da Silva Junior VA. Fluoxetine induces changes in the testicle and testosterone in adult male rats exposed via placenta and lactation. *Syst Biol Reprod Med* 2014;**60**:274–281.

Navarro B, Kirichok Y, Clapham DE. KSper, a pH-sensitive K+ current that controls sperm membrane potential. *Proc Natl Acad Sci USA* 2007;**104**:7688–7692.

Nedahl M, Johansen SS, Linnet K. Reference brain/blood concentrations of citalopram, duloxetine, mirtazapine and sertraline. *J Anal Toxicol* 2018;**42**:149–156.

Nieschlag E. *Scope and Goals of Andrology*. Berlin, Heidelberg: Springer Berlin Heidelberg, 2010, 1–10.

Norr L, Bennedsen B, Fedder J, Larsen ER. Use of selective serotonin reuptake inhibitors reduces fertility in men. *Andrology* 2016;**4**: 389–394.

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

Preskorn SH. How drug-drug interactions can impact managed care. *Am J Manag Care* 2004;**10**:S186–S198.

Publicover SJ, Giojalas LC, Teves ME, de Oliveira GSMM, Garcia AAM, Barratt CLR, Harper CV. Ca^{2+} signalling in the control of motility and guidance in mammalian sperm. *Front Biosci* 2008;**13**: 5623–5637.

Rahban R, Nef S. CatSper: the complex main gate of calcium entry in mammalian spermatozoa. *Mol Cell Endocrinol* 2020;**518**:110951.

Rehfeld A. Revisiting the action of steroids and triterpenoids on the human sperm Ca^{2+} channel CatSper. *Mol Hum Reprod* 2020;**26**: 816–824.

Rehfeld A, Dissing S, Skakkebæk NE. Chemical UV filters mimic the effect of progesterone on Ca^{2+} signaling in human sperm cells. *Endocrinology* 2016;**157**:4297–4308.

Rehfeld A, Egeberg DL, Almstrup K, Petersen JH, Dissing S, Skakkebæk NE. EDC IMPACT: chemical UV filters can affect human sperm function in a progesterone-like manner. *Endocr Connect* 2017;**7**:16–25.

Reis M, Aamo T, Ahlner J, Druid H. Reference concentrations of antidepressants. A compilation of postmortem and therapeutic levels. *J Anal Toxicol* 2007;**31**:254–264.

Relwani R, Berger D, Santoro N, Hickmon C, Nihsen M, Zapantis A, Werner M, Polotsky AJ, Jindal S. Semen parameters are unrelated to BMI but vary with SSRI use and prior urological surgery. *Reprod Sci* 2011;**18**:391–397.

Rennhack A, Schiffer C, Brenker C, Fridman D, Nitao ET, Cheng Y-M, Tamburrino L, Balbach M, Stölting G, Berger TK *et al.* A novel cross-species inhibitor to study the function of CatSper Ca^{2+} channels in sperm. *Br J Pharmacol* 2018;**175**:3144–3161.

Safarinejad MR. Sperm DNA damage and semen quality impairment after treatment with selective serotonin reuptake inhibitors detected using semen analysis and sperm chromatin structure assay. *J Urol* 2008;**180**:2124–2128.

Samplaski MK, Nangia AK. Adverse effects of common medications on male fertility. *Nat Rev Urol* 2015;**12**:401–413.

Santi CM, Martínez-López P, de la Vega-Beltrán JL, Butler A, Alisio A, Darszon A, Salkoff L. The SLO3 sperm-specific potassium channel plays a vital role in male fertility. *FEBS Lett* 2010;**584**:1041–1046.

Schaefer M, Hofmann T, Schultz G, Gudermann T. A new prostaglandin E receptor mediates calcium influx and acrosome reaction in human spermatozoa. *Proc Natl Acad Sci USA* 1998;**95**: 3008–3013.

Schiffer C, Müller A, Egeberg DL, Alvarez L, Brenker C, Rehfeld A, Frederiksen H, Wäschle B, Kaupp UB, Balbach M *et al*. Direct action of endocrine disrupting chemicals on human sperm. *EMBO Rep* 2014;**15**:758–765.

Schiffer C, Rieger S, Brenker C, Young S, Hamzeh H, Wachten D, Tüttelmann F, Röpke A, Kaupp UB, Wang T *et al*. Rotational motion and rheotaxis of human sperm do not require functional CatSper channels and transmembrane Ca^{2+} signaling. *EMBO J* 2020;**39**:e102363.

Semet M, Paci M, Saïas-Magnan J, Metzler-Guillemain C, Boissier R, Lejeune H, Perrin J. The impact of drugs on male fertility: a review. *Andrology* 2017;**5**:640–663.

Skakkebaek NE, Rajpert-De Meyts E, Buck Louis GM, Toppari J, Andersson A-M, Eisenberg ML, Jensen TK, Jørgensen N, Swan SH, Sapra KJ *et al*. Male reproductive disorders and fertility trends: influences of environment and genetic susceptibility. *Physiol Rev* 2016;**96**:55–97.

Smith JF, Syritsyna O, Fellous M, Serres C, Mannowetz N, Kirichok Y, Lishko PV. Disruption of the principal, progesterone-activated sperm Ca^{2+} channel in a CatSper2-deficient infertile patient. *Proc Natl Acad Sci USA* 2013;**110**:6823–6828.

Strünker T, Goodwin N, Brenker C, Kashikar ND, Weyand I, Seifert R, Kaupp UB. The CatSper channel mediates progesterone-induced Ca^{2+} influx in human sperm. *Nature* 2011;**471**:382–387.

Suarez SS. Regulation of sperm storage and movement in the mammalian oviduct. *Int J Dev Biol* 2008;**52**:455–462.

Suarez SS, Pacey AA. Sperm transport in the female reproductive tract. *Hum Reprod Update* 2006;**12**:23–37.

Sumigama S, Mansell S, Miller M, Lishko PV, Cherr GN, Meyers SA, Tollner T. Progesterone accelerates the completion of sperm capacitation and activates CatSper channel in spermatozoa from the rhesus macaque. *Biol Reprod* 2015;**93**:130.

Sylvester C, Menke M, Gopalan P. Selective serotonin reuptake inhibitors and fertility: considerations for couples trying to conceive. *Harv Rev Psychiatry* 2019;**27**:108–118.

Tamburrino L, Marchiani S, Minetti F, Forti G, Muratori M, Baldi E. The CatSper calcium channel in human sperm: relation with motility and involvement in progesterone-induced acrosome reaction. *Hum Reprod* 2014;**29**:418–428.

Tamburrino L, Marchiani S, Vicini E, Muciaccia B, Cambi M, Pellegrini S, Forti G, Muratori M, Baldi E. Quantification of CatSper1 expression in human spermatozoa and relation to functional parameters. *Hum Reprod* 2015;**30**:1532–1544.

Tanrikut C, Feldman AS, Altemus M, Paduch DA, Schlegel PN. Adverse effect of paroxetine on sperm. *Fertil Steril* 2010;**94**: 1021–1026.

Tanrikut C, Schlegel PN. Antidepressant-associated changes in semen parameters. *Urology* 2007;**69**:185.e5–7.

Tavares RS, Mansell S, Barratt CLR, Wilson SM, Publicover SJ, Ramalho-Santos J. P, p' DDE. activates CatSper and compromises human sperm function at environmentally relevant concentrations. *Hum Reprod* 2013;**28**:3167–3177.

Wang H, McGoldrick LL, Chung JJ. Sperm ion channels and transporters in male fertility and infertility. *Nat Rev Urol* 2021;**18**: 46–66.

Wang T, Young S, Krenz H, Tuttelmann F, Ropke A, Krallmann C, Kliesch S, Zeng XH, Brenker C, Strunker T. The Ca(2+) channel CatSper is not activated by cAMP/PKA signaling but directly affected by chemicals used to probe the action of cAMP and PKA. *J Biol Chem* 2020;**295**:13181–13193.

Williams HL, Mansell S, Alasmari W, Brown SG, Wilson SM, Sutton KA, Miller MR, Lishko PV, Barratt CLR, Publicover SJ *et al*. Specific loss of CatSper function is sufficient to compromise fertilizing capacity of human spermatozoa. *Hum Reprod* 2015; **30**:2737–2746.

Yuan Y, Ding X, Cheng Y, Kang H, Luo T, Zhang X, Kuang H, Chen Y, Zeng X, Zhang D. PFOA evokes extracellular Ca^{2+} influx and compromises progesterone-induced response in human sperm. *Chemosphere* 2020;**241**:125074.

Zeng XH, Yang C, Kim ST, Lingle CJ, Xia XM. Deletion of the Slo3 gene abolishes alkalization-activated K+ current in mouse spermatozoa. *Proc Natl Acad Sci USA* 2011;**108**:5879–5884.

Zhang X, Kang H, Peng L, Song D, Jiang X, Li Y, Chen H, Zeng X. Pentachlorophenol inhibits CatSper function to compromise progesterone's action on human sperm. *Chemosphere* 2020;**259**: 127493.