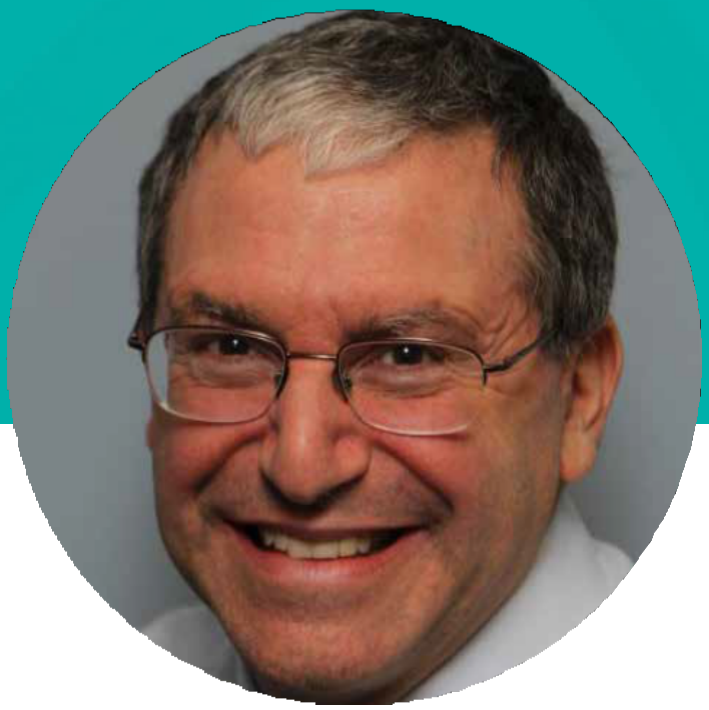

In Search of Success

At an exclusive visit at Maze Laboratories, Dr. Michael A. Werner shares with ATIME the background and figures on ESSM

One of the first terms that one gets acquainted with when setting out on the winding and exhausting journey of male infertility is "semen analysis." Typically, even before a diagnosis is given, the doctor will want a report of the count, motility and morphology, and only from there will the root of the issue be explored.

Most readers are intimately familiar with the procedure, the technical aspects and the pertinent halachic concerns, but here we present to you a revolutionary development in the field. It is thanks to this breakthrough technique that some patients were able to prevent more invasive procedures and surgeries in their quest to obtain sperm for use in an IVF cycle.

A diagnosis of a "zero sperm count" or "azoospermia" is frightening and disheartening, and usually leads to intensive interventions like surgical sperm extractions.

But when a team of Israeli doctors developed the revolutionary method of ESSM (Extended Sperm Search & Microfreeze), they forever changed the landscape of treatment of male reproductive medicine.

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An elegant office on the ninth floor of a luxurious Manhattan building is home to the prestigious Maze Health clinic, under the leadership of longtime urologist Dr. Michael Werner. And not far from it is the specialized ESSM laboratory under the supervision of Dr. Werner's assistant, andrologist Chaya Rothschild.

When A TIME's esteemed medical director, Rabbi Mordechai Koenig, introduced Dr. Werner to ESSM (a search method that, until then, was only performed in Israel) and suggested that he implement this revolutionary search method at Maze Labs, Dr. Werner was immediately on board. Dr. Werner, a urologist with over 25 years of experience servicing patients struggling with male factor infertility, was intimately aware of the crucial need for sperm search advances and was eager to bring this option to patients over the ocean. After learning of this method, he opened the first ESSM laboratory in the U.S. in November 2019.

As the first, and to date the only, laboratory employing this groundbreaking method, we reached out to Dr. Werner to share with you, the A TIME readership, the inside scoop on this advance.

Hello! Can you introduce yourself to our readers?

Hello to you! My name is Michael Werner. I was born and raised in Los Angeles and studied in Harvard Medical School as well as San Francisco University. Thereafter, I relocated to New York where I got married and launched my career at the male infertility department at Mt. Sinai Hospital.

When did you break into the field of infertility independently?

Twenty-six years ago, I opened my practice along with a laboratory for semen analyses, which I named MAZE (an acronym for the names of my four sons). About five years later, Maze expanded to include a new wing for sperm banking, where frozen sperm samples are banked when fertility preservation is warranted, such as before a male undergoes chemotherapy, or other procedures that can negatively impact fertility. Today, we are from the biggest centers in the field of sperm banking, and our client base includes Memorial Sloan Kettering and other prestigious medical institutions. In 2019, we opened our ESSM lab, in which we search and freeze isolated sperm using the new ESSM method.

How did you learn about ESSM?

ESSM was developed several years ago by a group of specialists in Israel. Until recently, they were the only center internationally to use this protocol and the only way to undergo the test was either to travel to Israel, or to get a sample to the country. In 2019, Rabbi Koenig approached me and suggested that I adopt this new method in my lab here in the U.S. I liked the idea and we immediately began to outfit our lab with the necessary tools and machinery and employed a staff that would enable us to launch this project. A few months later, we opened the first ESSM lab in the U.S.

How is ESSM more effective than a conventional semen analysis?

To answer this question, we first have to understand what the process of a conventional semen analysis is and why it is so.

A man with uncompromised reproductive function produces hundreds of millions of sperm cells per sample; every centimeter of such a sample includes a concentration of millions of microscopic sperm cells. Understandably, it is nearly impossible to analyze the entire sample and count the exact number of sperm cells swimming therein. Therefore the standardized system is to take a small droplet from the sample, analyze it under a microscope, and estimate how many cells are concentrated in that droplet. If, for example, about 10,000 sperm cells are found, it is assumed that it is proportionately true for the entire sample and that is how the sperm count is determined.

In a case where sperm is not found in the isolated droplet, the conventional school of thought is that the same is true for the entire sample, and this classifies the patient as azoospermic. Once the diagnosis of "zero sperm count" is determined, the only options are drastic measures, surgical sperm extraction procedures like TESE.

In truth, though, a large percentage of men who, based on a semen analysis, were determined to be azoospermic, actually do have minimal production; but since it is such a small number, it is often not found in the specific droplet that underwent the analysis.

This is where ESSM comes in.

ESSM — Extended Sperm Search & Microfreeze — employs two main components that make all the difference: a new method of searching for isolated viable sperm and a specialized technique for freezing what's found.

ESSM is a six-hour procedure in which each millimeter of a sample is thoroughly observed under a microscope in order to find the few isolated viable sperm swimming therein. Once those sperm are found, they are freezed for future IVF use in a manner that doesn't compromise those few precious finds, through a special technique that is the second component of ESSM.

Which type of patients are candidates for ESSM?

There are several categories within the scope of male infertility. Of course, the issue when faced with male fertility is not always that sperm cells were not found.

Specialized dish in which sperm cells are cryopreserved in small quantities

ESSM is mainly targeted for men who were determined to be azoospermic via a zero sperm count in a semen analysis.

Obviously, the reason for the azoospermia should be identified prior to pursuing ESSM. In a case of CBAVD (congenital absence of the vas deferens), for example, or other cases of obstructive azoospermia, there's no point in undergoing ESSM when the obstruction issue needs to be addressed. Therefore, a diagnosis is important before trying ESSM.

In some cases, patients with obstructive azoospermia approach us to inquire about ESSM, in their pursuit to try everything prior to proceeding to sperm extraction procedures. We explain to them that there is no real chance of finding anything in the ejaculate in those cases, but ultimately the decision is the patient's.

Is ESSM appropriate for patients with low motility issues?

In cases of a high sperm count but weak motility, the issue is usually that the sperm is getting compromised during ejaculation; and therefore the chances of finding healthy sperm in the ejaculate are low. In those cases, surgical sperm extraction may be more appropriate, since healthy sperm are often produced and only then damaged during ejaculation. Yet, there is a chance of finding isolated healthy sperm with ESSM, so it may be worth it to try it before going under the knife.

What is the average current success rate of ESSM?

It is hard to generalize, since success depends on the underlying issue. There are several levels under the umbrella term "azoospermia." The medical term for low sperm count is "oligospermia," and this reflects a count of between 5 and 15 million sperm cells. Thereafter is "cryptozoospermia," in which no visible sperm is observed in fresh semen, but only found once an extensive analysis is performed. In these aforementioned cases, our success rate is nearly 100%.

On the other end of the spectrum is virtual "azoospermia," in which sperm is only found through ESSM

and not a conventional search, and in those cases, our chances are lower. Overall, though, our success rate is over 40%.

When it comes to infertility data, success rates are calculated by live births achieved via a specific method. We only started the procedure over a year ago and then the coronavirus hit, which brought our operations to a standstill. So since we don't yet have enough data to determine the live birth rate, the abovementioned statistics are not a reflection of that, but of the cases in which sperm viable for IVF were found, and with that we've had great success.

Can a patient who has failed a TESE procedure try ESSM?

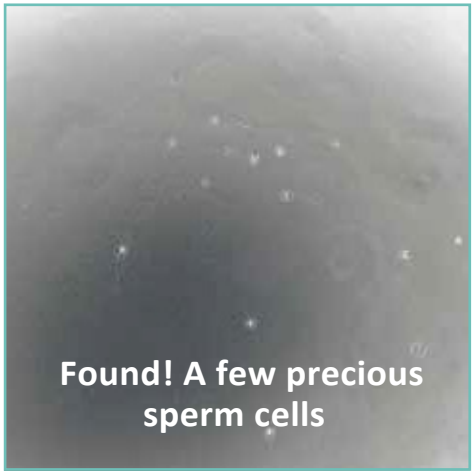
To date, we have not yet been successful to find sperm after a failed sperm extraction. This doesn't mean that it's not an option; in theory, success should sometimes be possible even in those cases — it depends on which doctor performed TESE, and though the chances may be low, the chances are there.

ESSM Explored

For the technical aspects of the procedure, Dr. Werner gave over the stage to andrologist Chaya Rothschild who oversees the daily operations as the lab technician at Maze's ESSM lab. Mrs. Rothschild led us to the lab where various machines, microscopes, test tubes, and needles of all colors and sizes were strategically placed over the work surface. Mrs. Rothschild explained to us the procedure step by step, as well as the role each instrument plays in the process.

The first thing a patient seeking ESSM needs to provide is a semen sample. As opposed to other facilities who request a sample produced after two to four days of abstinence, we request two samples; one produced between a seven- to ten-day abstinence and another one produced shortly thereafter. Sperm after a short abstinence will often be compromised in count and one after a long abstinence will often be compromised in motility; the dual sample gives us the best results.

When embryos or any material is vitrified, they are frozen with such speed that the ice doesn't crystalize, but instead forms into a smooth glass–like ice.



Found! A few precious sperm cells

Once the sample is transferred to the lab, the lab technicians get to work.

The first two tools used are the centrifuge (likened to the washing machine, it uses centrifugal force to separate different components of a fluid) and the gradient media (used in lieu of detergent in this comparison). In addition to the sperm cells that are (hopefully) swimming in the semen sample, there are hundreds of thousands of cells, bacteria, and other components. Before we can attempt to search for sperm, we need to eliminate all the other components in the semen sample.

The media is a specialized chemical; layering media of different densities in tubes and then centrifuging the tubes will allow the sperm cells (the tiniest components) to settle at the bottom while the larger components remain in the denser media layers, thus isolating the sperm from the other components that would interfere with a search. This centrifugation is repeated three times to ensure that no foreign object remains in the sample.

Once the sample is clean, the search begins. With a specialized needle, I remove one droplet of the washed sample and observe it under a microscope to search for the presence of sperm. If there's a considerable amount in this initial droplet, I will take another few droplets which should yield enough sperm for an entire IVF cycle.

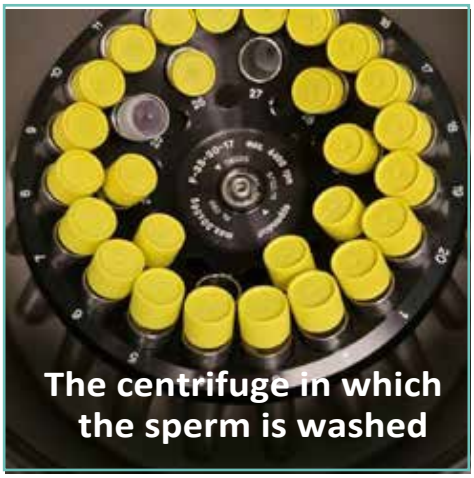
If the first drop doesn't contain any sperm, I take the entire sample and divide it into little droplets that I spread out over a dish. The dish is then transferred to a microscope equipped with a micro-manipulator (a robotic arm that selects the sperm) and then, I settle in for what will be a six-to seven-hour search…

I start at the corner and I examine each droplet separately. As soon as I see a sperm cell, I lift it with the super-thin needle attached to the micro-manipulator and place it in a tray called a spermVD, which is specially designed for our freezing method. Thereafter, we will freeze the sperm using the ESSM technique.

Do the sperm have to be frozen in all cases?

No, and it's great when we don't have to freeze; fresh sperm produced on the day of the retrieval is obviously optimal. With ESSM, though, the process is more complicated, because we don't know in advance how many viable sperm the search will yield. Therefore our protocol is to analyze a sample several days before retrieval, perform the search and microfreeze the sperm cells employing the ESSM method. If we find more than ten viable sperm, we are confident that we will also find that amount on the day of retrieval, and even in the worst-case scenario, if

The dish is then transferred to a microscope equipped with a micro–manipulator and then, I settle in for what will be a six–to seven–hour search…



The centrifuge in which the sperm is washed

we were not to have similar success on retrieval day, we have the frozen sample at hand.

Why is the traditional way of freezing not used in the case of ESSM?

When dealing with single sperm cells, each one is very precious, and traditional freezing methods damage about half of the sperm between freezing and thawing. Our microfreezing method results in 95% viable sperm after the thaw.

How does ESSM differ from standard sperm freezing protocols?

We use a vitrification process to freeze isolated sperm, and the conventional method is via slow-freezing. There are two significant differences between the two methods; the material in which the sperm cells are stored during the freezing process and the speed in which they are frozen. These are two significant factors in the quality of the sperm post-thaw.

Vitrification is a technique that has already been in use for a while, but was only used in the freezing of embryos; sperm is still generally frozen using slow-freezing methods.

The term vitrification has its roots in the latin word *vitrum*, meaning glass. When embryos or any material is vitrified, they are frozen with such speed that the ice doesn't crystalize, but instead forms into a smooth glass-like ice.

Prior to the use of vitrification, the embryos would be frozen slowly, first in the fridge, then in the freezer, and only then was it put into liquid nitrogen. This

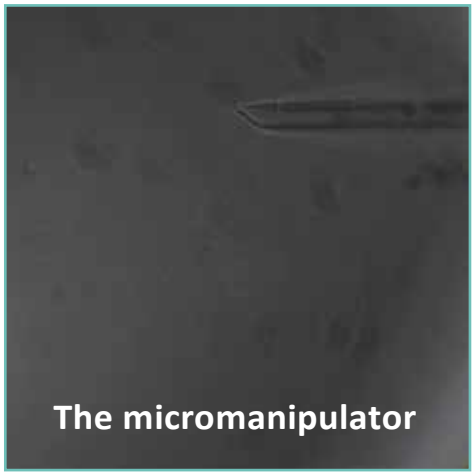
was an issue, since the fluid would freeze droplet by droplet, causing crystallization, and the crystals would damage the embryos, often compromising the viability in half of a given lot.

In the nineties, one doctor developed the method of vitrification: a method that removes the moisture of the cells using a special chemical and places the embryo in liquid nitrogen to achieve ultra-rapid cooling, enabling it to freeze as one solid glass-like piece. This process results in a 95% survival rate post-thaw. Vitrification is not generally used in the cryopreservation of sperm cells for several reasons, the prime one being that sperm cells usually get frozen by the ten thousands per tube, and so a 50% survival rate is not a concern.

However, when dealing with a single-digit count of sperm cells, it makes all the difference, and we cannot afford to lose even one. Therefore we cryopreserve sperm found via ESSM through vitrification. Obviously, the process had to be fine-tuned until the perfect method for vitrifying sperm was achieved, but currently our vitrified sperm cells have a 95% survival rate.

How did the IVF centers welcome this new method?

The reactions varied. Some centers were eager to hear about the breakthrough, while others were skeptical. There are also those centers where ESSM is not in line with their standard protocol and policies; for example, most centers won't use sperm cells that are immobile after thawing. This is a good approach when dealing with thousands of sperm,



The micromanipulator

but in cases with a limited number of sperm, immotile sperm is better than nothing! (And indeed, in this line of thought, some centers will focus on the viability *prior* to cryopreservation, using sperm cells that had displayed motility in their fresh state even when they are immotile post-freeze. We've seen success with such sperm cells.)

Other centers still maintain a policy of not employing any alternate method of obtaining sperm if the success rates don't match those of TESE. Although TESE definitely has an overall higher success rate than ESSM, there is no comparison between a complicated surgery and a non-invasive extensive analysis. And while it's definitely worth it to try a sample search before going under the knife, even when chances are minimal, the policy in some centers will not allow it.

There are also centers who don't want to hear about ESSM at all. They also refuse to accept any cells found via ESSM, with the claim that if we can find the sperm, so can they, despite the fact that the conventional method won't yield sperm in many cases where ESSM will.

However, there are many centers out there who've come to rely on our expertise and many are warming up to the idea of employing this groundbreaking search method.

What's keeping centers back from opening their own ESSM labs?

They are definitely free to do that! However small centers are not equipped to dedicate both a micromanipulator (which is typically used for ICSI) and an embryologist for a full day — for just one patient. As for the bigger centers who have more micromanipulators and larger embryology teams, they will proportionately also be servicing a larger client base and will often not find it worthwhile to dedicate the time, staff and resources necessary for ESSM.

Indeed, what do you do at Maze when you have more than one patient per day?

The first steps, sperm washing and preparation, are able to be performed by many of my colleagues, and we are in the process of training several "searchers" to be able to accommodate more patients per day.

When those few sperm cells are found, do you take motility and morphology into account?

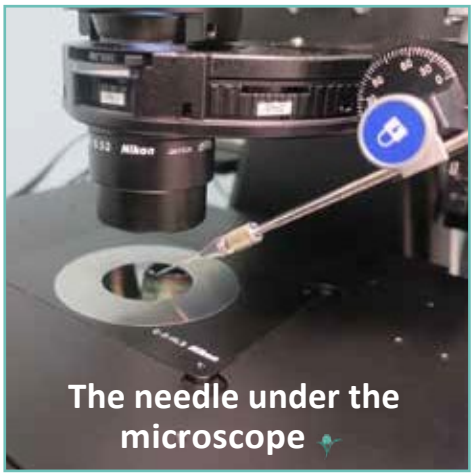
Of course, what use do we have from dead sperm? A sperm cell that isn't moving at all is not usable, but if I see the slightest sign of life, I will select the sperm cell for freezing. I divide the sperm cells among four dishes, ranked according to motility, from the best swimmers to the ones that are barely moving.

As far as morphology is concerned, the conventional school of thought is that motility trumps morphology; embryologists will usually choose motile sperm with a weak morphology over sperm with weak motility but good morphology. I take it case by case and base my selection on how bad the morphology is. If the head is entirely absent or without DNA, I'd definitely take that into account. However, if the head is simply misformed but the motility is good, morphology wouldn't be the deciding factor. This is because the main function of the head is to penetrate the oocyte and with ICSI, that function can be bypassed.

We realize that you refer to sperm cells with the words "he" and "they" as if you're referring to a human.

(Laughs). The truth is that it's not just a joke. Sperm cells are alive; they even have a sense of smell! The reason they swim in the direction of an egg is due to the smell that attracts them there. And since I observe sperm cells day after day, I actually see character traits in them! When they're transferred to another dish, for example, I can see sperms cells getting wild, almost drunken, while others freeze in place until they adapt to their new environment. Other times I can observe how a healthy sperm and egg refuse to fertilize, without any reason; they simply don't get along!

However, when dealing with a single-digit count of sperm cells, it makes all the difference, and we cannot afford to lose even one.



The needle under the microscope

Approximately how many ESSM procedures have you performed since the opening of Maze's ESSM lab?

The office was obviously closed for a while over the coronavirus wave, which impacted our pace. I'd estimate that since we've started we've had about 75 ESSM procedures. At this point, since ESSM is still little-known, a large percentage of our patients are couples referred to us by A TIME. We've also seen patients from across the USA who want to pursue all possible options before moving on to TESE. And I'm happy to report that we've been successful in preventing more invasive interventions for many of our patients using this method.

By the time we left the Maze Health clinic, the sun was setting and the office was preparing to close for the day. And so we thanked Dr. Michael Werner and Chaya Rothschild for the interesting and informative conversation and set out to bring the information to our dear readership.