

What to Expect While Your Embryos Are in The IVF Laboratory

The laboratory component of your IVF journey should not be mysterious, and we always welcome your questions. The science behind growing embryos *in vitro* is constantly evolving as we strive to make the culture environment as natural as possible to optimize your chance of success. It is now standard practice to grow embryos for 5 or 6 days before transfer and/or freezing (when they will have reached the “blastocyst” stage) as this has some very important advantages:

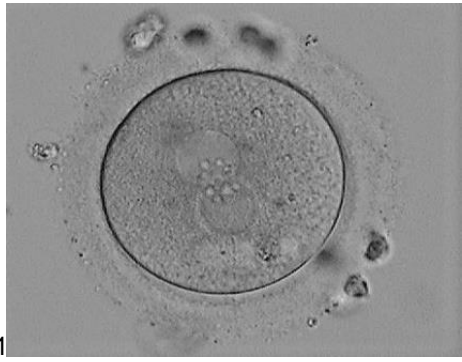
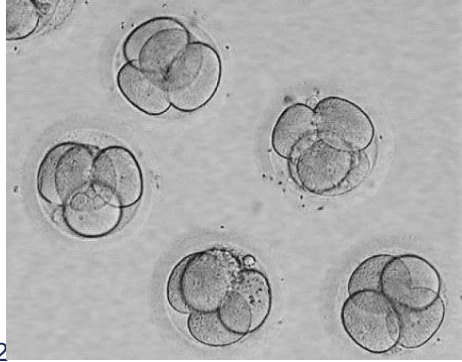
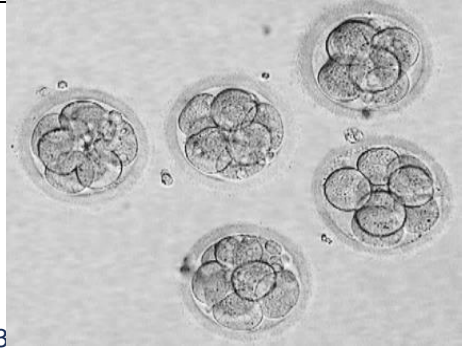

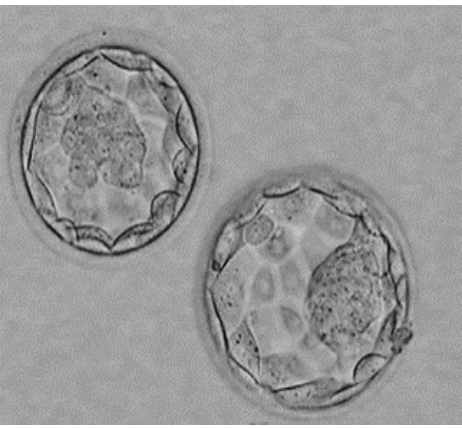
- 1) Growing embryos for 5 days instead of 3 is an excellent selection tool: only a proportion of embryos will become blastocysts, the others will stop growing.
- 2) If the embryo becomes a blastocyst, it has a much higher chance of implanting. This means fewer embryos can be transferred to achieve a healthy pregnancy, and the risk of multiples is subsequently reduced. It also allows us to eliminate poorer quality embryos which would not have made a pregnancy.
- 3) For successful implantation, the stage of the embryo and the lining of the uterus must be “in sync”, and this is more easily achieved when the embryo is at the blastocyst stage, matching more closely with what happens in a natural cycle.

At PCRM we have a reliable, reproducible culture system, and because of this we are happy to grow everyone's embryos to day 5 before transfer. There is no way to know for sure whether all embryos that fail in culture would also have failed in utero, but we believe that there is no harm caused to your embryos by five days of growing in the lab. What follows is a brief outline of what will be happening to your embryos for the six days that they will be in the lab.

When Will I Get an Update?

- We will provide you with an update through our messaging portal, Prelude Connect App. You will find these messages under “Embryo Update”.
- You will normally receive a message from the Embryology department between 8:00-10:00am with fertilization results the day after your egg retrieval. On the fourth day of your embryo culture, the Embryo Update will be sent to you between 11am-12:00pm. If you have signed up for genetic testing of the embryos, we will call on day 4 to confirm that you are comfortable proceeding with the genetic testing.
- Should anything unexpected arise with your embryos in culture, we will contact you by phone to discuss the progress.
- If our message is a bit delayed, especially on weekends, please don't worry. It simply means we are busy in the Lab, but rest assured, we won't forget to update you!
- Please allow 3 to 4 weeks after your cycle for a notification indicating that your “Cycle Summary Report” is available in your patient portal. You can view this document in your patient portal under the “Documents” tab. This report will provide you information about your fertilization, embryo development, photos of embryo(s) transferred, details of the semen, and the grades of your frozen embryo(s).

We will NOT provide any updates on day 2 or day 3.

Day 1		<p>(Day ZERO is the day of your egg retrieval).</p> <p>On day 1, normally fertilized embryos show 2 pronuclei (PNs) in the center (one from the male and one from the female). They will not divide until later today.</p> <p>PCRm will send a message that summarizes your fertilization results between 8 and 10am through Prelude Connect App.</p>
Day 2		<p>On day 2, we expect that most of the embryos will now have divided and will ideally be 2-4 cells. Around 98% of embryos which have shown normal fertilization on day 1 will divide and continue to grow.</p> <p>No patient update provided on Day 2.</p>
Day 3		<p>On day 3, we expect the embryos to have made another division from the previous day, and to ideally have 6-8 cells. Up until day 3, it is only the maternal DNA which has been contributing to the embryo's development. At some time on day 3, the male contribution kicks in, so there is sometimes a change in embryo quality after day 3, especially if there is a known "male factor".</p> <p>No patient update provided on Day 3.</p>
Day 4		<p>On day 4, we expect the embryos to have grown further, and to have started to compact – this is the morula stage (this just means that the individual cells can no longer be counted, and that the cells have started to fuse together).</p> <p>PCRm will send a message through the Prelude Connect App between 11am- 12pm with an embryo update and to confirm your transfer time, if that applies. However, if you are having your embryos genetically tested, we will contact you by phone to provide an estimate of how many embryos to be biopsied and frozen.</p>
Day 5/6		<p>On day 5, we expect a proportion of the embryos to have become blastocysts. The grades and quality of your embryos will be fully explained to you at the time of the transfer. Any surplus embryos which form GOOD QUALITY blastocysts on day 5 or day 6 may be cryopreserved for future use.</p> <p>Read the explanation of our blastocyst scoring system later in this booklet. Please allow 3-4 weeks for your "Cycle Summary Report" to be available in your patient portal. We will provide you with a message through the Prelude Connect App on day 6 for a final update.</p>

Do You Grade The Embryos?

Yes! From day 1 to day 4, we score cleavage embryos on a scale from 1 - 5.

1 = excellent

2 = good

3 = average

4 = a little below average

5 = below average (poor).

If embryos are graded 1-3, we are happy with their quality. If they are graded 4 and 5, we will caution you that those will likely have a lower chance of becoming blastocysts. The most important thing is that there is daily progression of the embryos. However, if an embryo is too far behind the ideal time points that we look for, it is less likely to be successful in causing a pregnancy. It is perfectly normal to have a mixture of embryo stages and grades, especially when you have a larger number of embryos.

Once the embryo becomes a blastocyst on day 5 or on day 6, its score describes the parts of the blastocyst, and consists of a number followed by 2 letters (for example 4AA). This scoring system and what it means will be fully explained to you before your transfer. Like most IVF Clinics, we use the Gardner Grading System for blastocysts. Read further in this booklet to see a visual explanation of both the cleavage and blastocyst grading system.

Embryo Transfer – Important Instructions

You will be given your transfer time when we send you a message through the Prelude Connect App on day 4. Transfer time is usually mid to late morning. Please remember to arrive at PCRM 30 minutes before the scheduled time. Take your medications as you normally would on the morning of your transfer, and make sure that your bladder is comfortably full - this will make it easier for the Physician to have a clear picture for your ultrasound-guided transfer. Drink 3-4 glasses of water 1-2 hours before the scheduled transfer time.

Will You Freeze Surplus Embryos?

On average, we expect around 50% of good quality day 4 embryos to develop into blastocysts (remember, this is an average for ALL patients therefore, some patients will have more embryos to freeze, and some may have less). On average, we freeze 70% of blastocysts. Blastocysts must be of good quality, or they will not survive the freezing or thawing processes. We will not freeze blastocysts of poor or sub-optimal quality, as they are not likely to survive.

What if my Embryos Are Not Blastocysts on Day 5?

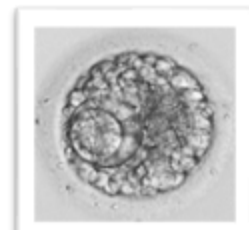
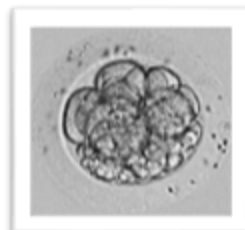
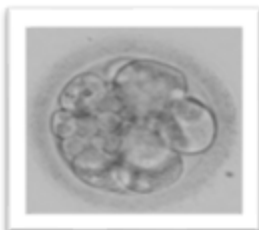
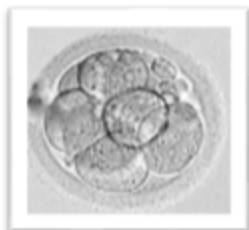
Sometimes embryos will not have reached the blastocyst stage at the time of your transfer on day 5- Do not worry, this happens often. If your embryos continue to grow each day, we will still transfer one or two of the most advanced embryos and grow all the others for one more day. You will receive a final message through the Prelude Connect App from the Laboratory on day 6 to inform you of any embryos that were frozen. Freezing can be done on day 5 or on day 6 – we wait until the blastocysts have developed the ideal number of cells before we freeze it.

On Day 4, How Do You Estimate How Many Embryos Will Be Frozen or Biopsied?

All of our Embryology staff have experience in estimating blastocyst formation numbers, using both clinic statistics and by observing how your embryos have developed over a four-day period. The estimate is based on our conservative “educated guess” and is the best estimate we can give at the time. The number being frozen or biopsied may end up being more or less than what we have estimated.

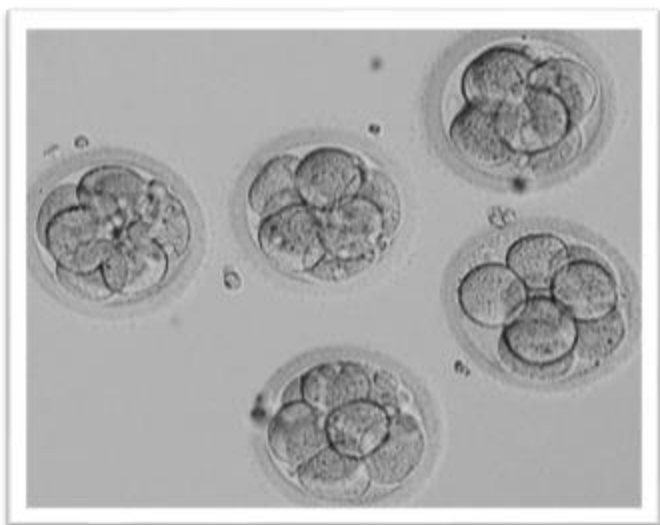
If you are having a “freeze all” cycle, or a PGT cycle, we will not update you on day 5, but we will update you sometime on day 6 with a final update.

Embryo Grading for Cleavage Stage Embryos (Day 2 to Day 4)

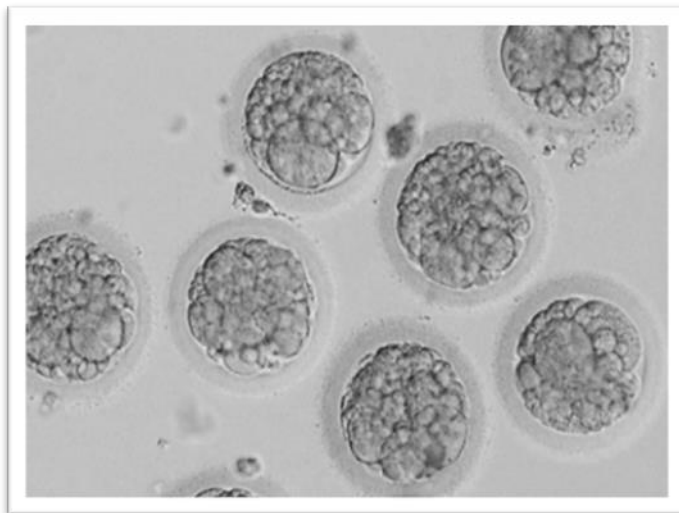


Grade 1 Excellent Quality	Grade 2 Good Quality	Grade 3 Average Quality	Grade 4 Below Average Quality	Grade 5 Poor Quality
No irregularities of membranes and/or cytoplasm	Minor irregularities of membranes and/or cytoplasm	Multiple minor irregularities OR a single moderate irregularity	Granular cytoplasm, dark color, irregular shape/membranes/zona	Multiple abnormalities of zona, membranes and/or cytoplasm.
Blastomeres of even shape and size	Blastomeres of slightly uneven size			
No fragmentation	≤10% fragmentation	>10 and up to 25% fragmentation	>25 and up to 50% fragmentation	>50% fragmentation.
No granularity of cytoplasm				

- If your embryos are graded 1, 2 or 3 we are happy with their quality, and these are the grades we see for most people each day in the lab.
- Most patients will have a mixture in the quality of their embryos, and this is normal, especially if you have a larger number of embryos.
- If your embryos are graded 4 or 5, they are of poorer quality. This does not mean they will not lead to a pregnancy, but we may caution you that they are less likely to make blastocysts, and less likely to implant.
- Remember, even if all of your embryos are perfect on day 4, on average only 50% of them will become blastocysts on day 5.



Good Quality Day 3 Embryos



Poor Quality Day 3 Embryos



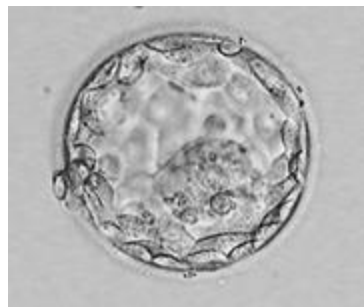
2AA



2BB



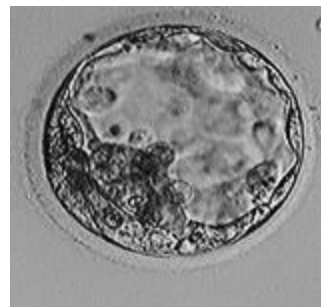
2CC



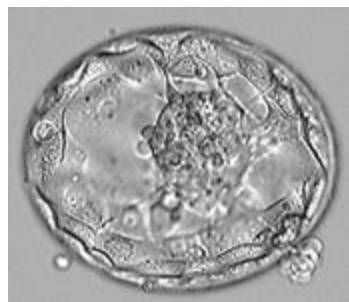
3AA



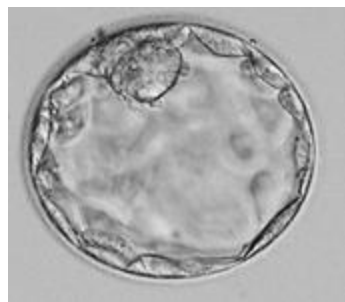
3BB



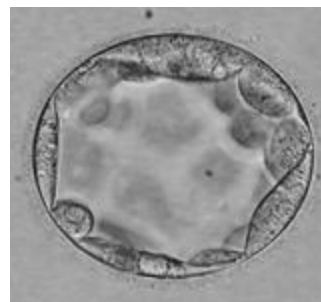
3CC



4AA



4BB



4CC

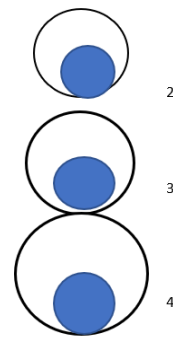


4DD



5AA- Hatching Blastocyst (Embryos that are assisted hatched)

Legend



Inner Cell Mass (ICM) Grading:

- A** Tightly packed, many cells.
- B** Loosely grouped, several cells.
- C** Very few cells. Poor
- D** No ICM visible

Trophoblast Grading:

- A** Many cells forming a cohesive epithelium.
- B** Few cells forming an epithelium.
- C** Fewer cells, borderline-below average quality
- D** Very few cells. Poor

- On average, 50% of embryos which are “good” quality on day 4 will progress to a blastocyst stage.
- The number in the score refers to the size of the blastocyst, and it changes quite rapidly on day 5.
- Inner Cell Mass (the first letter in the score) = the tight ball of cells inside the blastocyst which will eventually become fetal tissue.
- Trophoblast (the second letter in the score) = the surrounding cells which will become the placenta.
- Grades A and B, expanded to size 2, 3 and 4 are all good quality embryos and can be frozen. There is little difference in the potential to implant between blastocysts graded AA or BB.
- Blastocysts with a “C” grade in their trophoblast have a lower chance of implanting but can still make pregnancies. Grade C trophoblast embryos may be frozen depending on their development in the laboratory. These may have a lower survival rate during the freeze/thaw procedure.
- Blastocysts with a “C” grade in their ICM will not be frozen due to insufficient cells to form the future fetal tissues.