Cat**Flux**

Solutions for performing the Cat**Sper** Test

Frequently asked questions

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science for
the next generation

Dear Customer,

With these Frequently Asked Questions, we would like to introduce you to our product CatFlux, its application in the CatSper Test, and the scientific background behind it, helping you integrate this diagnostic tool into your practice.

For further questions, the Truion team is here to assist you!

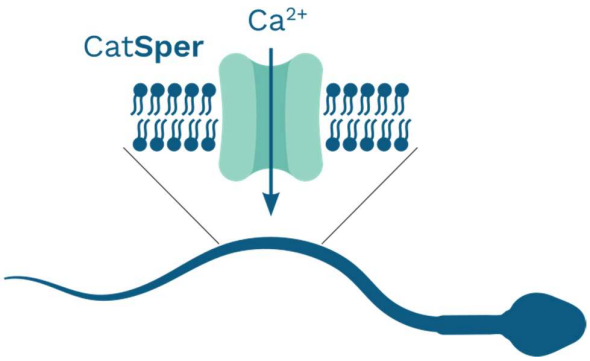
Frequently Asked Questions

1. Introduction to CatSper and CatSper-related male infertility

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

Sperm must traverse long distances and overcome barriers within the female reproductive tract to reach and fertilize the egg. Various physical and chemical cues assist fertilization by modulating sperm swimming behavior. At the molecular level, this behavior is regulated by the intracellular concentration of calcium ions.

The primary gateway for calcium ions is a sperm-specific ion channel located in the flagellum, known as CatSper (Cation channel of Sperm). This channel plays a central role in controlling sperm behavior. CatSper is activated by hormones in the fallopian tube, particularly progesterone, which is secreted by the cumulus cells surrounding the egg.



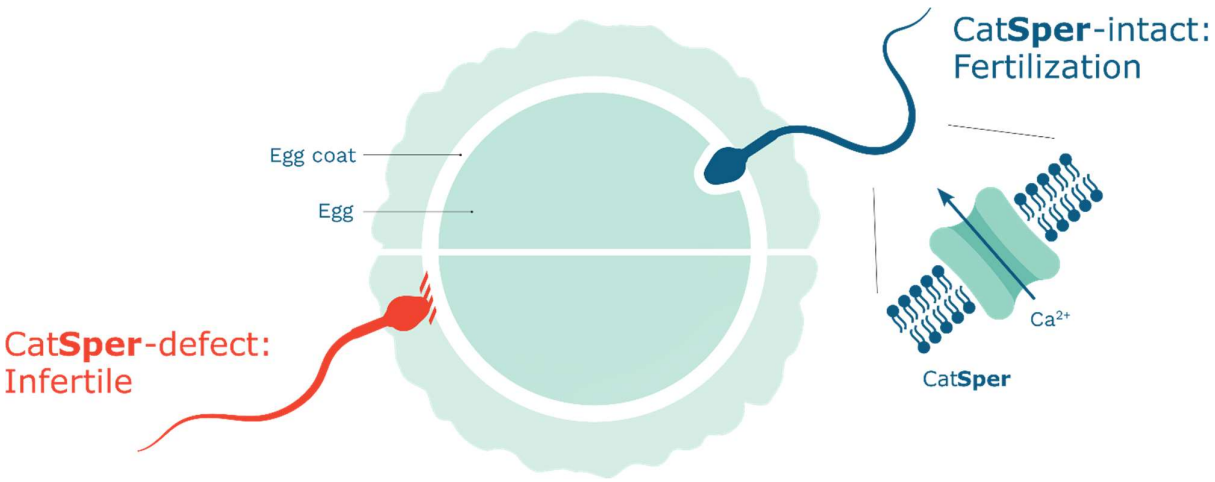
In proximity to the egg, activation of CatSper triggers a hyperactive flagellar beat. Hyperactivation is characterized by a vigorous, whip-like motion of the flagellum, which is crucial for generating the force necessary to penetrate the egg's protective coat (zona pellucida).

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.

The CatSper channel complex is comprised of more than 15 subunits encoded in the human genome by genes located across various chromosomes, making it the most complex ion channel currently known.. Although females also carry the CatSper genes, they are expressed exclusively in sperm. Therefore, defects in CatSper genes do not impact female fertility. Nonetheless, they can be passed on by the mother to the next generation – potentially leading to CatSper-related infertility of male offspring described below.

How is CatSper the "key to the egg" – and what does it mean for patients if it is defective?

CatSper triggers sperm hyperactivation, functioning as a "key to the egg": sperm with CatSper defects can bind to the egg coat but cannot penetrate it (Young et al., 2024). Consequently, men with a CatSper defect cannot conceive naturally, and most reproductive treatments remain ineffective. Hormonal ovulation induction (OI), intrauterine insemination (IUI), and conventional in vitro fertilization (IVF) all require sperm to penetrate the egg coat, a barrier CatSper-defective sperm cannot overcome. Only intracytoplasmic sperm injection (ICSI), which injects sperm directly into the egg, bypasses this requirement and is therefore the only viable treatment for CatSper-related infertility (see table below).



The challenge is that, although a CatSper defect prevents sperm from fertilizing an egg, standard semen analysis results are often unremarkable (see table). This is because standard semen analysis focuses only on superficial parameters like morphology, sperm count, and basic motility, without assessing deeper functional aspects where CatSper plays a critical role.

The semen analysis results and treatment histories of eight CatSper-defective patients.

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	teratozoospermia	failure			success

Abbreviations: OI – ovulation induction; IUI – intrauterine insemination; IVF – in vitro fertilization; ICSI – intracytoplasmic sperm injection. Empty cells: Couple did not undergo the respective treatment. “Failure”: All cycles of the respective treatment failed. “Success”: At least one cycle of the respective treatment succeeded in the fertilization of eggs. Taken from: Young *et al.*, 2024.

What is the prevalence of patients with a CatSper defect?

Patients with CatSper defects, for obvious reasons, accumulate in fertility clinics. Among infertile men with normozoospermia, the cohort described by Young *et al.* (2024), the prevalence of CatSper defects was 1.2%. A detailed breakdown of the study data is available there, though additional clinical studies are needed to more accurately estimate the true prevalence. This high prevalence among infertile patients with normozoospermia makes the CatSper defect in fact the most common known cause of “unexplained” male-factor infertility to date (Young *et al.*, 2024).

Can sperm with a CatSper defect be identified by semen analysis?

No. Men with a CatSper defect often present with completely normal semen parameters in standard semen analysis: CatSper defects do not impact sperm count, morphology, or basal motility. According to WHO criteria, these patients are typically classified as normozoospermic, leading to a diagnosis of “unexplained infertility.” However, a CatSper defect does not rule out other coexisting issues, such as teratozoospermia.

How does the CatSper Test address this situation?

At the Centre of Reproductive Medicine and Andrology at the University Hospital Münster, a test was developed that can simply and effectively assess the function of CatSper. This "CatSper Test" can be routinely performed as part of a semen analysis. The test was first described in a scientific study investigating over 2300 andrological patients found below.

Young & Schiffer et al., **Human fertilization in vivo and in vitro requires the CatSper channel to initiate sperm hyperactivation.** The Journal of Clinical Investigation 2024;134(1): e173564. www.jci.org/articles/view/173564

2. Integrating the CatSper Test in your clinic or practice

What does the CatSper Test have to offer?

Since all the methods capable of testing CatSper function (e.g. electrophysiology or fluorimetry) are essentially restricted to scientific research facilities due to their complexity, the CatSper Test represents the very first routine-diagnostic tool to test patients for CatSper-related infertility.

As the most common known cause of unexplained male-factor infertility, the CatSper Test is recommended along with the first or second semen analysis for infertile couples. This allows for the early detection of CatSper-related infertility, helping avoid prolonged attempts at natural conception or ineffective insemination or IVF cycles. A positive test result can promptly guide couples toward ICSI as a targeted treatment, providing a scientifically sound rationale for choosing the only viable option.

Which patients are the target group for the CatSper Test?

The CatSper Test is intended for infertile male patients who have not yet been able to conceive. For patients with an ICSI indication or have only conceived through ICSI, the test can provide an explanation as to why fertilization failed naturally and / or via other therapy options.

If the patient already has an ICSI indication, should I still perform the CatSper test?

First, an ICSI indication does and a CatSper defect can coexist. Second, the CatSper Test provides insight into an inherited infertility risk that may affect male offspring. If the father has a CatSper defect, there is a significant chance the son could inherit it, impacting his future fertility.

What is the procedure in case of a test result indicating a CatSper defect?

Although the CatSper test using CatFlux offers remarkably high sensitivity and specificity (see below), it is not, in itself, definitive proof of a disorder nor a deciding factor for therapy. Instead, it provides valuable information to guide physicians in their medical decisions within the context of the patient's individual medical history. Like for most diagnostic tests, multi-faceted confirmation is required as part of sound and responsible medical practice before establishing a definitive diagnosis or therapy recommendation. In the case of the CatSper test indicating a defect, further diagnostics, such as genetic analysis, are recommended.

Which kind of genetic analysis is recommended? Are specialized geneticists required to perform it?

Mutations or deletions of the CATSPER2 gene on chromosome 15 are the most common cause of CatSper defects. Therefore, the initial genetic test typically uses a PCR-based method called MLPA (Multiplex Ligation-dependent Probe Amplification) specifically

targeting CATSPER2. This technique is widely employed to detect copy number variations, such as deletions or duplications, in specific DNA regions. An MLPA test for detecting CATSPER2 deletions can be easily conducted with a commercial kit[1], making it straightforward for geneticists. However, if no deletion is detected, further, more advanced testing may be necessary. In such cases, consulting a specialized institute in reproductive genetics is recommended for additional support[2].

Are there other commercially available diagnostic tools for identifying CatSper-related male infertility?

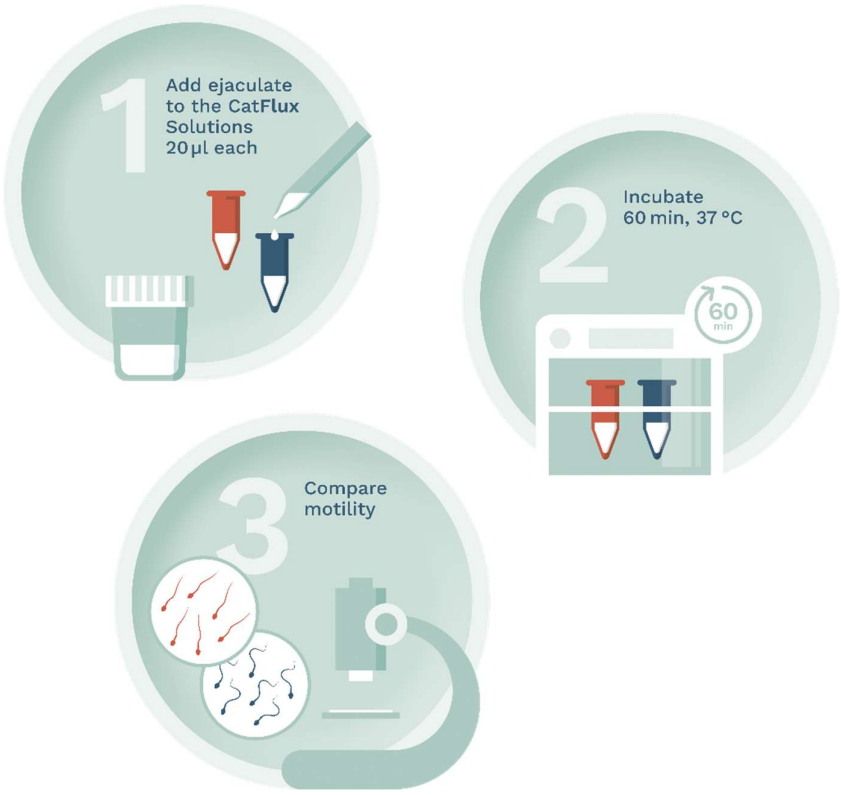
No. When it comes to testing the functional status of CatSper channels in a routine diagnostic laboratory, the CatSper Test stands alone.

[1] Available from MRC Holland: https://www.mrcholland.com/product/P461
[2] The Institute of Reproductive Genetics of the University of Münster in Germany, headed by Prof. Dr. med. Frank Tüttelmann is an example of one such institute.

3. Performing a CatSper Test with CatFlux in your laboratory

How does a CatSper Test work?

First and foremost, the test can be seamlessly integrated into the workflow of a standard semen analysis, requiring only a few minutes of hands-on time. Beyond standard laboratory equipment – such as a light microscope for assessing sperm motility, pipettes, and a 37 °C incubator – the test only requires the specially formulated CatFlux Solutions. A detailed instruction manual provides step-by-step guidance for professionals. The figure below outlines the workflow, but in no way replaces reading the instruction manual.



(1) Briefly, a small amount of a semen sample is added to the CatFlux Solutions and allowed to incubate at 37 °C. (2) After 60 minutes, samples are aliquoted onto a slide and (3) the fraction of motile sperm is assessed under a microscope.

What is the test principle?

The CatSper test translates the complex molecular function of CatSper – normally measurable in the lab only through highly sophisticated methods like electrophysiology – into a simple, easy-to-read cellular response: a loss of sperm motility. This is achieved by using CatFlux to disrupt the ion concentrations in sperm. However, this disruption only occurs if functional CatSper channels are present in the sperm's flagellum: If CatSper is absent or non-functional, sperm in the CatFlux Test Solution remain motile. To control for natural motility loss during the incubation period, sperm from the same semen sample are simultaneously incubated in the CatFlux Control Solution.

Does the patient have to provide an additional semen sample for the CatSper Test?

No, the test requires only 40 microliters of ejaculate, an amount that generally won't interfere with conducting a standard semen analysis in parallel.

Can the test be performed with cryopreserved sperm?

No, currently a CatSper test with CatFlux cannot be performed for diagnostic purposes using cryopreserved sperm, as there is not yet sufficient scientific data available.

Can the test be performed with processed ejaculate?

No, currently a CatSper test with CatFlux cannot be performed for diagnostic purposes using processed sperm (such as sperm purified from ejaculates via swim-up or density gradient centrifugation) because there is not yet sufficient scientific data available.

How many sperm need to be counted?

At least 200 sperm need to be counted to reliably determine the percecentage of motile sperm required for calculating the CatSper index (CI).

What is the shelf-life?

The shelf-life is 18 months from the date of manufacture. Truion GmbH guarantees at least one year of remaining shelf-life when shipped. The label on the product packaging provides the official expiration date of the respective CatFlux batch. After this date, the CatFlux solutions must no longer be used for diagnostic purposes.

What is the sensitivity / specificity of the test? Where do I find this information?

The key performance data for CatSper tests using CatFlux can be found in the product's instruction manual. It includes a table with the results from the performance studies conducted for CE certification. These studies reported a sensitivity of 100% (reliably identifying all CatSper defects) and a specificity of 98.3% (indicating that 1.7% of CatSper-intact patients were incorrectly identified as having a CatSper defect).

The Positive Predictive Value (PPV), which indicates the likelihood that a patient identified as having a CatSper defect truly has the defect, was estimated to be 42%, based on a prevalence of 1.2% (Young *et al.*, 2024). The Negative Predictive Value (NPV), representing the likelihood that a patient identified as CatSper-intact is indeed free of the defect, was estimated to be 100%. To achieve these results, however, the working protocol outlined in the instruction manual must of course be followed accurately.

What should I do if the CI is at or below the threshold (less than 80), meaning there are still motile sperm in the Test solution?

If this occurs, thoroughly mix the buffer solutions again, incubate for at least 15 minutes (but no longer than 60), and reassess motility.

CI < 80: Sperm remain motile in the Test Solution – indicative of a CatSper defect. Genetic analysis is recommended.
CI > 80: Sperm become immotile in the Test Solution – often indicates improper mixing. For example, high viscosity can prevent adequate mixing, or droplets may remain on the tube walls or lid. This can leave motile sperm in the sample, affecting the accuracy of the test.

Where can the CatFlux Solutions for performing a CatSper Test be obtained?

The CatFlux Solutions are manufactured by Truion GmbH, a spin-off from the University of Münster in Germany, and marketed through country-specific dealers. They are available in ready-to-use sets suitable for performing ten tests. The CatFlux Solutions are a CE-marked medical device in accordance with the European Union regulation on in vitro diagnostic medical devices (Regulation (EU) 2017/746 IVDR). Instructions for use are included.
Truion is happy to provide you with informational material for moderating a patient consultation. Please request it free of charge or find it online for download.

Find your local dealer at www.truion.de!

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