MODULE 4, CHAPTER 2

00:00:26
We will now continue with embryo culture.

00:05
So one of the exacting tests of the lab staff is to evaluate embryo quality. So morphologic evaluation by microscopy is a first line approach for assessing embryo quality and what the lab staff are looking at and what their evaluation parameters are, is that looking at the cell number, the degree of fragmentation, and that is debris that they will see in the cells or hope not to see too much of, the symmetry so the size and shape of the blastomeres and a blastomere is one of the cells in the ovary. I'm sorry, in the embryo. They look for cytoplasmic pitting and the absence of the vacuoles and multinucleation. Additional parameters for blastocyst stage involve looking at how the blastocoel is expanding and the status of the inner cell mass and the trophectoderm. Now, while we would like to look at the embryos constantly, it does take the embryo out of that nice environment that we are having them develop in. So once fertilization is done, the embryos are checked daily, but more commonly now only on certain days. So the embryos are disrupted less and not having to be taken out of the incubator as often.

01:32
There are other embryo techniques, primarily we do ocular evaluation that is just looking with eyeballs and the microscope and assessing the embryo. There is some subjectivity to this, although the lab staff is well experienced and well practiced at assessing embryos. There are other technologies that are being incorporated in the clinical setting with the goal of standardizing embryo quality and improving implantation success rates. In other words, decreasing the amount of subjectivity. One is aneuploidy screening, which is actually testing the chromosomal makeup of the embryo to discuss this in detail later, metabolomics profiling, which is testing the culture media to look at the metabolic products that the embryo is putting off to assess its development, and also time lapse imaging, which is like time lapse photography where the embryo is analyzed to see if it's developing normally.

02:41
So classification, this would be looking at a day 3 embryo and day 3 embryos are graded from A to D with A being the highest grade and D being the lowest. So let's just look at a grade A and look at what would we create that grade. So, we look at there's less than 10 percent fragmentation that is like debris in the cell. All the blastomeres are even there's no multi nucleation and no vacuoles.

To compare this to a grade D where a fragmentation would be greater than 35 percent. The blastomeres are uneven and there is multinucleation. That means more than one nucleus in the cells and there are vacuoles which are like little holes.

And you can see some examples of we see the 8 cell that has 15 to 20 percent scattered fragmentation, so this would be a grade B. And we see in the 4 cell embryo unequal blastomeres and one binucleated, which means it has 2 nuclei and then an 8 cell blastomeres with vacuoles. So those are not desired.

04:02
Grading a blastocyst is done under a different system where we look at grade 1 to 6 with 6 being the highest grade and in the other two columns we grade from 1 to C. I'm sorry, from A to C and grade 1 or A

would be the highest grade and the same for when we look at the trophectoderm morphology where 1 would be the highest and that would be an A.

So for example, if we look at 6 in the degree of expansion and hatching status and A or 1 a tightly packed with many cells and in the trophectoderm morphology column 1 or A, many cells forming a cohesive epithelium, that would be a 6 AA, which would be the highest grade possible.

04:57
So embryo grading of a day 5 to 6 blastocyst, if we look at these images here and try to guess which is which. Which has a high grade and which doesn't just going to go through them quickly. And just looking at this, see if you can guess which embryo was the highest grade or which embryo actually was successful implantation and live birth.

So we look at the first one. We can see this is an early blastocyst. And this one, we can see that there's a hatching blastocyst, which is good, and it has a grade of 5 AA. This one is a grade 3 AA. And remember, 6 AA would be the highest grade. This is a 5 BA. This is a 4 AA and this is a grade 3 CB. So which one do you think successfully implanted? It's this one, not the one that you might have imagined correct. Just goes to show that grading does not always relate to success or a higher grade or a lower grade. You could still have an implantation in a lower graded embryo, which is something that we can reassure our patients with.

06:25
So let's move on to assisted hatching. At the blastocyst stage, the embryo begins the hatching process. This is in vivo. So without reproductive technology and this allows the outer covering of the trophoblast cells to interact with the endometrium. Hatching is absolutely required for a successful implantation.

Failure of implantation may result from the inability of the blastocyst to escape from the zona pellucida which is the outer wall of the embryo, originally the oocyte cell wall. If warranted, assisted hatching is conducted prior to transfer. How this is done is that a defect is created in the zona pellucida to enhance implantation and it can be done by mechanical, chemical or laser. It's not a routine procedure, but selected patients may benefit.

07:24
Another thing that is done with the lab is the PGT-A, PGT-M and PGT-SR we will talk about this now. Preimplantation genetic testing is testing performed prior to implantation to identify genetic risks. We do this and this is also patient choice but patients who may benefit are those with a pre-existing genetic risk, for example, a known genetic mutation. So in family history, a higher risk for aneuploidy, such as for women who are 35 and older. If there's a known structural rearrangement, for example, a balance translocation. So PGT-M identifies a single, a monogenic gene disorder. For example, gene cystic fibrosis or Huntington's disease if there's a family history. PGT-A is a comprehensive chromosomal screening for aneuploidy, and this screens for extra or missing chromosomes similar to amniocentesis that would be done in pregnancy.

There's current development now to have a non-invasive PGT-A. So stay tuned as this is an emerging technology, PGT-SR is chromosomal structural rearrangements which can result in pregnancy loss.

08:56

Some of the things evaluated or syndrome's evaluated in PGT-M are X-linked disorders such as hemophilia, autosomal recessive disorders, for example, cystic fibrosis or Case X or autosomal dominant disorders, Huntington's disease and Marfan’s syndrome. Also BRCA or the breast cancer gene.

09:22
So the process of PGT requires an IVF cycle, including the usual ovarian stimulation. Once the embryo goes to blastocyst, a trophectoderm biopsy is performed. The trophectoderm cells become the placenta and this is something important when counseling patients to understand that this would be not the fetus that would be biopsied or the potential fetus. The cells are sent to a reference lab for analysis and this takes some time, which requires the blastocyst to be frozen and then be used at a later date. The patient consultation and decision making should be a collaborative process, and there are many difficult decisions for the patient to make whether to proceed with PGT or not, how many blastocysts are necessary to make PGT worthwhile and whether to transfer carrier embryos or not, or whether to transfer mosaic embryos or not. And the patient needs good, solid counseling during this time and a lot of support.

10:38
So embryo freezing or cryopreservation of embryos. We do this for patients who are high risk for OHSS. If implantation is temporarily compromised because of premature leutinization. Also for fertility preservation as an alternative to oocyte cryopreservation or for elective reasons. For example, during COVID we generally froze all embryos until we knew more about the complication, potential complications.

Also, if there are more embryos than are needed in the initial cycle and there are surplus embryos, they can be frozen for later use and avoid having to go through another IVF cycle. And there are other reasons to, for example, reasons that prevent a woman from carrying the pregnancy. If there is a uterine abnormality detected or if a gestational carrier is being used in the process. Also for PGT, as we already discussed, because of the turnaround time to receive results. The procedure is similar and that to freezing oocytes is that a cryoprotectant and vitrification are used to avoid the formation of ice crystals. And this really dehydrates the cells is quite hard on them, actually. So that's why the embryos with the higher grades would typically be frozen.

12:15
So in summary for IVF, IVF is a series of procedures used to assist with conception and include evaluation of mature oocytes and outside freezing, which are morphologically evaluated under light microscopy, sperm preparation, including the which includes semen collection and also using previously frozen sample. Fertilization for sperm with normal morphology, fertilization occurs in a petri dish by incubation of sperm with sight. In other words, spontaneous fertilization or using intracytoplasmic sperm injection with injection of a single sperm directly into an oocyte and then embryo culture. After 5 days of incubation, the transition from fertilized oocyte to blastocyst has occurred, although this can happen at a later time, for example, day 5 or 6.

13:18
Embryo selection and embryo freezing where the embryo is assessed to look at and we're discussing blastocyst here to look at the degree of expansion, the inner cell mass development and the trophectoderm morphology and cryopreservation which allows for the preservation of embryos by cooling and storing in liquid nitrogen for later use. Embryos are most commonly frozen at the blastocyst stage and this can reduce the need for multiple cycles of controlled ovarian stimulation.

PGT or prenatal or sorry, preimplantation genetic testing is performed prior to implantation to identify genetic risks and involves patient consultation and decision making prior to embryo transfer.

14:08
So now let's look at embryo transfer and embryo freezing.

14:14
Day 2 to 3 transfer generally occurs if there's concern about the embryo developing or making it to blastocyst stage or if there's very few embryos, for example, 1 or 2. Most commonly embryo transfer will occur at blastocyst stage. Just to note that blastocyst transfer may be associated with increased monozygotic twinning, which is a complication of pregnancy.

14:51
Embryo transfer technique, the mechanics of embryo transfer influence both implantation and pregnancy rates. It involves a transabdominal ultrasound guided technique with the placement of the catheter tip between 1 to 2 centimeters from the apex of the endometrial cavity, which is where generally normal implantation or natural implantation will occur.

We would want the endometrium to be have a thickness of greater than 8 millimeters for fresh embryo transfer or greater than 7millimeters for frozen embryo transfer to be receptive to the developing embryo and encourage implantation. And you can see the diagram here where a speculum is used to visualize the cervix, the catheter tip is introduced and the embryo or embryos are let deposited at the 1 to 2 centimeters from the apex or the fundus of the uterus.

15:47
So the steps of ultrasound guided embryo transfer. The blastocyst is first loaded into the catheter. And then under ultrasound guidance, the catheter is inserted into the uterine cavity. The embryo is deposited in the uterus and you can see in the ultrasound image, which is what the physician would be looking at, to see where the catheter is and when to deposit the embryo. The catheter then goes back to the lab who look at it under the microscope to determine that, yes, indeed, the blastocyst has been deposited in the endometrium.

16:33
So factors to consider in determining the number of embryos to transfer. The clinical factors are the age at a time of retrieval. Looking at the egg and embryo quality and implantation rates and pregnancy rates we know decline with age. A woman over 38 years is more likely to have more than one embryo transferred, patients may request more than 1embryo as it's they've determined this may be more cost effective to avoid of repeated IVF cycles. And this will occur more often for patients who are paying for their full treatment versus those with coverage. And we'll look at this more closely in a little bit. For example, in Ontario, the number of embryos transferred is mandated through the funding. So in Ontario, there is a set or which is a single embryo transfer policy for women who are up to age 35. Only 1 embryo is allowable to be transferred regardless of the embryo stage of development. For women who are age 36 to 37, 1embryo per transfer or 2 embryos per transfer. If it's a day 3 transfer for women who are 38 years or more, 2 embryos per transfer regardless of the embryo stage of development are allowable. The exception here is after 3 failed single embryo transfers, there may be an option to proceed to 2 embryos per transfer.

18:22
So in summary, for embryo transfer, embryo transfers can be done on day 2 to 3 at the cleavage stage or on day 5 to 6, the blastocyst stage. In clinical history and the number of good quality embryos available for transfer. Embryo transfer is best performed using transit dominated ultrasound guidance, where the catheter tip is generally placed 1 to 2 centimeters from the apex of the endometrial cavity done under ultrasound guidance and requires a full bladder. In patients with advanced maternal age more than 1 embryo may be transferred.

19:00
The next section in Module 4 is the implications of multiple births.

00:19:09:02
The implications of multiple births have risk to the neonate and the increased risks associated with even a twin gestation include a low birth weight, significantly increased risk of perinatal mortality, a 5-fold increased risk of cerebral palsy and higher rates of bronchopulmonary dysplasia, visual complications including blindness and necrotizing enterocolitis, also increase rates of learning difficulties and perceptual disabilities.

19:43
And there are also maternal risks to multiple pregnancies. The maternal morbidity is significantly increased, with multiple pregnancies and risk significantly more common than in singleton pregnancies include preeclampsia, myocardial infarction, heart failure, pulmonary edema, venous thromboembolism, caesarean section and hysterectomy, including blood transfusion. So these are not this is not a situation we would want to put our patients in.

20:18
Patients may request to transfer for multiple embryos for a variety of reasons, and there may be a higher pregnancy rate involved. However, the trend toward single embryo transfer has greatly reduced multiple births in Canada. In fact, multiple births pregnancies have been declining in Ontario since the IVF funded program and the SET, or Single Embryo Transfer policy. If you look here, if we look at 2012 as compared to 2018 we can see that definitely the rate of multiple pregnancy has reduced.

And if we compare multiples overall in Canada and multiples in Montreal, multiples in Ontario, we can see that with funding the multiple rate has reduced even more.

21:16
And again, if we look at Ontario and Quebec, both provinces that have funding and the rate with the rest of Canada, we can see that there is a much lower rate of multiple pregnancies even compared to the overall rate in all of Canada.

21:38
So worldwide countries with public funding, of which there is somewhat more than 39 currently, we do see that there are more countries without funding than with.

21:51
If we look at Canada, our funding, our provinces with funding are generally are all primarily located in the east, Ontario and Quebec have the most robust funding available. Manitoba and New Brunswick have

different forms of funding, such as a tax rebate and so on. There are developments in Quebec to even make their funding for IVF more robust, and Prince Edward Island is also looking at funding and we hope over time that all provinces would have funding.

22:35
So in summary, for implications of multiple births, the current standard of ART care involves elective single embryo transfer. Prior failed attempts may play a role in considering the number of embryos to transfer though funding dictates the number of embryos for transfer at times. Multiple pregnancies significantly increase maternal and fetal morbidity and mortality in addition to high health care costs.

Cryopreservation is the use of cryopreservation is important to facilitate multiple single embryo transfers if required.

23:16
Summary.

23:19
The development and advancement of IVF has brought the possibility of parenthood to many people. The 4 stages of ART include controlled ovarian stimulation, oocyte retrieval, IVF and all the laboratory procedures embryo transfer with or without cryopreservation. Assessment of embryo quality is important in the selection of the best embryos for transfer. Classification of day 3 embryos is based on the number of blastomeres, fragmentation, symmetry of blastomeres, multinucleation and vacuoles. Day 5 blastocysts are graded by degree of expansion and attributes of the inner cell mass and trophectoderm.

24:05
Oocytes and embryos may be cryopreserved for future use. Freezing is an option for fertility preservation in many circumstances, such as for cancer treatment, gender affirming treatment, and in combination with PGT for prevention of passing on genetic disorders. Also for some medical conditions, PGT involves a number of sophisticated techniques for the detection of genetic and chromosomal abnormalities. Multiple factors, including maternal age and funding requirements, play a role in determining the number of embryos to transfer. Complications of multiple pregnancy include premature birth, as well as a number of health risks to both the carrier and neonate.

24:52
Section completed. Please access the online classroom portal Digital Chalk to review and complete the exercises related to this content section.