



# **Guide to IVF Laboratory Results**

PACIFIC CENTRE FOR REPRODUCTIVE MEDICINE - EDMONTON  
3<sup>rd</sup> Floor, 9888 Jasper Avenue, Edmonton AB T5J5C6

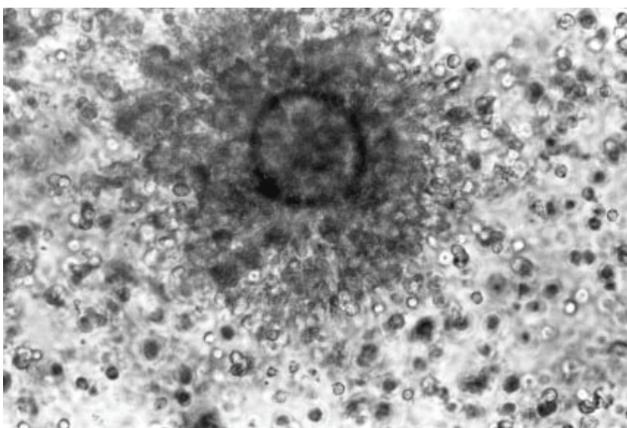
FRM-LAB-416E-20140122-1

The following information will guide you through what results to expect each day from the embryologists at PCRMC following your Egg Retrieval procedure. At the end of the document there are some Frequently Asked Questions (FAQs). It is very important that you read this section because there are some important details that may help you to understand the results that you are given by telephone each day.

### **Day 0 – day of egg retrieval**

Before your egg retrieval the embryologist will come out and introduce themselves to you, verify your identification and verify what laboratory procedures are being performed for your treatment cycle. The embryologist will also request a telephone contact number at which you can be reached for all embryology results during your treatment. After your egg retrieval procedure, the embryologist will again speak to you in the procedure or recovery room and let you know the final number of “egg masses” that have been found. Sometimes the number of “masses” is greater than the eventual number of eggs that the laboratory can use because the eggs may not be suitable or the mass did not contain an egg. The number of eggs retrieved is usually lower than the number of follicles that have been measured each day for your cycle monitoring. Many of the measured follicles are too small and the egg cannot be retrieved from them.

If a fresh semen sample is being used, your partner will be asked to provide a sample shortly before or after the egg retrieval. Insemination of the eggs (either by standard IVF or by ICSI) is 4-6 hours after the egg retrieval. With standard IVF, all of the egg masses are exposed to an aliquot of washed highly motile sperm and allowed to incubate overnight. If your treatment requires ICSI, only the mature eggs will be injected with a single washed sperm. Immature eggs are not yet competent and will not be injected because they do not fertilize properly.

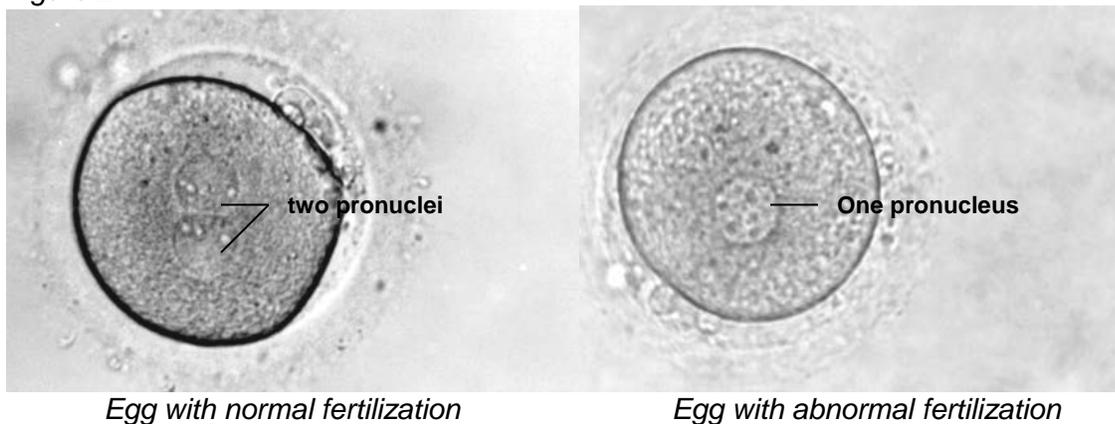


*Figure 1.  
Egg mass immediately following egg  
retrieval procedure. The dark circle in  
the centre is the egg.*

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

The fertilization process takes place overnight after the insemination (IVF or ICSI). The eggs are examined first thing the following morning and “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 or more than two of these “pronuclei” are seen. In both cases the egg is fertilized but is classified as “abnormally fertilized”. This means that the fertilization process has not progressed normally in the egg. Only those eggs that show normal fertilization will be grown in the lab to form embryos.

*Figure 2.*

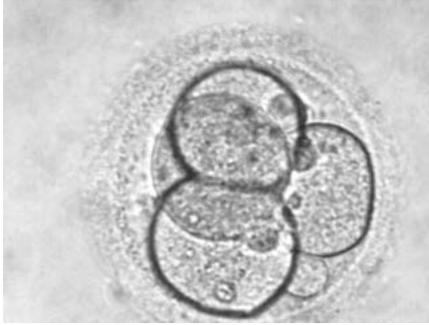


One of the embryologists will usually call you between 8 am and 10 am to let you know the results of the fertilization.

## **Day 2 – early embryo growth**

One of the embryologists will call you between by noon with your Day 2 results. On Day 2 we expect that the embryos have started to grow and that they have divided to have between 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.

If the total number of embryos is 3-4 or less, the embryologist may advise you that there is a chance of having a Day 3 embryo transfer and for you to be available first thing in the morning for a phone call between 8 and 8:30 am. In some cases the Day 3 transfer may already be confirmed with you on Day 2. For example, if you have 2 embryos and your wish is to have 2 embryos transferred, we already know which embryos to pick. In this case there is no need to grow the embryos to Day 5 for selection purposes.



*Figure 3.  
Good quality 4-cell embryo, 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 (possible embryo transfer day)**

All patients with less than 5-6 embryos should be available on the morning of Day 3 just in case an embryo transfer is required. If you were already cautioned on Day 2 that an embryo transfer may be scheduled for today, or if the embryos have changed overnight, the embryologist will call between 08:00 and 08:30 am and let you know if you need to come to the clinic. Proceeding with a Day 3 embryo transfer instead of a planned Day 5 transfer is not a negative outcome. In most cases it means that the embryologists are already able to select the best quality embryos and they do not need to keep the embryos in the lab for 2 more days for selection.

Should the plan be to proceed to Day 5 for ET, the embryologists will call you between 8 am and 10 am on Day 3 and update you on the progress and development of the embryos.

We expect 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.



*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 stages can be seen and this is normal. We expect the embryos to have more than 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 good quality embryos from Day 3 because of numerous changes occurring in the embryo at this stage of development. Only the “strong” embryos will progress through to morula formation.

One of the embryologists will call you by noon on Day 4 and update you on the progress and development of the embryos. The embryologist will also give you the appointment time for your embryo transfer the next day.

## Day 5 – blastocyst formation and embryo transfer

On Day 5 we expect a proportion of the embryos to become blastocysts. The grades and quality of the blastocysts will be explained at the time of embryo transfer. The embryologists will not call you today and you will receive the update regarding the embryos when you arrive at the clinic.



*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.*

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 **good quality** embryos from Day 3 will reach the blastocyst stage. This is the reason that there is the requirement for a minimum number of good quality embryos on Day 3 for continued progression to Day 5 for embryo transfer. 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.

The blastocyst grading system is based upon a number followed by two letters. The number refers to the amount of expansion the embryo has undergone and is not related to the morphological quality. The amount of expansion expected for each patient is very dependent upon when the oocytes were originally inseminated 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. The two letters refer to the quality of the two distinct areas of the blastocyst, as seen in Figure 5. The “clump” of 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 outer surface of the blastocyst is 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.

### **Embryo Cryopreservation (freezing) –**

**Day 3 embryo transfer patients:** If there are excess **good quality** embryos remaining after the embryo transfer procedure the embryologists will culture the embryos to Day 5 or Day 6 for possible cryopreservation. The embryologists will not call you each day anymore, they will call you on Day 6 to update you on whether or not they were able to cryopreserve any embryos.

**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, they 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 call you on Day 6 to give you the final results regarding how many embryos they were able to cryopreserve.

**Embryo “Freeze-all cases”:** If you are not having a fresh embryo transfer (OHSS, fertility preservation or other medical reason for no transfer), and all of your embryos are to be “cryopreserved”, there are 3 possible embryo stages when cryopreservation can be most effectively performed.

**Day 1** – usually done for fertility preservation cases and/or cases with very low embryo numbers

**Day 3** – usually done for fertility preservation cases and lower embryo numbers.

**Day 5/6** – usually done for “freeze-all” cases and for excess good quality embryos after embryo transfer that have reached the blastocyst stage.

## **FAQs**

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

Most likely your treatment involves ICSI if the normal forms are 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 sample, 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 PCRM, 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 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 75-85% 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 results that can be expected for a patient under 40 years of age.

### **I did not get a phone call from the embryology lab between 8 am and 10 am, is there something wrong?**

We will normally try our best to call between 8 am and 10 am but do not worry if we are later, it just means that we are busy and we will not forget to call at our earliest opportunity. Please also check your phone, your message box may be full or it may be turned off.

### **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 patient does have an effect on the eggs obtained and 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 with increasing age. It is important to note however that we do have many success stories with our patients over 40 years old and many healthy babies!

### **I just saw on the website that you can do genetic testing (PGD/PGS) on the embryos, when are you going to do this?**

PGD/PGS 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 PGD/PGS while you are in your current cycle. There is also an extra cost associated with the addition of this testing to your treatment. Any enquiries 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 calls 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 PCRM 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.

### **I am having acupuncture for my embryo transfer, how do I schedule this?**

All of the acupuncturists affiliated with PCRM are familiar with the scheduling. You will need to call them and give them the transfer time that we have passed on to you and the acupuncturist will arrange the pre-transfer acupuncture appointment time with you.

### **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 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 PCRM, 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 PCRM blastocyst culture (Day 5 transfer) is used as a selection tool. We know that a proportion of the embryos will advance in development to the blastocyst stage and others will not. Where there is a larger number of embryos in culture than are required for transfer 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 will develop.

### **If I am having a Day 3 transfer, does this mean that my chances of pregnancy are lower?**

If you have close to the exact number of embryos in culture that you would like transferred, it is very easy to select the embryos on Day 3 already. This does NOT mean that your embryos are of poorer quality, it only means that it is already easy for us to select the embryos.

***Best of luck with your IVF treatment and please do not hesitate to ask one of the embryologists 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)*



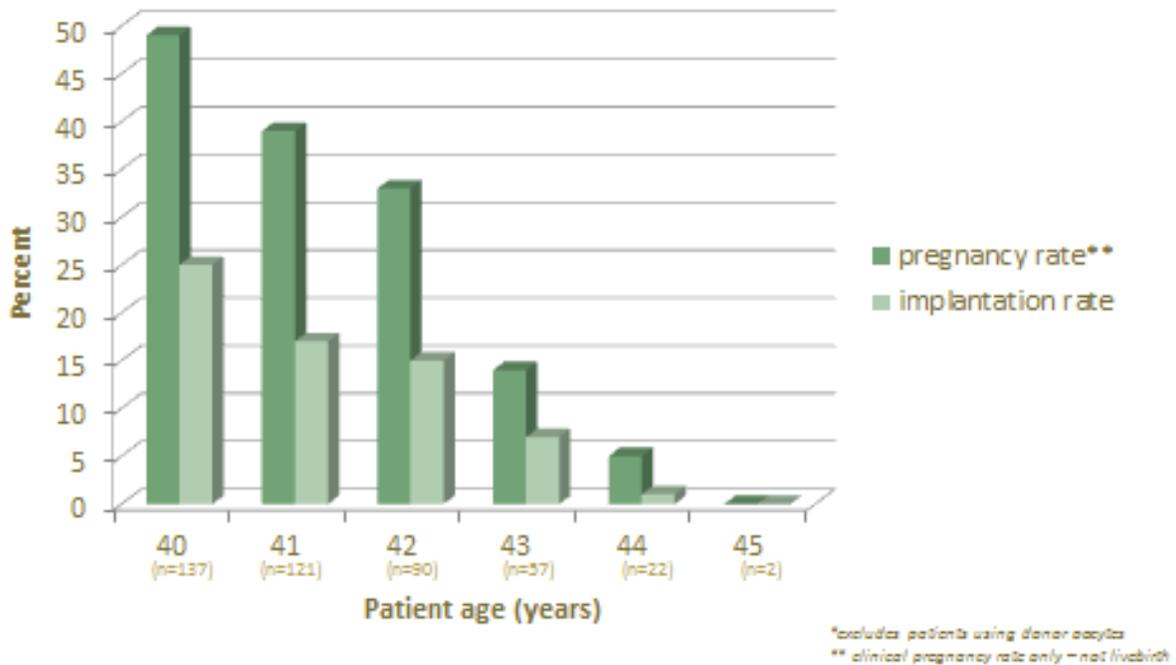
**Average number of blastocysts on Day 5/6**

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

## Pregnancy and Implantation rates in patients

### 40 years and older\*

(PCRM data 2007 to 2012 inclusive)



## Pregnancy and Implantation rates in patients 40 years and older

(PCRM data 2007 to 2012 inclusive)

Patient age (years)	Number of cases	Average # embryos transferred	Pregnancy rate	Implantation rate
40	137	2.8	49%	25%
41	121	2.8	39%	17%
42	90	3.1	33%	15%
43	57	3.0	14%	7%
44	22	3.1	5%	1%
45	2	2.0	0%	0%