Frequently Asked Questions of the ART Laboratory
INTRODUCTION

Welcome to the Center for Reproductive Medicine (CRM) at the University of Michigan and the services of the Assisted Reproductive Technologies (ART) Laboratory. As lab scientists, we most often work behind-the-scenes but want to take this chance to say hello and welcome. Please know that we are proud to be involved in your care and will do everything we can to make your visit pleasant. We do not take for granted the trust you have placed in us and we pledge to deliver high quality, state-of-the-art service as you start or expand your family.

About the lab

The ART program was founded at the University of Michigan in 1984. However, the CRM moved from the main campus hospital in 2005 to improve service and patient care. This move enabled the CRM and the ART lab to expand and upgrade its complex, which contains the most state-of-the-art facilities available. Each staff member works hard and is specially trained with many years of experience.

Rest-assured you are receiving top quality care. We are certified by the federal government, under the Clinical Laboratory Improvement Amendment (CLIA) and by the College of American Pathologists Laboratory Accreditation Program (CAP) for ART laboratories. Additionally, we are inspected by the FDA and the CRM is a member program of the Society for Assisted Reproductive Technology (SART) and, thus follows rules set forth by the American Society for Reproductive Medicine (ASRM) for IVF programs. All lab work is backed by ongoing quality improvement and control programs that ensure the safety of our patients.

About your care

Your treatment will be most effective when trust is formed between you, the clinical staff and the lab. This relationship requires knowledge of both your diagnosis and the reason for the treatment plan we have for you. Does this mean you must have in-depth knowledge of science of your condition and treatment? No, not at all. That, too, can become a burden. And, after all, that is our job. But you do need enough knowledge about your therapy to develop the trust that is needed for success. The lab is a crucial part of ART and we want you to know and trust this aspect of your care in the CRM. That is why we wrote this booklet - to give answers to you directly. It contains the most asked questions we encounter from our patients.

This book was developed “in-house”, based upon the educational needs of the patients of the CRM at the University of Michigan. It is designed as an educational tool to help you take an active role in your treatment, to avoid late-night worries, to give you direct access to the lab and to help improve your care.

How do I use this book?

The booklet was written in a question-and-answer format for one reason - so you do not have to read the whole book to find an answer to your question. Simply scan the table of contents, find the general subject, go to the correct page and look at the questions and answers under that topic. Even if you do not see your specific question in the table of contents, read over the general area of interest - the answer might be there. If not, you may still find enough facts to let you to answer your own question. The answers in this book reflect our opinions based upon our research, interactions with others in the field, and our own experience with IVF and other ART. Clearly, ART is a young field and many questions have no concrete answers. Some answers and opinions here may differ from others clinics and labs. Please keep this in mind - our answers can and, sometimes, will be different from what you have heard or read elsewhere. It is important, that this not upset or distract you. Reproductive medicine is too new to establish absolutes. In fact, one of the most important aspects of improving our pregnancy outcomes and your care has been a willingness to change our thinking when new scientific data and treatments emerge.

What if I want more information - Is the Internet a good source?
The internet is rich source of information, but not always an accurate one. Although some of the material is good, the information is not reviewed and often does not come from trusted sources. Much of it is word-of-mouth from various online chat rooms or forums. Though the source of some of these "facts" may be well meaning patients, they are sometimes misinformed. A key thing to keep in mind is that not all patients and cases are the same, even if they may appear to be similar on the surface. Thus, it is very hard to compare or draw any answers from comments online. If you would like more education material on a given subject, please contact us and we would be happy to provide or suggest better sources. Some good websites include:

http://www.reproductivefacts.org
http://www.sart.org/ARTPatients.html

What do I do if the answer is not here?

If you have a question and cannot find the answer here, you can ask doctor or nurse here and if they cannot answer the question directly, they will contact the lab.

We want the best outcome possible for you - please let us know how we can be of better service to you. Thank you for allowing us to help in your care.

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**Glossary**

**Abstinence** – period of time without ejaculating. Includes intercourse and masturbation.

**Blastocyst** – Advanced stage of embryo development characterized by differentiation of 2 cell types (Inner cell mass and trophectoderm) and formation of a fluid filled cavity.

**Diploid** – having the normal 46 pairs of chromosomes (2N)

**Ejaculation** – emission of semen from the penis

**Embryo** - formed following fertilization, or joining of egg and sperm

**Follicle** - small sac found on the ovary that hold the egg

**Gamete** - term for haploid cells, either sperm or egg

**Haploid** – having half the number of normal chromosomes (1N)

**ICSI** – (intracytoplasmic sperm injection) manual placement of 1 sperm into an egg to promote fertilization using a small glass needle and microscope

**ICM** – Inner cell mass. One of two cells types in the blastocyst. These cells are on the interior of the blastocyst and form the fetus with further development.

**Oocyte** – female gamete found within the ovary, also known as the egg

**Ovary** – Organ in the female that produces eggs and hormones

**Semen** – white fluid and included cells expelled during ejaculation

**Spermatozoa** - (sperm) male gamete produced in the testicle and usually present in semen

**Testicle** - male reproductive organ responsible for producing and storing sperm as well as hormones

**Trophectoderm** - one of two cells types in a blastocyst. These cells become tissues, such as the placenta with further development.

**Vitrification** - rapid cooling or freezing used to preserve oocytes and embryos

**Zona Pellucida** - Outer shell of the oocyte or embryo

**Zygote** – term used for the diploid cell formed following fertilization
# FREQUENTLY ASKED QUESTIONS

of the
Assisted Reproductive Technologies Laboratory
within the
Center for Reproductive Medicine
at the
University of Michigan

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**ANDROLOGY**

**SEmen Analysis:**

*The following are general questions regarding semen analysis:*

**Why do I need a semen analysis?**
Male infertility accounts for approximately one half of all infertility. The semen analysis is the single, most effective tool we have to assess men for fertility. It is also the most cost effective.

**When should I have a semen analysis?**
At the beginning of an evaluation of a couple for infertility. It needs to be done even if the man has had children in the past. Likewise, it should be done even when it is known that the woman has fertility problems. It is estimated that 10-15% of the time, both partners have fertility problems and both partner’s problems must be addressed.

**How often do I need a semen analysis?**
We require that you have a semen analysis performed by our lab sometime within 3 months before the start of your treatment. We require the sample be tested by our lab to ensure we have a current and accurate result.

**Can the results change with time?**
Yes, the results of the semen analysis can change from test to test. This can be due to different periods of abstinence (the time interval between intercourse or ejaculations), certain drugs, illness, trauma/blockage in the reproductive tract and natural changes in sperm count seen over the course of a year. However, if the test is done after a set period of abstinence (3-5 days), the results are similar most of the time.

**How do you keep track of my sample in the lab to ensure that the results are mine?**
Each sample is tracked with both patient name and a unique ID number. This means that every lab item, data sheet and report is labeled with these unique IDs. We also have a “chain of custody”, meaning we record who did each step of the test in the event that questions arise about the results. By law, we keep records of results for a two-year period and we can recall these results by name and date or by the ID number.

**Questions regarding collection of the semen sample:**

**What is meant by “abstinence”, and why is it important?**
Abstinence is most often thought of as the time you wait between sex. It really refers to the time between ejaculations in the male, which is measured most often as the time between intercourse, but also includes masturbation. We need to know how long it has been since you had ejaculation prior to giving us the semen sample for testing. An abstinence period that is too long or too short can have a negative impact on the semen sample. Waiting too long is as bad as not waiting long enough. The ideal abstinence period for most men is three days. If a semen analysis is ordered for Friday, you should have intercourse three days before. Do not feel that this must be exact - Monday night or Tuesday morning are both fine. Even two days or four days will be fine. However, one day is usually not enough and a week is too long.
How does the abstinence period affect semen quality?
A short abstinence period will lower the sperm count, but may increase the number of sperm that are swimming, which is known as motility. In some cases, you will be asked to have a short abstinence period to check for motility. In other cases, we may need to try to obtain more sperm, so we may ask you to observe a longer than normal abstinence period. When abstinence is too long, several properties of the sample degrade.

Is the container I use to collect the specimen important?
Yes. Using the wrong container used to collect the specimen can negatively affect sperm. We will give you specific cups that we have tested to ensure there is no toxicity to sperm.

How must I collect the sample?
Masturbation. We realize that this causes discomfort and embarrassment to some people. Please do not be worried about collecting a sample at the clinic. This is a common procedure and there is a private room available in our clinic. It is also the best chance that all of the sample will be collected and that the quality of the sperm will not be lowered. It is important that no lotions or gels be used during collection - these will have adverse effects on the sperm. Do not let water, soap or other items come in contact with the cup or sample. If it is not possible to collect by masturbation due to religious or other reasons, a special non-toxic condom can be used during intercourse. If you choose to collect at our office, the woman can join the man in the collection room. You can also collect at home if desired and bring the sample to us within 30 minutes.

Can I use a lubricant?
In most cases, no. Many lotions are toxic to sperm. If you must use a lubricant to collect, we can supply you with a special liquid to be used at the clinic that has been proven safe for sperm.

Are there special concerns in transporting the sample?
The major concern is if the specimen is collected at home or offsite. It is important that it not be exposed to high or low temperature and that the sample arrives at the lab within 30 minutes. Over time, the number of sperm that swim and the quality of the swimming begin to decline. Therefore, it is important that the sample be tested within one hour after collection. It is also important to ensure that the lid is tight on the cup. If any of the sample spills, the results will be incorrect and the test will have to be repeated. Mistakes can happen, but please make the lab staff aware if part of the sample was lost or if there was a spill. We recommend specimens brought from home be carried close to your body to avoid the cold and unnecessary exposure to light.

Do I collect differently if I have antisperm antibodies in my semen?
Yes, we will have you collect your sample and place it directly into a special liquid that we supply.

Results of the semen analysis:

When should I expect to have the results of the analysis?
The results are released from the laboratory within 5 days of the analysis. The exception to this may be on busy days for the lab or on short workdays, such as holidays. The requesting physician must review the results before they can be released to you, but we try to do that within two working days. It is important to know that clinical lab tests, like a semen analysis or a hormone assay, can only be ordered by a licensed physician using a written requisition - much like a script for a prescription.
You should also know that the test results can only be released to the requesting physician and, therefore, cannot be given directly to a patient by lab staff. To do so is a violation of federal law.

**What is measured in a semen analysis?**

A number of things are measured in a semen analysis - some that describe the sperm cells, some that describe other cells in the sample and some that describe the fluid fraction of semen. The range of values used to define “normal” may vary from lab to lab, so it is important to note which tests a lab uses. We use the standards set forth by the World Health Organization (WHO), third edition. The following are the major properties of semen that are measured.

- **volume** - the total amount of fluid in an ejaculate, usually 2-5 milliliters (abbreviated mL, which is the same as a cc).

- **count** - the number of all sperm cells (dead, alive, motile, non-motile), expressed as millions of sperm per mL. There should be 20 million per mL or more.

- **motility** - the percent of all sperm that move. Movement means anything from twitching through fast swimming. This should be 50% or more.

- **forward progression** - the swimming pattern or movement of the sperm.

- **morphology** - the percent of normal shaped sperm. Normal includes ≥15% using “Kruger's strict” morphology. A morphology of <4% is abnormal, while those samples in the range of 4-14% normal are borderline.

- **viscosity/liquefaction** - viscosity refers to the thickness of the fluid. It is normal for semen to be viscous when the sample is produced, but with time, the fluid should become liquid. The time required for this to occur is referred to as the liquefaction time and is usually one hour or less. In samples that fail to liquefy properly, it is possible to make the sample more liquid by special treatments in the lab.

- **color** - should be white or gray.

- **pH** - this is a measure of the acidity or alkalinity of the sample. A pH of 7.2 to 8.0 is normal. This is slightly alkaline (7 is neutral and below 7 is acidic). The pH of the sample rises with time after collection and as the pH moves out of the normal range, the motility of the sample can fall. This is a main reason that the sample should reach the lab within 30 minutes of collection, to allow the test to be complete within 1 hour.

- **debris/blood cells** - seminal fluid can contain different types of microscopic debris. The presence of these are noted on the test results. They rarely affect the sperm cells. Blood cells are also seen in semen but are of most concern when there are more than one million white blood cells per mL. This may mean there is an infection and may require the man to be treated with antibiotics. Immature germ cells or epithelial cells are also sometimes seen.

- **viability** - this is the percent of sperm that are alive. Viability is not always measured if the motility is normal. In samples with low motility, it becomes important to know if the sperm are non-motile and alive or dead. We do this with a special stain.

- **antisperm antibodies** - although the presence of antisperm antibodies is not always measured during semen analysis, we have found it to be a good time to do so. We
include this test in the analysis. Antisperm antibodies are defined in another section of this book (see Table of Contents for Antisperm Antibodies).

**Why would I be asked to repeat the semen analysis?**
You may be asked to repeat the semen analysis if the abstinence period is wrong or if some property of the semen appears abnormal. Sample quality sometimes varies and a repeated test allows us to tell if there is a real problem or just a sample variation. If the analysis was performed by an outside lab and something was reported as abnormal, we will want to repeat the test in our lab in order to confirm and study the problem more closely.

**What is male factor?**
Male factor is the term used to describe some aspect of the reproductive biology of the male that is not normal. Male factor is not always found in a semen analysis, but clearly exists when one or more of the variables measured in a semen analysis is out of range. The most common form of male factor involves one of the three of the variables: of low count (oligozoospermia), low motility (asthenozoospermia) and low morphology (teratozoospermia). All three together are sometimes referred to by the acronym “OAT”.

**INTRAUTERINE INSEMINATION (IUI):**

**What is an IUI?**
During natural reproduction, semen is placed in the vagina and sperm swim into the narrow opening of the uterus, known as the “cervix”. They swim into the uterus towards and then must enter the fallopian tubes (normally, there are two fallopian tubes - a left one and a right one). Once in the tubes, the sperm swim to the top of the tubes where the ovulated egg awaits or soon will arrive following ovulation. Fertilization then takes place in the tube. Intrauterine insemination, abbreviated “IUI”, is a procedure whereby sperm is loaded into a catheter (a “straw-like” tube of small diameter). The catheter is passed through the cervix and the sperm are placed into the uterus. This puts sperm inside the uterus, past the cervix and close to the openings of the fallopian segment prior to fertilizing the egg.
**Intrauterine Insemination:** Diagram of the female reproductive tract and intrauterine insemination (IUI).

**What are the main indications for IUI?**
IUI helps make sure enough sperm are near eggs at the right time to allow fertilization. This may be used for cases where the mucus or some other factor produced by the cervix is hostile to sperm. It also can be used to treat certain forms of male factor infertility and for unexplained infertility.

**Why would we be candidates for an IUI if there is no cervical factor and the semen analysis indicates all is normal?**
In a word, timing. The IUI is a simple tool to make sure that sperm and egg are at the right place at the right time for fertilization to occur. With ultrasound, hormone assays and ovulation inducing medicine, we can make a fairly good guess as to when ovulation will occur in the female. The placement of sperm via IUI allows us to improve the chance for a well-timed meeting of sperm and egg in the fallopian tube. Therefore, it is not unusual to use IUI strictly as a timing device, in cases where no male factor exists.

**Is there an optimal sperm count for IUI?**
We like to place somewhere between 10 to 20 million motile sperm during IUI, but it is not uncommon at all to have a pregnancy with fewer than 10 million motile sperm. The success becomes variable with fewer than 4 million sperm, but IUI may still be used. Using large numbers of sperm does not appear to increase the odds for pregnancy.
Is it better to have one or two inseminations in a cycle?
There are very good data that show that a single, well-timed insemination is as good as two or more inseminations to produce a pregnancy. It is not only time-effective for the patient, but less expensive as well.

Can you perform IUI using sperm that have been frozen?
Yes. In fact, this is the only way that donor sperm can be used safely. We can freeze sperm samples in our lab if the need arises during your treatment. There have been a few reports that the freezing process causes some sperm to be incapable of fertilization, but this may well be related to the freezing method. It should be stressed that recovery rates of live sperm depend upon the quality of the initial semen sample and that poor fresh samples will give poor recovery after freezing/thawing.

SPERM CRYOPRESERVATION (FREEZING):

How do you freeze sperm?
Sperm freezing, or cryopreservation, involves mixing the semen with various cryoprotectants. These cryoprotectants are chemicals that act as a sort of "antifreeze" and help prevent damage to sperm cells during the freezing process. Once the sperm has been mixed with the cryoprotectant, the sample is then cooled and frozen according to the specific protocol of the lab. There are various cryoprotectants and freezing protocols that are used with success. Two common types of freezing you may hear about include slow-rate freezing, or vitrification. These simply refer to the speed and manner by which the freezing has been performed. As the name implies, slow-rate freezing involves steps that slowly lower the temperature, while vitrification is a very rapid freezing. Both work well if used correctly. We use slow-rate freezing for sperm in our lab.

What are the dangers of freezing sperm?
As stated above, there are rare reports that sperm from certain fertile men are not able to fertilize after the freeze-thaw process. This is extremely rare. Also, the freezing process kills some sperm, so the recovery will not be 100%. There are no known freezing-related defects caused in children that are conceived using frozen semen. If the male has a normal semen sample, the survival rate, upon thawing, is about 50%. This is a good “rule of thumb” but you should know that the actual number might be slightly higher or lower. Again, the survival rate declines as initial sample quality and quantity decline. Samples from cases of male factor will have lower survival rates. Once frozen, good samples can be stored indefinitely if handled properly and maintained at the correct temperature.

Can I “bank” frozen sperm samples and use them together in order to overcome a low sperm count?
Though this sounds like a good idea, it works only rarely. The survival rate declines in male factor samples following the freeze-thaw process. For example, if survival is only 15%, a person would have to bank 7 frozen samples to end up with the same number of live sperm present in a fresh sample from the same person. Also, it appears that not all sperm are created equal - some can fertilize an egg and some cannot. The number of “good” sperm goes down in a sample with male factor. So not only are the total number of sperm reduced by freezing, but also the number of “good” sperm. The major problem in these samples is too few capable sperm in the population. Banking does not address this. In specific cases, we will freeze very low numbers of sperm, but this is for use with in vitro fertilization (IVF) and direct sperm injection into the egg (ICSI).
ANTISPERM ANTIBODIES (ASA):

What are ASA’s?
The surface of sperm, like all cells, is covered with a variety of proteins that form part of the outer layer. These proteins can detach from the surface, such as when a sperm cell dies and degenerates. The proteins or protein fragments are normally hidden from the bloodstream. However, in certain cases, these fragments make their way into the blood or other areas where they are “seen” as being foreign, much like a germ. The immune system mounts an attack to combat these “intruders” by making specific molecules, or “antibodies”, designed to bind and inactivate the intruders.

Who has the ASA’s, the male or the female?
Either partner can have ASA’s, but it is most common in the male. Once you have them, you will always have them.

How did I get them?
It is not clear how these proteins escape their normal location, but local trauma or infections are likely major causes. If small breaks in blood vessels occur in the reproductive tract in either the male or female, it is possible that the blood will “see” the foreign proteins. This happens with a vasectomy. During vasectomy, the reproductive tract and surrounding blood vessels are surgically cut, allowing exposure of sperm cells and proteins to the man’s blood. Nearly every man undergoing vasectomy develops ASA’s with time.

What harm are they?
It depends on how many you have (the concentration or amount) and where they bind to the sperm. ASA’s that occur in seminal fluid but do not bind to sperm are of no concern for fertility. Likewise, ASA’s that bind to the tail or middle portion of the sperm cell cause few, if any, problems. Those that bind to the sperm head, however, can block fertilization. In the case of female ASA’s, the sperm are not attacked until they enter the female reproductive tract. Antibodies are not “partner specific”, so if a woman makes ASA’s against sperm proteins from one partner, she will have ASA’s against all partners. Unfortunately, the relationship between the amount of ASA’s and their influence on fertility is not clear-cut. There is a large “gray” zone where the impact of ASA’s on fertility is not known.

How can I get rid of them?
Unfortunately, you cannot rid yourself of ASA’s. One treatment has been to suppress the entire immune system with drugs, but the results are not very successful. Another approach for female ASA’s has been to have a couple use condoms during intercourse to reduce the exposure of her system to sperm proteins. This again has not worked well since the immune system has a “memory” and does not require continued exposure in order to make ASA’s.

How does the lab deal with them?
As mentioned earlier, there is much debate in the field regarding the importance of ASA’s. As a rule of thumb, we assign little importance to their presence unless half of the sperm or more have ASAs. If all sperm have them and they are directed to the head of the sperm, in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) may be the best therapy. For IUI samples, we have the man collect the sample in a liquid to dilute and remove the seminal fluid and ASAs as quickly as possible.
DONOR SPERM:

When do you consider using donor sperm?
Donor sperm may be used in cases of very low sperm count (severe oligozoospermia) or in the total absence of sperm (azoospermia). There is no absolute sperm count that requires donor sperm, but men having fewer than 500,000 sperm will probably not fertilize an egg, even during standard IVF. Other reasons for donor sperm includes a history of inherited disease in the man that may be passed on to his children.

Is it medically safe to use donor sperm?
The short answer is yes. The major concerns with using donor sperm are the potential exposure of the woman to infectious diseases and the risk of a child inheriting diseases from the donor. We do not maintain a sperm bank in our center, but, instead, our patients order donor sperm from large, well-known national banks. These banks conduct thorough screening interviews and obtain complete family histories. Once a potential donor has passed this screening, he is tested for a wide variety of diseases, including hepatitis and HIV. Once these tests are shown to be negative, the donor sample is collected and frozen. The frozen semen is then held in storage, or "quarantined", for a minimum of 6 months. The donor is then re-screened for disease and, if he is again found to be negative, his frozen semen is made available for purchase. The six-month period provides ample time for any infectious disease(s) to appear in the donor. However, one factor to be aware of when using donor sperm is CMV. You should only order your donor sperm once you have received your CMV blood screening results. If you are CMV negative, it is recommended that you order CMV negative sperm.

Can I use CMV positive donor sperm?
CMV stands for cytomegalovirus. CMV is a member of the Herpes virus family, which also includes the virus that causes chicken pox and mononucleosis (Epstein-Barr Virus). CMV affects 50-80% of the population by time they reach age 40 and is generally silent, causing no problems in healthy people. CMV is transmitted through body fluids, and once contracted, is present for life. CMV is the most common virus transmitted from a mother to her unborn child and can lead to congenital infections and potentially to permanent disabilities. The greatest risk for transmission to an unborn child occurs when a woman who is negative for CMV becomes infected with CMV during her first pregnancy. This is why we recommend not ordering your donor sperm until you have been screened for CMV. If you are already CMV positive, you can order and safely use sperm from a CMV positive donor. However, if you are CMV negative, we recommend you order sperm from a CMV negative donor.

How do I know the sample I order will be good?
Sperm banks maintain records of thaw outcomes and pregnancy. Donors whose semen does not survive well are removed from the banks. Fertility data are also available from some but not all banks. Sometimes the sperm numbers or thaw survival seen in our center are lower than should be expected, based on rates from the sperm bank. This is rare and we will provide our data to the sperm banks and talk with them on your behalf so they can take appropriate actions, such as providing a replacement sample. We cannot be responsible for the sperm sample if thaw survival is low, if the count is low, if there is fertilization failure during IVF or if pregnancy is not attained using donor sperm.
SPERM FUNCTION TESTS:

What are sperm function tests?
These are tests to determine if sperm are capable of performing some of the basic steps in the overall process of fertilization. The majority of sperm function tests yield a result that is subject to interpretation and no sperm function test provides an absolute answer as to whether a man is fertile or infertile. Listed below are some of the more common sperm function tests and a brief description of each.

**Sperm penetration assay (SPA)** - this is also known as the hamster egg penetration assay, the zona-free penetration test or, simply, the hamster test. In this test, the outer layer of hamster eggs (the zona pellucida) is removed and the zona-free eggs are exposed to human sperm. After incubation, the eggs are squashed and examined under a microscope for evidence that they were penetrated by the sperm. The ability of human sperm to penetrate these eggs is a marker of the ability of the sperm to penetrate human eggs. Conversely, the failure of human sperm to penetrate the hamster egg carries a poor prognosis for the ability of the sperm to fertilize human eggs. Once highly regarded as a predictor of fertilization potential, this test is now used by very few centers.

**Hemizona assay** - fertilization is a process that requires the successful completion of a series of ordered steps. One of these is the tight binding of sperm head to the “shell”, or zona pellucida, of the egg. Poor fertilization outcomes during in vitro fertilization (IVF) are often accompanied by poor binding of sperm to the zona. In this test, the zona of an actual human egg is used to measure binding capacity of the man’s sperm. These eggs are usually obtained from ovaries that have been surgically removed for other medical reasons. The egg is cut into two equal halves in the IVF lab and the inside portions are discarded. The two remaining halves of the zona are then incubated with sperm - one with sperm from the man and the other with sperm from a fertile donor. After four hours, the zonae are washed and the number of tightly bound sperm on each zona is counted. The number of patient's sperm bound is compared to the number bound by the donor. This test is perhaps more useful than the SPA, but often produces results that fall into a large gray area where the fertility potential of the sperm remains unclear. Since it involves the use of human zonae, it is also an expensive test. This test is not widely used currently by IVF centers.

**Mannose binding** - this test is used as a refinement of the hemizona assay. Mannose is a sugar on the zona pellucida where the sperm bind. Rather than using whole zonae, this test simply measures the ability of sperm to bind mannose. Although promising in theory, it has proven no more predictive than other tests of sperm fertilizing potential and, thus, has not been widely adopted in fertility centers.

**Hypo-osmotic swelling test** - this test involves placing sperm in a very dilute solution to see if the sperm have intact and functioning cell membranes. If they do, the sperm tail will swell and this can be seen on a microscope. It is of limited use as an indicator of fertility potential, but may be valuable as an indicator of viability when all motility is lost, particularly when sperm are being analyzed for potential direct injection into an egg by ICSI.

**Acrosome reaction test/ARIC** - the acrosome is a “bag” or membrane-enclosed collection of enzymes located on the head of the sperm. They are membrane-enclosed to prevent them
from digesting internal parts of the sperm cells. These enzymes, once released from the acrosome, allows sperm to burrow through the zona. In order for these enzymes to be released, the acrosome membranes must first fuse with the membranes of the sperm cell. This process known as the “acrosome reaction”. A variety of tests are available to measure the ability of sperm to undergo this process. One popular form of the test involves the use of a substance known as an ionophore. It is therefore called the “acrosome reaction in response to ionophore challenge” test, thankfully most often called the ARIC test. The value of this test is questionable since only a small portion of sperm from fertile men undergo the reaction. Further, it is now known that this reaction does not occur in free-swimming sperm, but occurs only after zona binding. It is not widely used at present in many IVF centers.

**Do we ever order these tests?**
At one point, we offered most of these tests in our lab. With time, we have seen less and less utility in doing so. Even with the test results, our diagnoses rarely change and the treatment options usually do not change either. The results of these tests were used primarily to estimate whether or not fertilization would occur if we recommended conventional IVF to patients with male factor. However, with newer methods, such as intracytoplasmic sperm injection (ICSI), we do not need to make this decision before proceeding with IVF. Even in cases of borderline male factor, we usually prefer to not gamble with IVF but instead use ICSI. Clearly, if sperm function tests evolve that will direct us towards successful treatments other than ICSI; we will again use them in our lab.
A FEW BASIC QUESTIONS TO EASE YOUR MIND:

The following are general questions that all patients should ask -

How do I know that they are my embryos?
This question is one of the most common we receive. All patients should feel confident that the lab follows a foolproof method for tracking their sperm, eggs and embryos from start to finish.

The lab receives a written request from the clinic for an egg retrieval or semen preparation and this request contains a unique ID number. Unique means just that - the number has never before been used in our center. Therefore, all oocytes, semen samples and resulting embryos are identified by both patient name and the unique ID number. This double ID is written on all tubes and dishes, top and bottom, as well as on all paperwork associated with the samples. Names and ID numbers are also posted on the door and shelf of the incubator holding the material. When the caseload allows, patients have an incubator all to themselves. Even when the caseload is larger, patients have a shelf within an incubator all to themselves. There is another important rule in the lab - we never work with the gametes or embryos of two patients at the same time. Furthermore, when we perform critical steps, such as insemination or removing embryos from the incubator for transfer to a patient, it is a rule that the embryologists must confirm the ID of the material by reading both name and number off every container. This ensures that a sample matches the lab records before any action is taken. When it is time to perform the embryo transfer, we insist that two members of the staff positively identify the patient scheduled to receive embryos. It may sound silly, but you must tell us your name before we will remove any embryos from the incubator for transfer. Similar positive identification steps are taken during embryo freezing protocols.

What if the power fails in the middle of the night?
Do not worry - the CRM building has a generator and all of our incubators and critical equipment are connected. All incubators are also fitted with alarms and we are notified - 24 hours a day - if there has been any change in power, temperature, etc. in the incubators. A lab staff member is always available to respond to any alarms, 24 hours a day.

Who works in the embryology lab?
Our lab is staffed with biologists, specially trained in andrology, embryology, endocrinology and cryobiology. These workers do not perform routine clinical lab testing, but instead focus only on the methods and procedures of ART, bringing years of education and experience to the lab. This allows the lab to treat your gametes and embryos with the care and expertise they deserve and require.

I've been to other programs. What's so special about this IVF lab?
Again, this is a question we are often asked and one that has a subjective answer. Aside from our thorough training and commitment, (employee turnover is extremely rare), we also have state-of-the-art facilities that rival those of any center around the world. Though we may not be the largest lab, rest assured our equipment and protocols are cutting edge and you are receiving top quality treatment. Additionally, the lab has an active research program where we seek new methods for embryo culture and other methods for improving success rates. Thus, we are innovators in the field. This gives us the ability to stay on top of new developments in embryology and serves as further reassurance that you are receiving the best possible care.
**OOCYTE BIOLOGY 101 - SOME IMPORTANT THINGS TO KNOW:**

**How do oocytes (eggs) develop in the ovary?**
The current idea is that all of the oocytes that a woman has for her lifetime are produced while she is a fetus. The maximum number of oocytes reaches 6 to 7 million at about the 20th week of gestation of a female fetus. By birth, the number has already fallen to 1 to 2 million. By the time a woman begins to ovulate and menstruate, the number has fallen to about 300,000. During a normal reproductive lifespan, a woman can expect to ovulate around 400 to 500 oocytes. This means that far less than 1% of the oocytes a woman produces are destined for ovulation.

Oocytes are surrounded in the ovary by a layers cells known as granulosa cells. When stimulated to develop, a process that takes 3 menstrual cycles to complete, these surrounding cells multiply and form a fluid-filled cavity that surrounds the egg. The granulosa cells right next to the oocyte are specialized and are referred to as cumulus cells. The egg remains loosely attached to the wall of the fluid-filled cavity through these granulosa cells until ovulation, at which point it is released into the female fallopian tube. The surrounding cells, the fluid-filled cavity and the oocyte are called a "follicle". During the final 2 weeks of development, follicles become responsive to hormones. The amount of hormone in the blood early in the menstrual cycle can control the number of follicles that develop in a cycle as well as their rate of growth. The number and size of the follicles are measured during ultrasound examinations of the ovary.

**What is meant by oocyte "maturity"?**
Oocyte maturity refers to the status of the egg, and whether or not it has reached the stage of development that is needed for successful fertilization and resulting embryo development.

Oocyte maturation is a very complex process, but we will give a summary of its main points. Not only does the follicle grow in size during your hormone treatment, but the oocyte grows and develops as well, actually undergoing meiosis. The separation of the 2 cells is unequal so that only one of the cells receives the majority of the cytoplasm and, therefore, remains functional. The smaller cell is called a polar body. Although a polar body is non-functional, it is one sign that the egg has matured. This process is known as nuclear maturation, as it indicates the genetic material of the egg has been processed.

The oocyte also undergoes changes in the cytoplasm that helps with nuclear maturation, and plays a role in keeping more than one sperm from entering the egg during fertilization. These processes are commonly referred to as cytoplasmic maturation. Both nuclear and cytoplasmic maturation must occur for an egg to be considered “mature”. It is impossible to tell through simple visual analysis if an egg is fully mature. Though presence of a polar body is the most commonly used indicator of oocyte maturity and is very helpful, it is an imperfect assessment. Active research is ongoing aimed at more accurately assessing oocyte maturity.

Oocyte maturation is initiated in response to the surge of the hormone "LH", which stands for luteinizing hormone. LH also causes the cells surrounding the oocyte to loosen, thus releasing the egg from the ovary in an event called ovulation.

Only oocytes that have produced the first polar body are mature and only mature eggs can be fertilized. Immature eggs cannot be fertilized, either naturally or during IVF, even if a sperm is injected into the immature egg with ICSI (discussed elsewhere in detail). When oocytes are collected for IVF, they are surrounded by the cumulus cells, making it hard to determine egg maturity. In many cases, the presence of the cumulus cells can be used as a vague indicator of egg maturity, although this not a precise assessment.
Oocyte maturation. The immature oocyte on the left contains a nucleus, called the germinal vesicle (GV). In the oocyte in the middle, which has cumulus cells around it, the germinal vesicle has disappeared, indicating that maturation is proceeding but the absence of a polar body means that the process is not complete. The oocyte on the right has a polar body (located at 5 o’clock) and is mature.

What causes genetic problems in oocytes?
It is thought that the aging of oocytes is responsible for some of the chromosomal problems that are seen, given the amount of time (years) between egg production and ovulation. Remember that all of the oocytes present in the adult ovary are actually produced before birth, meaning that some eggs can be 35 to 45 years old. However, many oocytes contain chromosome errors from the time they are produced. There are estimates that the baseline incidence of problems in chromosome number (aneuploidy) in oocytes in healthy women may be as high as 30 - 40%. This oocyte chromosomal aneuploidy, either having too many or too few chromosomes, can lead to embryonic aneuploidy, which is the leading cause of miscarriage. Other factors may influence chromosomes in the oocytes, but age is a primary factor.

THE OOCYTE RETRIEVAL (TRANSVAGINAL OOCYTE RETREIVAL (TVOR)):

How do you know when to retrieve the eggs?
During your IVF cycle, we will make two kinds of measurements at various intervals to judge your response to the medications. One form of measurement is the ultrasound exam where we will count the number of follicles on each ovary and determine their size. We will also draw a small sample of blood in order to measure the amount of estradiol (E2) that your ovaries are producing. By tracking both follicular growth and serum E2 levels, we can judge the readiness of your oocytes for ovulation. Once the follicles have achieved the right size and are producing the correct amount of E2, you will be given a final injection to induce follicular maturation and ovulation. This medication is called hCG (human chorionic gonadotropin) and it is similar to LH so that when injected, it induces final egg maturation. The retrieval is scheduled about 35 hours after the injection of hCG is given. It is crucial that the proper amount of hCG be taken and that the injection is given at the precise time assigned by the nursing staff. Remember, the injection of hCG starts a clock for ovulation. Thus, taking your hCG injection too early or too late can have negative effects on the maturity and quality of your oocytes.

What happens in the lab during the retrieval?
Using ultrasound,, a biopsy needle penetrates each follicle through the vaginal wall. The fluid from within the follicle is gently drawn into a sterile test tube. This tube holding the follicular contents is
given to an embryologist within the lab located only a few steps away. The embryologist pours the fluid into a Petri dish and quickly examines the fluid under a microscope. After all eggs are collected, they are moved into dishes containing a special type of culture medium and placed in the incubator. Importantly, all of this occurs within the lab in a clean environment, where conditions, such as pH, light exposure, and temperature are closely monitored.

What do the eggs look like?
The human oocyte is an egg. Thus, it has an outer shell surrounding an inner cytoplasm. This outer shell is not hard like the shell of a bird's egg, but instead clear and flexible. This shell is known as the zona pellucida. The space between the egg and the zona is known as the perivitelline space. The egg is in fact the largest cell in the body. It measures ~ 120µm wide and though images don't depict it well, the oocyte is in fact a sphere. The oocyte and zona pellucida are found within a group of cells called cumulus cells. These cells are sometimes referred to as the cumulus oophorus or cumulus oophorus complex (COC). Cumulus cells immediately next to the zona pellucida are referred to as the corona radiate. Both the coronal cells and the cumulus cells are specialized cells derived from the wall of the follicle. Their presence makes it difficult to see details within the oocyte, but their appearance often correlates with the maturity of the egg. Sperm must pass through these layers - the cumulus, the corona and the zona – to cause fertilization.

How many eggs should I expect to have retrieved?
This is a hard question to answer, as each patient is different. As you might expect, the more eggs retrieved, the better the chance of getting pregnant. This tends to hold true, simply because with more eggs there are more embryos. With more embryos, the best quality embryos can be selected within the lab for later transfer. However, it is important to remember that the quantity of eggs does not relate to quality of eggs. Not all of the eggs retrieved will be able to make a healthy embryo or produce a pregnancy. There are cases where patients have dozens of eggs retrieved but the resulting fertilization and embryo development are poor. Conversely, there are patients with only a few eggs retrieved who have perfect fertilization and great embryo development. Therefore, the quality and maturity of the eggs are the most critical factors. Remember, it only takes 1!

When do you mix the sperm and the eggs?
Anywhere from 3 to 5 hours after egg retrieval.
FERTILIZATION - INSEMINATION / SPERM INJECTION (ICSI):

How many sperm are used and how are the eggs inseminated?
We use 100,000 motile sperm per mL of media to inseminate eggs during in vitro fertilization. We use a sterile pipette to draw up a small, measured volume of media containing the pre-counted sperm and this amount is then placed in the dish with the eggs. We then look at the dish under the microscope to ensure that the sperm are swimming in the presence of the eggs. As mentioned earlier, the source of both the sperm and eggs before they are mixed. The sperm-egg mixture is then placed into the incubator for ~17h.

What is ICSI?
ICSI is a treatment for male factor infertility and the acronym stands for "intracytoplasmic sperm injection". It is a method whereby we inject a single sperm into the interior of an egg by piercing the zona and egg membrane with a glass pipette. This is done under a special microscope with devices called micromanipulators. The egg is held for the injection procedure using suction with a glass holding pipet. As in IVF, only mature eggs can be fertilized via ICSI. The fertilization rate and pregnancy rates with ICSI are close to those of IVF.

What are the reasons for ICSI?
Low sperm count, low motility, low morphology, high levels of antisperm antibodies, prior failed fertilization with IVF or when using sperm from the epididymus (microsurgical epididymal sperm aspiration [MESA], percutaneous epididymal sperm aspiration [PESA] or testis (testicular sperm extraction [TESE]). In any situation where the outcome from standard insemination (IVF) appears in doubt, we suggest that at least some of the eggs be injected via ICSI.

Are there risks associated with ICSI?
Yes. First of all, ICSI is a microsurgical procedure and there is the risk of potential egg damage simply from manipulating them. Some oocytes are more fragile than others are and do not survive the injection process. There is also some thought that embryos derived from ICSI do not develop to late-stage embryos (blastocyst) as readily as those produced by IVF. This may be related to poor sperm quality and not to the technique however it points out that fertilization does not always equal embryo development or indicate the quality of the embryo. Therefore, using ICSI for all patients isn't always a good thing. This is why we try to use the procedure sparingly and only when required.

Also, there are further genetic concerns for offspring produced from ICSI. If a couple is being treated with ICSI because of congenital absence of the vas deferens in the man (CAVD), the woman will need to be screened for cystic fibrosis. It is now thought that most, if not all cases, of CAVD are
mild forms of cystic fibrosis. Therefore, if the woman is a carrier, there is a chance that a child would develop cystic fibrosis. Also, certain types of severe oligozoospermia (low sperm count) are due to microdeletions of part of the Y-chromosome in the man. If a male offspring is produced with these sperm, the same microdeletion can be expected in the male child. There are other slight increases in sex chromosome abnormalities being reported with ICSI. All-in-all, however, ICSI has improved the treatment of male factor infertility, allowing fatherhood in cases that would have been previously impossible.

EMBRYO CULTURE AND EMBRYONIC GROWTH IN VITRO:

What is the "fertilization check"?
It takes about 15 hours to tell if an egg has been fertilized. This coincides with 7 a.m. or 8 a.m. of the morning after the egg retrieval. At this time, an embryologist removes any remnants of the cumulus cells from outside of the egg with a small micropipette. The bare or denuded egg is then looked at under a microscope for the presence of two nuclei, termed pronuclei. One pronucleus contains the genetic material provided by the father and the other contains that from the mother. The presence of pronuclei means the egg has now formed a zygote. In other words, it means normal fertilization. After a few hours, the pronuclei will align within the egg and will disappear as the zygote prepares to divide into a 2-cell embryo. Sometimes an egg will contain more than two pronuclei indicating that either more than one sperm entered the egg or that the egg failed to expel a maternal set of chromosomes after sperm entry. In either event, a fertilized egg containing more than two pronuclei is genetically abnormal and must be discarded. Sometimes an egg will contain only a single pronucleus. This may indicate that the egg was activated in the early steps of fertilization, but that the process was not completed. Other scenarios can produce oocytes with single or many pronuclei. It is crucial that we examine each egg at the proper time for the presence of only two pronuclei since eggs with either single or many pronuclei can make normal-appearing embryos, despite their genetic abnormality.

Fertilization: Appearance of fertilized oocytes (zygotes) at the fertilization check. The zygote on the left has two pronuclei pressing against one another in the center of the cytoplasm and is considered normal. The zygote on the right is abnormal and contains three pronuclei.

How are the embryos grown?
The embryos are grown in a special culture medium in a CO2-incubator. The culture medium is designed to provide nutrition to the growing embryo. The incubator controls the temperature, humidity and pH of the embryos to ensure a healthy growing environment. If embryos are going to
be cultured for a long period to produce blastocysts, a second culture medium will replace the first after three days of culture.

**What is meant by cleavage-stage embryos?**
The cell divisions of an embryo are called cleavage-stage divisions. This is because in early development, the volume of the egg is parceled out to the cells of the embryo. In other words, the embryo is the same size as the egg, even though it has more cells. Embryos from the 2-cell stage are called cleavage-stage embryos until around 3 or 4th cell division (8-16-cell stage) and you can count the number of individual cells.

![Cleavage stage embryos: 2-cell, 4-cell and an 8-cell cleavage stage embryo.](image)

**What is a morula?**
Around day four of culture, the embryo undergoes a process known as compaction and the cells appear tightly pressed together. The junctions between cells are so tight that the embryo appears as a single cell. The embryo is called a morula at this stage, a term that is derived from the Latin word morus, mean "mulberry".

![Morula: A) 8-cell embryo with individual blastomeres still visible. B) Early morula, as indicated by compaction, or increased contact between blastomeres.](image)

**What is a blastocyst?**
On around five days of culture following fertilization, a fluid-filled cavity appears in the solid cluster of cells that form the morula. This cavity, called the blastocoel, signifies that the morula has developed into the next embryonic stage, the blastocyst. As the blastocoel continues to expand, the cells of the blastocyst organize into two distinct groups. The outer layer of cells unite to form a continuous layer and this layer is called the trophectoderm. These cells become the extra embryonic material, such as the placenta. On one side of the embryo, just inside of the trophectoderm, there is a collection of cells called the inner cell mass (ICM). It is the ICM that forms the actual fetus after the embryo is...
implanted into the uterine wall. At some time late on day 5 or on day 6 in culture, the embryo may expand, forcing the zona pellucida to tear. The embryo will escape the confines of its shell in a process known as hatching.

![Image of blastocyst stages](image)

**Blastocyst Development:** A) An early blastocyst B) an expanding blastocyst C) a hatching blastocyst and D) a completely hatched blastocyst outside of the zona pellucida. TE-trophectoderm, ICM-inner cell mass.

**Why do some embryos fail to grow to the blastocyst stage?**
Some embryos are derived from eggs that were never destined to make viable embryos, due to genetics or other problems. Remember that less than 1% of the eggs in the fetal ovary are ever even ovulated in nature and not all of these have the genetic potential to make a fetus. Thus, simply because we retrieve several eggs does not mean that each egg will make a viable embryo. Also, the embryo is controlled by the genes of the mother until about day three of development. It is at this time that the genes of the embryo, contributed by both the mother and the father, awaken and take control of later development. Not all embryos make this important transition in development. Also, although our embryo culture systems are greatly improved over the earlier versions we used as IVF, research is ongoing aimed at further improving success rates.

**Can you tell if my embryos are chromosomally abnormal?**
Not simply by looking at them, at least not usually. There are certain, subtle signs that embryos are not normal - the egg from which the embryo develops is unusually large or the cells (blastomeres) of the embryo are have several nuclei - but most chromosomal problems cannot be detected by simple visual means.

There are other methods for looking for specific genetic abnormalities, but these require that a cell be removed from the embryo by biopsy and then analyzed (see Pre-implantation Genetic Diagnosis or PGD).

**Can you tell the sex of my embryos?**
Not by simply looking at them. This, too, requires PGD.
EMBRYO TRANSFER:

When do you transfer the embryos to my uterus - day 3 or day 5?
This question really asks if the transferred embryos will be cleavage-stage embryos (day 3) or blastocysts (day 6). So first, what are the advantages of doing a blastocyst transfer? As indicated above, a blastocyst is a late-stage, pre-implantation embryo that differs from cleavage-stage embryos in many ways. The blastocyst is under the genetic control of embryonic chromosomes and genes. Cleavage-stage embryos are still under the genetic regulation of maternal genes from the oocyte. Therefore, when evaluating cleavage-stage embryos, only the quality of the oocyte is evaluated, not the genetic potential of the embryo. Blastocysts have also undergone some major changes in cellular organization, and have both trophoderm which become the extra-embryonic material and inner cell mass cells (ICM) which becomes the fetus. This cellular differentiation gives more endpoints by which to select the best embryos for transfer. Additionally, as a general rule the number of blastocysts that will implant in the uterus is higher per embryo transferred than that of cleavage-stage embryos. Thus, the most attractive feature of blastocyst transfer is that there are added selection methods available and fewer embryos can be transferred while still keeping a good chance for pregnancy. This reduces the chance of a multiple pregnancy.

Why, then, are not all embryo transfers done on day 5 at the blastocyst stage? The main reason is that not all embryos will form blastocysts in culture and the possibility that there will be no embryo transfer is increased. Predicting which embryos will make blastocysts has been somewhat difficult, although strides are being made in this area. More and more blastocyst transfers will likely be made as predictive abilities improve. Also, though we provide the best growth environment for your embryos in the lab that we can, our methods are far from perfect and are no substitute for the uterus. Furthermore, some research has suggested that prolonged culture of embryos in the lab could introduce certain abnormalities. Therefore, one line of thinking is that putting the embryos back into the uterus earlier, rather than later, may give the embryos the best chance of continuing development. It is also important to remember that the pregnancy rates in programs that do all blastocyst transfer are not always better than those programs doing day 3 transfers - in fact, some are lower. To date, the only real advantage demonstrated for blastocyst transfer is the ability to put back fewer embryos in order to reduce the number of high order multiple gestations (triplets or higher) while still maintaining a high pregnancy rate.

How do you select the best embryos for transfer?
Let's start with a day 3 transfer of cleavage-stage embryos. The classic approach in IVF has been to have an embryologist examine the embryos prior to transfer, then assign a quality score to each embryo based upon their microscopic appearance. This scoring procedure assumes that we know the features of embryos that are likely to implant. The number of times pregnancy occurs from the transfer of embryos with a low score tells us that scoring embryos solely upon their appearance – a beauty contest of sorts – is not as predictive as we might like. Therefore, it is our opinion that becoming tied to a quality score and, even worse, getting our patients tied to quality scores is truly an unnecessary, emotional exercise. Instead, we assess each embryo for several qualities that have historically had predictive value in our fertility center. These are discussed below.

Growth rate - the rate at which an embryo grows has more predictive power for potential implantation than any other characteristic we score. You should realize that human embryos do not divide synchronously (meaning that you can have an embryo with an odd number of cells) so that appropriate growth does not occur at a given instant, but rather within a window of time. We look for embryos that are on the front-end of the window. It is possible, however, for embryos to develop too rapidly and this can have a negative impact upon implantation.
Cell-to-cell contact - the degree to which the cells of an embryo interact with one another is also important. Embryos having blastomeres that touch over broad areas implant at a higher rate than do those having rounded blastomeres that either do not touch or touch each other only slightly.

Fragmentation - some embryos tend to have cells that do not remain intact, but rather partition into small bubbles of cytoplasm called fragments. The exact cause of fragmentation has long been a source for speculation, but it is now known that several mechanisms can cause an embryo to fragment. Also, remember that cell division is an active process. When we briefly examine embryos for development, we are simply taking a snapshot of development. Time-lapse photography has shown that embryos fragment and then re-absorb these fragments. Thus, fragmentation can be a temporary process and fragmentation at one point in time may be reflected at a later point in time. The important thing to keep in mind is that fragmentation is not necessarily bad and that embryos with some degree of fragmentation are fully capable of producing healthy offspring. Embryos with a large degree of fragmentation, such as those having 50% or more of the area of the embryo occupied by fragments, have a reduced chance of implantation. These embryos are typically selected for transfer only if no others are available. Highly fragmented embryos also have a reduced chance for continued development in culture, greatly lowering the chance that they will form blastocysts.

When patients are scheduled for blastocyst transfer, the embryologist selects blastocysts with a high cell number, with a clearly delineated inner cell mass and expanded blastocoel.

<table>
<thead>
<tr>
<th>Cavity Size</th>
<th>ICM</th>
<th>TE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - very early</td>
<td>A – many cells, tight</td>
<td>A – many cells, tight</td>
</tr>
<tr>
<td>2 - early</td>
<td>B – many cells</td>
<td>B – many cells</td>
</tr>
<tr>
<td>3 - full</td>
<td>C – few cells</td>
<td>C – few cells</td>
</tr>
<tr>
<td>4 - expanded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 - hatching</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 - hatched</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Blastocyst Morphology: Blastocyst quality is graded based on cavity size and appearance of the inner cell mass (ICM) and trophectoderm (TE) cells.
How many embryos should be transferred to my uterus?
This is, by far, the hardest question to answer in this entire booklet. The two primary ways we determine how many embryos to transfer are using (a.) maternal age and (b.) embryo quality. One way to approach this question is by considering the implantation rates of embryos. This is simply the chance, expressed as a percent, that an embryo will implant and make a sac in the uterus. Recently, we have determined by looking our data that good rates are obtained when two embryos, selected from a larger group, are transferred, as long as at least one embryo has reached the 8-celled stage of development by the morning of the third day after egg retrieval. We adjust our recommendations based upon quality of the embryos available, the growth rates of the embryos and certain patient characteristics, such as age and medical history. As a patient, there are two things you must know: First, although to many infertile couples the idea of having twins seems desirable, even ideal, it is not and it is our goal to help you conceive one baby at a time. Secondly, although we will set a maximum number of embryos that we are willing to transfer, the decision to reduce the number for transfer is solely yours and one that we will honor. There is usually no reason to transfer more than two blastocysts if we are doing a day 5 transfer. This typically defeats the purpose of blastocyst transfer.

You need to know all of your options and that it is possible to reduce the number of embryonic sacs in the uterus through a reduction method conducted under ultrasound. This is available through referral for patients with three or more sacs, but there is risk associated with the procedure. We realize that embryonic reduction is not an option that all couples want to consider and we want you to understand that it is not a method we employ as an excuse for poor decision-making on the day of the embryo transfer. This is why we encourage you to give the decision of how many embryos to transfer detailed, specific thought before the day of embryo transfer. If for no other reason, doing so will make the transfer go more quickly and, since transfers are done under ultrasound guidance, a full bladder is needed to image the uterus properly. It is unpleasant to have to take the time to make a critical decision with a full bladder. Of course, you will need some information about the embryos to make that decision and we won't have the information until the day of transfer, but you can make tentative plans. You will be given the information about the growth and status of your embryos when you are taken into the transfer room. Feel free to ask any questions you wish at that time.

One final note for couples who have been through IVF before. There is a natural tendency to think that the solution for failure to conceive in a prior cycle is to transfer more embryos. If embryonic growth rate, cell-cell contact and fragmentation are within normal limits, there is usually no reason to think that increasing the number of embryos for transfer is the answer. Transferring more embryos does not necessarily increase your chance for pregnancy, but certainly increases your chance for multiples.

What is assisted hatching?
This is a process where an artificial hole is created in the zona pellucida of an embryo. One way to do this is to dissolve a hole in the zona with acid, although the hole can be made mechanically with a glass micro-needle (similar to ICSI) or more often, with the micro-beam of a laser. At this point in time, laser is the preferred method of assisted hatching to prevent stress to the embryo. The idea for creating a hole in the zona goes back to earlier studies on human embryos in culture and the observation that at least one half of embryos do not hatch (escape the zona pellucida) in vitro. Remember, the embryo must escape the zona in order to implant, so it was inferred that the failure to hatch in vitro meant these same embryos likely did not hatch in the uterine cavity. Why didn't they hatch in vitro? It has been suggested that the zona hardens under a variety of circumstances, including exposure to culture medium, increased maternal age and cryopreservation (freezing). Valid experimental support for any of these is limited in the scientific literature. Recent studies with
a large number of participants have made it clear that only certain portions of women potentially benefit from assisted hatching and that it should not be applied full-scale to all.

EMBRYO FREEZING (CRYOPRESERVATION) AND STORAGE:

How do you freeze an embryo?
Similar to sperm, there are several methods by which to freeze embryos. These procedures all expose embryos to various compounds known as cryoprotectants. As the name implies, cryoprotectants serve to protect the embryo from damage induced by freezing, such as ice crystal formation, by slowly removing water from the cells. The embryos are slowly cooled and eventually reach the temperature of liquid nitrogen, the medium in which they are stored. This final temperature is −321° F (−196° C). The embryos are frozen in small straws. We usually freeze two embryos per straw to save space in our nitrogen storage tanks and, thus, expense to the patient. Each straw is labeled to ensure proper identification upon thawing. Thawing of embryos is performed in a step-wise manner raise the temperature of the embryo safely, but to also remove the cryoprotectant to avoid any potential toxic effects.

Can you freeze eggs?
Yes. The slow-freezing approach used to freeze embryos does not produce high survival rates when applied to unfertilized eggs. This is largely due to the size of the oocyte, as well as unique properties of its membrane. We therefore use oocyte vitrification, or ultra-rapid cooling, to freeze eggs. Vitrification avoids damage induced by slow-rate freezing, such as ice crystal formation.

Are there birth defects that arise from embryo freezing?
No. None have been reported in the medical literature for human embryos and none are known for the millions of embryos frozen from animal species.

At what stage should embryos be frozen?
The first thing to note is that embryo cryopreservation is a stress on the embryo and that there is no guarantee they will survive the process. With embryo freezing, there is always a "trade-off" for each embryo stage. Pronuclear stage embryos (zygotes) can be frozen with a very high capacity to both survive the freeze-thaw process and to continue development. That is the advantage. The disadvantage is that freezing zygotes removes them from the group of embryos cultured for fresh embryo transfer. It is possible, then, that the very best embryos will be frozen and not available for fresh transfer. This also applies to day 2 embryos (2 to 4-cells). If you wait until after the fresh transfer on day 3, then freeze the remaining embryos, you will have all embryos produced that cycle available to use for the fresh transfer. That is an advantage. Day 3 embryos, however, do not freeze as readily as other stages. Remember that the cells do not divide at the same time and some cell structures present in dividing cells are cold intolerant. This is the disadvantage. Morulae also do not freeze well. However, blastocysts tolerate the freeze/thaw process very well. In fact, some of our highest survival is obtained with frozen blastocysts. In addition, these are later stage embryos and in theory should implant at a higher rate. This is why we prefer to freeze blastocysts. Not all embryos will make blastocysts, and this is the "down-side" of freezing blastocysts - a reduced number of frozen embryos or even no embryos available for freezing. However, our feeling is that by only freezing the highest quality blastocysts, that this will optimize the value of the frozen embryo transfer (FET).
When should an embryo not be frozen?
Embryos that are very slow in developing, those that have too much fragmentation and those that appear unhealthy (grainy, vacuolated) should not be frozen. Remember that the freezing and thawing processes are a physical challenge to cells. Therefore, we only freeze high quality embryos/blastocysts that meet predefined criteria.

How long can frozen embryos be stored?
Indefinitely. There is no shelf life or loss of viability at –196°C if handled correctly.

FROZEN EMBRYO TRANSFER (FET) CYCLES:

What strategies are used for FET cycles?
There are two basic strategies for the transfer of frozen embryos. You can either elect to thaw only the number of embryos you wish to transfer or you may thaw more than you will transfer, then select the best looking embryos for transfer.

How many embryos should be thawed?
It depends upon the plan you have selected. The answer is obvious for the first scenario - thaw only the number you will transfer. If you wish to apply selection in culture, we usually thaw several embryos and select the best two or three for transfer. This most commonly occurs when cleavage stage embryos have been frozen. We can then culture the remaining embryos for an additional 2-3 days and refreeze them as blastocysts if they grow well. Successful pregnancies have occurred from this strategy of double freezing.

If you have frozen blastocysts, we most often thaw two. We only freeze high quality blastocysts. This ensures that only high quality embryos are frozen, which improves their likelihood of surviving the freeze/thaw process and increases the chance of success following the FET. In this case, the FET is sometime referred to a frozen blastocyst transfer (FBT).

When are the embryos thawed?
Zygotes (2PNs) are thawed, then cultured for two days prior to transfer. Day 3 embryos are thawed and then either transferred immediately, or cultured for 2-3 days prior to transfer to reach the blastocyst stage. Blastocysts are thawed several hours prior to transfer.

PRE-IMPLANTATION GENETIC DIAGNOSIS (PGD):

What is PGD?
A single cell can be removed from an embryo (day 3 cleavage-stage embryo) with micromanipulation in a process known as embryo biopsy. Similarly, a piece of trophectoderm from a blastocyst, which contains several cells can also be biopsied. There is enough genetic information and development potential in the remaining cells to form a normal fetus upon implantation. The biopsied cell/cells can be analyzed in a host of different ways using molecular methods. This information can be obtained quickly enough so that the genetic status of an embryo for chromosome number or a specific gene can be established prior to embryo transfer. In this way, the transfer of affected or abnormal embryos can be avoided.
Though biopsy is performed by skilled embryologists in the lab, it is an invasive procedure. Thus, there is the risk of damage to the embryos. Additionally, there are other risks involved that include lack of genetic diagnosis. These risks are discussed with the patient during the specialized consent process that is used when PGD has been determined to be an appropriate approach.

**Embryo Biopsy:** A cell or cells can be removed from an embryo by a small glass pipette and then analyzed for genetic disorders. This is known as PGD, or sometime, PGS, depending on what genetic analysis is being performed. Unaffected embryos can then be selected for transfer to the uterus.

**CONCLUDING REMARKS...**

That concludes our brief review. The experience of infertility is really not that uncommon, so you should never feel that you are alone or that you are somehow defective or incomplete. Also, the medical reasons for infertility do not have to be a major deviation. Some seemingly very minor “glitches” - an unseen problem in a single step of a simple biochemical pathway or the failure of sperm to bind to the shell of the egg, for example - can have dramatic impact in a couple’s quest for pregnancy. For people wrestling with infertility, frustration often becomes an unwelcome visitor in their relationship. We have written this booklet with you in mind, hoping that the information helps to describe some of the biological basis for infertility, some of the lab aspects of reproduction. This information guides the plan to combat your infertility issues. We want you to share in this knowledge so that we can become better partners.