



Guide to IVF Laboratory Results

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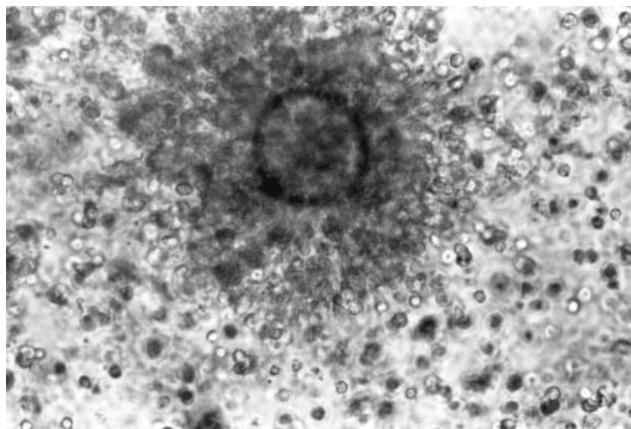
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This booklet has been created to help with understanding the laboratory results following the Egg Retrieval procedure for IVF at PCR. At the end of the document there are also some Frequently Asked Questions (FAQs). It is very important to read this FAQ section because there are some details or perhaps answers to questions that each patient has during treatment.

Day 0 – day of egg retrieval

On the day of and just before the egg retrieval, the embryologist will come out to the pre-op waiting area and introduce themselves, verify the identification on the lab documents and verify what laboratory procedures are being performed for the treatment cycle. After the egg retrieval procedure, the embryologist will come out to the recovery room and update the final number of “egg masses” that have been collected. Sometimes the number of “egg masses” collected is greater than the actual number of eggs present. Some of the eggs may not be suitable for use due to quality, they may not be mature or the egg mass did not actually contain an egg. The final number of eggs retrieved is usually lower than the number of follicles that have been measured each day during cycle monitoring. Many of the measured follicles are too small and the egg cannot successfully be retrieved from them.

If a fresh semen sample is being used, the sperm provider will be asked to provide the sample shortly before or after the egg retrieval. If a frozen semen sample is to be used, the sample will be thawed later in the morning. Insemination of the eggs (either by standard IVF or by ICSI) is 4-6 hours after the egg retrieval. For standard IVF, all of the egg masses are exposed to an aliquot of washed, highly motile sperm and allowed to incubate overnight. If the treatment requires ICSI, each of the mature eggs will be injected with a single washed sperm. Immature eggs will not be injected.

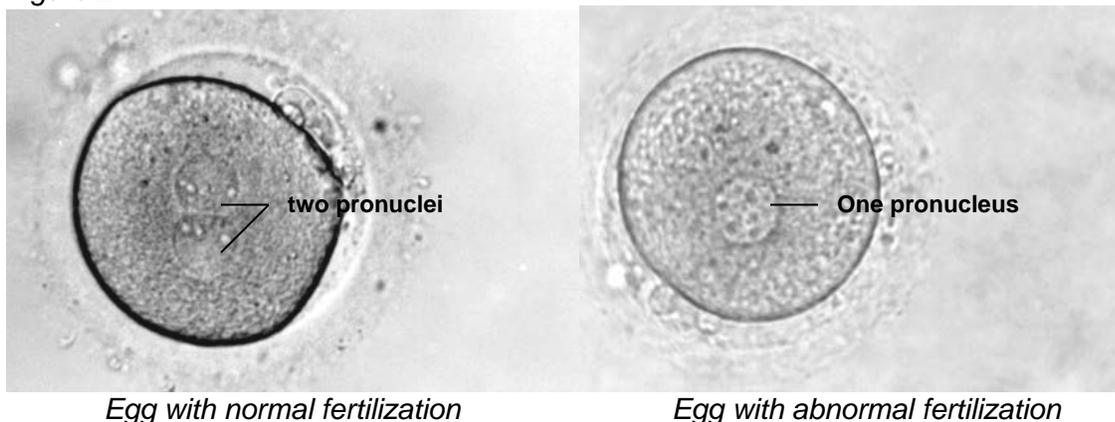


*Figure 1.
Egg mass immediately following egg
retrieval procedure. The dark circle in
the centre is the egg.*

Day 1 – fertilization day (the day following egg retrieval)

The fertilization process takes place overnight after the insemination (IVF or ICSI) of the eggs. The eggs are examined first thing in the morning and then a second time later in the morning. “Normal fertilization” of an egg is confirmed by the presence of a “nucleus” from the egg and another “nucleus” from the sperm. Fertilization cannot be classified as normal if these structures are not seen. Sometimes only one nucleus is seen or there are more than two of these nuclei seen. In both cases the egg is fertilized but is classified as “abnormally fertilized. Only those eggs that show normal fertilization (i.e. with the nucleus from the egg and the nucleus from the sperm) will be grown in the lab to form embryos.

Figure 2.



One of the embryologists will send a **portal message** with the results of the fertilization.

Day 2 – early embryo growth

One of the embryologists will send a portal message with the Day 2 results. On Day 2, the embryos should have started to grow and should have divided to form an embryo of 2 cells to 4 cells. The important milestone for Day 2 is that the embryos have progressed from the 1-cell, fertilized egg stage to an embryo with 2 or more cells.

In addition to the stage of growth, the embryos will each receive a grade from 1 to 5 (1 being the best quality and 5 being the lowest quality). On average, most embryos are grades 2 and 3. Embryos will sometimes have grades 4 and 5. The grade does not determine the ultimate fate of the embryo. Many grade 4 embryos can successfully form blastocysts and therefore all grades of embryos are always kept in culture. Grade 5 embryos usually indicate that the embryo is not surviving or it is not growing as it should.

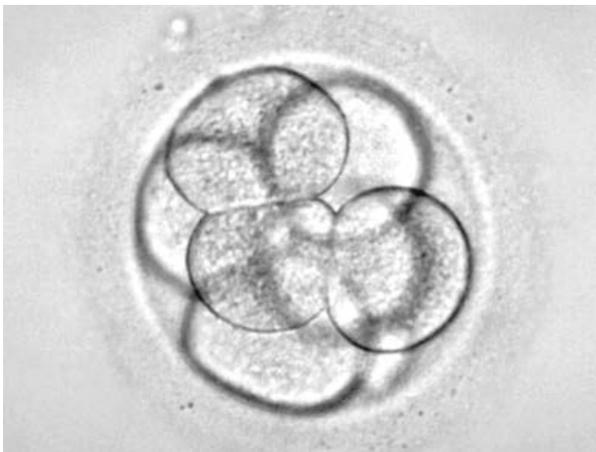


*Figure 3.
Good quality 4-cell embryo (grade 2), with cells (blastomeres) of slightly different sizes. There are a few fragments (the small “bubbles” seen). This is an example of a grade 2 embryo.*

Day 3 – early embryo growth

It is expected that most embryos will have divided to have 6 to 8 cells on Day 3, however it is not unusual to have some that are slightly slower or some that are more advanced. As long as the embryos are of average to good quality (grades 1-3) and have grown since Day 2, there is usually no cause for concern. The embryos will again receive a grading from 1 to 5 on Day 3. Remember, the grade does not necessarily predict the ultimate fate of the embryo. The cohort of embryos is progressing well if the embryos are mostly in the grades 1 to 3 range.

One of the embryologists will provide an update via the portal regarding the Day 3 embryo progress.



*Figure 4.
Excellent quality Day 3 embryo with 7 cells present. This is an example of a grade 1 embryo*

Day 4 – morula development

The embryos should be progressing to the “morula” stage later today, however a large range of embryo development can be seen today and this is normal. The embryos should have developed to have 10 cells or more on Day 4. During this phase of development cells are also all starting to “compact” together and form a ball in which the individual cells are not visible. It is important to know that it is not unusual to lose up to half of the average to good quality embryos from Day 3 to day 4. This is because of the complex changes

occurring in the embryo at this stage of development. Only the “strongest” embryos will progress through to morula formation.

One of the embryologists will send a portal message to update on the progress and development of the embryos. **If there will be a fresh embryo transfer, the embryologist will also include, in the message, the appointment time for the embryo transfer the next day.**

For patients who have chosen preimplantation genetic testing, the embryologists may call on Day 4 if an embryo transfer instead of embryo biopsy might occur (only for PGT patients on luteal support medication).

Day 5 – blastocyst formation and embryo transfer

On Day 5 the expectation is that a proportion of the embryos have begun to form blastocysts. For patients having a fresh embryo transfer, the embryologists will not send a message today. The progression and quality of the embryos will be discussed just prior to the embryo transfer procedure.



*Figure 5.
Very good quality expanded blastocyst. The “scalped” inner edge of the blastocyst is called the trophoblast and the “clump” of cells at the bottom of the blastocyst is the inner cell mass (ICM). Both of these structures must be present and normal for the blastocyst to be transferred or cryopreserved. This blastocyst is a grade “4AA”*

Most of the embryos that formed morula on Day 4 should be progressing to the early blastocyst stage on the morning of Day 5. It is normal that only 40-50% of the **average to good quality** embryos from Day 3 will reach the blastocyst stage. Some embryos may be slightly slower and others more advanced, each patient is very different and the number of blastocysts formed may be more or less than average. The most advanced, best quality embryo(s) will be chosen for the embryo transfer.

Any **GOOD QUALITY** surplus blastocysts after the embryo transfer will be cryopreserved on Day 5 or Day 6 as they develop to the appropriate stage for cryopreservation.

For PGT patients, the embryologist may call, early in the morning of Day 5, if a decision must be made regarding proceeding to embryo biopsy or undergoing embryo transfer.

The blastocyst grading system uses a number followed by two letters. The number refers to the amount of expansion the embryo has undergone and is not related to the quality. The amount of expansion expected for each patient is very dependent upon when the oocytes were originally inseminated, back on Day 0, and at what time of day on Day 5 the embryos are being transferred. There should be no interpretation of embryo quality read into this number that is assigned to the degree of blastocyst expansion. The subsequent two letters refer to the quality of the two distinct areas of the blastocyst, as seen in Figure 5. The “clump” of cells (inner cell mass – fetal cells) inside the hollow ball shaped blastocyst is scored as A, B, C or D depending upon the number of cells and the size and shape of the clump of cells. The cells on the outer surface of the blastocyst (trophectoderm – placental cells) are scored using the second letter, again A, B, C or D and this letter refers to the number of cells in this area and the amount of stretching that is occurring. All blastocysts with any combination of As and Bs are considered good quality for embryo transfer or cryopreservation. Sometimes blastocysts with an A or B quality in one area and a C quality in the other, may be considered for embryo transfer depending upon the judgement of the embryologist.

Expansion number:

2- early blastocyst 3- blastocyst 4- expanded blastocyst
5- hatching blastocyst 6- hatched blastocyst (5 or 6 rarely seen on Day 5)

Quality letter:

A- good to excellent B- average to good C- borderline D- poor

Example: 3BA is a moderately expanded blastocyst with an average to good inner cell mass cells and good to excellent trophectoderm cells

Occasionally there are cases where there are no blastocysts forming for transfer on Day 5 or on Day 6 for freezing. The embryologist will confer with the doctor to determine if the embryos are growing, but slowly, and an embryo transfer is possible or if the embryos have stopped growing and are not suitable for embryo transfer or freezing.

If the embryos have stopped growing, the embryo transfer and embryo freezing will be cancelled. This situation is very frustrating and confusing and most patients will want answers right away. The laboratory cannot interpret the embryo results as there are many possible determining factors that may have contributed to the outcome (i.e. age, medical history, male factors, etc.). The doctor is the best person to speak to regarding the cycle outcome. The doctor’s medical office assistant will help you to schedule an appointment as soon as possible.

Embryo Cryopreservation (freezing)

Day 5 embryo transfer patients: Any **good quality** embryos that remain after the embryo transfer procedure will be cryopreserved on Day 5 or Day 6. In order to maximize the potential for a blastocyst to survive cryopreservation and thawing, the embryos need to be at a very specific stage of growth. Sometimes they are ready on Day 5 and sometimes they need to be grown to Day 6. The quality and success of the cryopreservation and thawing will not be compromised if the embryos need to be grown to Day 6. **The embryologists will send a message through the portal on Day 6 with the final results regarding how many embryos they were able to freeze (cryopreserve).**

Embryo “Freeze-all” patients: For patients not having a fresh embryo transfer (OHSS, fertility preservation or other medical reason for no transfer), and the embryos are to be “cryopreserved”, the embryologists will cryopreserve (freeze) good quality blastocysts forming on Day 5 and Day 6. Any embryos that have not formed blastocysts on Day 5 or Day 6 will not be cryopreserved.

Occasionally blastocysts with an A or B quality in one area and a C quality in the other, may be considered for embryo cryopreservation depending upon the judgement of the embryologist.

PGT Patients: will be updated via the portal regarding the total number of blastocysts biopsied and cryopreserved, usually on Day 6.

FAQs

My partner has 0% normal forms for his sperm assessment, does this mean that the fertilization will not work?

In some cases, the doctor and the embryologist may recommend ICSI if the normal forms (morphology) is 0%. The embryologist performing the ICSI will search for the most normal looking sperm in the washed sample. We frequently have sperm samples with 0% normal forms and the ICSI results are usually as expected with normal fertilization rates.

How do you know which sperm to use for ICSI?

The embryologists will always pick the sperm with the most normal looking morphology and the best and most normal motility pattern

Is this enough sperm, or do I have to fill the cup?

IVF and ICSI require only a portion of a normal ejaculated sample. A volume of 2-5 mL is considered normal and this volume will usually look small in the sample container.

How long do I have in the collection room to produce my sample?

We do prefer the semen samples to be produced on site so that we can assess and process them in a timely manner. We recognize that this is not the most comfortable environment and do not expect you to be quick. We will however become concerned if you have been in the collection room longer than 30 minutes. If you are longer than 30 minutes, please check back in with the lab staff and they may suggest that you take a break or see if your partner is available to assist you.

Can I use saliva?

Saliva contains many digestive enzymes so it is not to be used to assist with lubrication. We provide packets of lubricant in each collection room. If there are no packets, please advise the lab staff and they will provide you with some.

Is it better to abstain and save up my sperm?

No. We normally recommend that you abstain between 2-5 days before producing a sample but do not worry if it has only been one day of abstinence, you will still have plenty of sperm in your sample. Abstaining for too long will increase the number of dead sperm and other artifacts in the sample. Therefore it is preferable to have a shorter abstinence than a longer one.

I have been sick, how will this affect my sperm sample?

A sustained elevated temperature may have an effect on sperm motility and some drugs and antibiotics will affect sperm quality. You will complete a questionnaire at the time of sample collection that will let us know if there are any circumstances which will affect the sample.

How long will it take to get the result and what are the normal parameters?

Because we are using the semen sample for IVF treatment, we do not do a complete semen analysis at this time. We process and wash the sample in preparation for inseminating the eggs obtained from your partner. A complete diagnostic test will have been done prior to the treatment and your physician will have these results and how they compare to the normal values.

How do you know that you will not mix up my eggs or the sperm sample with someone else's?

This is one of the most common fears of all IVF patients. At PCR/M, we take great care in the proper identification of all patient eggs, sperm and embryos. We follow a system of double identification and "chain of custody". This process begins with the identification check with you and your partner, at the time of egg retrieval and sperm sample collection. Once the eggs and sperm are in the laboratory, we label the dishes and tubes with a minimum of two identifiers unique to you and your partner. Witnessing is performed by two embryologists every time that the eggs and sperm are matched together. The double witnessing is then performed every time the resulting embryos are handled or moved. At the time of embryo transfer, a second identification check will be performed with you and your partner. A verbal and visual identification is also made from the identifiers on the embryo dish before the embryos are brought into the procedure room for the transfer.

How many of my eggs will fertilize?

On average most patients will have 70-85% of their mature eggs fertilize. Immature eggs cannot be injected (ICSI) and will not fertilize in standard IVF. If the eggs are poorer quality and/or the sperm used is very poor, the fertilization may be lower than for the average patient. Please refer to the chart at the end of the FAQ section in order to see the average egg and embryo development results that can be expected for a patient under 40 years of age.

I did not get a portal message from the embryology lab first thing in the morning, is there something wrong?

We will normally try our best to message between 8 am and 10 am but do not worry if we are later, it just means that we are busy and we will message as soon as we are able to. Our first priority is handling and checking eggs and embryos and we always want to do this safely and without rushing before updating you.

What grading system do you use for the embryos?

For embryos prior to the blastocyst stage (days 1-4) we use a scale from 1 to 5. 1=excellent, 2=good, 3=average, 4=slightly below average, 5=below average/poor.

What if I do not have any embryos that are grade 1 or grade 2?

If the embryos are graded 1,2 and 3, we are happy with their potential and consider these embryos to be relatively equal. If the embryo is graded 4 or 5, we consider these to have lower chance of success however, patients have achieved pregnancy with grade 4 and 5 embryos.

My embryos are not at the same stage as my friend's embryos were on the same day, is there something wrong?

Every patient is different and every patient's embryos can grow at a different rate each day. The important thing is that there is daily progression of the embryos. It is also normal to have a mixture of grades and stages of development especially with a large group of embryos.

I have heard that the success of IVF is lower the older that you are but I am really fit and healthy, will this improve my chance of success?

Unfortunately the age of the eggs is the best and most significant predictor of the number of and/or quality of the subsequent embryos. It is indeed best to be active and healthy and not have any detrimental lifestyle habits but the egg lifespan is biologically predetermined for each patient and unfortunately success does decrease as women get older. It is important to note however that we do have many success stories with our patients over 40 years old and many healthy babies! Please check our website: pacificfertility.ca > Resources > Success Rates for the most updated statistics.

I just saw on the website that you can do genetic testing (PGT) on the embryos, when are you going to do this?

PGT cycles involve a significant amount of extra work-up and counselling prior to the initiation of your IVF cycle and because of this we cannot, at the last minute, perform PGT while you are in your current cycle. There is also an extra cost associated with the addition of this testing to your treatment. Any questions regarding genetic testing of the embryos are to be made to your physician or the nursing team.

How do you grade the embryos on Day 5 before the transfer?

Once the embryo forms the blastocyst, the score describes the blastocyst. The score is a number (unrelated to the grade numbers previously described) followed by two letters (for example 4AA). The embryo score for Day 5 is previously described in this booklet.

What information will I need to know for my embryo transfer?

You will be given your transfer time when the embryologist messages you with your Day 4 embryo update. Embryo Transfers are usually performed mid to late morning but can vary due to the number of other patients that may be booked. You will be required to arrive at PCR 30 minutes prior to your transfer time and must prepare by having a fairly full bladder. The instructions for the transfer preparation and the continuation of your medications are in the written instructions already given to you by the nurses.

When I went to another clinic they had 14 embryos and transferred 2 on Day 3 and froze 10 embryos for me. Why do I have fewer embryos to freeze after Day 5 transfer?

On average, we expect 40-50% of average to good quality Day 3 embryos to develop to the blastocyst stage. This total number that develop to blastocyst will not change with the thawed Day 3 embryos, they will experience the same development after thawing as the fresh embryos; only 50% will be able to form blastocysts. With Day 3 frozen embryos, more embryos may be frozen in your fresh cycle but more embryos will have to be thawed for your FET. At PCR/M, we prefer to freeze only high potential embryos that have already shown us that they can form blastocysts. We will not freeze blastocysts of poor or sub-optimal quality as they are not likely to survive the process or form a healthy implantation.

My sister went to another clinic and they do Day 3 embryo transfers and do not do Day 5 blastocyst transfers, why is this?

At PCR/M blastocyst culture is used to determine that the embryos are able to grow and develop beyond the internal embryo changes that occur on Day 3. In order to identify these high potential embryos, we perform Day 5 embryo transfers. We know that a proportion of the embryos will advance to the blastocyst stage and others will not. In addition, where there is a larger number of embryos in culture and the quality is similar or mixed, we prefer to grow the embryos to Day 5 in order to best select the most advanced and better quality embryos that develop. Even if there are only one or two embryos, growing them to Day 5 for embryo transfer or to Day 5 or 6 for cryopreservation, helps to determine if the embryo is viable and suitable for transfer or freezing.

Best of luck with your IVF treatment and please do not hesitate to ask one of the embryologists or your doctor if you have a question that has not been explained here or if there is any information that requires clarification. Please also refer to the diagram on the next page as a guide to the average cycle expectations

Average number of eggs and embryos per patient (less than 40 years of age)



Average number of eggs retrieved (11)

(the exact number varies per patient – all patients respond differently to the stimulation and the number of eggs retrieved may be more or less than the average)



Average number of mature eggs (9)

egg must be mature in order to fertilize



Average number of eggs that fertilize (7)

approximately 80%



Average number of fertilized eggs that will form embryos (98%)



Average number of embryos on Day 3 of culture

(50% of good quality embryos on Day 3 of culture will have the ability to grow and form a blastocyst on Day 5/6)



Average number of blastocysts on Day 5/6

(fewer eggs retrieved than average will result in fewer than average blastocysts on Day 5/6).