MODULE 4, CHAPTER 1

00:01
Module 4 overview of assisted reproductive technologies.

In this module, our objectives will be to discuss the procedures used in assisted reproductive technology or ART and the selection of patients, to outline the oocyte retrieval process, to review and understand the five components of the IVF laboratory, including oocyte evaluation, sperm preparation, fertilization, embryo culture and embryo selection. Also, to review the medical and societal implications of multiple and high order multiple gestations. Finally, to introduce the ethical and legal aspects of reproductive technologies.

00:47
So let's start with an introduction to ART

00:51
We can't discuss it without talking about ethical aspects and reproductive technologies, and although ART has given hope to individuals experiencing infertility or situational infertility, the process is introduced several ethical, social and legal challenges. One is multiple gestation pregnancy. The transfer of more than one embryo is associated with health risks for both the carrier and child, the financial aspects where costs may be prohibitive for some individuals and creates inequality in access to care and creates reproductive privilege and challenges the ethics of health care, resources and funding.

Also, preimplantation genetic testing or PGT, we can characterize the genetic composition of the embryo, but what conditions are we right to screen for?

01:48
Also, reproductive autonomy, sometimes someone will present at our clinic wanting treatment, but we realize the futility of treatment and that person wants to proceed against medical advice. What do we do?

Refusal to initiate treatment, for example, treatment for menopausal women, medical risk of premature birth. We do have the Federal Assisted Human Reproduction Act, which is the AHR Act, which prevents discrimination on the basis of sexual orientation or marital status and protects gender identity rights. Also non-gatekeeping counseling. Reproductive tourism, where one travels internationally to pay foreign surrogates, may create financial coercion.

02:41
And then, third party reproduction brings its own suite of ethical aspects. For donors, donors face potential legal, medical and psychological issues involved in donation under the AHRA, the minimum age for a donor is 18 and donors cannot be paid for their donation. It must be altruistic. Donating gametes or embryos does not provide legal rights or duties for any resulting children. Also, information sharing is not guaranteed and the possibility of offspring contacting donors in the future.

For surrogacy under the AHRA, the minimum age is 21 years, also under the act, a surrogate cannot be paid for carrying the pregnancy, as with donors. And what rights does a surrogate have?

Under the act, the sale of sperm donor eggs or embryos is prohibited. Sex selection is illegal except to prevent, diagnose or treat a sex-linked disorder. Under the new Health Canada regulations, specific rules for reimbursement of expenses and screening are outlined, and this act became available in May of 2020.

The CFAS recommends that all individuals involved in third party reproduction and their partners of appropriate should undergo counseling prior to treatment, and most clinics will abide by this.

04:16
So what is ART? ART refers to a number of techniques in which sperm and oocytes are handled in the laboratory, the stages which we will discuss in detail involve controlled ovarian stimulation or COS, oocyte retrieval, in vitro fertilization with or without intracytoplasmic sperm injection or ICSI, embryo culture with or without assisted hatching, PGT preimplantation genetic testing, embryo freezing and finally, embryo transfer.

04:55
The indications for using ART are several. We see that there are a number of factors here. Please note that the male factor, the bar for male factor, seems much higher than it does for a female. And this is because the male factor is not been differentiated as we see on this slide for female. In fact, slightly more than 50 percent of reasons for infertility are female.

05:26
The treatment options for ART candidates. We have to screen our candidates to make sure that they have adequate ovarian reserve and good egg quality, that there's viable sperm and a responsive, supportive endometrial cavity to ensure their best option of success. Those without adequate ovarian reserve or adequate egg equality may benefit from egg donation, and those without viable sperm may benefit from sperm donation. Those without a responsive or supportive endometrial cavity may benefit from a gestational carrier.

06:07
To evaluate ovarian reserve, ovarian reserve refers to the number of follicles that may respond to stimulation by gonadotropins and can vary from cycle to cycle. When we see diminished ovarian reserve and you may see this as DOR, it refers to a low number of follicles such as seen on a basal antral follicle count. There are several tests that may be used to assess ovarian reserve to determine if the patient is a viable candidate for ART with the most common that we see are basal FSH levels with its companion test estradiol, a basal antral follicle count, or AFC, which is done by ultrasound and another serum test, anti-müllerian hormone, which is AMH, which has greater utility than a basal FSH.

07:01
The next section in Module 4 are the four stages of ART.

07:07
The four stages of ART include controlled ovarian stimulation, followed by oocyte retrieval, then IVF and embryo culture, and lastly, embryo transfer with or without embryo freezing.

07:25
So let's start with controlled ovarian stimulation.

07:32
In controlled ovarian stimulation, follicular development is carefully monitored to adjust medication dose and the time of retrieval mature oocytes. This is done by serial follicular ultrasound and hormone assays, primarily estradiol during the stimulation. High-responder patients are at risk for ovarian hyperstimulation syndrome, primarily if human chorionic gonadotropin or HCG is used as a trigger for ovulation. In these patients the safest approach is a short protocol, using a GnRH antagonist to prevent premature ovulation and then a GnRH agonist as the trigger.

08:18
So let's just stop for a moment to look at ovarian hyperstimulation syndrome. This is an iatrogenic complication of COS which is entirely due to the administration of gonadotropins. The reported OHSS incidence in moderate group is 3 to 6 percent and for severe it is 0.1 percent to 2 percent. The pathophysiology of this complication results from the release of vasoactive peptides, for example, VEGF, from the hyperstimulated ovaries. And this is characterized by a shift of intravascular protein rich fluid to the abdominal cavity, which can produce ascites which is fluid in the peritoneum or even pleural effusions in the lungs.

How we identify risk factors is when we see a high basal antral follicle count on ultrasound similar to the number of follicles we'd see for PCOS with a large number of follicles growing. Or if at retrieval, there's a large number of oocytes retrieved greater than 15 oocytes. If there's a previous history of OHHS or if we've used hCG in the trigger prior to egg retrieval. There are two phases of OHHS, the first occurs early, which within the first 7 days after trigger and late, which occurs if the patient is pregnant.

09:54
To diagnose a OHSS, firstly, the patient would have a recent history of ovarian stimulation, followed by ovulation or hCG administration. The classic symptoms of moderate to severe OHSS or a sensation of bloating, abdominal pain, rapid weight gain, often more than 2 pounds a day and decreased urine output. Also, the symptoms can include shortness of breath, great ovarian enlargement seen on ultrasound, nausea, vomiting and diarrhea. So in other words, the patient feels very unwell.

Other clinical evaluation to diagnose are the ultrasound to look at the panel of electrolytes and the coagulation profile. Alternative diagnosis should be kept in mind also because the symptoms may also be represent pelvic infection, intra abdominal haemorrhage, ectopic pregnancy, appendicitis and other complications such as ovarian cysts.

11:06
So the 2016 Canadian guidelines to classify OHSS from moderate to critical, if we look in this table under mild will see mild abdominal pain, abdominal bloating and ovarian size that is less than 8 centimeters. Although the patient in mild OHSS is feeling uncomfortable, it's not serious. Moderate would have moderate abdominal pain, nausea and vomiting possibly, and ultrasound evidence of ascites which is fluid in the peritoneum and ovarian size that's 8 to 12 centimeters. For severe there would be clinical ascites. In other words, ascites could be palpated, oliguria which is greatly reduced urine output, hemoconcentration with a haematocrit that's greater than 45 percent and hyperproteinemia and ovarian size would usually be greater than 12 centimeters. For critical OHSS there'd be tense ascites or large pleural effusion and this would be the abdomen would be very tense on palpation for this. Haematocrit would be greater than 55

percent, a white count greater than 25,000 and either oligouria or anuria, anuria is no urine output, thromboembolism and also acute respiratory distress syndrome.

12:35
For management of OHSS in the mild to moderate, these cases can be managed on an outpatient basis and to maintain adequate hydration, we would need to instruct the patient to, even though they feel bloated and uncomfortable to what we call push fluids and add in things such as Gatorade to help their electrolyte balance. Paracentesis, which can be done an outpatient patient basis to relieve the ascites and to avoid vigorous activity. Also pain relief for the discomfort of the symptoms. Some patients may require in patient management to push or give them IV fluids to maintain adequate hydration. Severe OHSS often will involve hospitalization and to assess their general condition, hematocrit and laboratory testing are critical at this point.

The complications may lead to intensive care that is being admitted to an ICU in the hospital, IV hydration with a crystaloid solution, plasma expander, for example, the administration of heparin and to aspirate acidic fluid, paracentesis as well as not only daily monitoring, but very close monitoring.

14:01
To prevent OHSS, first of all, is to recognize the risk factors and tailor the COS protocol, for example, lower doses of gonadotropisn and using an antagonist cycle with a GnRH agonist trigger. Secondary preventive measures, which would be if a patient is already on treatment, would be to cancel the cycle if OHHS is apparent, to withhold exogenous gonadotropins, which is also called coasting, although there's a low quality of evidence for this approach. Also, an agonist trigger and then freeze-all, either all embryos or all oocytes, the use of IV albumin or hydroxyethyl starch, plasmic expander at the time of oocyte retrieval and also post retrieval dostinex.

14:59
All right, so now let's move on to oocyte retrieval.

15:05
So oocyte retrieval is typically performed at 35 to 36 hours following medical induction of final mature oocytes, that would be what we call using a trigger to trigger ovulation, although we don't want that to happen. If the oocyte retrieval is done less than 34 hours after this oocyte maturation may be incomplete and oocytes may be still attached to the wall of the follicle, making them impossible to retrieve.

If oocyte retrieval happens more than 30 hours after the trigger or the medical induction, the patient may ovulate. And of course, this is not what we want to happen because we want to capture all those lovely oocytes. The criteria for trigger or induction is typically done when a certain number of follicles reach 17 millimeters in diameter or more. However, this is both clinic specific and patient specific. In some patients, mature oocytes may be harvested from follicles that are greater than 12 millimeters in diameter, but this is unlikely in most cases. They do need to be that 17 millimeters or more.

16:23
So going back in history in the early days of IVF, oocyte retrieval was done with laparoscopy, which required general anesthesia, poor oocyte retrieval rates. And because this preceded the use of GnRH antagonists and agonists was often done as an emergency at all hours of day and night. So you can imagine how challenging that would be. Unfortunately, now it's it is exclusively done with transvaginal

ultrasound guided needle aspiration which requires minimal intravenous sedation, and it is an outpatient procedure and can be timed precisely with agonist, antagonists and trigger medications.

17:09
In this sagittal view, we see the example of the guided oocyte retrieval and you can see the ultrasound probe, which is in the vagina for visualization. Attached to the ultrasound probe is a needle guide where a needle passes through and each ovary is punctured. And within the ovary, each follicle is punctured. The follicles are fluid filled. So while we cannot see oocytes on ultrasound, we definitely can see the follicles and drain that fluid. And you can see the test tube there and the fluid goes into the test tube. And when the test tube fills to a certain amount, it's passed off to the lab. And this process continues until all follicles of appropriate size, are drained and typically takes only about 10 minutes or so. So that's one thing that we can teach our patients that most will report during their procedure, they feel a little bit of pinching as the needle enters the ovary and sometimes some cramping and pressure. And these are normal and can be mediated by the IV sedation.

18:24
So our next stage we are going to discuss is IVF and embryo culture.

18:32
So now that oocyte retrieval has taken place and the follicular fluid has been passed off to the lab now the lab staff works their magic. So, their process involves evaluation of the oocytes, sperm preparation, fertilization, embryo culture and embryo selection and freezing.

18:56
First of all, as the fluid is passed off to the lab, the fluid is put under a light and a microscope and the eggs are extracted from the fluid but examined in the process. The egg is immediately placed in culture medium and incubated after being retrieved from the follicular fluid. So the evaluation criteria that the lab uses to assess the oocyte is to look at the oocyte and cumulus mass morphology, the polar body morphology and the meiotic spindle detection. In this photo, you can see on the screen, you'll see that ring around the oocyte, which is the cumulus cells. This is a beautiful egg, by the way, and the lab would be very happy with it. You'll see a little bump on the right hand side of the oocyte and that’s the polar body. So the first meiotic division has occurred, which is normal. And that's what the lab would like to see. It's very difficult to see the meiotic spindle, but the trained eyes of the laboratory staff would identify this and know that this was a normal egg.

20:09
So at this point, egg freezing would occur for patients who have elected to have oocyte cryopreservation and we've discussed this earlier, but the indications for fertility preservation can be for personal or social reasons. Also, prior to chemotherapy and radiotherapy, to preserve fertility, also prior to gender affirming treatment and can be an alternative to embryo freezing when a sperm sample is not available.

The process of cryopreservation involves evaluation of the oocyte to make sure that they are mature and high quality and able to withstand the freezing process. The oocytes are frozen by vitrification and stored long term in liquid nitrogen until the patient is ready to use them. Vitrification uses high initial concentrations of a cryoprotectant and ultra rapid cooling to solidify the cell into a glass like state without formation of ice crystals. This process does create a hardening of the zona pellucida, which means that ICSI must be done if that when the eggs are being used in the future.

21:30
The other process that the lab will be doing usually at the same time is the eggs are being retrieved from the follicular fluid and so on is to do sperm preparation. Sperm is absolutely required on the day of oocyte retrieval if we're not doing oocyte freezing and will be ejaculated sperm or surgically retrieved sperm and can be previously frozen sperm also.

The process involves a technique called swim up, where the highly motile sperm are separated from the seminal plasma. The collection methods involve masturbation or using a special condom through intercourse, can be surgically retrieved or done retrieved through a electroejaculation.

22:23
So the sperm retrieval techniques, the ones that are being used, will be different for non-obstructive azoospermia and they can involve testicular sperm aspiration which is a fine needle aspiration, a testicular sperm extraction, which is an open testicular biopsy, something called micro-TESE which is a very small bit of testicular tissue that is removed and using a microscope at great magnification.

If there's obstructive azoospermia that is, if there's no sperm in the ejaculate then microsurgical epididymal sperm aspiration or percutaneous epididymal sperm aspiration may be the sperm retrieval method of choice, or TESA using more of an open biopsy in the testes.

23:17
And this diagram shows us the various ways that sperm are aspirated. If you look at the bottom left testicular biopsy, there's a larger area of tissue that is removed and sperm extracted. But whatever method is used, sperm are extracted and then cryopreserved and ready to use at the time of oocyte retrieval.

23:46
Also for men who have erectile or ejaculatory disorders, vibrostimulation or electroejaculation may be used most commonly for spinal cord men who have experienced spinal cord injury.

Also, in some instances, men may have a retrograde ejaculation. This can be helped by sympathomimetics which are similar to cold medications which help strengthen the bladder neck and reduce the chance of a retrograde ejaculation. But if there is a retrograde ejaculation, the sperm can be harvested from the bladder after the patient urinates after ejaculation.

24:37
So fertilization, another process that takes place in the lab. If we're doing what we call spontaneous fertilization, so ICSI is not being done. 25,000 to 100,000 sperm can be incubated with a single oocyte. It's not necessary to add more than this as higher concentrations do not improve fertilization rate.

The insemination is typically performed a few hours after oocyte retrieval and approximately 18 hours after that, the oocytes are examined to determine if there is successful fertilization. A pre-zygote with two pronuclei and two polar bodies is evidence of normal fertilization. So, if you look at the image on the right, you'll see that there are two nuclei and the nuclei have fused. So that would be the nuclei of the sperm and the nuclei of the oocyte that have fused. And you now have a full complement of chromosomes of 46. And this is now a zygote. You can also see at the top left hand of the oocyte there are the two polar bodies. So this would be a normally fertilized oocyte.

25:56
So if we do fertilization via intracytoplasmic sperm injection or ICSI, this involves selecting a single sperm and injecting it directly into the oocyte. The oocyte can withstand this, it is similar to putting a needle in a rubber stopper and pulling it out and it seals immediately. And this is the ultimate option for successful treatment of severe male factor infertility.

26:30
The indications for ICSI are if there is a low sperm count, motility and or abnormal morphology, and in previous treatment there's been fertilization failure or low fertilization rate. If there's surgically retrieved sperm because there will be a small number of sperm through this method where ICSI is required. If there's frozen sperm but it's not adequate for spontaneous fertilization. Also frozen sperm from patients with cancer because remember we can just select one single sperm and inject it into the oocyte or if preimplantation genetic testing for monogenetic diseases, we'll discuss this later.

27:19
Also, with epididymal sperm extraction which is done in the case of a congenital absence of the vas deferens or obstruction of ejaculatory ducts or if there's a failed vasectomy reversal. And testicular spermatozoa, which is retrieved because of it's noted there's an absence of sperm in the ejaculate or if testing has shown that there's increased sperm DNA fragmentation.

27:53
Next step in the lab is embryo culture. So once the oocytes are fertilized, they undergo a characteristic transformation in morphology. They're incubated most commonly until day 5 or day 6 when blastocyst has developed. They may be transferred on day 2 or 3 if there is at the 4 cell or 8 cell stage, maybe done have indicated but is less common.

So let's look at these images. On day 1, we see fertilization, as we saw in the previous picture of the fused nuclei and the two polar bodies. By day 2 the embryo has started to divide and we call this cleavage. We see a 4 cell embryo. On day 3 we see an 8 cell embryo. Day 4 to 5 is termed morula, where there's too many cells to count under the microscope. And day 5 or day 6 we have blastocyst development.

28:58
So in the lab, the culture parameters, that is the culture media where the embryos are growing and dividing are meant to mimic natural conditions. For example, as the embryo is developing, it would require more proteins and sugars or different ones as it goes from stage to stage. And often the media will be changed to mimic this. The lab environment is critical to the development of the embryo. Air quality requires regular air quality checks. And you will not see alcohol-based hand cleansers in the lab. Only soap and water. Any scents can be damaging to the developing embryo and that's why you'll see it is a scent free area with no one using perfumes or perfumes or any scented products. In fact, most clinics will be entirely scent free.

29:55
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