

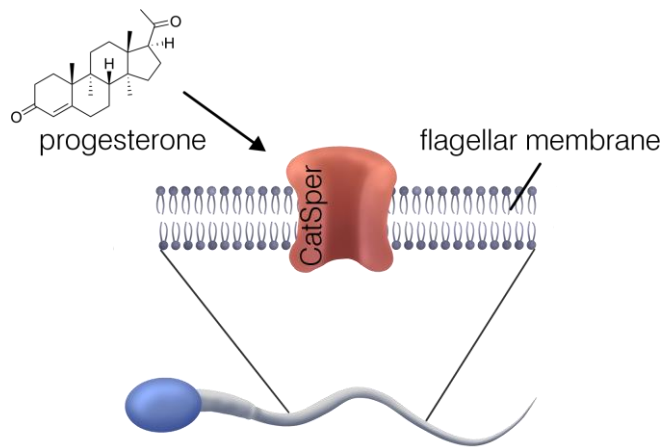
## CatFlux

Buffer reagents for performing the CatSper-Test

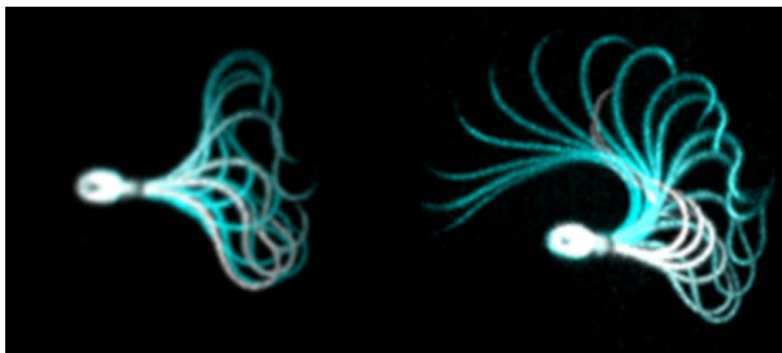
**What is CatSper and what role does it play in fertilization?**

Sperm must navigate long distances and overcome obstacles in the female reproductive tract to reach and fertilize the egg. Various physical and chemical cues have been proposed to assist fertilization by modulating sperm’s swimming behavior. On a molecular level, swimming behavior is controlled by the intracellular concentration of calcium ions.

The primary gate for calcium is a sperm-specific ion channel in the flagellum known as CatSper, and thereby, takes center stage in orchestrating sperm behavior. CatSper is activated by hormones in the fallopian tube, in particular by progesterone, which is released by the cumulus cells.

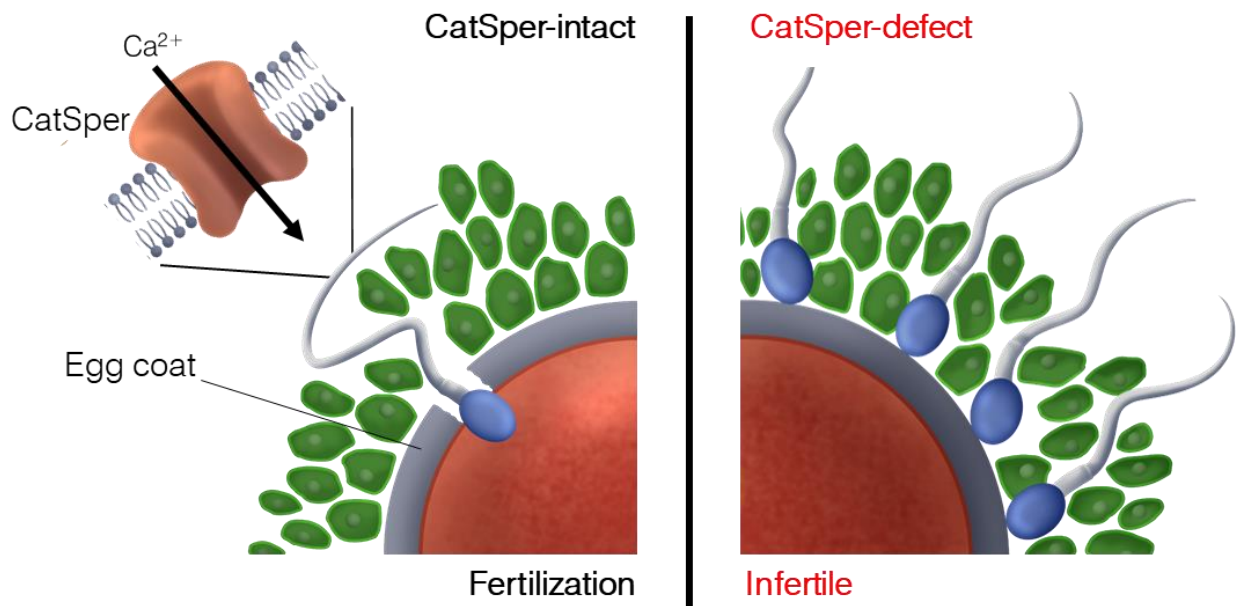


In proximity to the egg, activation of CatSper triggers so-called hyperactivation. Hyperactivation is characterized by a particularly forceful, whip-like flagellar beat, which facilitates penetrating the protective layer (i.e., *zona pellucida*) of the egg.



Superimposed image series of two flagellar beat cycles of a single sperm cell before (left) and after stimulation with progesterone (right). In contrast to the symmetrical, low-amplitude flagellar beat (left), the hyperactivated flagellar beat (right) is asymmetrical and features a high amplitude.

Proper function of CatSper is an essential component for successful fertilization: A large-scale study (Young *et al.*, 2024) demonstrated that sperm lacking CatSper function cannot hyperactivate when stimulated with progesterone; sperm with this dysfunction could only bind to the egg coat, but were unable to penetrate it, resulting in total fertilization failure of an in vitro fertilization (IVF).



A CatSper dysfunction, therefore, results in affected men not being able to conceive naturally, via hormonal ovulation induction (OI), intrauterine insemination (IUI), or IVF, as indicated by the history of the patient cohort identified in the study named above.

**Are couples affected by a CatSper dysfunction able to conceive?**

Men with a CatSper dysfunction were still able to father a child via intracytoplasmic sperm injection (ICSI) treatment, presumably because sperm are injected directly into the egg, forgoing the necessity to penetrate the egg coat unassisted (see Table below of the treatment history of eight CatSper-deficient patients described in Young *et al.*, 2024)

Patient	Semen analysis	OI	IUI	IVF	ICSI
1	normal	failure		failure	success
2	normal	failure		failure	success
3	normal	failure		failure	success
4	normal		failure		success
5	normal		failure	failure	success
6	normal	failure			success
7	normal				
8	teratozoo-spermia	failure			success

Abbreviations: OI – ovulation induction; IUI – intrauterine insemination; IVF – in vitro fertilization; ICSI – intracytoplasmic sperm injection.

### **Can sperm with a CatSper dysfunction be identified (microscopically)?**

No. Men with a CatSper dysfunction can have completely normal ejaculate parameters as determined by a standard semen analysis: A CatSper defect does not affect sperm count, morphology, or basal motility. According to WHO criteria, patients with a CatSper defect are often classified as normozoospermic. This does not exclude, however, that other underlying issues are coincidentally present, e.g. teratozoospermia.

### **Are there currently any routine diagnostic procedures to test for CatSper function?**

No. Methods capable of investigating CatSper function (e.g. electrophysiology or fluorimetry) have largely been restricted to scientific research facilities due to their complexity. No method has proven suitable for the demands and constraints of a routine diagnostic laboratory – until now.

### **How does the CatSper-Test change this situation?**

At the Centre of Reproductive Medicine and Andrology at the University Hospital Münster (chief physician: Prof. Dr. med. Sabine Kliesch), a test was developed in the work group of Prof. Dr. Timo Strünker, which can simply and effectively assess the function of CatSper. This "CatSper-Test" can be routinely performed as part of a semen analysis. The test procedure was published in a scientific study with a cohort of nearly 2,300 patients:

Young *et al.* (2024):

**Human fertilization in vivo and in vitro requires the CatSper channel to initiate sperm hyperactivation.** J Clin Invest. 2024;134(1):e173564



<https://doi.org/10.1172/JCI173564>

### **What does the CatSper-Test have to offer?**

A CatSper dysfunction in sperm can hamper natural conception as well as medically assisted fertilization, e.g. IVF. When performed on patients first presenting with suspected fertility disorders (see section on target group), the CatSper-Test can contribute to early-diagnosing a CatSper defect, thereby, avoiding loss of time anticipating natural conception and / or frustrated insemination or IVF treatment cycles. Additionally, a CatSper-Test can serve as a valuable diagnostic aid for the treating physician to select the most suitable therapy option for a couple.

### **Which patients are the target group for the test?**

The CatSper activity test is intended for patients with a desire to have children who have not yet been able to conceive. For patients who have achieved fertilization only through ICSI therapy, the test may be able to provide an explanation as to why fertilization failed naturally and / or via other therapy options.

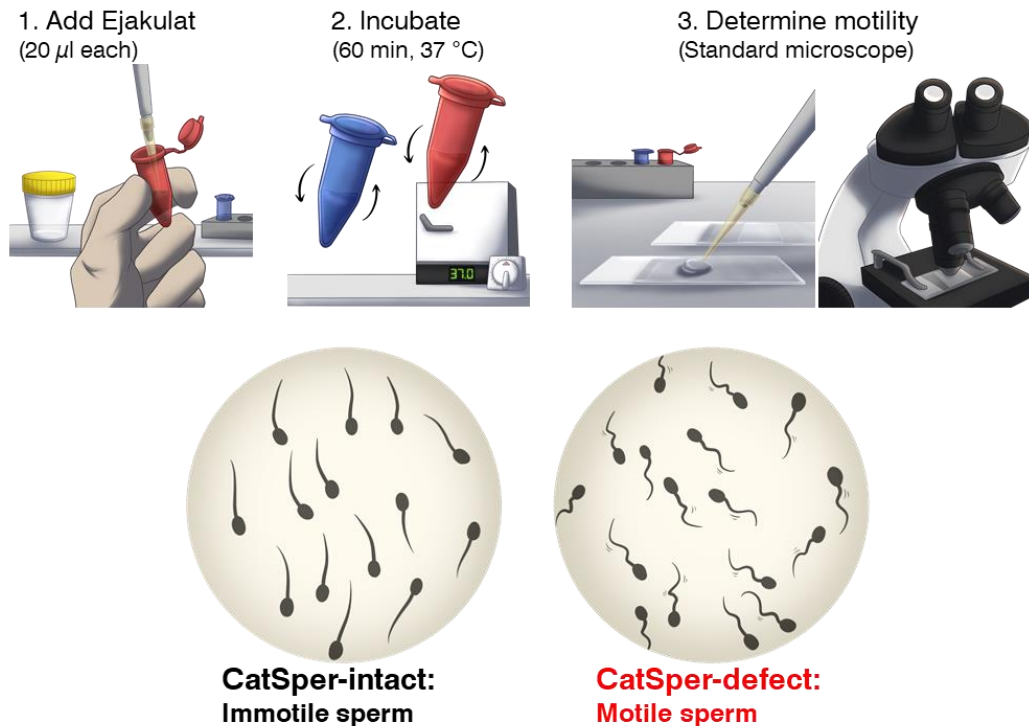
### **What is the procedure in case of a test result indicating a CatSper dysfunction?**

A CatSper-Test, like most diagnostic tests, is in and of itself neither "proof" of a disorder nor a deciding factor for a therapy option, but rather provides information that is useful to the physician to aid in medical decisions within the context of the patient's individual medical history. In the case of an abnormal test result further diagnostics such as genetic testing from an institute specializing in reproductive genetics is advisable. One example

of an institute, which provides such consultation is the Institute of Reproductive Genetics of the University Hospital Münster in Germany.

### How does a CatSper-Test work?

First and foremost, the test can easily be integrated into the workflow of a standard semen analysis and requires only a few minutes of hands-on time. Beyond common laboratory equipment such as a light microscope for determining sperm motility, pipettes, and an incubator (37 °C), the test only requires the use of the specially formulated CatFlux Buffer solutions. A detailed instruction manual guides the professional user step-by-step through the procedure which is outlined in the figure below.



Briefly, a small amount of a native semen sample is added to the CatFlux Buffers and allowed to incubate at 37 °C. After 60 minutes, samples are aliquoted onto a slide and the fraction of motile sperm is assessed under a microscope. See instructions for use for more details.

### Does the patient have to provide an additional semen sample for the test?

No. The test only requires a total of 40 microliters, an amount that mostly will not compromise performing a standard semen analysis in parallel.

### What is the sensitivity / specificity of the test?

Performance studies have determined a sensitivity of 100 % and a specificity of 98.3%. Detailed results can be found in the instructions for use.

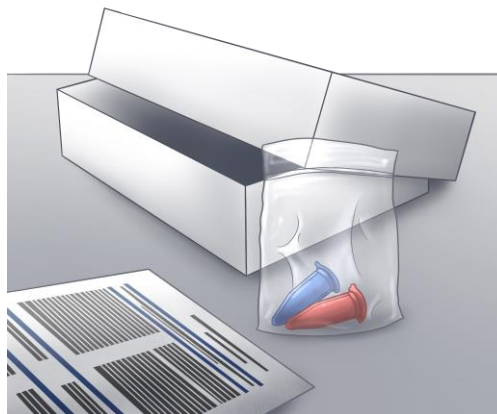
### What is the prevalence of patients with a CatSper dysfunction?

In the target group of the patient cohort described in the study by Young *et al.*, 2024, the prevalence was approximately 1 %; a breakdown of the study data can be found there (link to study on page 3). However, further clinical studies are required to determine the true prevalence in the target population. Right now, a forecast is not possible.

### **Where can the CatFlux Buffers for performing a CatSper-Test be obtained?**

The CatFlux Buffers are manufactured and distributed by Truion GmbH, a spin-off from the University of Münster in Germany. They are available in sets suitable for performing ten tests. The CatFlux Buffers are CE-marked medical devices in accordance with the European Union regulation on in vitro diagnostic medical devices. Instructions for Use are included (can also be found at [www.truion.de](http://www.truion.de)). To date, a delivery outside the European Union is not possible due to medical device regulations.

Informational material for moderating a patient consultation can be requested free of charge and is also available at [www.truion.de](http://www.truion.de).



### **Truion GmbH**

CEO: Vincent L. Fischer

Hüfferstraße 62  
48149 Münster  
Germany

Tel. +49 251 83 54858

[info@truion.de](mailto:info@truion.de)  
[www.truion.de](http://www.truion.de)

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