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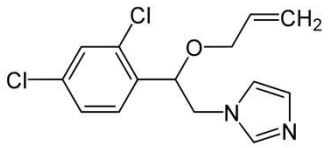
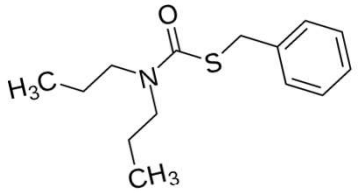
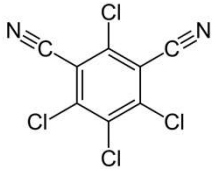
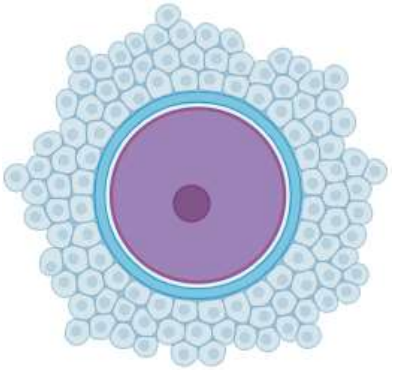
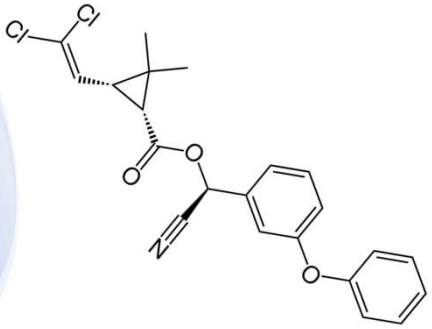


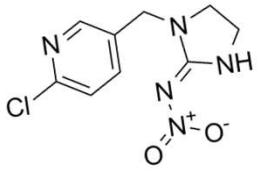






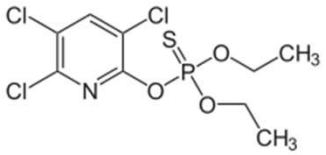


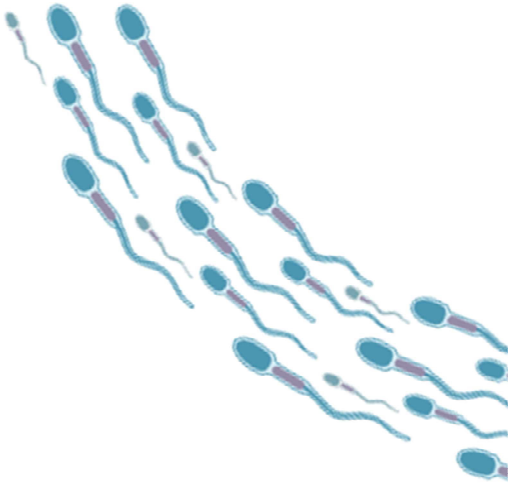
Direkte effekter af pesticider og biocider på CatSper Ca^{2+}-kanalen i humane sædceller



Anders Rehfeld

Rigshospitalet





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Prime Minister Mette Frederiksen's New Year's Address on January 1, 2024

- "Today, every 8th child in Denmark is born following fertility treatment."
- "Unintended childlessness affects too many people."

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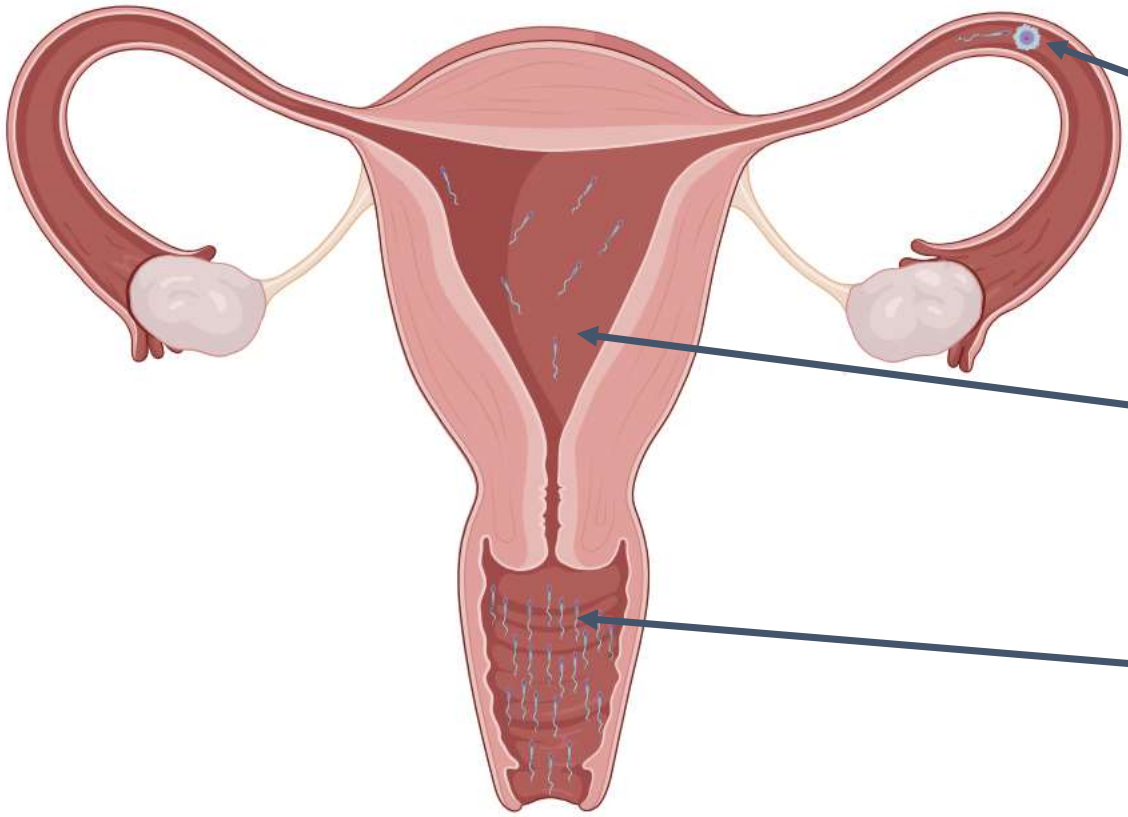
The journey towards the egg



Chemotaxis

Capacitation

Motility

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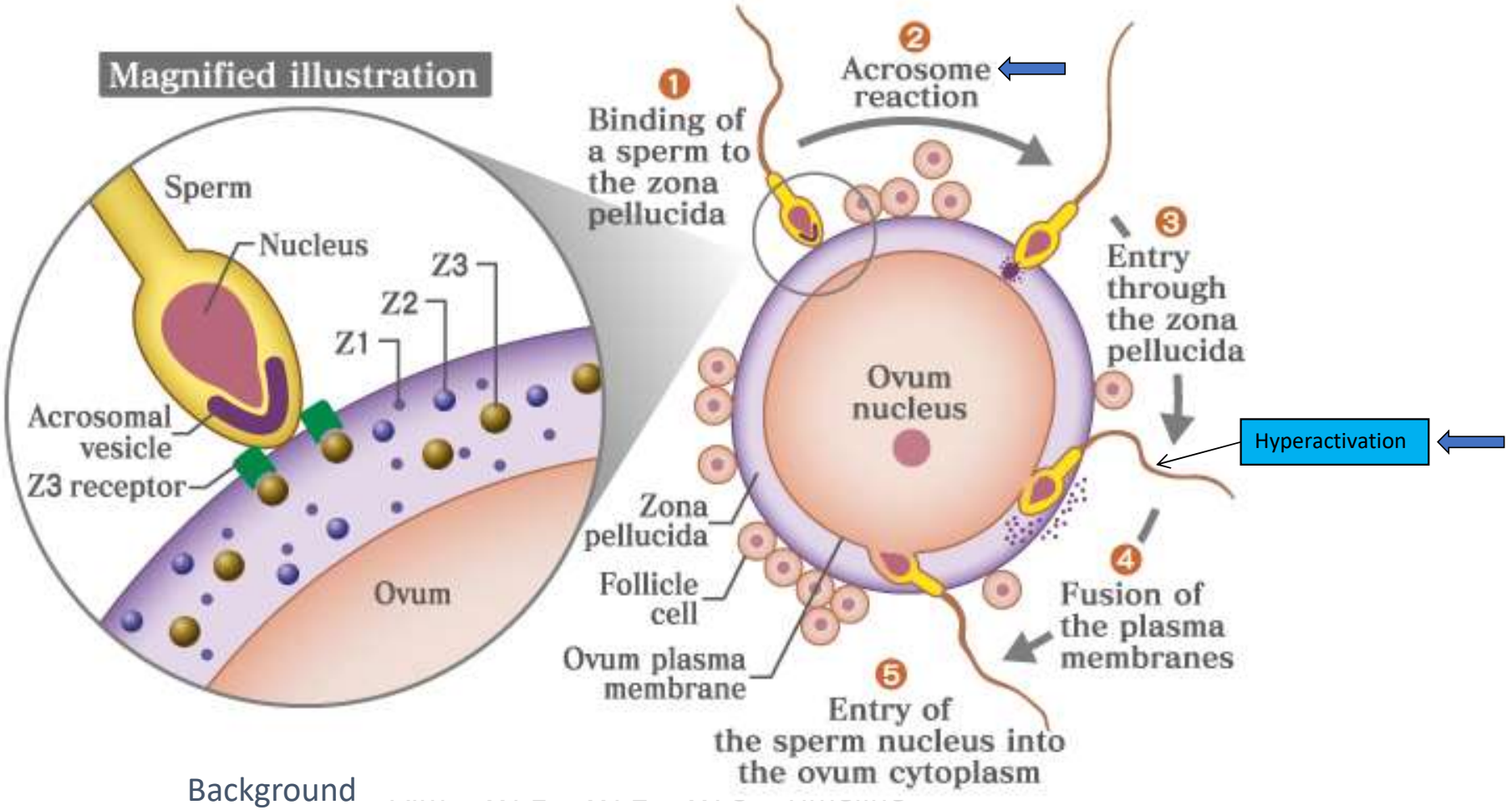
The journey towards the egg



Background

From Nature Reviews Molecular Cell Biology 7, 276-285 (April 2006). doi:10.1038/nrm1893 and http://csls-text.c.u-tokyo.ac.jp/

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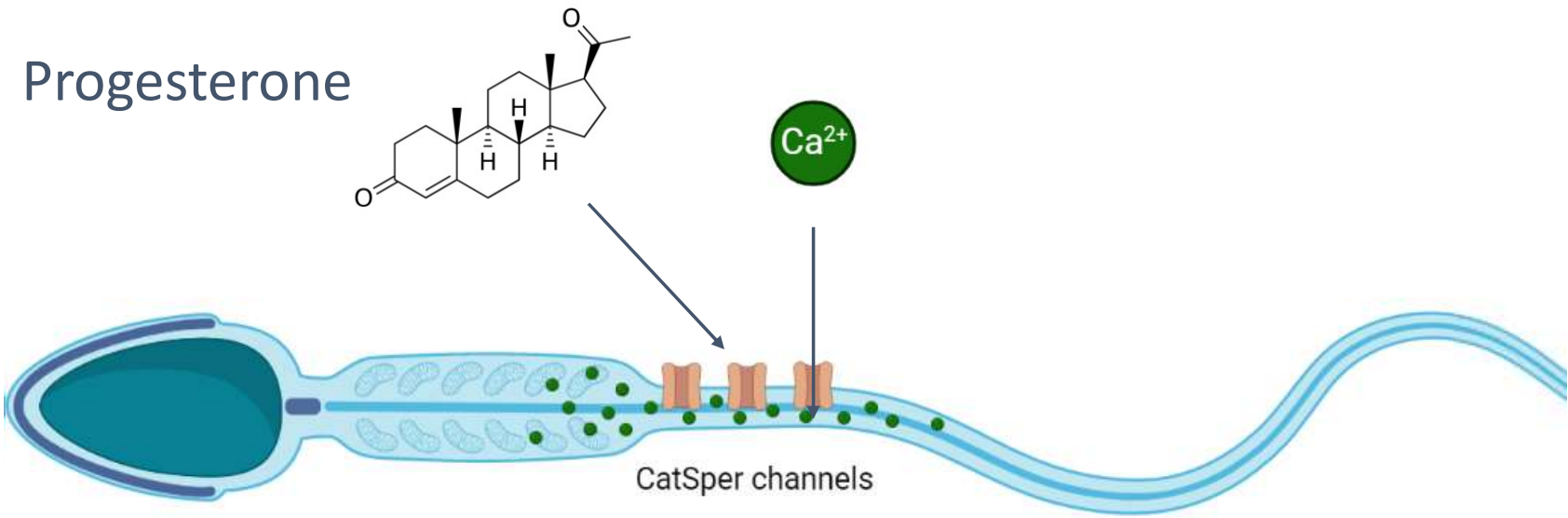
CatSper: The principle Ca^{2+} channel in human sperm

Progesterone



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Normal CatSper function is essential for male fertility

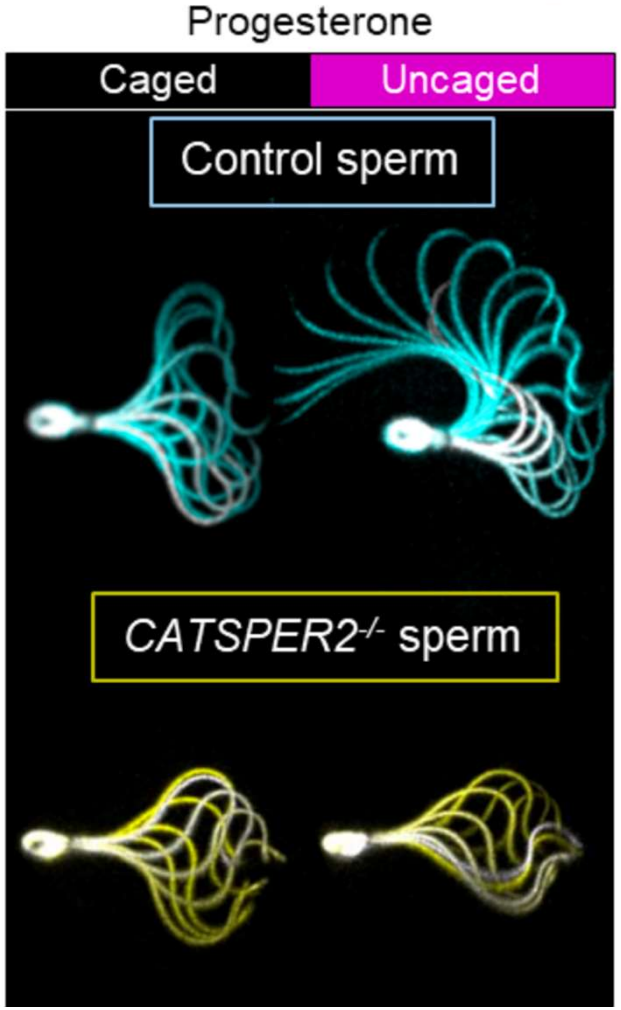

Table1: Semen-analysis parameters and history of medically assisted reproduction of the eight *CATSPER2*$^{-/-}$ patients (C1-C8) and the *CATSPERE* $^{c.536G>A / c.2394-2399del}$ patient (C9)

Semen Parameters	Reference Values (WHO)	Patient C1	C2	C3	C4	C5	C6	C7	C8	C9
Semen volume (ml)	≥1.5	3.0	3.4	3.1	3.5	2.0	2.7	4.4	2.9	2.2
pH	≥7.2	7.7	8.1	7.9	8.1	8.9	7.9	7.9	8.3	8.1
Total sperm count (10^6 per ejaculate)	≥39	440	337	94	90	158	157	224	48	65
Sperm conc. (10^6 per ml)	≥15	147	99	30	26	79	58	51	16	30
Total motility (PR + NP, %)	≥40	54	47	61	64	63	61	61	56	56
Success of medically assisted reproduction										
Ovulation induction (OI)		--	--	No	--	--	No	--	--	--
Intrauterine insemination (IUI)		--	--	--	No	No	--	--	--	--
In-vitro fertilization (IVF)		No	No	No	--	No	--	--	--	--
Intracytoplasmic sperm injection (ICSI)		Yes	Yes	Yes	Yes	Yes	Yes	--	Yes	--
Live births (thus far)		Yes	Yes	--	Yes	--	Yes	--	--	--

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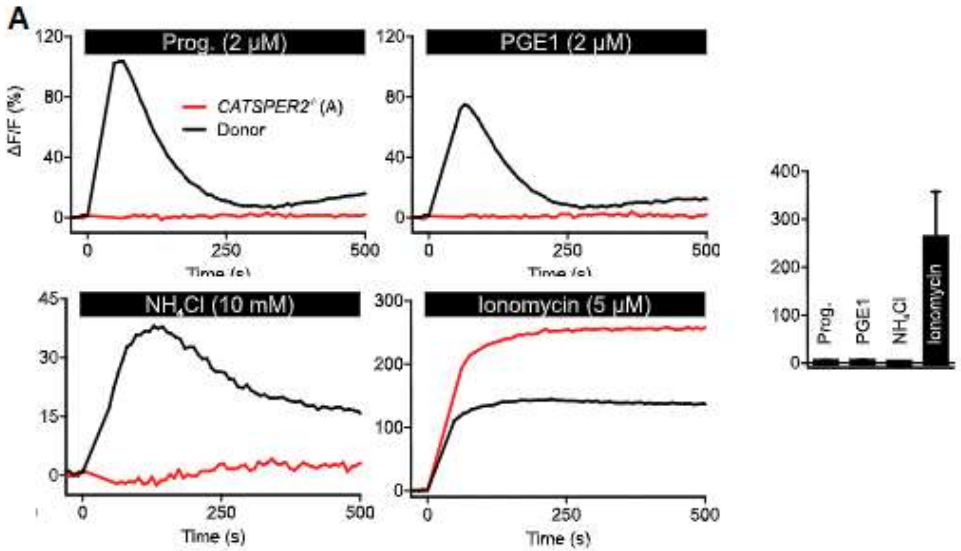
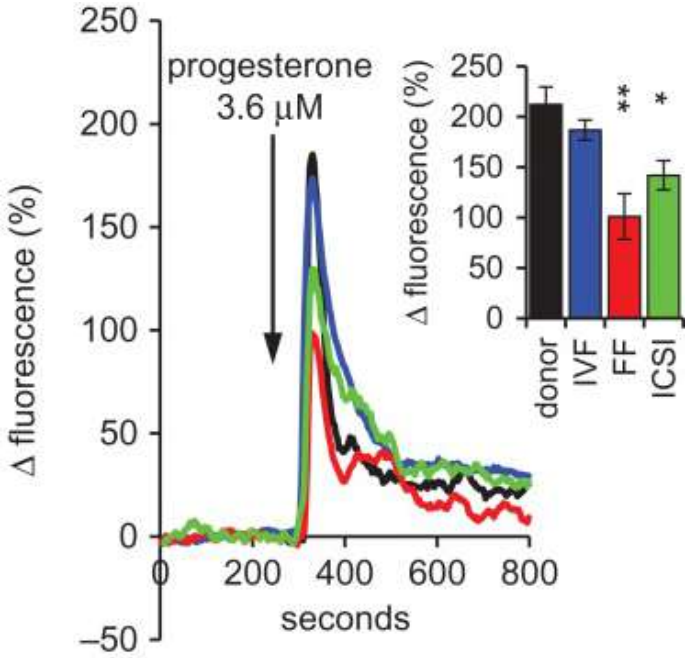
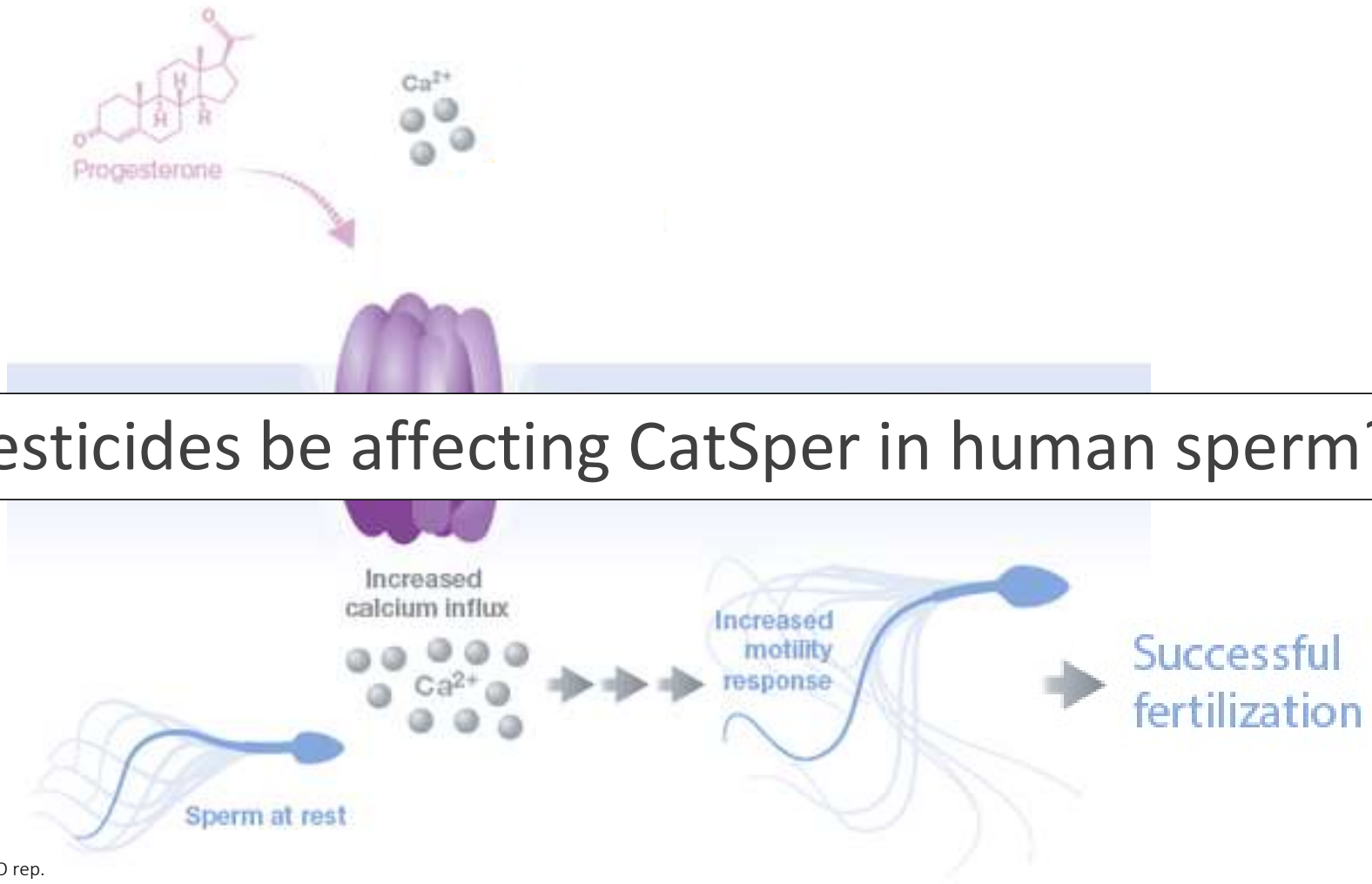
Normal CatSper function is essential for male fertility





Background



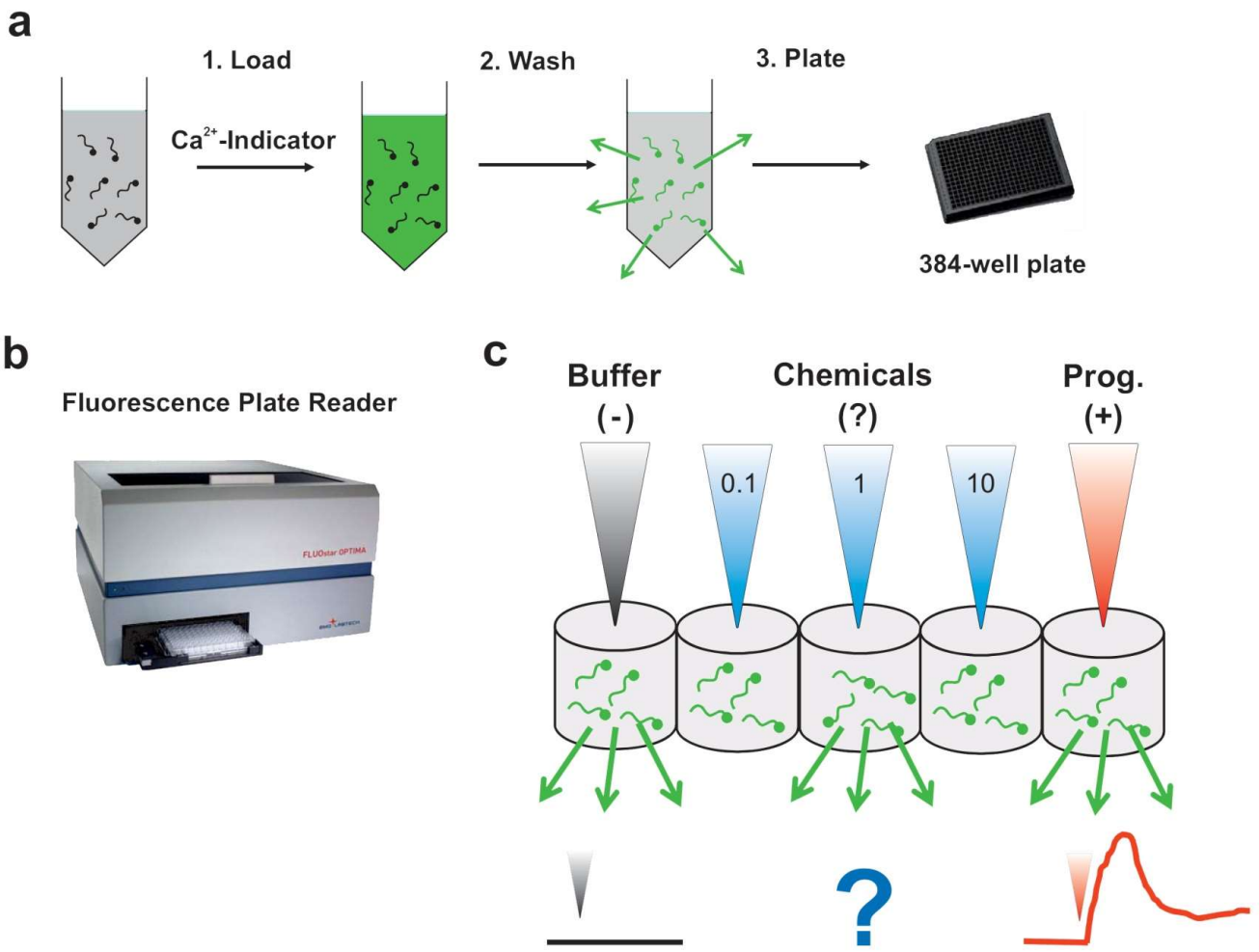
Could pesticides be affecting CatSper in human sperm?

Schiffer et al. 2014 EMBO rep.

Aims of this project

- WP 1-2: Investigate high exposure pesticides and biocides for their effect on Ca^{2+} signaling in human sperm and characterize their pharmacological mode of action.
- WP 3: Investigate additive and/or synergistic actions of pesticides, biocides and inert additives in chemical mixtures.
- WP 4: To assess the effect of pesticides and biocides directly on human sperm motility and acrosome reaction.

WP1-2:
Fluorescence based Ca^{2+}-signaling assay



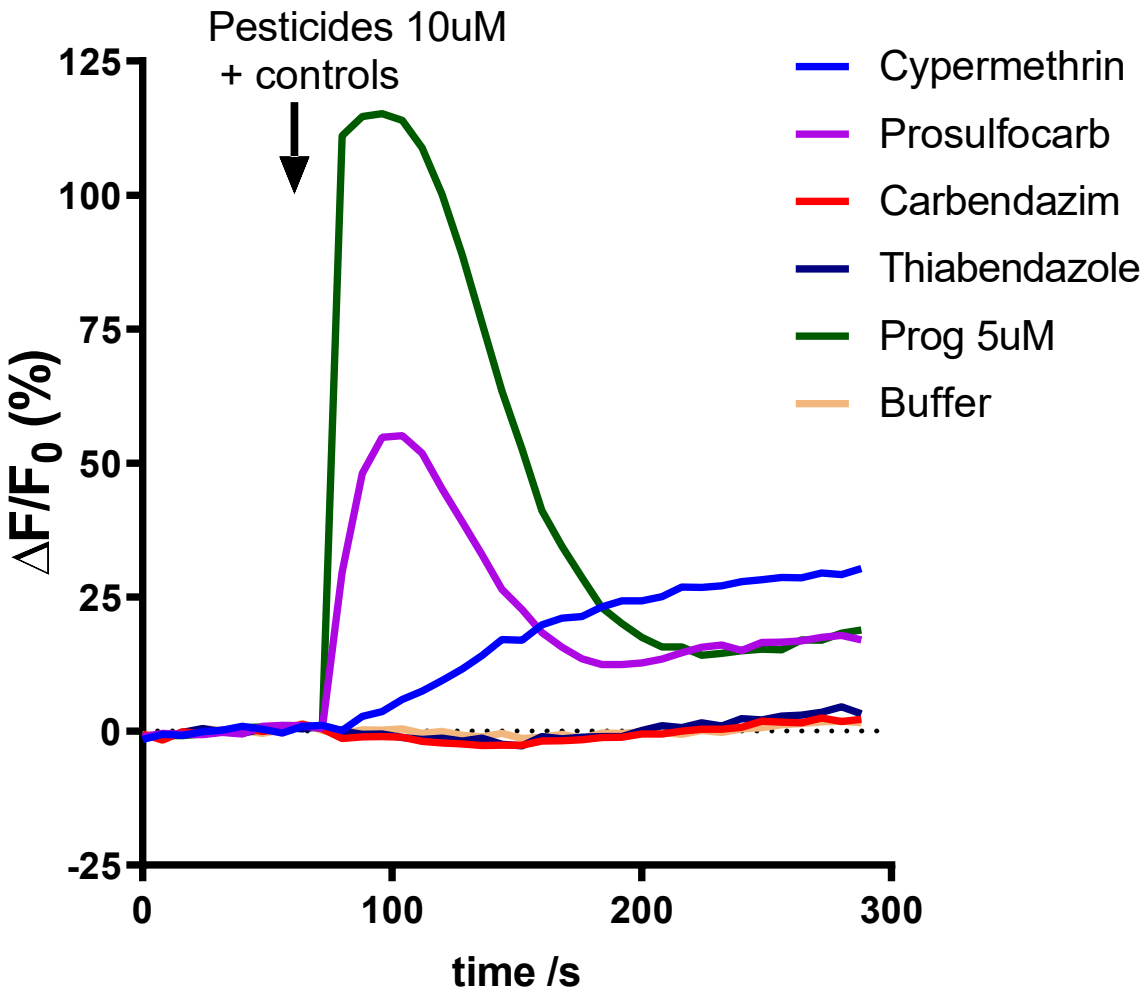
53 pesticides/biocides/ metabolites tested

Based on the interested of the Danish EPA and/or due to their occurrence in an environmental biomonitoring study on hair samples from pregnant women (Béranger *et al.*, 2018).

- **Milbemectin A4**
- **Milbemectin A3**
- Chlorpyrifos
- **Prosulfocarb**
- **Fipronil Sulfone**
- Trifluralin
- Endosulfan
- Hexachlorophene
- **Metofluthrin**
- **Imazalil**
- **Pyraclostrobin**
- Fenitrothion
- Oxadiazon
- Lindane
- Pentachlorophenol
- Prochloraz
- **Cypermethrin**
- Propiconazole
- Chlorothalonil
- **Permethrin**
- **Deltamethrin**
- **Tebuconazole**
- **Desthioprothioconazole**
- Boscalid
- Triticonazole
- **3-Phenoxybenzoic acid**
- Cyprodinil
- **Prothioconazole**
- **CL2CA**
- TCPy
- Bifenthrin
- Pyrimethanil
- **Dichloroprop-P**
- Metolachlor
- **Diuron**
- **1,2,4-triazol**
- **Azoxystrobin**
- **Imidacloprid**
- **Glyphosate**
- 3Me4NP
- Propamocarb
- Dimethyl phosphate
- **Terbutryn**
- p-Nitrophenol
- **Fludioxonil**
- **Carbendazim**
- MCPA
- Mecoprop
- **Fipronil**
- 2,4-Dichlorophenoxyacetic acid
- Diethyl phosphate
- IMPy
- **Thiabendazole**

Birch et al. 2022 Env. Int..

WP1-2 screening



WP1-2 screening:

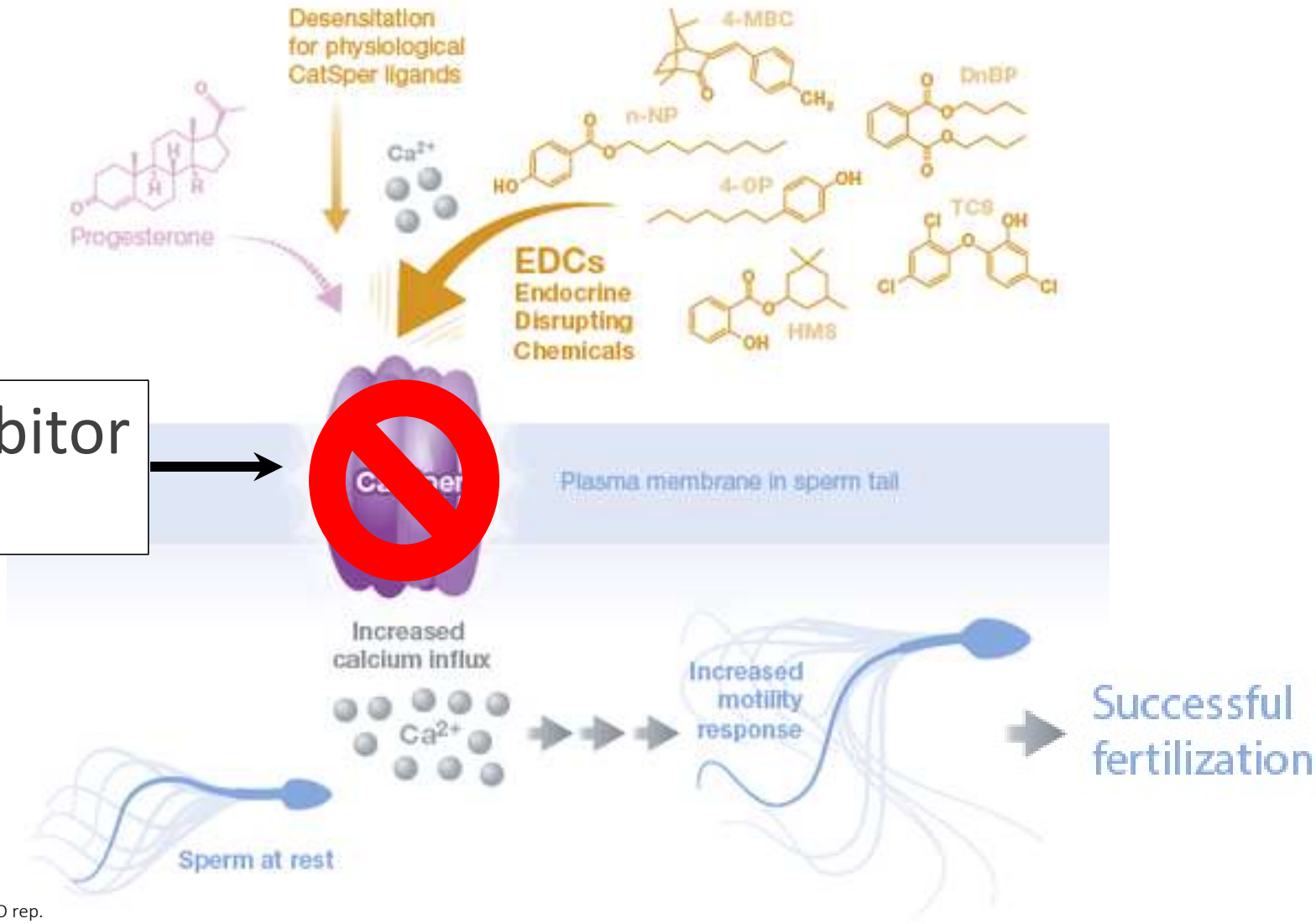
28 hits = Induced signal > negative control +3SD (8.2%*)

Rank:	Compound:	CAS #:	Mean signal at 10 µM (in %)* (n=3):	Rank:	Compound:	CAS #:	Mean signal at 10 µM (in %)* (n=3):
1	**Milbemectin A4**	51596-11-3	87.8	15	Pentachlorophenol	87-86-5	24.6
2	**Milbemectin A3**	51596-10-2	85.6	16	Prochloraz	67747-09-5	22.3
3	Chlorpyrifos	2921-88-2	76.3	17	**Cypermethrin**	52315-07-8	20.0
4	**Prosulfocarb**	52888-80-9	53.0	18	Propiconazole	60207-90-1	19.3
5	**Fipronil Sulfone**	120068-36-2	46.4	19	Chlorothalonil	1897-45-6	17.7
6	Trifluralin	1582-09-8	44.2	20	**Permethrin**	52645-53-1	17.7
7	Endosulfan	115-29-7	42.4	21	**Deltamethrin**	52918-63-5	16.7
8	Hexachlorophene	70-30-4	41.5	22	**Tebuconazole**	107534-96-3	16.6
9	**Metofluthrin**	240494-70-6	37.1	23	**Desthioprothioconazole**	120983-64-4	15.6
10	**Imazalil**	35554-44-0	36.6	24	Boscalid	188425-85-6	11.8
11	**Pyraclostrobin**	175013-18-0	32.3	25	Triticonazole	131983-72-7	10.6
12	Fenitrothion	122-14-5	31.2	26	**3-Phenoxybenzoic acid**	3739-38-6	9.5
13	Oxadiazon	19666-30-9	26.9	27	Cyprodinil	121552-61-2	9.2
14	Lindane	58-89-9	26.8	28	**Prothioconazole**	178928-70-6	8.7

*: compared to paired progesterone-response in specific experiment.

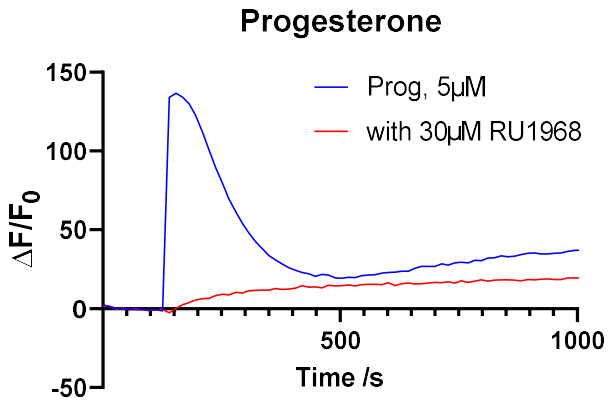
WP1-2 Mode of action - CatSper inhibitor RU1968

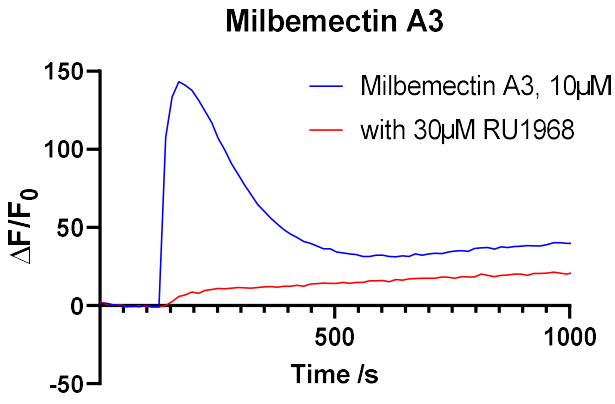
CatSper-inhibitor RU1968

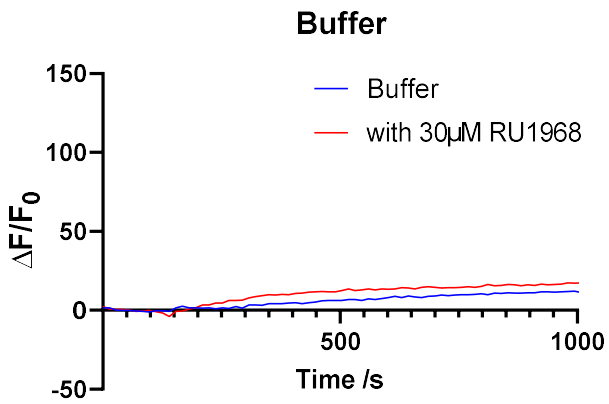


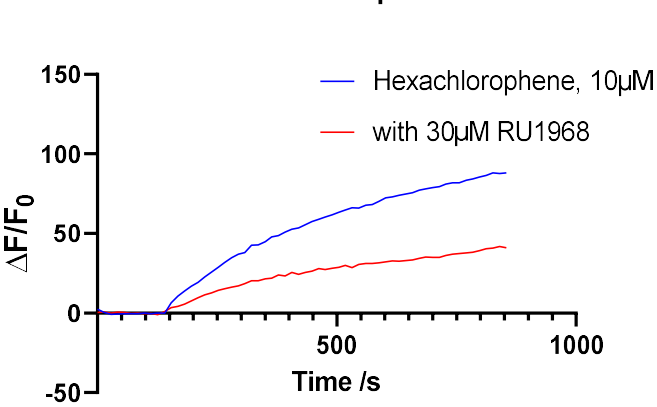
Schiffer et al. 2014 EMBO rep.

WP1-2 Mode of action - CatSper inhibitor RU1968

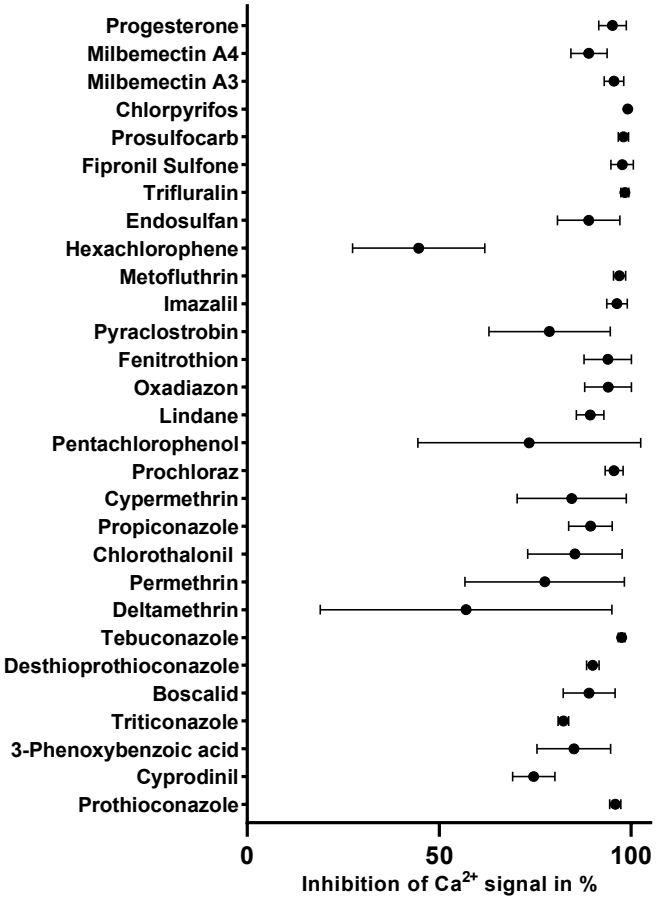






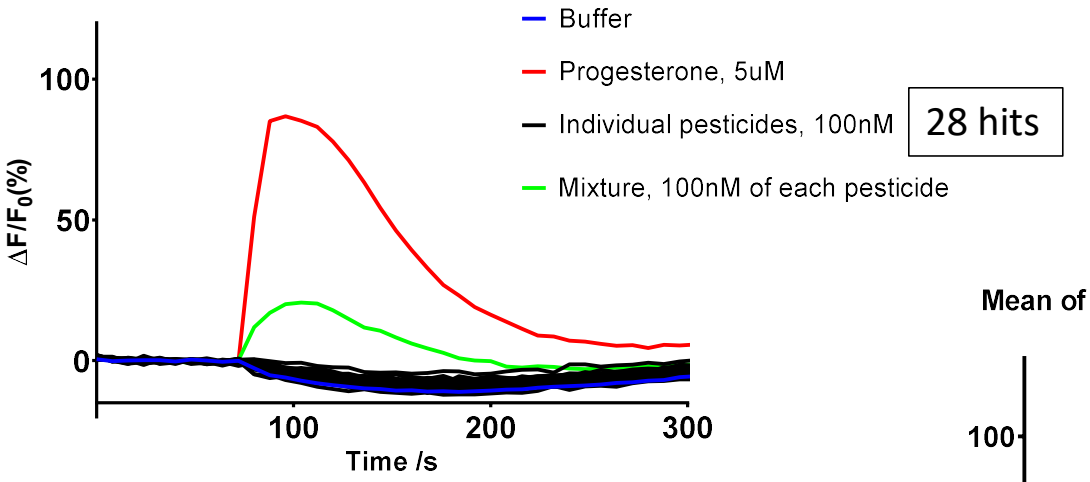


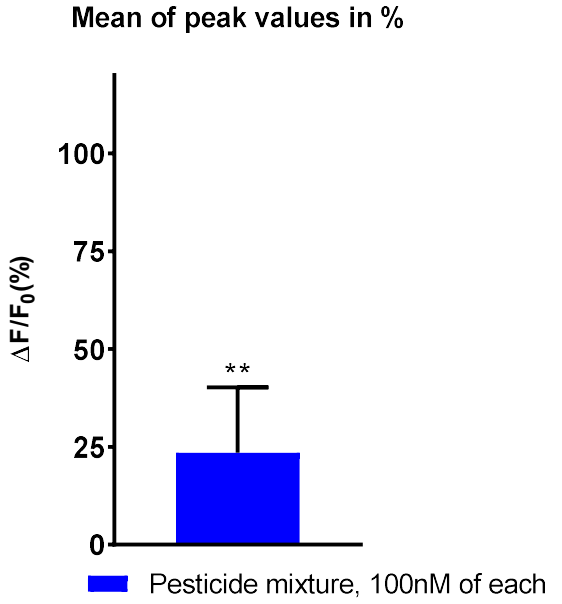
WP1-2 Mode of action - CatSper inhibitor RU1968



Elimination of Ca^{2+} signals for the majority of the compounds -> Suggests effects via CatSper.

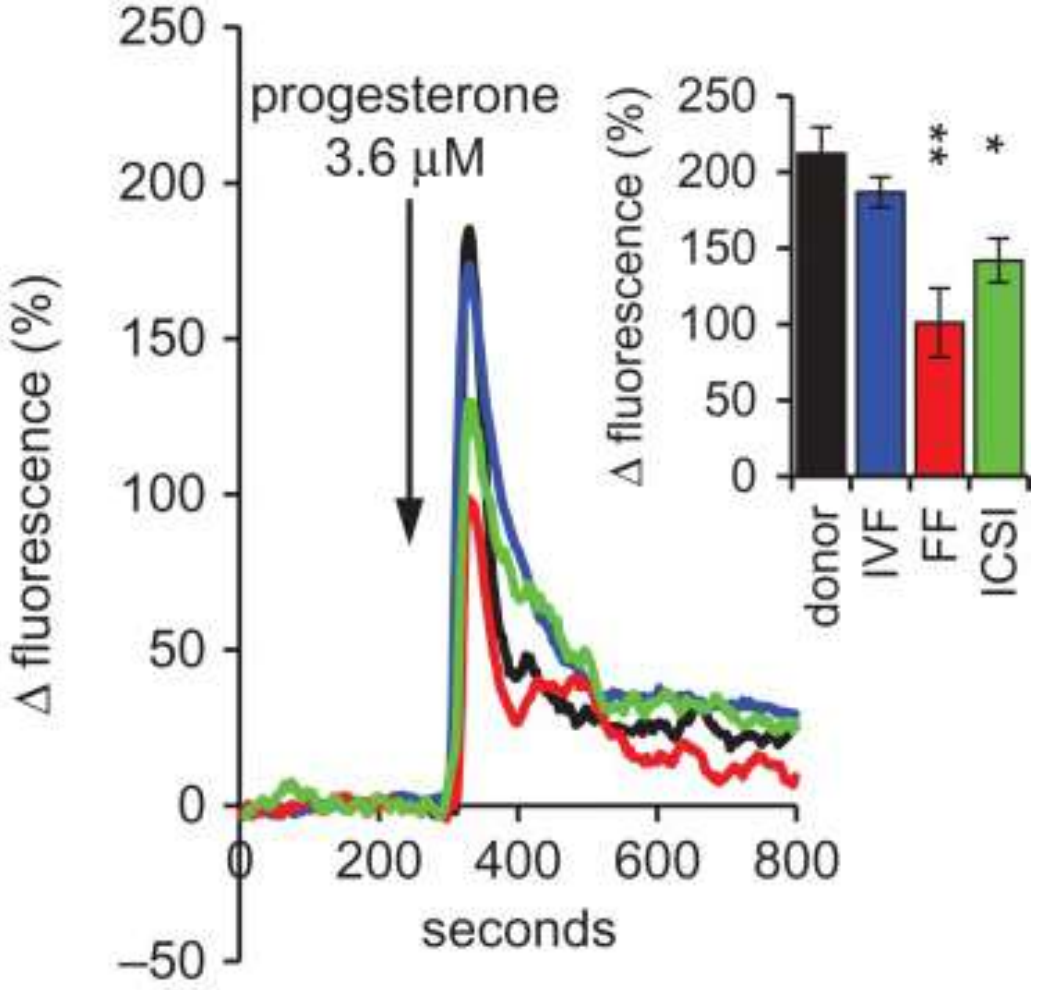
WP3 additive and/or cooperative actions of chemical mixtures

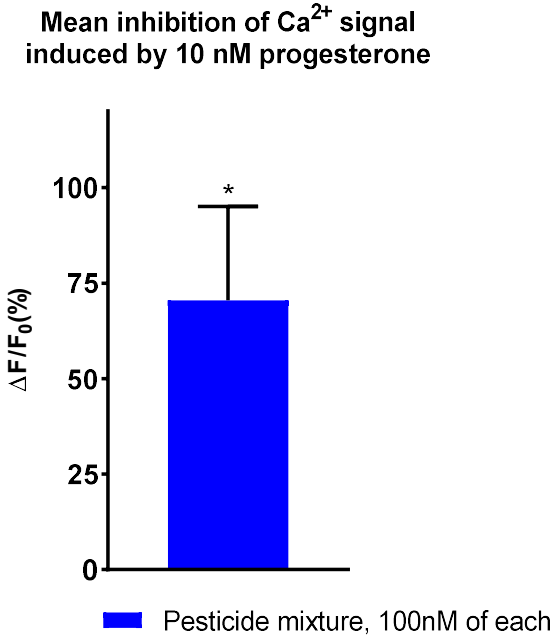




** Significantly different from the negative control

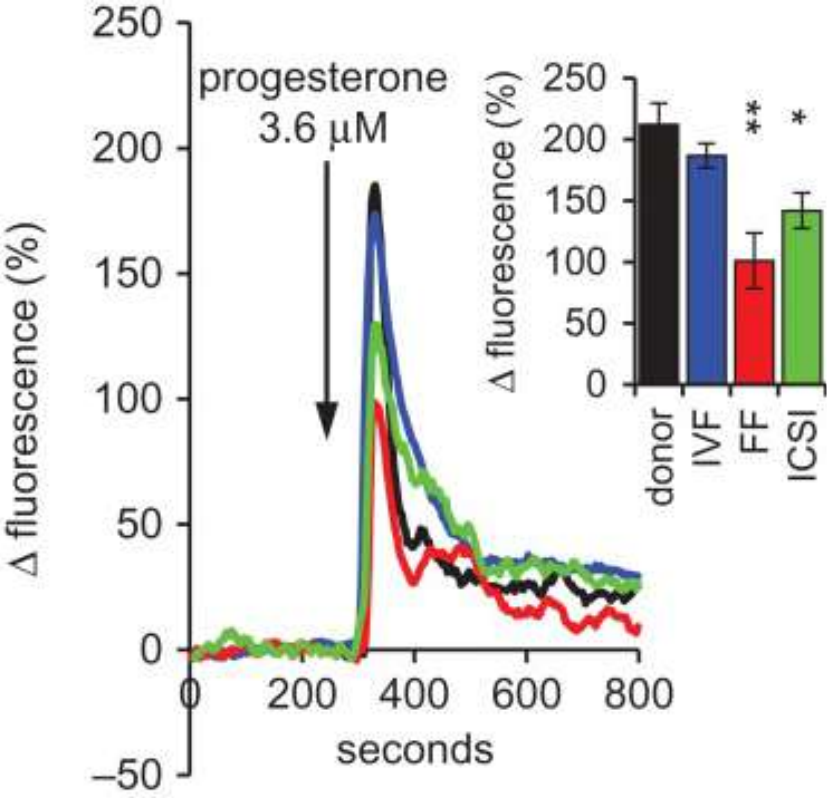
WP3 inhibitory actions of chemical mixtures

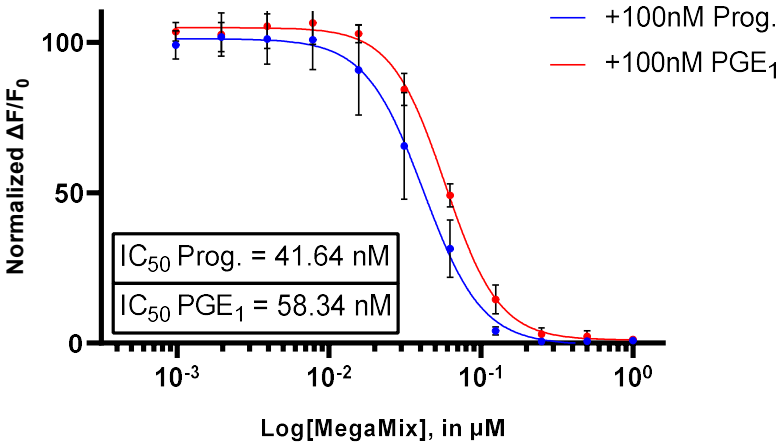




* Significant inhibition of 10nM progesterone signals

WP3 MegaMix

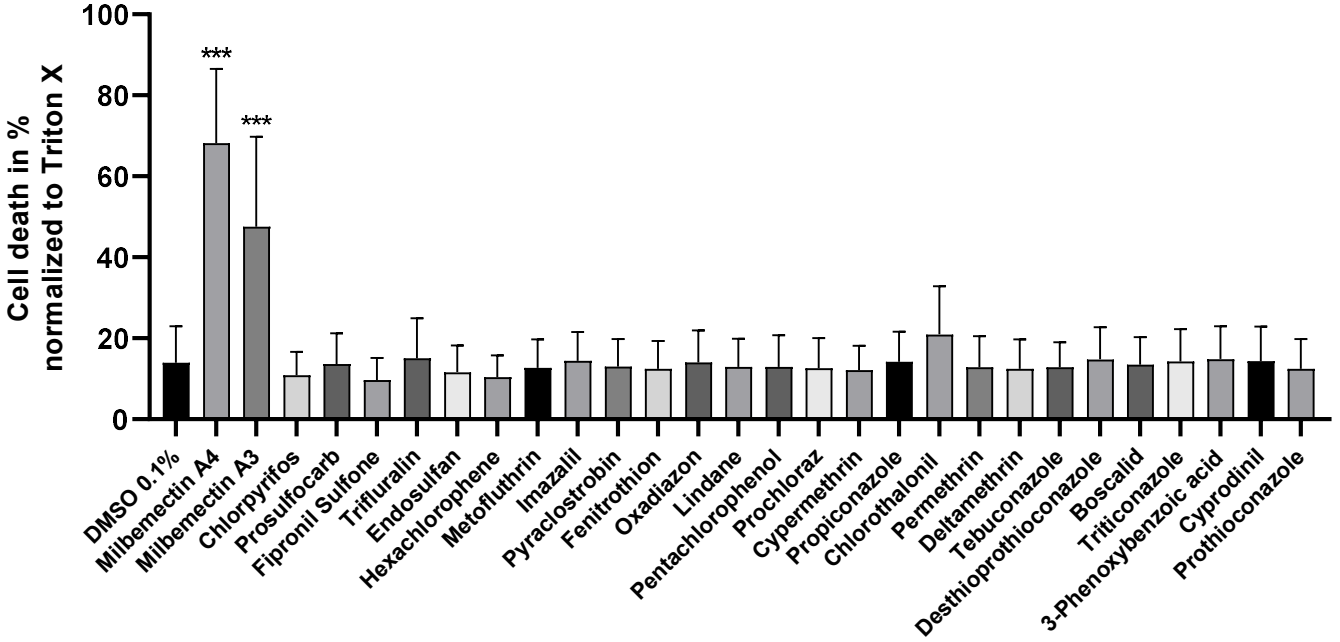

progesterone
3.6 µM
Δ fluorescence (%)
250
200
150
100
50
0
–50
0
200
400
600
800
seconds
Δ fluorescence (%)
250
200
150
100
50
0
**
*
donor
IVF
FF
ICSI


+100nM Prog.
+100nM PGE1
Normalized ΔF/F0
100
50
0
IC50 Prog. = 41.64 nM
IC50 PGE1 = 58.34 nM
10-3
10-2
10-1
100
Log[MegaMix], in µM

WP4 human sperm function - Viability

All compounds were tested at 10 µM.

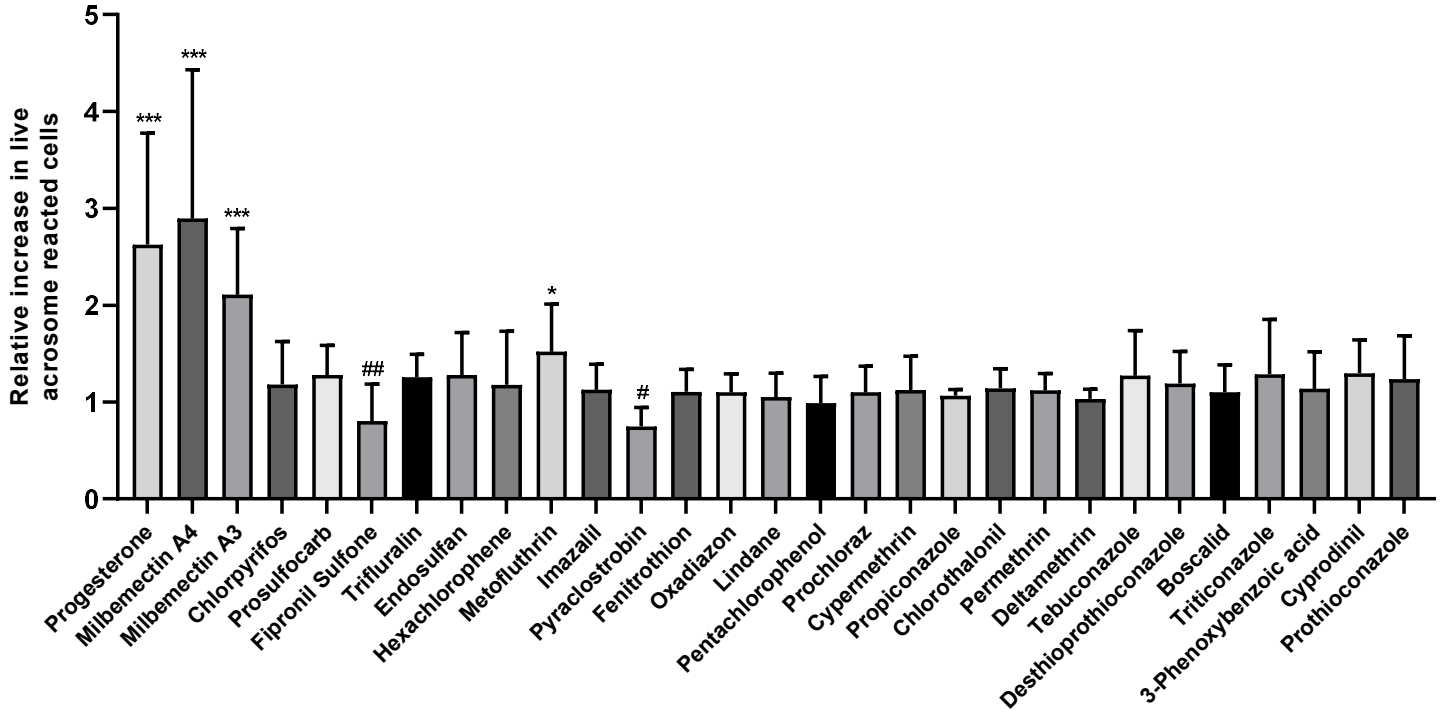
Viability was measured after 21h



Significant increase in cell mortality after 21h: ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05

WP4 human sperm function - Acrosome Reaction

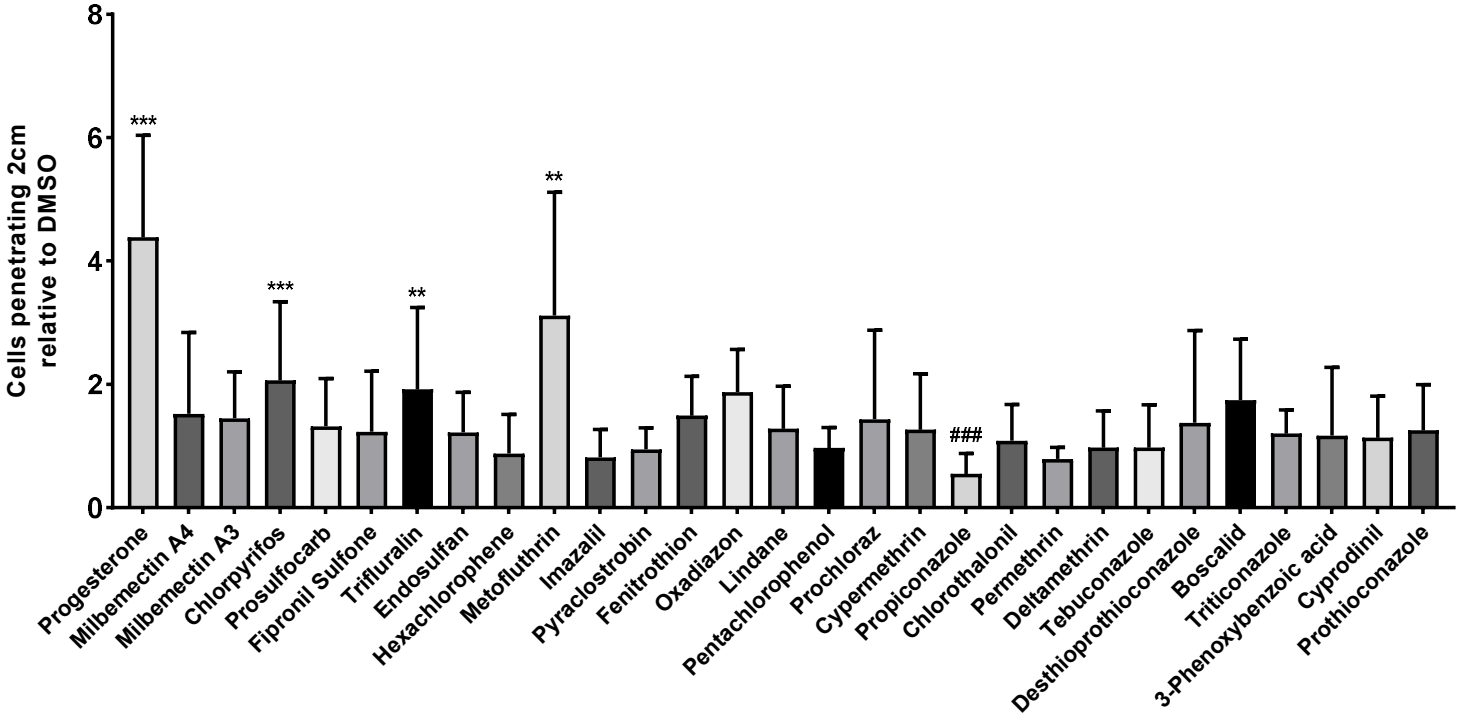
All compounds were tested at 10 µM.



Significant increase in live acrosome reacted cells: '***' 0.001 '**' 0.01 '*' 0.05
Significant decrease in live acrosome reacted cells : '**###**' 0.001 '**##**' 0.01 '**#**' 0.05

WP4 human sperm function - Sperm Penetration

All compounds were tested at 10 µM.



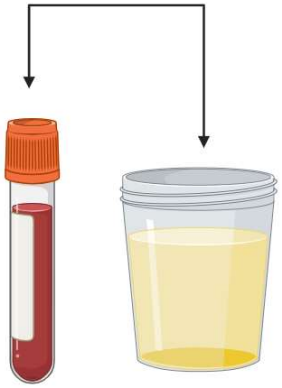
Significant increase in cell penetration: '***' 0.001 '**' 0.01 '*' 0.05
Significant decrease in cell penetration: '**###**' 0.001 '**##**' 0.01 '**#**' 0.05

Discussion – Relevance of results?

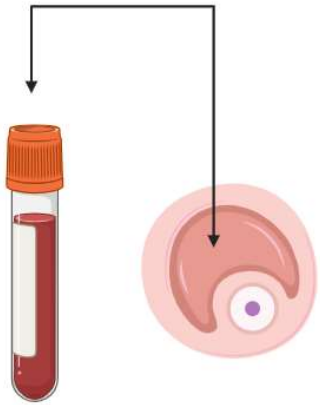
- Are the “active” concentrations of the pesticides and biocides physiologically relevant?
  - Almost no information on the levels of these chemicals in human reproductive fluids (ongoing MST project)

Measure PFAS, pharmaceuticals, and polar metabolites (e.g. azol-fungicide metabolites), if we obtain funding.

Female samples

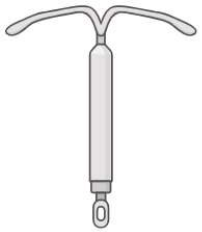


N=12



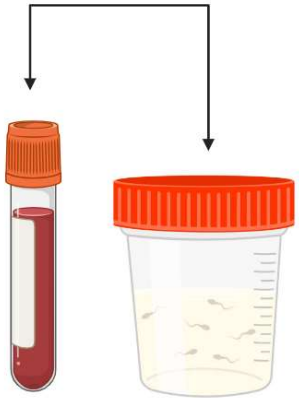
N=12

Extracts



N=12

Male samples



N=12

Discussion – Relevance of results?

- Are the “active” concentrations of the pesticides and biocides physiologically relevant?
  - Almost no information on the levels of these chemicals in human reproductive fluids (ongoing MST project)

- Do the interference with Ca^{2+}-signaling and function of human sperm translate into negative effects on human fertility?
  - Difficult to answer in non-primate animal models (differences in regulation)

Discussion –
Relevance of testing in human sperm

All 5 EDCs induce Ca^{2+}-signals in human sperm

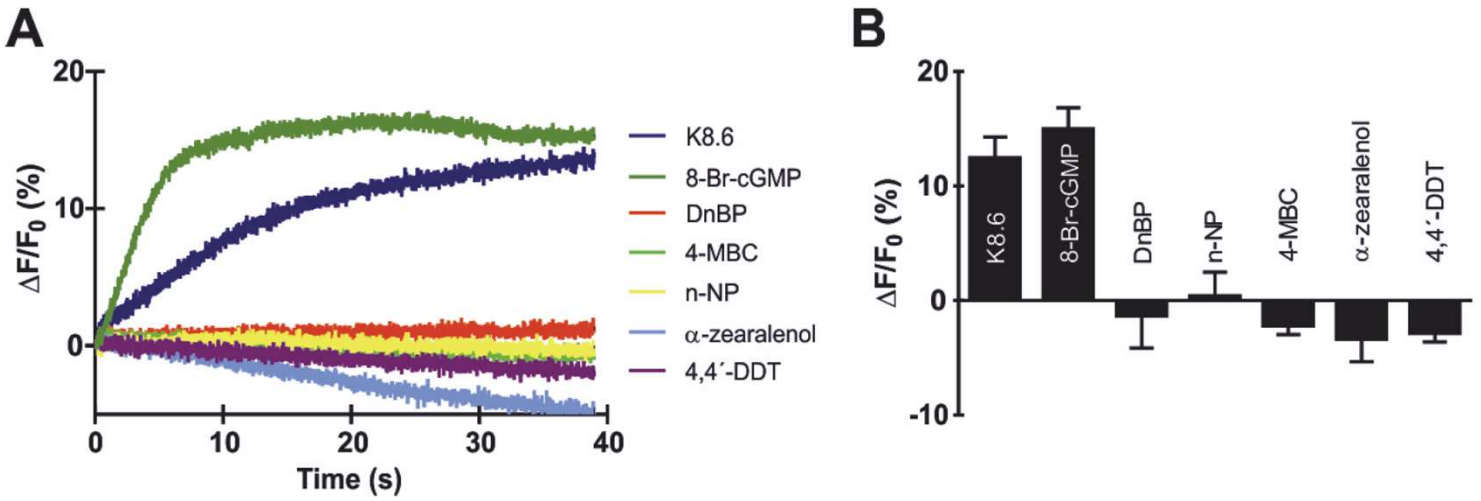
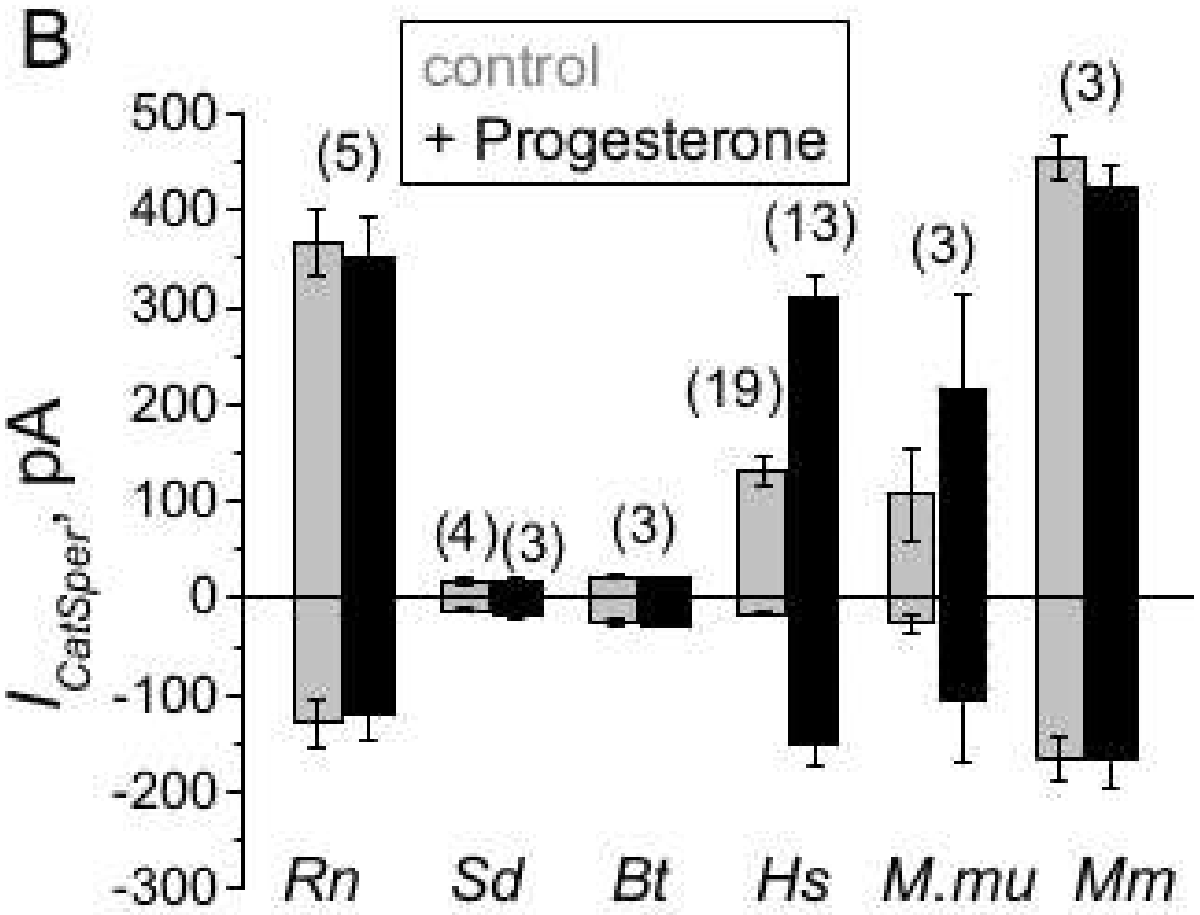


Figure S5. CatSper in mouse sperm is largely insensitive to EDCs.

Schiffer et al. 2014 EMBO rep.

Discussion –
Relevance of testing in human sperm



Liu B et al. *JoVe 2021.*

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journal homepage: www.elsevier.com/locate/envint

Full length article

In vitro investigation of endocrine disrupting effects of pesticides on Ca^{2+}-signaling in human sperm cells through actions on the sperm-specific and steroid-activated CatSper Ca^{2+}-channel

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Miljøministeriet
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