## CLINICAL STUDY PROTOCOL

# A Sibling Oocyte Study- Comparison of ZyMotTM Microfluidics Device to Density Gradient for Sperm Selection During ICSI

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# Synopsis

**Primary Objective**
The primary objective of this study is to evaluate whether the percentage of good quality embryo formation following ICSI is improved with the use of ZyMot method of microfluidic sperm separation compared to density gradient.

**Secondary Objectives**
The secondary objectives will be to evaluate:

1. Sperm parameters prior to and following sperm separation with ZyMot compared to density gradient
2. Normal fertilization rates following ICSI comparing sperm separation with ZyMot with density gradient
3. Euploid rates, in the event that PGT-A was performed, following ICSI comparing sperm separation with ZyMot with density gradient
4. Embryo transfer results including:
5. Not pregnant
6. Biochemical pregnancy, defined as BHCG value > 5 mIU/ml without ultrasound evidence of gestational sac within the uterus
7. Clinical miscarriage, defined as loss of a pregnancy following a positive BHCG value and ultrasound revealing presence of a gestational sac within the uterus
8. Clinical pregnancy, defined as positive BHCG with a gestational sac within the uterus
9. Ongoing Pregnancy, defined as a positive BHCG value with ultrasound evidence of intrauterine gestational sac at or past 8 weeks gestation
10. Live birth defined as birth of a baby at 20 weeks or later, showing signs of life, including breathing, beating of the heart, pulsation of the umbilical cord, or definite movement of the voluntary muscles.
11. Ectopic Pregnancy, defined as positive BHCG and presence of pregnancy outside of the uterus.

**Study Duration**
The study will be completed once 408 oocytes are randomized, which is anticipated to be in approximately 1-2 years.

**Study Design**
Subjects meeting inclusion criteria will be approached for inclusion into the study and informed consent will be obtained from the patient and the sperm source. Controlled ovarian hyperstimulation will be performed followed by oocyte retrieval as per our standard protocol. On the day of oocyte retrieval, sperm samples will be split into two samples and processed via density gradient (our standard sperm separation technique) and ZyMot (our experimental arm). Sperm parameters will be evaluated pre and post-sperm separation following ZyMot and density gradient. Following oocyte retrieval, the oocytes will be assessed as per our standard fashion. In the event that the oocytes will be inseminated via intracytoplasmic sperm injection (ICSI) and there are 6 or more mature oocytes, half of the oocytes will be inseminated via ZyMot processed sperm and half with sperm processed via density gradient. In the event that there are less than 6 mature oocytes or the insemination technique will be IVF, the oocytes will only be inseminated with sperm processed via density gradient. Additionally, in the event that the sperm sample is insufficient for ZyMot processing (<0.5 million total motile sperm and/or no sperm observed with progressive forward motility), the sperm will not be processed with ZyMot and the patient will be withdrawn from the study.

The primary outcome will be percentage of good quality embryo formation resulting from oocytes inseminated following ZyMot separated sperm compared to density gradient separated sperm. Secondary outcomes will include percentage of euploid embryos in the

event that PGT-A is performed, pregnancy rates, miscarriage rates, live birth rates, and sperm parameters following sperm processing. Decision to transfer an embryo will be made as per our standard protocols regardless of the sperm processing technique.

**Study Population**
The study population will include patient/sperm donor dyads where all patients over 18 years of age are undergoing controlled ovarian hyperstimulation for IVF with ICSI using ejaculate sperm and having at least 6 mature oocytes retrieved. The sperm source/donor will provide informed consent for the use of their sperm sample in this research study. The relationship of the sperm source will can be the intended father, a known sperm donor in a same-sex couple or a known donor in the case of a single patient. The identity of the donor cannot be anonymous.

**Number of Participants**
Following power calculation, 408 oocytes will be needed to randomize (see details in the statistics section). The number of patient/sperm source dyads enrolled will depend on the number of oocytes obtained from each patient. The maximum number of subjects will be 68 (in the event that 6 oocytes were obtained from every patient enrolled in the study).

**Number of Study Sites**
This study will take place at NYU Langone Reproductive Specialists of NY.

**Primary Outcome Variables**
The primary outcome of our study is to evaluate whether good quality embryo formation is significantly improved with ICSI following sperm separation using ZyMot compared to density gradient.

**Secondary and Exploratory Outcome Variables**
The secondary objectives will be to evaluate:

1. Sperm parameters prior to and following sperm separation with ZyMot compared to density gradient
2. Normal fertilization rates following ICSI comparing sperm separation with ZyMot with density gradient
3. Euploid rates, in the event that PGT-A was performed, following ICSI comparing sperm separation with ZyMot with density gradient
4. Embryo transfer results including:
    a. Not pregnant
    b. Biochemical pregnancy, defined as BHCG value > 5 mIU/ml without ultrasound evidence of gestational sac within the uterus
    c. Clinical miscarriage, defined as loss of a pregnancy following a positive BHCG value and ultrasound revealing presence of a gestational sac within the uterus
    d. Clinical pregnancy, defined as positive BHCG with a gestational sac within the uterus
    e. Ongoing Pregnancy, defined as a positive BHCG value with ultrasound evidence of intrauterine gestational sac at or past 8 weeks gestation
    f. Live birth, defined as birth of a baby at 20 weeks or later, showing signs of life, including breathing, beating of the heart, pulsation of the umbilical cord, or definite movement of the voluntary muscles.
    g. Ectopic Pregnancy, defined as positive BHCG and presence of pregnancy outside of the uterus.

# Abbreviations

| Abbreviation | Explanation |
|---|---|
| ART | Assisted reproductive technologies |
| DNA | Deoxyribonucleic acid |
| ICSI | Intracytoplasmic sperm injection |
| IVF | In vitro fertilization |
| PGT | Preimplantation genetic testing |
| PGT-A | Preimplantation genetic testing for aneuploidy |
| PI | Principle investigator |
| RSOFNY | Reproductive Specialists of New York |

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# 1 - Introduction

## 1.1 Introductory Statement

The primary objective of this study is to evaluate whether the percentage of good quality embryo formation following ICSI is improved with the use of ZyMot method of microfluidic sperm separation compared to density gradient.

# 2 - Background

## 2.1 Background/prevalence of research topic

Sperm integrity and function is critical to embryo formation and IVF success rates. Male factor infertility accounts for approximately 1/3 of infertility diagnosis and is the single most common diagnosis for IVF cycles in the US. During an IVF cycle, oocytes retrieved are inseminated with sperm via IVF or ICSI. IVF is the process by which approximately 50,000-100,000 motile sperm are incubated with one or more oocytes, whereas ICSI is the process of selecting and injecting a single sperm into an oocyte. ICSI was originally developed to treat male factor infertility, however, its use has been greatly expanded in ART including insemination of cryopreserved oocytes, previous fertilization failure or poor fertilization results following IVF, unexplained infertility, in vitro maturation, cases of PGT when paternal contamination is of concern, and other clinically relevant indications at the physician's discretion (1). Selection of healthy sperm for use in ICSI is critical to ICSI success. Density gradient is a commonly used method to select sperm for ICSI, which depends on centrifugation, and is the standard of care for sperm processing prior to ICSI in our institution. This technique requires mechanical and chemical processing that can increase oxygen free radical formation, which can cause sperm DNA damage and increase in DNA fragmentation (2, 3). Although conflicting data exists as to whether elevated levels of sperm DNA fragmentation compromises live birth rates in assisted reproductive technology (ART) treatments, some data has revealed that increased sperm DNA fragmentation decreases fertilization rates, decreases progression to good quality embryos, decreases pregnancy rates, and increases miscarriage rates (4-10). Studies have shown elevated levels of sperm DNA fragmentation in infertile males with abnormal semen parameters (11-13), however, interestingly data has revealed that increase levels of DNA fragmentation can also be found in infertile males with normal semen parameters (14).

Separation of sperm with microfluidics prior to ART resembles the physiologic environment that sperm encounters in the female reproductive tract more closely compared to density gradient, and has been proposed to decrease sperm DNA fragmentation. Indeed, separation via ZyMot, a microfluidic technique has been shown to significantly decrease sperm DNA fragmentation compared to the traditional density gradient technique (15). In a non-randomized study with a small sample size enrolling couples with recurrent ART failure and male factor infertility with high sperm defragmentation, outcomes with density gradient versus microfluidic sperm separation using ZyMot were compared and revealed that embryo euploid rates following PGT-A and pregnancy rates were significantly improved with the use of ZyMot (16). A retrospective study evaluating pregnancy rates using ZyMot sperm separation compared to density gradient for intrauterine insemination (IUI) revealed significantly higher ongoing pregnancy rates with use of ZyMot compared to density gradient (17). Additionally, a prospective randomized study evaluating ZyMot sperm separation with the swim up technique for patients undergoing ICSI due to unexplained infertility revealed a significant increase in good quality embryo formation with ZyMot sperm separation, however, this study included a significant proportion of embryos evaluated and transferred at day 3 or 4 as opposed to day 5, which is our standard of care (18).

The aim of our study is to evaluate whether good quality embryo formation is significantly different following ICSI comparing sperm separated using ZyMot with density gradient.

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### 2.2.1 Device Preclinical Experience

The ZyMot Multi Sperm Separation Device (850µl) is used to prepare motile sperm for use in ART. ZyMot separates motile sperm from the semen based on the motility of the sperm within a microenvironment. The ZyMot Multi sperm separation device has an inlet port for applying the semen sample to the lower sample chamber and an outlet port for collecting the motile sperm from the upper collection chamber. The chambers are separated by a microporous filter or membrane. After incubation at 37C for 30 min, the culture medium containing motile sperm is collected from the outlet port.

The device is made from Polymethylmethacrylate, borosilicate glass, flash-spun high-density polyethylene fibers. The devices are gamma radiation-sterilized devices with a sterility assurance level (SAL) of 10-6. They are individually packaged and for single-use only.

This device has been FDA approved for our proposed use (see attached).

### 2.2.2 Device Clinical Experience

Separation via ZyMot has been shown to significantly decrease sperm DNA fragmentation compared to the traditional density gradient technique (15). In a non-randomized study with a small sample size enrolling couples with recurrent ART failure and male factor infertility with high sperm defragmentation, outcomes with density gradient versus microfluidic sperm separation using ZyMot were compared and revealed that embryo euploid rates following PGT-A and pregnancy rates were significantly improved with the use of ZyMot (16). A retrospective study evaluating pregnancy rates using ZyMot sperm separation compared to density gradient for intrauterine insemination (IUI) revealed significantly higher ongoing pregnancy rates with use of ZyMot compared to density gradient (17). Additionally, a prospective randomized study evaluating ZyMot sperm separation with the swim up technique for patients undergoing ICSI due to unexplained infertility revealed a significant increase in good quality embryo formation with ZyMot sperm separation, however, this study included a significant proportion of embryos evaluated and transferred at day 3 or 4 as opposed to day 5, which is our standard of care (18).

# 3 - Rationale/Significance

### 3.1 Problem Statement

Commonly used methods for separating and processing sperm prior to ICSI involves mechanical and chemical processing such as density gradient separation. Density gradient sperm separation can increase sperm oxygen free radicals and DNA fragmentation. Sperm oxygen free radicals and DNA fragmentation can adversely affect sperm quality which can adversely affect ART success rates. Sperm separation via microfluidics separates sperm in a more physiologic fashion and has been shown to decrease DNA fragmentation as compared to the density gradient.

### 3.2 Purpose of Study/Potential Impact

We intend to evaluate whether formation of high-quality embryos following ICSI are improved with ZyMot microfluidic sperm separation compared to density gradient. Potential impact of the study could be that good quality embryo formation is improved with ZyMot compared with density gradient, which could have a positive impact on ART success rates.

### 3.3.1 Potential Risks

Potential risks of the study include the possibility that good quality embryo formation may not be improved with the use of ZyMot or may be inferior compared to density gradient. Inferior embryos could negatively impact the ability of the embryo to lead to yield a liveborn fetus. To our knowledge, existing literature has not revealed any detrimental effect to the quality of embryo formation with the use of the ZyMot device. Additionally, enrollment in the study can incur possible psychological distress due to study participation.

Procedures to limit psychological distress will be a thorough informed consent process, ability to discuss with any participating provider questions related to the study, and ability to withdraw enrollment at any point prior to insemination of the oocytes.

### 3.3.2 Potential Benefits

Potential benefit to study enrollment could be that good quality embryo formation is improved following sperm separation with ZyMot compared to density gradient, which could improve the participant's ART success rates.

# 4 - Study Objectives

### 4.1 Hypothesis

We hypothesize that the percentage of good quality embryo formation following ICSI will be improved with sperm separated via ZyMot compared to density gradient.

### 4.2 Primary Objective

The primary objective of this study is to evaluate whether the percentage of good quality embryo formation following ICSI is improved with the use of ZyMot method of microfluidic sperm separation compared to density gradient.

### 4.3 Secondary Objectives

The primary objective of this study is to evaluate whether the percentage of good quality embryo formation following ICSI is improved with the use of ZyMot method of microfluidic sperm separation compared to density gradient.

The secondary objectives will be to evaluate:

1. Sperm parameters prior to and following sperm separation with ZyMot compared to density gradient
2. Normal fertilization rates following ICSI comparing sperm separation with ZyMot with density gradient
3. Euploid rates, in the event that PGT-A was performed, following ICSI comparing sperm separation with ZyMot with density gradient
4. Embryo transfer results including:
    a. Not pregnant
    b. Biochemical pregnancy, defined as BHCG value > 5 mIU/ml without ultrasound evidence of gestational sac within the uterus
    c. Clinical miscarriage, defined as loss of a pregnancy following a positive BHCG value and ultrasound revealing presence of a gestational sac within the uterus
    d. Clinical pregnancy, defined as positive BHCG with a gestational sac within the uterus
    e. Ongoing Pregnancy, defined as a positive BHCG value with ultrasound evidence of intrauterine gestational sac at or past 8 weeks gestation
    f. Live birth defined as birth of a baby at 20 weeks or later, showing signs of life, including breathing, beating of the heart, pulsation of the umbilical cord, or definite movement of the voluntary muscles.
    g. Ectopic Pregnancy, defined as positive BHCG and presence of pregnancy outside of the uterus.

As per our standard of care, patients will be contacted by embryology staff via phone after their expected due to date for their pregnancy outcome. Pregnancy outcomes will be recorded in the patient’s medical record.

# 5 - Study Design

### 5.1 General Design Description

This study will be a randomized sister oocyte (oocytes from the same patient within the same cycle) study at a single academic institution. By randomizing sister oocytes, we will eliminate any potential confounding variables.

### 5.1.1 Study Date Range and Duration

The anticipated study duration is anticipated to be approximately be 1-2 years following initiation of subject recruitment.

### 5.1.2 Number of Study Sites

This study will take place at NYU Langone Reproductive Specialists of NY.

## 5.2 Outcome Variables

### 5.2.1 Primary Outcome Variables

The primary objective of this study is to evaluate whether the percentage of good quality embryo formation following ICSI is improved with the use of ZyMot method of microfluidic sperm separation compared to density gradient.

Good quality embryos will be defined as blastocyst stage embryos on day 5 or day 6 of culture with an overall quality grade of good or fair.

### 5.2.2 Secondary and Exploratory Outcome Variables

The secondary outcomes will be to evaluate:

1. Sperm parameters prior and following sperm separation with ZyMot compared to density gradient.
2. Sperm parameters that will be evaluated are sperm concentration, percent motility, and grade of forward progression.
3. Normal fertilization rates following ICSI comparing sperm separation with ZyMot to density gradient. Normal fertilization will be defined as visualization of exactly two pronuclei between 16-18 hours post-insemination.
4. Euploid, aneuploid, and mosaic rates in the event that PGT-A was performed following ICSI comparing sperm separation with ZyMot to density gradient. PGT-A biopsy samples will be sent to and analyzed by Cooper Genomics.
5. Euploid embryo will be defined as 46XX or 46XY. Mosaic rates will be defined as presence of euploid and aneuploid cells and be reported as high or low mosaic. High mosaicism will be defined as embryos having 41 - 80% aneuploid cells, whereas, low mosaicism will be defined as 20 - 40% aneuploid cells.
6. Embryo transfer results including:
    a. Not pregnant
    b. Biochemical pregnancy, defined as BHCG value > 5 mIU/ml without ultrasound evidence of gestational sac within the uterus
    c. Clinical miscarriage, defined as loss of a pregnancy following a positive BHCG value and ultrasound revealing presence of a gestational sac within the uterus
    d. Clinical pregnancy, defined as positive BHCG with a gestational sac within the uterus
    e. Ongoing Pregnancy, defined as a positive BHCG value with ultrasound evidence of intrauterine gestational sac at or past 8 weeks gestation
    f. Live birth defined as birth of a baby at 20 weeks or later, showing signs of life, including breathing, beating of the heart, pulsation of the umbilical cord, or definite movement of the voluntary muscles.
    g. Ectopic Pregnancy, defined as positive BHCG and presence of pregnancy outside of the uterus.

## 5.3 Study Population

The study population will include all patient/sperm source dyads over 18 years of age undergoing controlled ovarian hyperstimulation for IVF with ICSI using ejaculate sperm from known sperm source and having at least 6 mature oocytes retrieved.

### 5.3.1 Number of Participants

As this study will be randomizing oocytes, and therefore the number of participants will depend on the average number of oocytes retrieved per patient. Following power calculation,408 oocytes are needed for randomization in order to detect a significant difference in our primary outcome. If 6 oocytes are obtained from each patient enrolled in the study, then 68 patient/sperm source dyads will be enrolled.

Based on our 2020 data, there was a total of 1,680 mature oocytes from 137 retrievals that produced 6 or more mature oocytes for ICSI insemination, from 121 different patients. The average number of mature oocytes within ICSI cycles with 6 or more mature oocytes obtained was 12 mature oocytes. Based on this data, we anticipate 138 patient cycles are needed for this study and this could include up to 138 patient/sperm source dyads.

### 5.3.2 Eligibility Criteria/Vulnerable Populations

Eligibility will be determined by treating physician, nurse practitioner, or research assistant involved in the care of the subject.

Inclusion criteria for patients will include:

1. Patient(s) over 18 years of age
2. Patient(s) capable of providing informed consent
3. Use or possible use of ICSI for oocyte insemination
4. At least 6 mature oocytes at time of insemination via ICSI

Exclusion criteria for patients:

1. Patient under 18 y/o
2. Patients not capable of providing informed consent
3. Use of IVF for insemination
4. Less than 6 mature oocytes at time of retrieval
5. Anonymous donor sperm source
6. Surgically retrieved sperm
7. Sperm sample not sufficient for use with ZyMot device

Inclusion criteria for donors:

1. Donor(s) over 18 years of age
2. Donor(s) capable of providing informed consent
3. Use of ejaculate sperm, fresh or frozen, for insemination
4. Sufficient sperm for use of ZyMot

Exclusion criteria for donors:

1. Anonymous donors

This study will not enroll vulnerable subjects; however, we routinely follow patients through their pregnancy to determine the outcome of embryo-transfer and will use the data from their standard of care follow-up calls for this study. The follow-up is considered minimal risk as it only involves data collection. No inducements, monetary or otherwise, will be offered to terminate a pregnancy. Individuals engaged in the research will have no part in any decisions as to the timing, method, or procedures used to terminate a pregnancy. Individuals engaged in the research will have no part in determining the viability of a neonate.

# 6 - Methods

### 6.1 Treatment – Device

### 6.1.1 Intended use for the device

The intended use for the ZyMot device is to separate sperm prior to ICSI insemination alongside and comparing it to sperm separation with use of density gradient.

### 6.1.2 Device administration and schedule

Patient/sperm source dyads enrolled in the study and meeting inclusion criteria will have their sperm sample prepared prior to, or near the time of, oocyte retrieval. Semen parameters will be recorded prior to separation. Semen will be split and separated via ZyMot and density gradient.

**Density gradient will be performed as follows:**

- Density gradient centrifugation will be performed using a one-layer preparation of 90% Isolate (Irvine Scientific) in 15 mL conical tubes. Semen will be layered over 1 mL of gradient and then centrifuged for 15 min at 300xg. The supernatant will be removed and discarded. The sperm pellet will be washed by mixing with Multipurpose Handling Medium Complete (Irvine Scientific) and centrifuging the sample for 5 min at 400xg. After the wash, the supernatant is removed and discarded and the pellet is re-suspended in culture medium (Continuous Single Culture-NX Complete, Irvine Scientific), assessed for sperm parameters, and held at room temperature until insemination.

**ZyMot separation using the Multi (850 uL) microfluidic separation device will be performed as follows:**

- 850 uL of untreated semen will be directly deposited into the inlet port of the ZyMotTM Multi device, followed by placement of 750 uL culture medium (Continuous Single Culture- NX complete, Irvine Scientific) in the outlet port and throughout the upper collection chamber. The device will then be incubated in a humidified 37C CO2 incubator for 30 minutes. During incubation, the healthiest and most motile sperm will swim through the microporous filter and into the upper collection chamber, where they will be recovered via the outlet port. 500 uL of the sperm sample will be removed and placed in a separate tube for analysis and insemination.
- After sperm processing, sperm parameters will be evaluated and recorded for each preparation, including sperm concentration, percent motility, and grade of forward progression. The density gradient preparation results will be recorded as Sample 1 in the electronic medical record (eMR) system gamete sheet. The ZyMot preparation results will be recorded as Sample 2 in the eMR system gamete sheet.
- Mode of oocyte insemination is ordered by the physician of record in the patient's chart. In the event that ICSI is ordered, the oocytes will be inseminated via ICSI. In the event that the physician orders ICSI per embryologist, the decision to perform ICSI will be a clinical decision based on the final prepared semen sample and/or other relevant clinical data. The sample must meet the following minimal criteria below in order to be eligible for IVF:

| Parameters | Initial Sample | Final Sample |
|---|---|---|
| Volume | ≥ 1.0 mL | ≥ 0.25 mL |
| Concentration | ≥ 15 mil/mL | NA |
| Motility | ≥ 35% | ≥ 70% |
| Forward Progression | ≥ 2 | ≥ 3 |

- In the event that any of the parameters above are not met and/or other clinical parameters favoring ICSI insemination over IVF including, but not limited to, microscopic appearance of the oocytes, review of previous insemination cycles, and previous morphology grading of sperm, ICSI will be performed.
- In the event that the parameters above are met and there are not any other factors favoring ICSI insemination, the oocytes will be inseminated via IVF from sperm separated by density

gradient and the oocytes will not be inseminated via ZyMot separated sperm, however, the pre and post sperm parameters following ZyMot separated sperm and density gradient separated sperm will be recorded as per our secondary objectives. Similarly, in the event that there are less than 6 mature oocytes, the oocytes will only be inseminated via density gradient separated sperm, however, pre and post sperm parameters following ZyMot separated sperm and density gradient separated sperm will be recorded as per our secondary objectives.

- As per our standard verbal and written consenting protocols, patients are aware that their oocytes may undergo ICSI the day of oocyte retrieval based on above criteria. The decision to perform ICSI will not be affected in any way by enrollment into this study.
- Following oocyte retrieval, oocytes will be denuded of their cumulus cells per standard protocol**.** If at least 6 mature oocytes (determined by visualization of a polar body) are present, half the oocytes will be ICSI inseminated with sperm prepared via ZyMot device and half will be ICSI inseminated with sperm prepared with density gradient. Patients whose patient identification number (PID) is even will have the group of oocytes inseminated with sperm separated via ZyMot device recorded first in the eMR gamete sheet (beginning with oocyte #1.) Patients with an odd PID will have the oocytes inseminated with sperm separated via density gradient recorded first in the eMR gamete sheet (beginning with oocyte #1.) In the event of an odd number of oocytes, the patients with even PIDs will have the extra oocyte inseminated with sperm separated via Zymot device and patients with odd PIDs will have the extra oocyte inseminated with sperm separated via density gradient. Inseminated oocytes will be grouped into separate wells of a LifeGlobal 4 well GPS dish (Cooper Surgical) by the sperm separation method — one well will contain all oocytes inseminated with sperm selected by ZyMot device and a separate well will contain all oocytes inseminated with sperm selected by density gradient centrifugation.

**Control Group of Oocytes:**
Half of the eggs will be inseminated via ICSI using sperm selected by conventional gradient centrifugation.

**Treatment Group of Oocytes:**

- Half of the eggs will be inseminated via ICSI using sperm selected using ZyMot.
- Fertilization assessment will be performed between 16 and 18 hours after insemination and normal fertilization will be considered visualization of exactly two pronuclei. Normally fertilized oocytes (embryos) will then be cultured in Continuous Single Culture Medium — NX Complete (Irvine Scientific) in separate, numbered droplets in LifeGlobal microdrop GPS dishes (Cooper Surgical.) The numbers of the droplets will correlate to oocyte/embryo number in the eMR gamete sheet and, thereby, also correlate to the type of sperm used for the insemination. Development of the embryos will be assessed on culture days 5 and 6 and embryo stage and grade will be recorded for each embryo.
- Decision as to which embryo to transfer will be made by the clinical team irrespective of type of sperm preparation for insemination. Transfer of embryo is generally based on ploidy status (if PGT-A was performed) or blastocyst grade (if PGT-A was not performed). In the event that two embryos of similar quality are available for transfer, priority is given to lowest embryo number (correlated to oocyte/embryo number). The decision as to which embryo to transfer will be made regardless of whether the sperm used to inseminate the oocyte was processed with density gradient or ZyMot and will be as per our standard protocol.

### 6.1.3 Method of Assignment/Randomization

Patient/sperm source dyads meeting inclusion criteria and enrolled in the study will have their sperm sample prepared prior to or near the time of oocyte retrieval. Semen parameters will be recorded prior to separation. Semen will be split and separated via ZyMot and density gradient.

Following oocyte retrieval, oocytes will be denuded of their cumulus cells per standard protocol**.** If at least 6 mature oocytes (determined by visualization of a polar body) are present, half the oocytes will

be ICSI inseminated with sperm separated via ZyMot device and half will be ICSI inseminated with sperm prepared with density gradient. Patients whose patient identification number (PID) is even will have the group of oocytes inseminated with sperm separated via ZyMot device recorded first in the eMR gamete sheet (beginning with oocyte #1.) Patients with an odd PID will have the oocytes inseminated with sperm separated via density gradient recorded first in the eMR gamete sheet (beginning with oocyte #1.) In the event of an odd number of oocytes, the patients with even PIDs will have the extra oocyte inseminated with sperm separated via Zymot device and patients with odd PIDs will have the extra oocyte inseminated with sperm separated via density gradient.

**6.1.4 Device Calibration**
No calibration of the device is required. Each device is single use and disposable after use.

**6.1.5 Storage Condition**
The device will be stored at room temperature within our embryology laboratory as per manufacturer protocol**.** Receipt of the device, including number of devices received, lot number(s) of the devices, open and close dates for each lot, and certificates of analysis for each lot will be recorded and/or filed. Unused devices at the conclusion of the study will be returned to the study sponsor.

**6.1.6 Concomitant therapy**
None

**6.1.7 Restrictions**
Restrictions for use of the device will include severe male factor defined as an initial ejaculate sample with <0.5 million total motile sperm and/or no sperm observed with progressive forward motility (forward progression grade of 2.) Any surgically derived sperm will be excluded from the study. Additionally, when orders are written for ICSI per embryologist, any specimen not meeting above criteria for ICSI (see section 6.1.2), will be inseminated via IVF and therefore, their oocytes will not be inseminated with ZyMot separated sperm.

**6.2 Assessments**

**6.2.1 Efficacy**
The efficacy of the device will be evaluated by our primary objective, percentage of good quality embryos available following ICSI insemination with ZyMot separated sperm compared to insemination with density gradient separated sperm.

**6.2.2 Safety/Pregnancy-related policy**
Not Applicable.

**6.2.2.1 Adverse Events Definition and Reporting**
**Definitions**
Unanticipated Problems Involving Risk to Subjects or Others Any incident, experience, or outcome that meets <u>all</u> of the following criteria: Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)

<u>Related or possibly related to participation in the research</u> (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)

Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

**Adverse Event**

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

**Serious Adverse Event**

**Adverse events are classified as serious or non-serious. A *serious adverse event* is any AE that is:**

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event
- Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

**Preexisting Condition**

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

**General Physical Examination Findings**

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

**Post-study Adverse Event**

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

**Recording of Adverse Events**

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of

the case report form. All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to study participation should be recorded and reported immediately.

Reporting of Serious Adverse Events and Unanticipated Problems

**For Narrative Reports of Safety Events**

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

| | |
|---|---|
| • Study identifier<br>• Study Center<br>• Subject number<br>• A description of the event<br>• Date of onset | • Current status<br>• Whether study treatment was discontinued<br>• The reason why the event is classified as serious<br>• Investigator assessment of the association between the event and study treatment |

Investigator reporting: notifying the IRB Federal regulations require timely reporting by investigators to their local IRB of unanticipated problems posing risks to subjects or others. The following describes the NYULMC IRB reporting requirements, though Investigators at participating sites are responsible for meeting the specific requirements of their IRB of record.

**Report promptly, but no later than 5 working days:**
Researchers are required to submit reports of the following problems promptly but no later than 5 working days from the time the investigator becomes aware of the event:

**Unanticipated problems including adverse events that are unexpected and related**
Unexpected**:** An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.

Related to the research procedures**:** An event is related to the research procedures if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.

Harmful: either caused harm to subjects or others, or placed them at increased risk

**Other Reportable events:** The following events also require prompt reporting to the IRB, though *no later than 5 working days*:

***Complaint of a research subject*** when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.

***Protocol deviations or violations*** (includes intentional and accidental/unintentional deviations from the IRB approved protocol) for any of the following situations:
one or more participants were placed at increased risk of harm
the event has the potential to occur again
the deviation was necessary to protect a subject from immediate harm

***Breach of confidentiality***

***New Information indicating a change to the risks*** or potential benefits of the research, in terms of severity or frequency. (e.g. analysis indicates lower-than-expected response rate or a more severe or frequent side effect; FDA labeling change or withdrawal from market)

**Reporting Process**
The reportable events noted above will be reported to the IRB using a Reportable New Information submission and will include a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution, and need for revision to consent form and/or other study documentation. Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

**6.3.1 Study Schedule**
Subject time devoted to this study will be the reviewing and signing the informed consent.

The timepoints for involvement in the study will be:

1. The process of informed consent for patient/sperm source dyads
2. The oocyte retrieval- After retrieval in the event that there are 6 or more mature oocytes, the couple will be enrolled into the study
3. The sperm separation- The sample will either be obtained via fresh production from sperm source the day of oocyte retrieval or will be cryopreserved sperm. The sample will be assessed the day of oocyte retrieval and if the sperm sample is sufficient, it will be processed via ZyMot and density gradient. Following processing of the sperm sample, in the event that there are 6 mature oocytes available to inseminate, the patient will be enrolled into the study. Oocytes will be randomized to insemination via density gradient separated sperm and ZyMot separated sperm.
4. In the event that the orders were written for ICSI per embryologist, if the patient does not meet criteria for IVF the day of oocyte retrieval (as per section 6.3), the oocytes will be inseminated via IVF with density separated sperm only, however, the pre and post sperm parameters following ZyMot separated sperm and density gradient separated sperm will be recorded as per our secondary objectives. Similarly, in the event that there are less than 6 mature oocytes, the oocytes will only be inseminated via density gradient separated sperm, however, pre and post sperm parameters following ZyMot separated sperm and density gradient separated sperm will be recorded as per our secondary objectives. Insemination- Following oocyte retrieval, those enrolled into the study will have their oocytes split and inseminated with ZyMot separated sperm and density gradient separated sperm.
5. Fertilization check- Approximately 16-18 hours following insemination the embryos will be assessed for fertilization.
6. Five and 6 days following insemination, the embryos will be assessed for stage of growth and those reaching blastocyst stage will be given a morphological grade.
7. In the event that patient will undergo a fresh transfer the patient will be transferred 5 days following oocyte retrieval. In the event that pre-implantation genetic testing is performed or the embryos are cryopreserved for any other reason, the patient may undergo a frozen embryo transfer in standard fashion.
8. Nine days following embryo transfer, the patient will follow up for a pregnancy test. In the event its is positive, the patient will follow up approximately 2-3 days later for a repeat pregnancy test. Depending on the value, the patient will either follow up again for a pregnancy test or follow up approximately 1 week later for an ultrasound. In the event of a normal intrauterine pregnancy, the patient is generally referred to her Obstetrician for obstetric care.

As per our standard of care, patients will be contacted by embryology staff via phone after their expected due to date for their pregnancy outcome. Pregnancy outcomes will be recorded in the patient’s medical record.

### 6.3.2 Informed Consent

Consent and other informational documents provided to participants consent forms describing in detail the study agent, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study product.

Consent procedures and documentation informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants. Consent forms will be IRB-approved and the participant will be asked to read and review the document. All discussions about this research with patient/sperm source dyads will occur in private consultation offices, exam rooms, or a secure WebEx platform. The patient/sperm source dyad will have the consent emailed to the patient/sperm source dyad to be signed during the WebEx meeting or in person. The study investigator and or research assistant will explain the study protocol and details of the patient/sperm source's role and anticipated experience during the procedure, allowing the patient/sperm sources to ask questions, which will be answered to the subject's satisfaction. The consenter will also ask questions of the prospective patient/sperm sources to assess that they have understood the information presented to them. The participants should have the opportunity to discuss the study with their relatives or think about it prior to agreeing to participate. The patient/sperm sources will sign the informed consent document prior to any procedures being done specifically for the study. The investigator or research assistant who obtained consent will sign the consent form. A signed copy of the consent form will be provided to the patient/sperm source dyad. The day of the oocyte retrieval, the sperm will be processed as explained above and used to inseminate the occytes with both sperm processed using the density gradient and ZyMot microfluidics as long as they are eligible for inclusion. Subjects can withdraw from the study at any time if they no longer wish their data to be collected and used for study purposes. However, they will be informed that if they withdraw after insemination, the insemination process cannot be reversed. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

If, during this interview, it becomes apparent that the prospective patient / sperm source dyad lacks sufficient knowledge and comprehension of the information represented by the elements of informed consent to enable them to make an informed and enlightened decision on whether or not to participate in the study, they will be excluded from participating in the study.

A copy of signed informed consent document will be obtained and stored in a binder locked in the investigators' office. The consent process, including the name of the individual obtaining consent, will be thoroughly documented in the patient/sperm source dyad's research record. Any alteration to the standard consent process (e.g., use of a translator, consent document presented orally, etc.) and the justification for such alteration will likewise be documented.

See attached informed consent.

### 6.3.3 Screening

All patients/donor dyads potentially undergoing ICSI and meeting inclusion criteria for the study will be approached to participate in the study. Screening will be performed by study investigators at NYU Langone Reproductive Specialists of NY.

### 6.3.4 Recruitment, Enrollment and Retention

Recruitment into the study will be performed by a study investigator at NYU Langone RSOFNY. Patient/sperm source dyads are recruited from the study investigators' practice (RSOFNY) during a routine clinical visit. If patient/sperm source dyads desire to enroll in the study, it will be denoted in their medical record chart. If they meet eligibility criteria on the day of oocyte retrieval (no evidence of severe male factor infertility or surgically derived sperm, at least 6 mature oocytes available for randomization, and possibility for ICSI insemination) the study will be performed as per study protocol.

In the event that the patient consented to the study but the provided semen sample on the day of oocyte retrieval shows severe male factor infertility. The patient/sperm source dyad will then be withdrawn from the study since the sample will not qualify for preparation via ZyMot nor density gradient.

In the event that the patient/sperm source dyad consented to the study but does not meet the inclusion criteria of ICSI insemination or at least 6 mature oocytes available for insemination, insemination will occur only with the sperm separated via density gradient, however, the sperm parameters following ZyMot and density gradient sperm separation will be recorded. The data on sperm parameters after sperm separation via ZyMot and density gradient will still be collected but the patient/sperm source dyad will have no further participation in the study.

**6.3.5 On Study Visits**
Not applicable.

**6.3.6 End of Study and Follow-up**
If the patient/donor dyad is enrolled into the study, the primary and secondary data endpoints will be documented as they become available.

No further follow up for those subjects withdrawn from the study will be needed as they will undergo ART procedures as per our standard of care.

**6.3.7 Removal of subjects**
Following enrollment into the study, the patient/sperm source dyads may withdraw from the study at any point prior to insemination of the oocytes.

In the event that the patient/sperm source dyad consented to the study but the provided semen sample on the day of oocyte retrieval shows severe male factor infertility as per above criteria, the patient/sperm source dyad will be withdrawn from the study since the sample will not qualify for preparation via ZyMot nor density gradient.

In the event that the patient/sperm source dyad consented to the study but does not meet the inclusion criteria of ICSI insemination or at least 6 mature oocytes available for insemination, insemination will occur only with the sperm separated via density gradient, however, the sperm parameters following ZyMot and density gradient sperm separation will be recorded. The data on sperm parameters after sperm separation via ZyMot and density gradient will still be collected but the patient/sperm source dyad will have no further participation in the study.

**6.4 Statistical Method**
**6.4.1 Statistical Design**
All data will be de-identified and then will be analyzed by a statistician. Descriptive statistics of the study sample will be calculated (mean ± standard deviation or median [$25^{th}$, $75^{th}$ percentiles] for continuous variables; frequency and percent for categorical data. Paired analyses will be performed. A result will be considered statistically significant at the $p<0.05$ level of significance. All analyses will be performed using SAS version 9.4 (SAS Institute Inc., Cary, NC).

**6.4.2 Sample Size Considerations**
For a two-tailed McNemar's test with alpha level 0.05, the following relation yields an approximate power of 80% to detect a difference *d* between proportions $p_1$ and $p_2$:**$n = 16pq\ (1 - r)/d^2$** where n is the size of the sample (i.e. number of eggs), $p = (p_1 + p_2)/2$, $q = 1 - p$, $d = |p_2 - p_1|$, and r is the phi

correlation coefficient[1].We will assume a conservative estimate for the correlation r=0.5. Assuming a 48% good quality blastocyst development rate and a 7% improvement in good quality blastocyst development with ZyMoT, we would need a total of 408 oocytes randomized to either density gradient or ZyMoT separation, which would equate to 68 patient/sperm source dyads each with ≥6 eggs retrieved.

### 6.4.3 Planned Analyses

#### 6.4.3.1 Primary Analyses

Percentage of good quality embryo formation between control and experimental arm will be analyzed using McNemar's test.

#### 6.4.3.2 Secondary Objectives Analyses

Sperm parameters following ZyMot and density gradient sperm separation, as well as Euploid rates in the event that PGT-A was performed following ICSI comparing sperm separation with ZyMot to density gradient, will be assessed using the paired t-test, Wilcoxon signed rank test, ora generalized linear mixed model (GLMM). A random subject-specific intercept and an unstructured correlation structure will be used to account for within-subject correlation between rates between the two groups. Data will be reported as least squares means with corresponding 95% confidence intervals.

Pregnancy outcomes will be assessed using McNemar's test. The difference in good quality blastocyst development rate and pregnancy outcomes between the ZyMot and density gradient separation groups will also be analyzed separately using a conditional logistic regression model with generalized estimating equations to account for both correlation between observations from the same patient and the size of the cohort of eggs from each patient.

#### 6.4.3.3 Safety/Pregnancy-related policy

Not applicable.

#### 6.4.3.4 Analysis of Subject Characteristics

Subject characteristics will be documented including female age, male age, infertility diagnosis, stimulation protocol, medications used, gonadotropin dosage, method of trigger, infertility diagnosis, sperm parameters prior to sperm separation, gravity, parity, and previous pregnancy outcomes.

#### 6.4.3.5 Interim Analysis

An interim analysis will be performed at the midway point of oocyte recruitment. Interim analysis will evaluate percentage of good quality embryo formation. Study will be terminated in the event that there is a significant decrease in good quality embryo formation in the study arm compared to the control arm.

#### 6.4.3.6 Health economic evaluation

Not applicable.

#### 6.4.3.7 Other

Not applicable.

### 6.4.4 Subsets and Covariates

This study will be randomizing sister oocytes (oocytes from the same patient within the same cycle) and thus limiting the impact of confounding variables.

### 6.4.5 Handling of Missing Data

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[1] Lehr, R. G. 2001. Some practical considerations and a crude formula for estimating sample size for McNemar's test. Drug Information Journal, 35: 1227–1233.

Missing data will be maintained as missing in the analysis datasets, unless specified otherwise. If it is deemed that imputing data is necessary, the analysis dataset will contain a new variable with the imputed value and the original variable value will be maintained as missing.

# 7 - Trial Administration

### 7.1 Ethical Considerations: Informed Consent and HIPAA Authorization

The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. Informed consents will be scanned into the patient's chart and hard copy will be stored in a locked file draw/cabinet at NYU Langone RSOFNY. The PI and sub-investigators will have access to the locked storage unit.

Data will be stored using codes assigned by the investigators in an excel spreadsheet. The data will be collected and stored on REDCap. Only investigators will have access to the samples and data.

There is not any foreseeable possibility that a previously unknown condition (disease, genetic disposition, etc.) will be discovered as the result of the study procedure.

Additional information as a result of this study will be added to the patient's medical record and include the use of ZyMot to process the sperm specimen and post-processing sperm parameters.

### 7.2 Institutional Review Board (IRB) Review

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form will be obtained prior to enrolling any participants. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved.

### 7.3 Subject Confidentiality

All data will be stored and analyzed in accordance with NYU's policy on retention of and access to research data. Potential identifiable information (patient and cycle ID) will be recorded as a numerical number. All research data will be maintained in tangible form and will be retained by the principal investigator and other researchers in confidential and secure storage space owned or controlled by NYU Langone Health and approved by NYU Langone Health's Medical Center Information Technology (MCIT) department. The data will be stored for 6 years after manuscript completion. It will be destroyed by deleting it from the storage space. All Research Data containing protected health information will be maintained in accordance with New York State laws and regulations.

All hard copies of the informed consents will be stored in a locked file cabinet at the study site and only the investigators will have access to the cabinet.

### 7.4 Deviations/Unanticipated Problems

Protocol deviations are any noncompliance with the clinical trial protocol. The deviation may be either on the part of the participant, the investigator, or the study site staff.

It will be the responsibility of the principal investigator to use continuous vigilance to identify and report deviations promptly from the time the investigator becomes aware of the event. All protocol deviations will be addressed in study source documents, and reported to the sponsor and to the local IRB per the guidelines.

### 7.5 Data Collection

Data collection will be the responsibility of the study staff at the site under the supervision of the site PI. The investigator will be responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. Information pertinent to this project will be available in the electronic

medical records, clinic records, and a subject datasheet. A study investigator will record demographic data and related medical data on the subject datasheet.

All data will be stored and analyzed in accordance with NYU's policy on retention of and access to research data. Potential identifiable information (patient and cycle ID) will be recorded as a numerical number. All research data will be maintained in tangible form and will be retained by the PI and other researchers in confidential and secure storage space owned or controlled by NYU Langone Health and approved by NYU Langone Health's Medical Center Information Technology (MCIT) department. The data will be stored for 6 years after manuscript completion. It will be destroyed by deleting it from the storage space. All Research Data containing protected health information will be maintained in accordance with New York State laws and regulations.

All hard copies of the informed consents will be stored in a locked file cabinet at the study site and only the investigators will have access to the cabinet.

**7.6 Data Quality Assurance**
The study investigators will be responsible for implementing and maintaining quality assurance and quality control systems to ensure that the trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirement(s).

The study investigators will be responsible for securing agreement from all involved parties to ensure direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by domestic and foreign regulatory authorities.

Quality control will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

Agreements, made by the sponsor with the investigator/institution and any other parties involved with the clinical trial, will be in writing, as part of the protocol or in a separate agreement.

**7.7 Study Records**
Study records will include patient consents, protocols, electronic medical records, and data related to the study.

**7.8 Access to Source**
Access to study records will be limited to IRB-approved members of the study team. All data will be stored and analyzed in accordance with NYU's policy on retention of and access to research data. Potential identifiable information (patient and cycle ID) will be recorded as a numerical number. All research data will be maintained in tangible form and will be retained by the PI and other researchers in confidential and secure storage space owned or controlled by NYU Langone Health and approved by NYU Langone Health's Medical Center Information Technology (MCIT) department. The investigator will permit study-related monitoring, audits, and inspections by the IRB/EC, the sponsor, government regulatory bodies, and university compliance and quality assurance groups of all study related documents (e.g., source documents, regulatory documents, data collection instruments, study data etc.). Data may be shared with the sponsor of the study; however, data will be de-identified prior to sharing the data.

**7.9 Data or Specimen Storage/Security**
All data will be stored and analyzed in accordance with NYU's policy on retention of and access to research data. Potential identifiable information (patient and cycle ID) will be recorded as a numerical number. All research data will be maintained in tangible form and will be retained by the PI and other researchers in confidential and secure storage space owned or controlled by NYU Langone Health and approved by NYU Langone Health's Medical Center Information Technology (MCIT) department.

All Research Data containing protected health information will be maintained in accordance with New York State laws and regulations.

All hard copies of the informed consents will be stored in a locked file cabinet at the study site and only the investigators will have access to the cabinet.

**7.10 Retention of Records**
All data will be kept for 6 years following completion of the study. If permission is needed to move or destroy the records, it will be the PI's responsibility.

**7.11 Study Monitoring**
Halfway through recruitment of sample size, preliminary results will be analyzed to by the principal investigator and co-investigators. Information related to the study will be periodically evaluated for accuracy by one or more of the study investigators.

**7.12 Data Safety Monitoring Plan**
Halfway through recruitment of sample size, preliminary results will be analyzed by the principal investigator and co-investigators. If the primary endpoint is adversely affected by study arm, the study will be terminated. The primary endpoint will be percentage of good quality embryos comparing those inseminated with density processed sperm and ZyMot processed sperm. In the event that there is a statistically significant ($p<0.05$) percentage of higher quality embryo formation in the embryos created via sperm inseminated with density processed sperm compared to ZyMot processed sperm, the study will be terminated. Preliminary analysis of the results will be communicated promptly to the IRB.

**7.13 Study Modification**
If at any points study modifications are to be made, the protocol will be updated and changes submitted to the IRB. Changes to the protocol will be implemented once approved by the IRB.

**7.14 Study Discontinuation**
In the event that preliminary analysis reveals significantly reduced primary outcome in the experimental arm, the study will be discontinued.

**7.15 Study Completion**
Study will be completed when 408 oocytes are randomized which is anticipated to be in approximately 1-2 years from study commencement.

**7.16 Conflict of Interest Policy**
Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) will have the conflict reviewed by the NYU Langone Conflict of Interest Management Unit (CIMU) with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All NYULMC investigators will follow the applicable conflict of interest policies.

**7.17 Funding Source**
The study will be funded by DxNow Inc, manufacturer of the ZyMot device.

**7.18 Publication Plan**
Publication procedures and authorship will be addressed in the Clinical Trials Agreement for this study.