Nature's Principles. Proven Success.

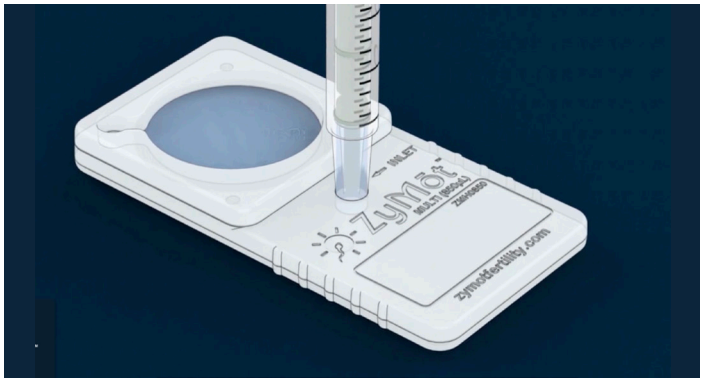
Understanding ZyMōt® Sperm Separation Devices

Better Sperm Selection by Mimicking Nature

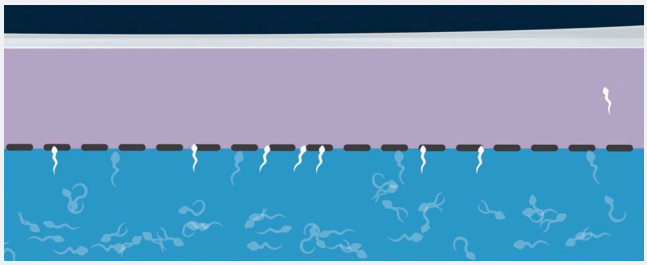
ZyMōt Fertility, Inc., has developed novel devices for use in ART procedures conducted by fertility clinics and OB/GYN practices. ZyMōt Multi Sperm Separation Devices prepare motile sperm from semen for use in IUI, IVF and ICSI procedures. These devices are the first of their kind and available worldwide.

Our revolutionary tools simulate the natural barriers of the cervical and uterine pathway that sperm must overcome to fertilize an egg. We enable separation of optimally functional sperm without the use of damaging chemicals or density gradient centrifugation (DGC).

ZyMōt Multi Device



The ZyMōt Multi device is available in two processing volumes, 850µL and 3mL. A sample is applied through the device's inlet port, connected to a lower sample chamber. This chamber is separated from an upper collection chamber by an 8µm microporous filter. Filter size was determined after comparison between 3µm, 5µm and 8µm pore sizes. Incubation times of 15, 30 and 45 minutes were evaluated, with sperm saturation achieved at 30 minutes.[1] These parameters yielded optimal sperm collection efficiency and motility, with the 8µm pore demonstrating the highest degree of normal morphology.[2]



During sample incubation, the most motile sperm migrate upward through the filter, leaving less motile sperm behind. Separated sperm are then collected from the upper chamber for subsequent use in IUI, IVF and ICSI procedures.

Simplifying and Standardizing Workflow

Easy to adopt and simple to use, ZyMōt Multi Sperm Separation Devices provide considerable time savings and standardization over traditional methods. ZyMōt devices avoid damaging DGC and preserve normal sperm morphology. Contact us for more information about how to evaluate ZyMōt devices in your clinic. We offer comprehensive support with experts who are ready to help you incorporate our tools into your practice and extend your success. **Learn more at** zymotfertility.com.

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Revolutionizing Sperm Preparation

ZyMōt® Sperm Separation Devices: Better for your patients. Better for your practice.

A Better Way to Prepare Sperm

Quality, accuracy and efficiency are central to the success of a fertility practice. Traditional sperm preparation methods are not only time-consuming and laborious, but could cause additional sperm DNA fragmentation[1] and cellular stress,[2] lowering the odds of success.[3,4] ZyMōt Sperm Separation Devices are a better way to prepare sperm for use in IUI, IVF and ICSI procedures. It's that simple.

Work on Your Timeline

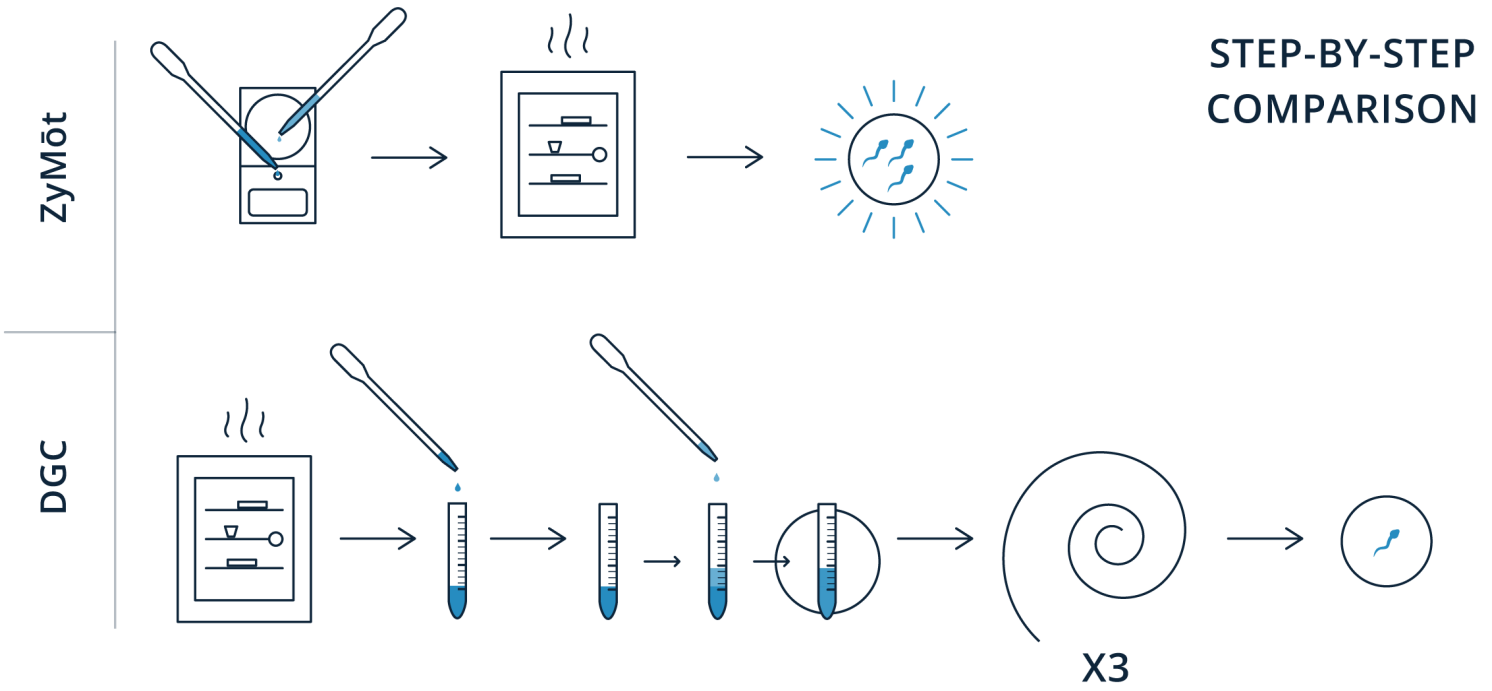
ZyMōt devices enable processing whenever a sample is ready, eliminating delays caused by an equipment bottleneck. With **only 5 minutes of total hands-on tech time per sample**, every ZyMōt-processed specimen represents a significant time savings over traditional methods. Using ZyMōt devices frees staff for other critical tasks and improves lab productivity.

Simple to Adopt. Easy to Use.

Available worldwide, ZyMōt devices efficiently isolate healthy, rapidly-progressive sperm, to help achieve outcomes that matter.[5,6] Minimal training is required, with simple, standardized procedures that help users **quickly achieve optimal performance.**

Fewer Steps. More Confidence.

A shorter chain of custody – fewer movements per sample – means that ZyMōt devices help minimize mismatching risk, reducing the potential for costly error. **Processing sperm with ZyMōt devices gives providers more confidence and gives patients more peace of mind.**



Comparison of major sperm separation steps when using ZyMōt Sperm Separation Devices (top) versus using the traditional method (bottom). ZyMōt requires fewer movements per sample, improving efficiency and productivity while reducing risk of costly errors.

Natural. Simple. Effective.

Try ZyMōt Sperm Separation Devices and realize immediate savings of time and resources, while providing premium quality sperm separation for your patients. **Learn more at zymotfertility.com.**

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Publication Spotlight: Utilizing Spermatozoa with Higher Genomic Competence Improves ICSI Outcomes

Understanding the latest science in the ZyMōt® revolution

ZyMōt Device Helps Select Sperm with High Genomic Integrity

Building on research from Parrella *et al* in 2019[1], scientists at Weill-Cornell Medical College have been examining various impacts of genomic integrity. Specifically, these Weill-Cornell researchers have studied sperm chromatin fragmentation (SCF) and the effects on clinical outcomes.

In a study presented at ASRM 2021, Keating's objective was to demonstrate that selecting spermatozoa with the highest genomic integrity utilizing the ZyMōt Multi 850µl Sperm Separation Device would enhance ICSI outcomes. In this follow-on to the Parrella *et al* 2019 study[1], the first step was to look at sperm parameters in men known to have SCF >15% after processing by, in parallel, density gradient centrifugation (DGC) and the ZyMōt device.

FIG. 1 SEMEN PARAMETERS (N=126 PATIENTS)

	SELECTION METHOD		
	RAW	DGC	ZYMŌT
MOTILITY (%)	33.7±14	61.2±33	96.3±13
MORPHOLOGY (%)	2.2±1	2.0±1	3.0±1
SCF (%)	23	18	1.4

The SCF results collected from these studies, as shown in **Fig. 1**, highlighted the need to continue to focus on genomic integrity for male-factor patients. In particular, the encouraging results using the ZyMōt Multi 850 device supported taking a further look at clinical outcomes in additional patients.

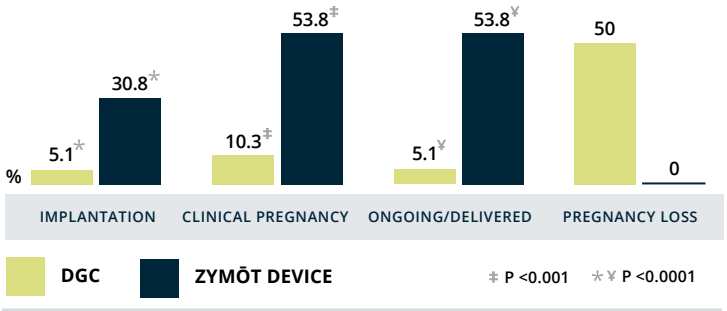
Low SCF with ZyMōt Device Leads to Better Clinical Outcomes vs. DGC

Based on the significant improvement in SCF utilizing the ZyMōt Multi 850 device, the researchers next looked at the clinical outcomes of this device compared to DGC (see **Figs. 2-3**). A total of 21 men (aged 43.3±8 years) had an average SCF in their raw semen of 22.1±10%, which decreased to 19.1±7% after DGC sperm preparation. These men underwent 39 ICSI cycles with their female partners (aged 38.0±4 years).

FIG. 2 SEMEN PARAMETERS (N=21 PATIENTS)

	SELECTION METHOD		
	RAW	DGC	ZYMŌT
SCF (%)	22.1±10	19.1±7	1.2±1

FIG. 3 CLINICAL OUTCOMES - FRESH EMBRYO TRANSFER



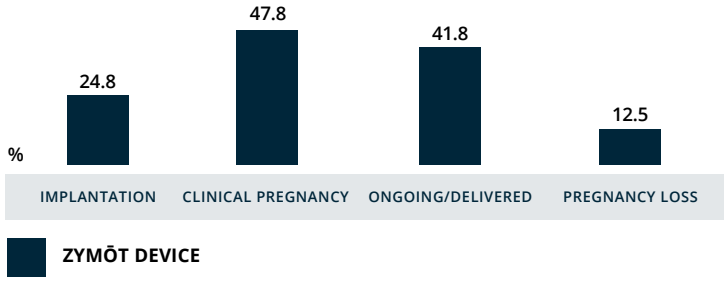
Subsequently, these couples underwent 26 ICSI cycles utilizing sperm preparation with the ZyMōt Multi 850 device. The SCF after ZyMōt device use was 1.2±1%, substantially lower than the raw sample and DGC. All cycles were fresh embryo transfers[2].

ICSI Treatment with ZyMōt Device for Men with High SCF Enhances Clinical Outcomes

After seeing the significant results comparing the ZyMōt device to DGC, Keating sought to evaluate the clinical outcomes solely using the ZyMōt device in couples where the men were known to have elevated SCF. Fifty-five (55) men (aged 42.3±8 years) were treated in 69 ICSI cycles with their female partners (aged 38.3±5 years). The SCF in their raw samples was 22.3±10%, which fell to 3.0±4% (p <0.0001) following ZyMōt sperm sample preparation (see **Fig. 4**).

As Keating *et al* concluded, "Compared to the more conventional DGC, MFSS (the ZyMōt device) is capable of selecting the most progressively motile spermatozoa with the highest genomic integrity. Treatment by ICSI with MFSS (ZyMōt) for men with high sperm DNA fragmentation enhances fertilization, embryo development, and clinical pregnancies."

FIG. 4 CLINICAL OUTCOMES - ZYMŌT DEVICE ONLY



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Publication Spotlight: Utilizing Spermatozoa with Higher Genomic Competence Improves ICSI Outcomes

Understanding the latest science in the ZyMōt® revolution

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FIG. 2 SEMEN PARAMETERS (N=21 PATIENTS)

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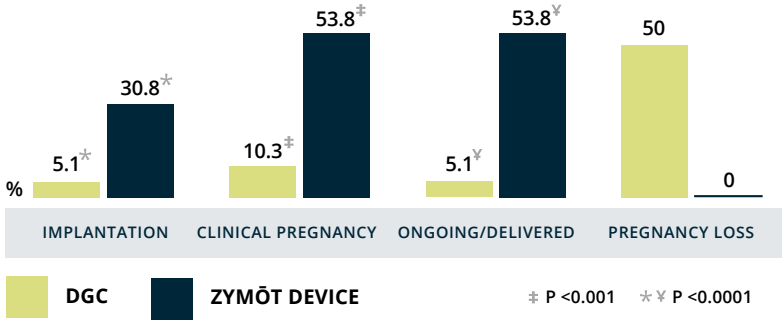

ICSI Treatment with ZyMōt Device for Men with High SCF Enhances Clinical Outcomes

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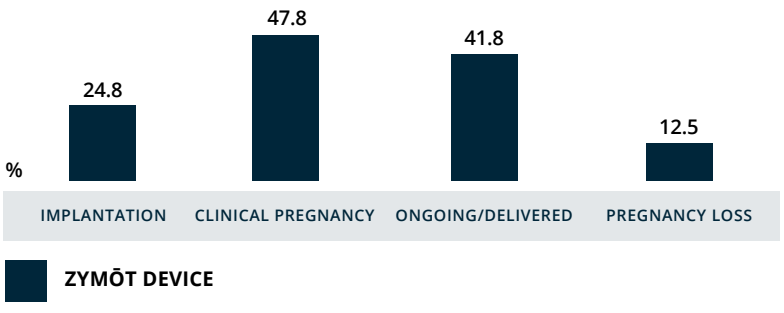

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Publication Spotlight: A Sperm Selection Technique to Improve Embryo Ploidy

Understanding the latest science in the ZyMōt® revolution

OBJECTIVE

To assess the role of an enhanced sperm selection method in mitigating paternal contributions to embryo aneuploidy.

MATERIALS AND METHODS

Over the last 4 years, 57 couples underwent ICSI with sperm selected by density gradient centrifugation (DGC), resulting in few frozen embryo transfers (FETs) due to consistent embryo aneuploidy following preimplantation genetic testing for aneuploidy (PGT-A).

These men consented to sperm chromatin fragmentation (SCF) assessment, inclusive of double-stranded DNA breaks (dsDNA) in their raw semen, as well as post-DGC and post-microfluidic sperm selection with the ZyMōt Multi Sperm Separation Device (ZyMōt). These couples underwent subsequent ICSI cycles with ZyMōt. Outcomes of cycles processed by DGC and ZyMōt were analyzed and compared.

SCF was assessed by terminal deoxynucleotidyl dUTP transferase nick-end labeling (TUNEL) on ≥500 spermatozoa per patient, with a normal threshold of ≤15%. A neutral Comet assay was used to assess dsDNA on ≥200 spermatozoa, utilizing a modified in-house protocol and a normal threshold of ≤3%.

RESULTS

A total of 57 men had the following semen parameters: concentration of 40.0±32x106/mL, 37.1±11% motility, and 2.2±1% normal morphology. After selection by DGC or ZyMōt, the concentrations were 3.3±3.4x10^6/mL and 8.0±13x10^6/mL, with 58.0±29% and 96.9±9% motility, respectively ($p<0.0001$). The SCF decreased from 21±14% in raw specimens to 18±6% following DGC and to 1.9±1% following ZyMōt ($p<0.001$). The dsDNA fell from 3.6±2% in raw specimens to 3.1±1% after DGC and to 0.3±0.2% after ZyMōt ($p<0.001$).

These men (aged 40.9±6 years) underwent DGC selection for 71 ICSI cycles with their female partners (aged 36.5±5 years), achieving a fertilization rate of 58.4% (403/690) and a blastocyst euploidy rate of 28.5% (47/165). Only 19 FET cycles were performed, with a 6.7% implantation rate (2/30) and two clinical pregnancies resulting in miscarriage.

Subsequently, these men had their specimens selected by ZyMōt in 71 ICSI cycles, resulting in a higher fertilization rate of 75.9% (647/852; $p<0.0001$) and a much improved ($p<0.0001$) blastocyst euploidy rate of 48.9% by PGT-A (192/389). In 48 FET cycles, 51 embryos were replaced with an increased implantation rate of 60.8% (31/51; $p<0.0001$), a CPR of 64.6% (31/48; $p<0.001$), and an ongoing/delivery rate of 62.5% (30/48; $p<0.0001$).

FIG. 1 PATIENT DEMOGRAPHICS PGA-T (N=57 COUPLES)

	SELECTION METHOD	
	DENSITY GRADIENT	ZYMŌT DEVICE
CYCLES	71	71
MATERNAL AGE (M±SD)	36.5±5	37.2±15
MATERNAL AGE (M±SD)	40.9±6	41.1±7

FIG. 2 EUPLOIDY RATES - PGT-A

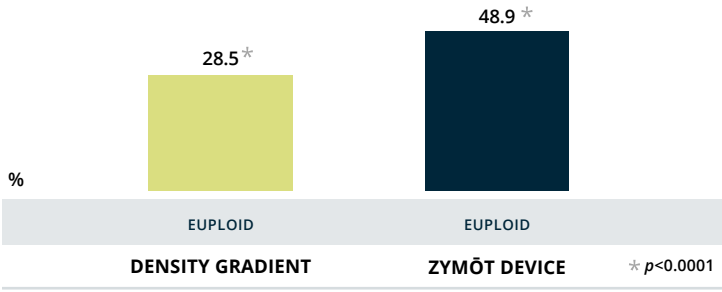
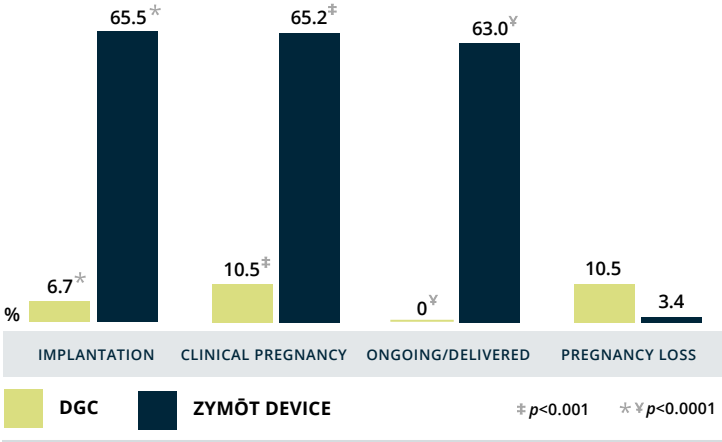


FIG. 3 CLINICAL OUTCOMES



CONCLUSIONS

With its dsDNA component, SCF tangibly contributes to embryo structural chromosomal abnormalities. An enhanced spermatozoa selection method for ICSI appears to remarkably increase the proportion of euploid blastocysts with consequent successful clinical outcomes.

IMPACT STATEMENT

Sperm genomic integrity is associated with the ploidy of the conceptus, and a high SCF inclusive of dsDNA can be mitigated by proper sperm selection.

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Publication Spotlight: DNA & ROS Levels After ZyMōt® Sperm Prep

Understanding the latest science in the ZyMōt revolution

The Need for Healthy Sperm

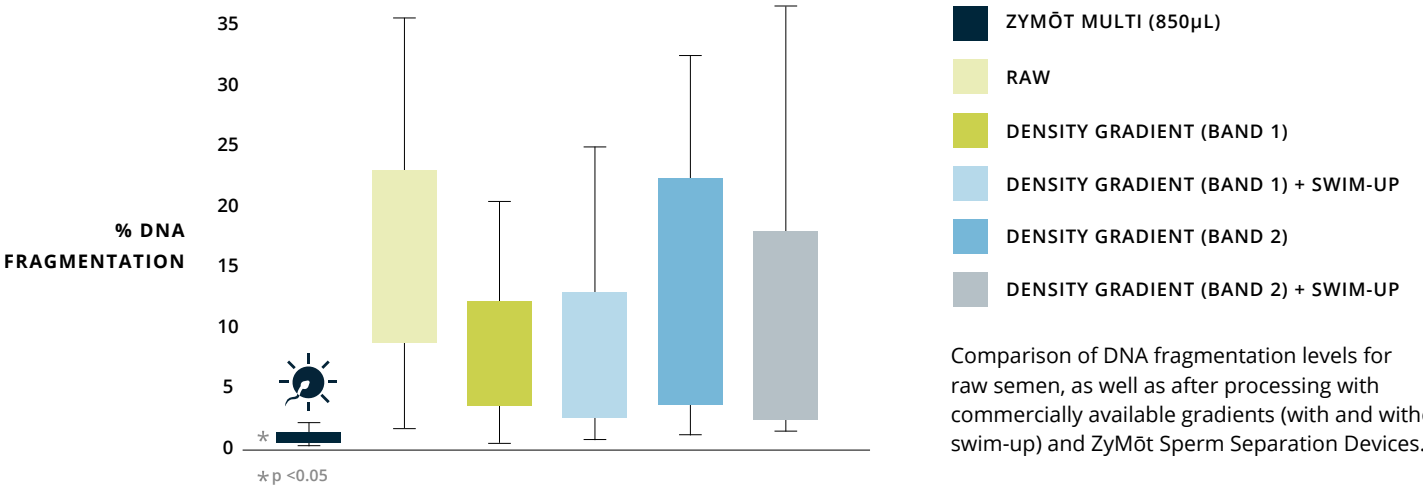
Using healthy sperm for IUI, IVF and ICSI procedures is more important than ever. In new research[1] from scientists at Imperial College London, recurrent pregnancy loss was directly connected to the presence of elevated sperm DNA fragmentation and reactive oxygen species, along with a lower percentage of normal morphology. This follows a growing body of evidence that links improved sperm health to better pregnancy outcomes.[2] ZyMōt Sperm Separation Devices enable the preparation of sperm with low levels of DNA fragmentation and oxidative stress.

Avoiding DNA Fragmentation and Oxidative Stress

ZyMōt devices have been shown to separate sperm with near-zero DNA fragmentation, compared to density gradient centrifugation.[3] In an independent study from Midwest Fertility Specialists, ZyMōt Multi (850µL) Sperm Separation Devices were directly compared to traditional sperm preparation techniques.[4] This clinical research determined which approach resulted in improved DNA fragmentation index (DFI) and other sperm health biomarkers such as oxidative stress adducts (OSA) and high DNA stainability (HDS).

Results: Using ZyMōt devices significantly reduced DFI ($P<0.05$) compared to standard protocols: two commercially available gradients, and gradients followed by swim-up. The device also effectively reduced ($P<0.05$) OSA levels, a measurement of oxidative stress, and HDS, a measurement of immature cells and high histone retention. "**Overall, the quality of the sperm obtained post-processing was improved by the use of the separation device**," wrote the study author.

Conclusion: Using ZyMōt devices shows statistically significant improvements in three DNA- and stress-focused indicators of sperm health and function, when compared to traditional methods.



Comparison of DNA fragmentation levels for raw semen, as well as after processing with commercially available gradients (with and without swim-up) and ZyMōt Sperm Separation Devices.

Improving Efficiency and Outcomes

ZyMōt devices are easy to adopt and simple to use, helping labs quickly achieve optimal performance. With only 5 minutes of total hands-on tech time per sample, every ZyMōt-processed specimen represents a significant time savings over traditional methods. In addition to increased efficiency, ZyMōt devices deliver improved sperm performance to help achieve the best possible outcomes in IUI, IVF and ICSI procedures. **Learn more at zymotfertility.com**.

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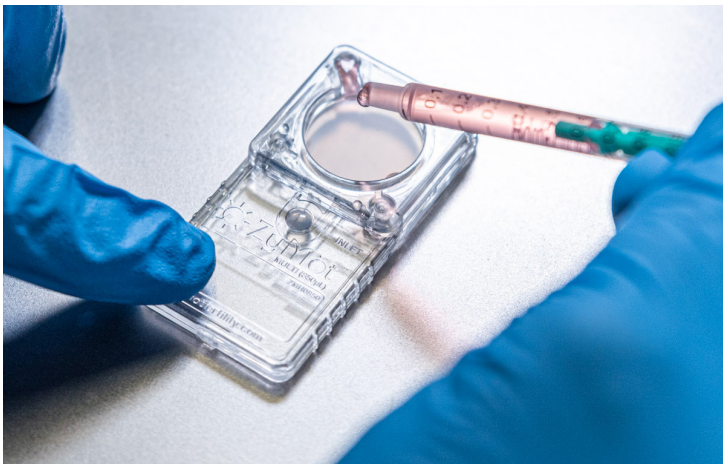
Publication Spotlight: Improving Genomic Integrity & Patient Outcomes

Understanding the latest science in the ZyMōt® revolution

Not All Sperm are Equal

Using the best sperm helps increase the odds of a successful fertility treatment cycle. Not all sperm are created equal: up to 11% of men with a "normal" semen analysis have a measurable problem with sperm chromatin (DNA) fragmentation, and thus reduced motility.[1] Double-stranded sperm DNA damage is a cause of delay in embryo development and can impair implantation rates.[2]

ZyMōt Sperm Separation Devices are a new way to process sperm. ZyMōt devices enable the separation of sperm with nearly undetectable levels of DNA fragmentation and oxidative stress.[3] Improved sperm health means better clinical outcomes.[4-6]



Results: Sperm DNA Damage Lowers the Odds of Success

Work from Keating showed that double-stranded DNA breaks in sperm were a major factor in chromosomal abnormalities, embryo aneuploidy and pregnancy loss.[7] This highlights the need to focus on genomic integrity – not just for male-factor patients, but for every sample.

Results: Improved Outcomes for Challenging Patients

In an update to her 2019 publication[1], Parrella and colleagues studied patients with histories of ART failure and high sperm chromatin fragmentation (SCF) (≥22%). This research asked if microfluidic sperm separation was able to select sperm with higher chromatin integrity.[8]

One patient group underwent fresh embryo transfer (FET) after processing with DGC. Initially, this group saw low levels of clinical pregnancy and high levels of loss. These patients then had their semen specimens processed with ZyMōt in a subsequent ICSI cycle, yielding significantly higher implantation rates, clinical/ongoing pregnancy rates, and decreased pregnancy loss.

In another group, patients also had both high SCF and a history of high embryo aneuploidy rates. Patients underwent PGT-A and frozen embryo transfer, after sperm processing with either DGC or the ZyMōt device. Euploidy rates were significantly higher with the ZyMōt device compared to DGC processing. Implantation rates, clinical pregnancy rates, ongoing/delivered rates (there were none with DGC) were all significantly higher with ZyMōt compared to DGC processing with greater pregnancy loss, respectively.

The ZyMōt Difference

The science is clear: it's essential to do everything we can to improve sample quality by selecting sperm with the lowest possible levels of DNA fragmentation. Processing with the ZyMōt device enhances sperm sample motility, and progression and morphology, along with providing a "remarkable reduction" of DNA fragmentation.[1] ZyMōt devices yield sperm with higher genomic competence, demonstrated by their improved euploid rate and ability to establish healthy pregnancies, even for couples with histories of previous ART failure. Learn more at **zymotfertility.com.**

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Publications Spotlight: Comparison of Microfluid Sperm Sorting Chip and Density Gradient Methods for Use in Intrauterine Insemination Cycles

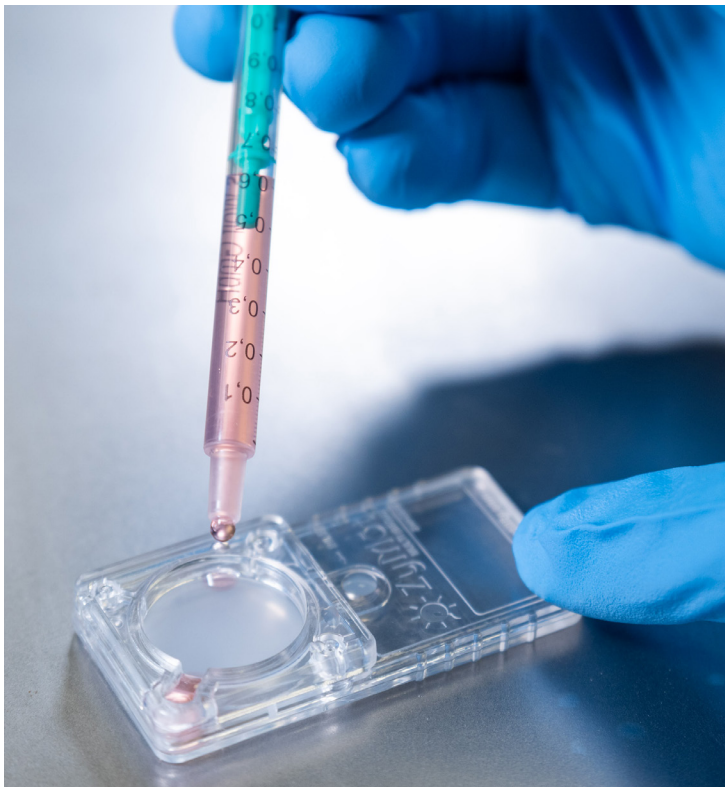
Understanding the latest science in the ZyMōt® revolution

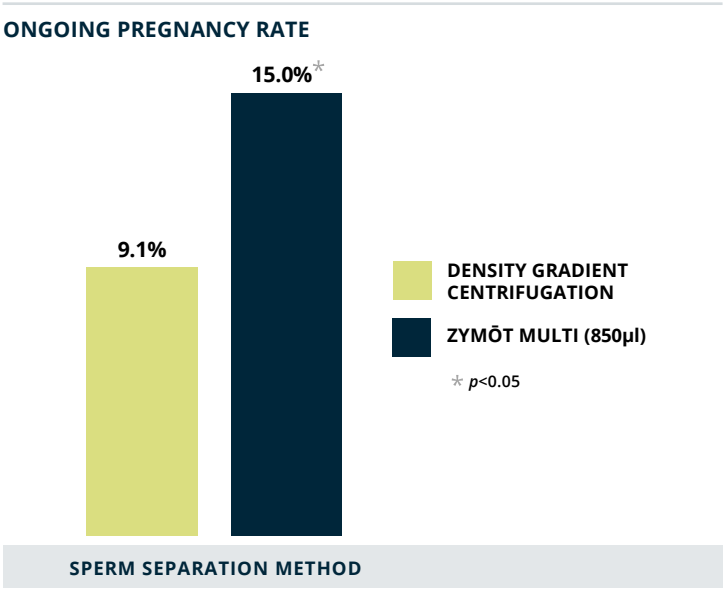
A Better Way to Process Sperm for IUI

ZyMōt Sperm Separation Devices offer an alternative preparation method that allows for simple, natural, and effective isolation of motile sperm.

Results: In a retrospective study of 265 IUI patients with unexplained infertility, patients whose semen samples were processed using ZyMōt Sperm Separation Devices were 3.5 times more likely to achieve an ongoing pregnancy than the age-matched control group, where sperm was processed with the traditional, centrifugation-based method.[1] In this study, the ZyMōt IUI treatment group also experienced a reduced miscarriage rate when compared to patients whose semen samples were processed by density gradient (0% vs. 5% respectively).[1]

Conclusion: Processing sperm with ZyMōt devices can improve a patient's IUI treatment prognosis at the onset of their infertility journey, offering a greater chance of success with a lower risk treatment option.





The effect of IUI sperm preparation method on pregnancy outcomes: ZyMōt improved treatment prognosis.

Simple to Adopt. Easy to Use.

FDA-cleared, CE-certified and available worldwide, ZyMōt Sperm Separation Devices efficiently isolate the healthiest, rapidly progressive sperm, to help achieve outcomes that matter.[2] Minimal training is required, with simple, standardized procedures that help users quickly achieve optimal performance. ZyMōt Sperm Separation Devices are a better way to prepare sperm. It's that simple. Learn more at **zymotfertility.com**.

References

1. Gode, F., *et al*. Fertil Steril (2019). doi: 10.1016/j.fertnstert.2019.06.037
2. Parrella, A., *et al*. J Assist Reprod Genet (2019). doi: 10.1007/s10815-019-01543-5

ZyMōt FERTILITY, INC.

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Publications Spotlight: Improved Patient Outcomes after ZyMōt® Device Prep

Understanding the latest science in the ZyMōt revolution

Recent Studies Examined Euploidy and Ongoing Pregnancy Rates

ZyMōt Sperm Separation Devices have been designed and developed to aid reproductive medicine professionals in the selection of motile sperm for use in assisted reproductive technology (ART) procedures. In new research presented at ASRM 2020, investigators examined euploidy and ongoing pregnancy rates, and saw significant improvement when processing samples with ZyMōt devices.

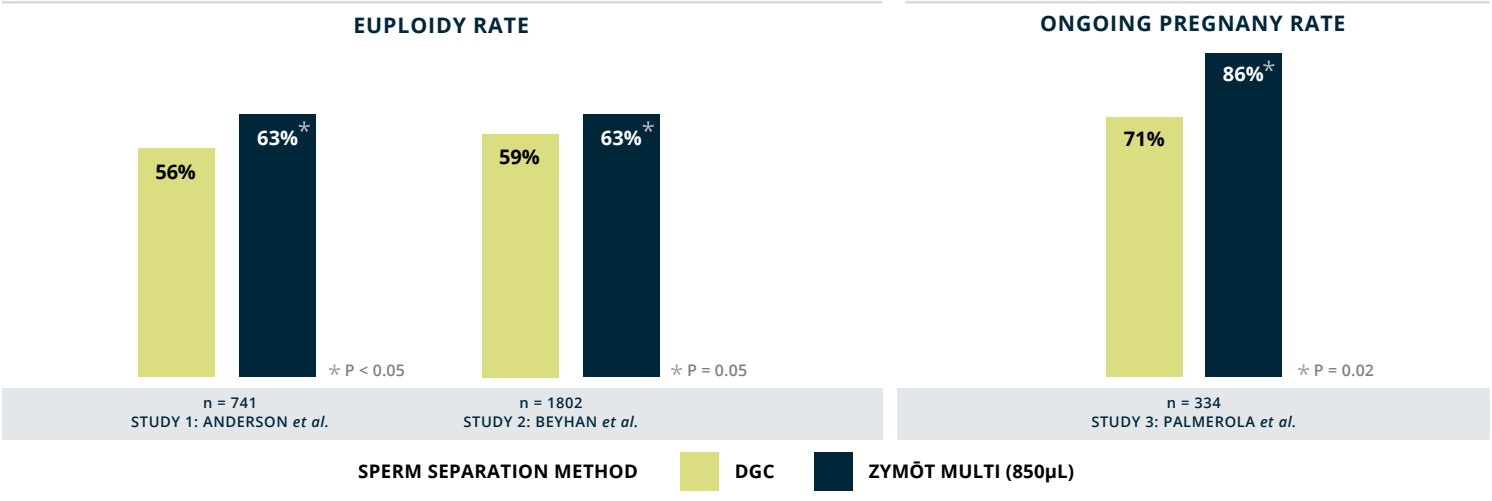
Results: Improved Euploidy Rates

Anderson and colleagues conducted a prospective cohort study[1] that compared the impact of sperm prepared utilizing density gradient centrifugation (DGC) or sperm separation with the ZyMōt Multi (850µL) device on euploidy and pregnancy outcomes. The D5 euploid rate was significantly higher using ZyMōt compared to DGC (below, left). Anderson also presented results based on a Six Sigma-style evaluation of time and showed that ZyMōt saves procedural steps and time.

Beyhan and colleagues conducted a retrospective study[2] that examined preimplantation development following ICSI after ZyMōt or DGC, in presumed normal to moderate male infertility patients. Similar fertilization and blastocyst conversion rates between the cohorts were observed. An increased euploid rate was observed for the ZyMōt-processed samples (below, middle).

Results: Improved Ongoing Pregnancies

In another retrospective study, Palmerola and colleagues[3] compared ongoing pregnancy rates for two cohorts that used either DGC or ZyMōt device preparation. A significant improvement in ongoing pregnancies following single, euploid embryo transfer was observed (below, right). Fertilization, usable blastocysts and D5 and D6 biopsy rates were similar between the DGC and ZyMōt groups.



Improving Efficiency and Outcomes

ZyMōt devices are simple to use, helping labs quickly achieve optimal performance. With only 5 minutes of total hands-on tech time per sample, every ZyMōt-processed specimen represents a significant time savings over traditional methods. Learn more at **zymotfertility.com.**

References

1. Anderson T., *et al*., Fertility and Sterility (2020), Volume 114, Issue 3, O-104
2. Right: Beyhan Z., *et al*., Fertility and Sterility (2020), Volume 114, Issue 3, P-48
3. Palmerola K., *et al*., Fertility and Sterility (2020), Volume 114, Issue 3, P-45

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A Vital Innovation
for Fertility Patients.

ZyMōt® FERTILITY, INC.

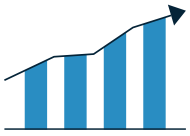
A GAME-CHANGER FOR YOUR LAB.

ZyMōt® Sperm Separation Devices

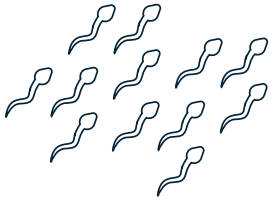
MORE THAN
50%
FEWER STEPS

ZyMōt devices require less than half the sample handling steps compared to average sperm prep, greatly reducing the risk for error.

MORE EMBRYO TRANSFERS
Increased euploidy rates mean more opportunities for embryo transfers.



ALL-TIME HIGH DEMAND
Patient demand for ZyMōt devices is at an all-time high. Making them available in your lab will help attract more patients.

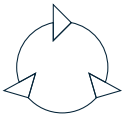


CONSISTENCY
Ensure you're always getting the best sample every time, regardless of tech experience.

UP TO 80%
GREATER EFFICIENCY

Reduce hands-on prep time by up to 80% and free up your lab technician's time for other tasks.

90%
LESS WASTE



Reduce the amount of sperm preparation media, tubes, pipettes, and other equipment by up to 90%.

90%
CUSTOMER RETENTION

More than 90% of labs that have used ZyMōt devices continue to use them.

SCALABILITY

ZyMōt devices streamline a typically laborious process, which saves you time and positions your lab for growth.

ZyMōt FERTILITY

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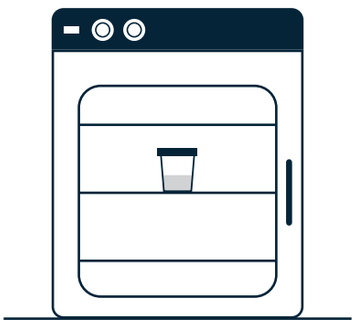
ZyMōt®

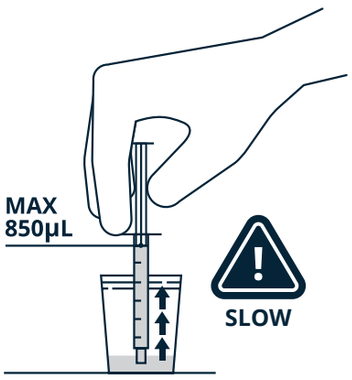
MULTI (850µL)

Sperm Separation Device | ZMH0850

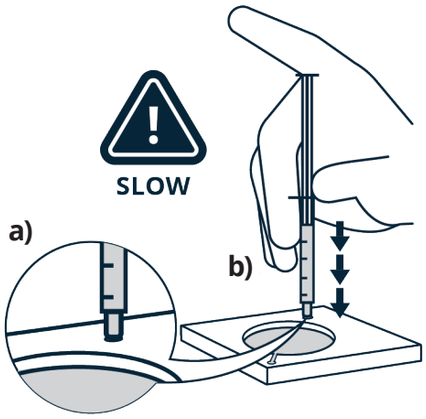
INSTRUCTIONS FOR USE



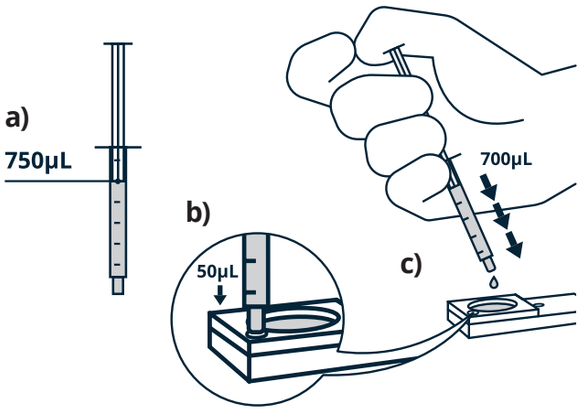
1 Allow sample to liquify



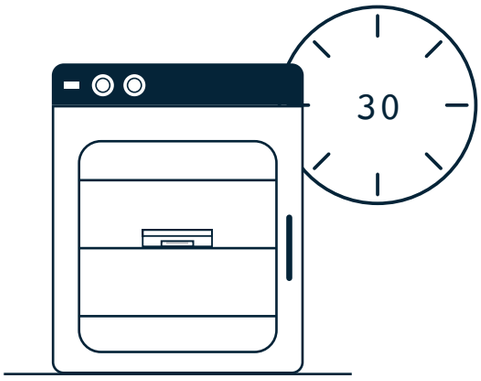
2 Draw 850µL of the sample.



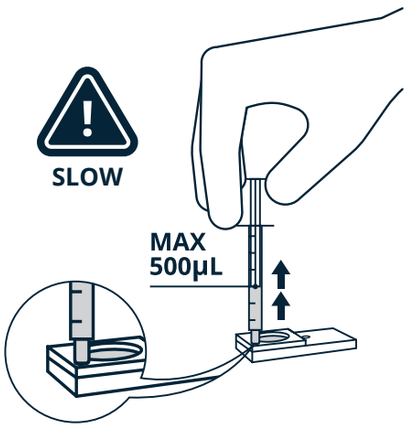
3 **a)** Achieve seal. **b)** Slowly inject sample.



4 **a)** Draw 750µL of media. **b)** Prime outlet channel. **c)** Cover membrane surface.



5 Incubate at 37°C for 30 minutes.

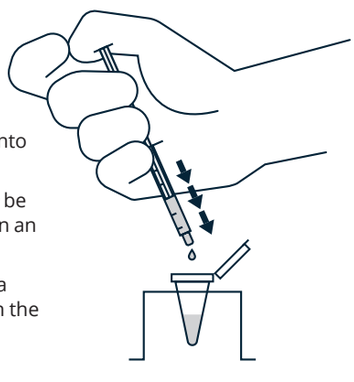


6 Slowly aspirate a maximum of 500µL

ICSI and IUI
Transfer the collected sample to an appropriate culture tube: a 4mL round bottom culture tube with a snap top or into the bottom of a 15mL conical tube.

Tubes using HEPES-buffered media may be held on the benchtop or tightly capped in an incubator.

Tubes using bicarbonate-buffered media should be st ored in a CO_2 incubator with the lid loosely closed.



7 Sample Handling After Collection – **ICSI** and **IUI**

IVF
Transfer the collected sample into a 15ml conical tube.

Add 3ml of bicarbonate-containing media (whatever media is usually used for the final suspension of sperm for conventional insemination) to the conical tube. Mix gently.

Centrifuge the conical tube for 5min at 300 x g.

Remove the supernatant, being careful to not disturb the lower pellet.

Perform count and motility as usual and dilute if needed to achieve appropriate final insemination concentration.

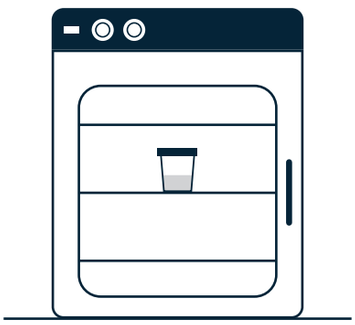
Store tube in a CO_2 incubator until insemination. Insemination should occur more than 1hr, but less than 4hrs after preparation.

7 Sample Handling After Collection – **IVF**

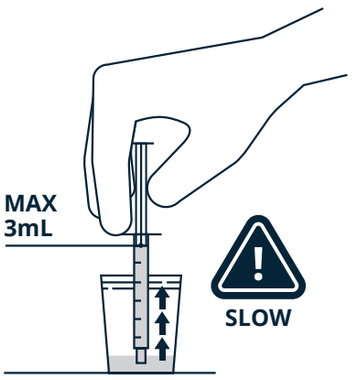
ZyMōt®
MULTI (3mL)

Sperm Separation Device | ZMH3000

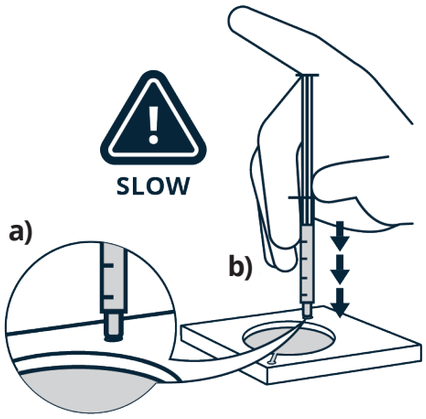
INSTRUCTIONS FOR USE



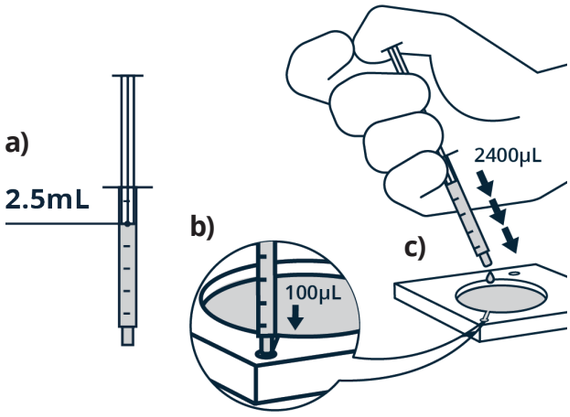
1 Allow sample to liquify



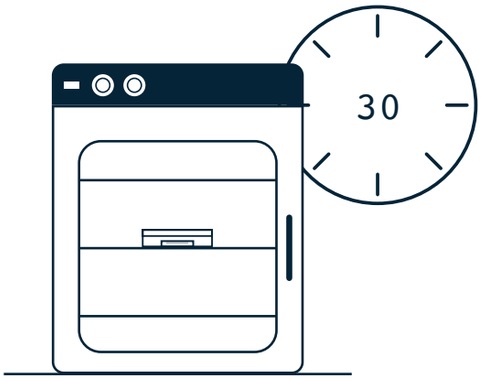
2 Draw 3mL of the sample.



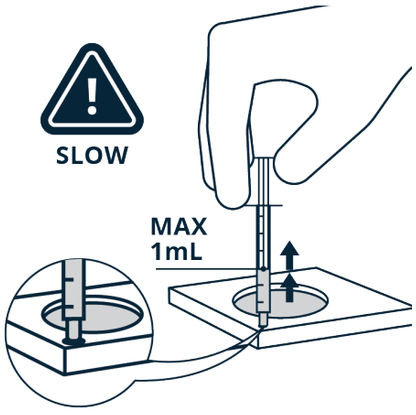
3 **a)** Achieve seal. **b)** Slowly inject sample.



4 **a)** Draw 2.5mL of media. **b)** Prime outlet channel. **c)** Cover membrane surface.



5 Incubate at 37°C for 30 minutes.

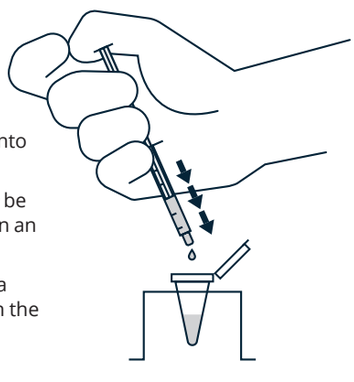


6 Slowly aspirate a maximum of 1mL.

ICSI and IUI
Transfer the collected sample to an appropriate culture tube: a 4mL round bottom culture tube with a snap top or into the bottom of a 15mL conical tube.

Tubes using HEPES-buffered media may be held on the benchtop or tightly capped in an incubator.

Tubes using bicarbonate-buffered media should be st ored in a CO_2 incubator with the lid loosely closed.



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7 Sample Handling After Collection – **IVF**