ABCs of ART
Module 6.1 – 6.9

English Transcripts

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Module 6.1_ ABCs of ART_1st Trimester Pregnancy Care_EN Transcript

Dr. Stacy Deniz

Slide 1 – Note to Audience slide [00:00:00]

Slide 2 -Title slide [00:00:08] My name is Dr. Stacy Deniz, and I am an Assistant Clinical Professor at McMaster University and a practicing physician at ONE Fertility. I have the privilege of taking you through the ABCs of ART Module Six: First Trimester Pregnancy Care.

Slide 3 [00:00:29] A pregnancy can be divided into three trimesters, each lasting approximately 13 weeks. Today, our focus will be on the counseling and management of that first trimester care.

Slide 4 [00:00:44] We will begin by reviewing how to confirm and date a pregnancy. We will then review early pregnancy symptoms and advice that we can offer to patients, pregnancy loss and management, and most importantly, the supportive care and counseling that we can provide our patients, particularly in the infertility world.

Slide 5 [00:01:09] So confirming and dating a pregnancy.

Slide 6 [00:01:14] Having accurate dating is key to the management of a pregnancy. It is what we use to estimate the expected date of delivery and plan numerous preventative interventions. When a patient is suspected to be pregnant, it's important to confirm the last menstrual period. This is most important in a patient who has regular menstrual cycles and a predictable time of ovulation. We can do blood work to confirm beta human chorionic gonadotropin hormone or an HCG level, which we can then use to help track the pregnancy and help predict viability.

Slide 7 [00:01:56] Once that pregnancy is confirmed, we can repeat the HCG level every 48 hours. HCG levels should rise every two to three days, depending on the level that it initially starts at. When beta levels do not rise appropriately, it is one red flag that this may be a pregnancy at increased risk for a non-viable or ectopic pregnancy. It is no means diagnostic of a bad outcome. It's just a flag that should suggest that closer follow-up is needed to ensure that the pregnancy and the patient is safe.

Slide 8 [00:02:36] Last menstrual period or date of ovulation is one tool that can be used on a pregnancy wheel to help establish the expected due date. Fertility wheels have now been published where we can use the date of ovulation or the date of embryo transfer for a more accurate prediction of the estimated date of delivery. In the absence of a pregnancy wheel, we can calculate this date by using the last menstrual period, adding a week, subtracting three months and then adding a year. This can only be used in patients who have regular predicted menstrual periods.

Slide 9 [00:03:20] Once the HCG level reaches about 1500, ultrasound becomes the best modality to confirm the age of the pregnancy and its location. Confirming a location of pregnancy is one of the most important steps of early pregnancy care and often falls under the fertility clinic realm as we see these patients and we care for them very early in their pregnancy.

Slide 10 [00:03:48] So early pregnancy care and advice.

Slide 11 [00:03:54] Patients often call in with questions in anticipation of pregnancy, or once they become pregnant, with questions regarding how to best manage and care for

their early pregnancy. It is recommended that every pregnant woman, or actually pre-pregnancy, start at least 400 micrograms of folic acid and 300 milligrams of elemental iron. Ideally, particularly for planned pregnancies, women should be on this supplement several months prior to conceiving. When women are at higher risk of a neural tube defect, defined as having a neural tube defect themselves, or a child who has a neural tube defect, we do recommend a higher dose of folic acid.

Slide 12 [00:04:41] Patients have frequent questions about lifestyle modifications. We generally advise smoking cessation or risk reduction. It may not be feasible for everybody to stop smoking at the onset of pregnancy. However, it is a highly motivating factor. When given the opportunity to counsel patients pre-pregnancy, we can advise smoking cessation programs to work with them to try to decrease the overall nicotine intake. There is no safe limit of alcohol, cannabis or illicit drug use, and the uptake of cannabis is much more significant in our population than we often expect. There is no safety data showing a level that is acceptable in pregnancy, and so we recommend to our patients that all alcohol, cannabis and illicit drug use be stopped at any onset of pregnancy.

Slide 13 [00:05:36] Weight gain is a common question for patients, as they are often concerned about gaining too much or too little, but weight gain really should be guided by their preconception body mass index. So once a body mass index is calculated, patients can be advised of how much weight to gain. Most of this weight is generally gained in the second and third trimester, as in the first trimester, this baby often measures a few millimeters or centimeters at most. So for those women who are underweight, gaining 28 to 40 pounds. For those who are a normal weight to start their pregnancy, 25 to 35 pounds. When women get overweight or obese, the amount they have to gain is much less than an underweight or normal weight woman and counseling as to proper nutrition in order to achieve that is very important.

Slide 14 [00:06:31] Physical activity is recommended for women who are pregnant. It is recommended of at least 150 minutes per week. We often get questions at our clinic about how much to do and what activities to do, and this changes over the course of a pregnancy. So in the initial parts of pregnancy, we advise based on the patient's particular risk and history of pregnancy loss. For women with lower risk, we do recommend regular activity. They can maintain the same level activity as before, keeping in mind that they may feel unwell or they may feel lightheaded or dizzy, and they need to listen to their body. As the uterus grows, those recommendations change because the body's center of balance is also very different. So isolating certain groups in terms of weight lifting exercises may come in handy, and modifying exercises to accommodate for the change in physiology is important. One of the biggest points about physical activity is hydration. During that first trimester, women’s body volume increases significantly, and maintaining that hydration in and outside of exercise is really important to them feeling well, maintaining energy levels and being able to exercise or workout.

Slide 15 [00:08:01] Pregnancy advice regarding food is probably one of the most hot topics or questions that we get questions about. So one thing to remember is that most foods can be enjoyed in pregnancy and are enjoyed around the world. So there are more things that people can enjoy than we generally tell them to restrict, but they are some guidelines of what to restrict. First thing is raw meat. So when it comes to things like steak, we do recommend that people cook them all the way through. Same goes for raw shellfish or fish. There is, when it comes to things like sushi, sushi can be safe as long as you go to a place with high turnover of fish. There are people who eat sushi all through their pregnancy in Japan and still remain healthy. So this is a matter of fresh, good quality fish to make something like this safe. Deli meats are generally avoided, raw egg or products that contain raw egg. These are things that tend to come out in things like mayonnaise or

raw cookie dough, or things that have been on the counter in something like a potluck for hours at a time that kind of have a higher risk of infection. Unpasteurized milk or cheese or other dairy products should be avoided. Any milk products sold in a grocery store, particularly in our area is pasteurized. But some products used at restaurants are not pasteurized, so when in doubt, just ask.

Slide 16 [00:09:38] Travel is safest in the second trimester, but it really depends on the environment and the timing to which you wish to travel. So when it comes to our patients, I tell them, you know, in the first trimester, there's more of a chance of pregnancy loss. And so I like them to stay close so that we can manage them. And in the third trimester, many airlines have policies that do restrict travel. And so it's important to review airline policies and look into travel restrictions so there are no surprises at the time of flight. It's important to have travel insurance because pregnancy is a pre-diagnosed medical condition, and many preexisting plans may or may not cover this, as well as trip cancelation insurance, should there be a complication in pregnancy, then pregnancy would not be advised. Ensure the area of travel is safe from a physical perspective, but also from an infectious disease perspective. The world right now is changing very quickly in terms of infectious disease, and so your safety with regard to those agents is really important. You also need to be aware, or patients need to be aware, of where and when to seek medical care should they be in a foreign country, particularly with a provider that can speak the same language and how to leave that country should they need to.

Slide 17 [00:11:09] Pregnant women should avoid changing cat litter of an indoor and outdoor cat. The evidence isn't completely there for a strictly indoor cat. However, we do recommend it to our patients just for consistency in message. Pregnant women who work on a farm or directly with animals should speak to their medical team about additional precautions, depending on their actual involvement with the animal and the type of risk which may be specific to the animal that they work with, or the type of contact that they have.

Slide 18 [00:11:41] Intercourse is safe in all three trimesters unless directed by a medical professional. We do tell people to avoid intercourse in certain situations. For example, in some patients with recurrent pregnancy loss or with early trimester bleeding or threatened missed abortion, or they may restrict intercourse when placentation is in question or there is concern for the pregnancy.

Slide 19 [00:12:13] Prescription and over-the-counter medications should be reviewed with the medical team and the pharmacist. Ideally, patients review their medications pre-pregnancy and have a chance to optimize their medications prior to becoming pregnant. This is one real advantage of working with fertility patients, who, for the most part, have pre-planned pregnancies. We have the opportunity to refer them to high-risk physicians or have their medications reviewed to ensure that when they enter pregnancy, their medication profile is optimized and they're ready to have the safest pregnancy possible. There are some medications where we do have to do risk reduction, and it's best to have that planned ahead of time or as early in the pregnancy as possible.

Slide 20 [00:13:02] We get our data about safety from Health Canada and the U.S. Food and Drug Administration. And these are really valuable tools in helping to counsel patients about the risk of medication and also the level of supporting evidence. Most pregnant women are removed from a lot of studies, and so it is about a risk reduction, about keeping the patient safe, keeping their medical conditions controlled to allow for the safest pregnancy possible. Category A medications are ones that fail to demonstrate risks to the fetus in the first trimester, and they tend to be the ones that we recommend or feel to be safe for pregnant women. For those in Category B, C or D, it is a matter of risk reduction,

and it will depend on why we are using that medication in the first place. If medication can be avoided, then regardless of the category system, it's probably best to avoid that medication.

Slide 21 [00:14:02] So management of pregnancy symptoms.

Slide 22 [00:14:08] Every pregnancy is unique and can vary over the course of that first trimester. Patients often get concerned because one day their symptoms will magically go away or they didn't have any symptoms or their symptoms are very different than a previous pregnancy. But to be very honest, we don't know what causes people to have certain symptoms. We don't know what caused them to go away almost instantly, and we don't know what makes them come and go or vary between pregnancies. So we tell our patients, or I tell my patients, that every pregnancy is unique and symptoms are poorly correlated with the health of the pregnancy because the heart of their concern is, well, what does this mean for my actual pregnancy?

Slide 23 [00:14:52] The most common symptoms that you will encounter are nausea and vomiting, fatigue, breast changes, constipation, gas or bloating, frequent urination, backaches, headaches, mood swings, abdominal cramping, leg cramps or anxiety or heightened anxiety.

Slide 24 [00:15:16] When it comes to nausea and vomiting, I usually tell my patients that this does tend to be self-limiting. Around 10 weeks, people do tend to start feeling better, and there's only a small subset of people that continue to have nausea and vomiting after that 10 to 12-week mark. They should try to avoid strong smells, as this is one thing that tends to provoke nausea and vomiting, get sufficient rest, which is easier said than done, eat small meals and snacks and particularly carbohydrates and salty foods do tend to help the nausea a little bit better. Stay hydrated, which can be quite challenging when you are feeling nauseous or vomiting. One trick that my patients tend to like is the use of popsicles, either store-bought or homemade if we're trying to reduce the sugar, because a cold, slow drip tends to be much better tolerated than drinking back large amounts of water. Acupuncture bands have shown to have some evidence, as well as mindfulness and CBT, in overcoming that initial nausea and vomiting. And by CBT, I mean cognitive behavioral therapy.

Slide 25 [00:16:33] For women who’s nausea and vomiting is ongoing, despite these lifestyle medications, we can substitute their prenatal vitamin for folic acid early on in the pregnancy. The iron in the prenatal vitamin can tend to irritate some people early on in pregnancy, and we generally move it back to the prenatal vitamin at that 12-week mark, when majority of that nausea and vomiting has resolved. Pharmaceutical interventions can be considered if there's no improvement. I generally start my patients on pharmaceutical interventions if they're really not able to do their daily functions anymore or significantly impacting their day-to-day life, then it's time to consider some other options to make them more comfortable.

Slide 26 [00:17:19] Bleeding remains one of the most distressing symptoms to patients, particularly in early pregnancy. It is probably the most common call that we get. And I always remind patients that a third of pregnant women do have bleeding in the first trimester. And even though when you just see the symptom of bleeding it is hard to know if this is a concerning sign or it is a normal finding, it is important to remember that many people who have bleeding in the first trimester go on to have healthy pregnancies.

Slide 27 [00:17:53] Whenever a patient presents with bleeding, it is important to clarify that source of bleeding. So we presume that most bleeding comes from the uterus and is

coming from that pregnancy, but that's not always the case. Other sources are things like the cervix or the vagina or even rectally. So when it comes to the cervix, vessels of the cervix tend to become more superficial and dilated in pregnancy. So with intercourse, or if they've recently had an ultrasound, sometimes these cervical vessels can bleed. Fertility patients tend to be using a lot of vaginal hormonal support, and the constant introduction of these hormones can cause some bleeding and hemorrhoids are really common in pregnancy, and more so in patients who have constipation. Unfortunately, miscarriage does occur in 15 to 20 percent of pregnancies and is the source of anxiety for most patients when they present with bleeding. It is unfortunate and probably the hardest thing that patients have to work through, but there is no treatment to stop a miscarriage if miscarriage is truly what's happening.

Slide 28 [00:19:06] One of the most important things to remember if a patient does have bleeding is to look into their blood type. If a patient has RH antibody negative, so they are, you know, A negative or B negative or O negative, we do need to give RH immunoglobulin and ideally should be administered within 72 hours. This is offering them protection for future pregnancies so that they don't mount a response against the fetal blood cells that they are now seeing in their circulation. A dose of 120 micrograms is recommended prior to 12 weeks, and 300 micrograms can be administered if the lower dose is not available.

Slide 29 [00:19:50] Constipation is very uncomfortable for patients. We encourage hydration. Patients often don't anticipate how much fluid is needed, but usually we say eight to 10 glasses at a bare minimum of fluid is really important. Eat lots of fiber rich food, and there are many modifications of supplements that we can take in pregnancy that can help keep patients more regular and deal with constipation. And of course, there is always pharmaceutical interventions, if needed, if this is an ongoing concern that patients have.

Slide 30 [00:20:29] So pregnancy loss.

Slide 31 [00:20:34] Early pregnancy loss is distressing for many people and very distressing for fertility patients. With the loss comes the loss of so much emotional grief and hope and desire, and it takes into account their journey that they've had to try to get pregnant. There are many ways to manage it, and we'll talk more specifically about the pregnancy and then we'll go more detailed into dealing with that psychosocial support that we can offer patients to really help them along this really difficult time. So when we want to manage that pregnancy, it can be done expectantly, with the assistance of medication or surgical care. And how we decide the route that we take really depends on the patient's preference and often their journey and experiences in the past.

Slide 32 [00:21:29] For expectant management, we let the pregnancy be, and we allow the body to resolve the pregnancy at its own time. Spontaneous resolution does occur in about 90 percent of patients within four weeks. Complications are quite rare, but we do monitor them closely for signs of infection. So any signs of foul smelling odor or fever would allow us to talk to the patient and decide if we need to then move on to a different management style, and patients can switch to a medical or surgical approach at any point in time. It's my experience that with fertility patients, many don't take the expectant route because they can't begin to deal with the emotional burden that this loss has had until the physical burden has been dealt with.

Slide 33 [00:22:21] There are many different medical protocols, often based on the center to which you work. People do use misoprostol 800 micrograms, often vaginally and with a repeated dose 24 hours later. Women often get increasing vaginal bleeding till they reach a peak in flow, and then it more abruptly stops. One more recent protocol change that we have employed is the combination of mifepristone with misoprostol because it has a higher

degree of resolution of all products and a lower chance of retained products that patients then need to be followed for. We do follow our pregnancy hormones down to zero and we do repeat an ultrasound following to make sure that the cavity is completely clear.

Slide 34 [00:23:13] Patients need to be counseled on what to expect in terms of the amount of cramping and bleeding. And it is an alarming amount of bleeding that you have when you are passing tissue, so they need to expect this. They need to know when is it too much? And I usually tell patients if they're bleeding or filling a pad for more than an hour and this is lasting, or they start to feel lightheaded, they start to feel dizzy, they need to seek emergency care. They need to know what services are available in their area. And I often provide this instruction to patients in writing because in the heat of the moment at the time of the loss, it is difficult to process and understand all these details. RH immunoglobulin needs to be administered once again, and patients need to be followed for their beta HCG to ensure a complete resolution of that pregnancy. We do see a drop in levels of 80 percent from pretreatment to the first beta at seven to 14 days, usually indicates a successful resolution. At fertility clinics, or at least at our fertility clinic, we do have the access to blood work and so we do follow these pregnancy hormone levels right down to zero.

Slide 35 [00:24:24] We can complete a D&C as well or an aspiration and curettage. And this is often done under a general anesthetic or conscious sedation in which the contents of the cavity are aspirated to ensure that the physical loss of the pregnancy is resolved.

Slide 36 [00:24:46] Ectopic pregnancy is one that is most concerning to many providers. It is more common in patients undergoing fertility treatments, and so it is very important that we look for it and we look closely for it. An ectopic pregnancy is one in which the pregnancy occurs outside of the uterus, and the most common location is the fallopian tube. The fallopian tube is thin and it's fragile, and it's not meant to house a pregnancy. So in situations where that pregnancy gets big and has the potential to rupture, it is a medical emergency that requires urgent hospital care. In the setting of fertility treatments where we often have access to both ultrasound and blood work, early diagnosis is key because these patients can be treated medically and avoid a medical emergency or urgent surgical intervention.

Slide 37 [00:25:41] Now supporting care for pregnancy loss, so something that I think is actually probably the most important thing that we could do during this time.

Slide 38 [00:25:52] When a patient has a loss, it encompasses both the physical and symbolic loss of that pregnancy, even when patients try to remain as neutral as possible or as cautiously optimistic, you start to visualize dreams and hopes of what this pregnancy could be and the loss of a pregnancy is a loss of all that vision and all that hope and all that desire.

Slide 39 [00:26:18] Women and men who attend a fertility clinic have a high rate of mental health conditions even before the loss, especially things like anxiety and depression. It is possible that their journey of their fertility, because patients often try for years before they ever get to the fertility clinic, has heightened this, and they see this as their hope and their vision of trying to get the family that they so desire. When a patient has a loss, those heightened symptoms of depression and anxiety, they last for years after and the grief lasts for years after. And so addressing that concern and being there and being supportive for that patient is really important.

Slide 40[00:27:00] There are many techniques that are described in the literature to help council patients or to support them, because I often hear from people that they just don't

know what to say and they don't want to say the wrong thing. Patients often remember the words that we choose to use and where they were at the time bad news was provided. And so keeping all these things in mind and having tools in your tool belt that you can use is really handy. There are several common themes when we go through all these different techniques, and I've pulled out a few of those themes more specific to fertility counseling for us to discuss here.

Slide 41 [00:27:41] Setting is really important, and in the ideal world we would have reviewed or discussed this bad news in person. We would allow the patient to have a nice, quiet environment with the support person of their choice should they wish to have that. They would be speaking with somebody who has information to provide them and can fully answer their questions. And they would have ample amount of time to ask any questions they have and get the right answers that they need so that they can leave there with all the information that they want. Some people actually prefer doing this in two separate appointments where they get delivery of the bad news, but they are so upset, are filled with grief, that they can't ask the questions that they need. And so bringing them back at a separate visit might actually be helpful for many patients. Unfortunately, with the world as it is and COVID as it is, we do have to give some bad news often to patients over the phone. And so addressing those and using nice conducive language to deliver this bad news is really important. So I often ask patients, you know, is now a good time to talk? It's possible that we call them at a time when they're in the middle of a meeting or they're in a public setting and it isn't the best time for them to get this bad news. Are you in a quiet space where you can discuss freely? You want to make sure that they're in a place where they can ask questions and nobody else is listening, or they're feeling conscious about what they're saying or the emotion that they're expressing. Or is there anyone else you would like to be with you today? And maybe there is, and maybe it's worth calling them back at a later time when they can ensure that everyone who they wish to be on the call is present.

Slide 42 [00:29:32] Whenever possible, prior to speaking with a patient, whether it is in clinic or over the phone, try to best know the patient's history or journey. We do have the option of following patients, often for months or years, and so we can know that they've gone through losses before or they've had several ectopics before and or these have been really challenging for them. Or maybe this is their first time undergoing it. And that context that we can provide to patients allows us to best support them because we can allow them to feel seen and feel heard. We want to use simple, honest, clear language. If patients are already in a heightened state of grief or anxiety, we want to make sure that the communication that we have with patients is as clear as possible. We want to take the time to actively listen to their concerns in a non-judgmental environment, and these concerns may not feel expected to us and might not be what we are thinking that they're concerned about. But it is really valuable to them, and it's something that's really bothering them. For example, you know, patients often worry about, you know, I've had this loss now and we tried for this for eight months. It's going to be another eight months before I get pregnant again and I'm just getting older or how am I going to tell my family? We're meeting for the holidays. Or, you know, I can't look at another social media post where people talk about how happy they are with their pregnancy. And listening to all these things, even if you have no advice to provide them to try to get over it, it helps them to be seen or heard because they often don't have anywhere else to express these emotions.

Slide 43 [00:31:20] Whenever you are speaking to a patient and you are hearing what they're saying, try to help by identifying the emotion and normalizing it for patients. For example, you know, I'm hearing that you're feeling very overwhelmed or I'm hearing that you're feeling angry that this has happened to you. I can hear your grief as you speak. It helps patients feel that you are actually listening to them and they are feeling heard. Then offer them some understanding. You know, there's a lot going on right now. How can I help

you or, you know, I'm sure doing this alongside your work or I'm sure coming to the fertility clinic for this long period of time is really challenging. How can I help you or how can I best support you? Patients also need to know that, you know, if this encounter isn't enough and if their grief is lasting much longer than they would like or have hoped for, then there are additional supports that they can seek to best help them through this time and help them through their fertility journey. It's my experience with fertility patients that fertility is a rollercoaster and there are ups and downs. And what determines if a patient makes it through to have that family that they so desire is ensuring that they have that social and that support network to help them get through this journey.

Slide 44 [00:32:51] Patients continue to have distressing psychiatric symptoms. They do benefit from a variety of different services, as I just mentioned. Psychiatry, social work, psychology or group settings. Even in a world of COVID where everything tends to be online, there are now virtual group sessions that patients can be part of, and there are ones specific to what the patients are going through. So there are infertility group sessions, but there are also pregnancy loss or infant loss group sessions that can provide more specific care to patients and allow them to not feel so alone. And for some of my patients, they really don't want to be talking to other people or seeing what other people's experiences are like. They prefer a more individual approach, in which case one-on-one counseling is going to be most useful.

Slide 45 [00:33:44] And finally, a self check-in. Provider burnout, particularly in the world of fertility, is real. We spend a lot of our time listening to other people's or patients' grief and their anxieties and their fears. And it is important to constantly check in on yourself and how you're feeling and how you're able to provide care. Professionals or health care professionals often become the target of patients' negative emotions. They're angry at the clinic, they're angry at the person on the phone. And really, this is misdirected anger when their true emotion is their anxiety or fear about what's happening to them and the journey that they're on. So have insight into that. This is maybe not directed specifically to you but be aware of your own limits so that you can take care of yourself and continue to provide care for people for years to come.

Module 6.2_ ABCs of ART_Unexplained Infertility_EN Transcript

Dr. Yasmine Usmani

Slide 1 – Note to Audience slide [00:00:00]

Slide 2 – Title Slide [00:00:07] Hello, my name is Yasmine Usmani, and I am a fertility specialist at the Reproductive Care Center in Mississauga, Ontario. I'm very happy to be talking to you about unexplained infertility as part of Module six for The ABCs of ART.

Slide 3 [00:00:25] I'd like to start off with a case study. I had a 28-year-old woman and her partner present to my clinic for fertility assessment. They had been trying to conceive for approximately 15 months without success. We embarked on a standard fertility workup, which included cycle monitoring, where we were able to identify an ovulation. There was an adequate lining that was grown. All of the hormonal profile investigations that were returned were normal. She had a medium ovarian reserve. Her sonohysterogram demonstrated a normal cavity and bilateral tubal patency, and her partner's semen analysis was well within the normal range. Upon hearing that everything was normal, the couple wasn't sure where to go next. Because on one hand, having a completely normal fertility workup is a positive thing, but when there's nothing identified as to why trying to conceive for 15 months just hasn't worked, where do you go from here?

Slide 4 [00:01:31] It's important to let your couples know that of couples experiencing infertility, up to 30 percent are diagnosed with unexplained infertility after a standard evaluation. And even though there may be some element of subfertility, the cause may not be identified due to the limits of the technology we have at this time.

Slide 5 [00:01:54] The objective of this Module six presentation is to review the definition of unexplained infertility, to discuss the investigations that can lead to this diagnosis, what some postulated etiologies are, what types of counseling are indicated, and then we'll continue on with reviewing the case study.

Slide 6 [00:02:15] The definition of unexplained infertility refers to the absence of a definable cause for a couple's failure to achieve pregnancy, despite a thorough evaluation. Typically, this investigation would be sought after 12 months of attempting conception in the 35 years or less age group. And in women who are of advanced reproductive age, typically, they would be referred to a fertility clinic for more thorough workup after six months of trying without success.

Slide 7 [00:02:48] A fertility workup includes cycle monitoring to track an ovulation and to assess lining of the uterus. A sonohysterogram, or an HSG, is typically arranged to look at the uterine cavity and to make sure it's free of any structural issues that could be complicating achieving pregnancy, and also to assess tubal patency. It's important to include a semen analysis, as well, as part of the basic fertility workup to see if there's any male contributing factor, as well as to assess the ovarian reserve.

Slide 8 [00:03:28] The evaluation typically demonstrates at least one patent fallopian tube, as well as a normal ovulation, and the semen analysis that typically has an adequate number of motile sperm for the male partner.

Slide 9 [00:03:45] Since no treatable cause is identified in the unexplained infertility population, treatment is by necessity just empiric in nature. And so what that means is counseling patients and ensuring that they're aware that 30 percent of the fertility population is in the same boat and that they're not alone. And also discussing all of the options that are open to the couple in a supportive way. These patients typically will

appreciate close follow up just to discuss how certain treatments are going and ways to optimize each step in the treatment pathway.

Slide 10 [00:04:23] Possible etiologies that are difficult to discern could be implantation failure, certain subtle cervical factors, problems with egg and sperm transport or interaction, a combination of multiple factors like a female partner who is over 35 years in the advanced reproductive age category with decreased ovarian reserve and a male partner with low normal semen parameters, or a defect in endometrial receptivity.

Slide 11 [00:04:56] Part of the initial counseling for couples, once they have a diagnosis of unexplained infertility, is openly discussing lifestyle issues and ensuring that these can be addressed and optimized. These can include such things as ensuring the couple is of a healthy BMI, addressing any sort of nicotine use, smoking, marijuana use, other recreational drug uses, alcohol intake, caffeine intake and providing the couple with resources to help optimize themselves from this perspective. Another detailed discussion should have a very thorough discussion of efficacy, cost, safety, and risks of various treatment alternatives. And it's important to offer any indicated treatments to your patient and discussing where they might feel comfortable starting as a jump-off point for fertility treatment to facilitate pregnancy.

Slide 12 [00:05:59] Historically, it's a stepwise progression and treatment that is initiated, and typically couples will feel most comfortable starting with least invasive or least expensive option within a subsequent gradual progression to assisted reproductive therapies, if the initial treatments fail.

Slide 13 [00:06:19] In all circumstances, the therapeutic approach should involve counseling so that the patient is clear on what expected outcomes are at that level of fertility treatment, any related adverse events, and you'd want to ensure that individualized treatment plans based on age and duration of fertility are made. So, for example, if a woman of advanced reproductive age and decreased ovarian reserve wanted to start off with a natural cycle timed intercourse, you wouldn't want her to linger at that treatment level. You'd want to follow up with her, and if we weren't having success, you'd want to progress in a timely manner, given that we know chances of success decrease with age and the decline in age-related egg quality.

Slide 14 [00:07:11] Expectant management has been a long-standing option for management of unexplained infertility. In the appropriate population, evidence suggest it's an effective approach for good prognosis couples. So retrospective data have shown that a cumulative pregnancy rate over two years can be as high as 72 percent in young women. So that would be women aged less than 35. Once a woman's older than 35, there's a decline in success to 45 percent. And if a woman has experienced five years of infertility, there's a further decline to 30 percent.

Slide 15 [00:07:53] Part of the counseling, you know, in the appropriate good prognosis population is that a new study tracking hundreds of couples with diagnosed unexplained infertility has found that there's a 64 percent success rate at conceiving one child without additional medical treatment, and only 6.6 percent of the population did not have a child over the course of their reproductive lifetime. So in the proper population where expectant management could be indicated, there is success to be found.

Slide 16 [00:08:32] This study was a long-term follow up to the FASTT trial, and in that trial, it was found that 81 percent of the couples achieved at least one live birth. And the majority of the couples who tried to conceive spontaneously rather than medically assisted

were successful, 64 percent. And studies showed that most of the patients were satisfied with their ultimate family size.

Slide 17 [00:09:02] When a thorough literature review has been done, the best initial therapy that is suggested for couples with unexplained infertility tends to be insemination. Now, insemination can be done with either a tablet form of ovarian stimulation, so that would be using clomiphene or letrozole, to then have more than one egg present at the time of insemination. Or it can be done with the use of injectable FSH medications. The studies have shown that the pregnancy rates between the tablet form of stimulation or injectable form of stimulation are not statistically different. But what is statistically different is the rate of multiple pregnancy when you use the FSH injectable medications. So therefore, you know, the literature suggests that for unexplained infertility, three to four cycles of tablet ovarian stimulation with IUI are a good jumping-off point. To then progress on to the injectable medications is not recommended, simply because of the higher risk of multiple pregnancy, with no statistically significant increase in pregnancy rate that is associated. If there's no success after three to four cycles, the studies demonstrate that moving on to IVF for couples who have been unsuccessful in achieving pregnancy would be the next best step. This would be an evidence-based approach, and it's suggested that advancing to IVF in terms of looking for improved live birth rates and reduced multiple pregnancy rates is appropriate. However, we all know that IVF is invasive and costly.

Slide 18 [00:10:51] We know that IVF does result in the highest per cycle pregnancy rate in the shortest time interval. And you look to IVF to not only be therapeutic and to achieve that wanted pregnancy, but you also look to IVF to be diagnostic. This is the opportunity where we have the oocytes in the lab and we can see them. We can discern certain egg quality parameters. We can see the egg-sperm interaction and we can watch the embryology. So we do know that from studies, couples with unexplained infertility do demonstrate reduced oocyte fertilization embryo cleavage rates compared to couples without a diagnosis of unexplained infertility. And there's also higher rates of complete fertilization failure that are seen. Sometimes, these answers do help couples understand why they've experienced such long-standing subfertility.

Slide 19 [00:11:51] For couples in whom IVF is not desired or not feasible, there is a school of thought that offers diagnostic laparoscopy to these patients. The purpose of this is to see if endometriosis is present. Endometriosis can cause scarring or, you know, alterations in anatomy internally and you can also be asymptomatic with endometriosis. So in diagnostic laparoscopy, any scarring can be taken down, any anatomy can perhaps be restored to normal, and there can be a benefit to this type of next step in treatment for the unexplained infertility population. There can be some consideration given to donor-egg therapy, gestational surrogacy, adoption, or even just cessation of treatment if the couple does not want to pursue any other avenues.

Slide 20 [00:12:52] In terms of my case study, the patient after counseling and the diagnosis of the unexplained infertility, the couple decided that they wanted to try one to two more cycles of expected management now that they were confident that all of the ingredients for a successful pregnancy were present. After the one to two cycles, they followed up and they had not had success. So they were most comfortable progressing on to letrozole with timed intercourse, and we had decided that we were going to regroup in two to three cycles just to review how the cycles had gone. We always review each cycle, even if we don't speak to the patient, to adjust dosing, et cetera, and so we had done that. But now, after two to three cycles of escalating doses of letrozole to a level of comfort, we didn't go very high because she's a young woman with presumably good quality eggs, they're ready to move on to insemination. So at this point in their treatment, you know, I'm happy to follow up with them each cycle just because we're getting to the point where

there's unexplained infertility, we haven't had success yet, they're moving on to insemination, and they've already expressed that if there's no success within the upcoming cycles, that they will progress likely onto in-vitro fertilization. Again, we would look to this IVF cycle to be not only therapeutic, but hopefully diagnostic, and shed some light on why there's been this long-standing subfertility in this very young and healthy, otherwise normal from a fertility perspective, couple.

Slide 21 [00:14:34] So in conclusion, unexplained infertility is a common problem affecting many Canadian couples. It is a diagnosis of exclusion and while positive in that there's no identifiable cause, you know, it is stressful for couples because that means there's nothing directly to address to treat. So in all circumstances, the therapeutic approach should involve individualized counseling. There is a role for less invasive treatment options, especially in patients that are considered good prognosis, so young with good ovarian reserve. And typically, it is all right to start with expectant management and then from there, moving on to insemination and typically with letrozole or clomid would be the next appropriate step. There is evidence-based medicine to suggest that there's a benefit to advance to therapy with IVF, as it does improve live birth rates and it does reduce multiple pregnancy rates. What's important to know is that there's a pressing need for additional therapies to bridge the wide gap between IUI and IVF. Typically, it's quoted that insemination has a 15 percent success rate associated with it. And in terms of IVF, if a genetically normal embryo is identified, it's typically quoted that there's a 70 percent implantation success rate associated with that embryo. So that's a wide no man's land between the 15 percent and the 70 percent. So additional therapies to fill that gap would be very helpful to the fertility population in general, including the unexplained infertility population. So thank you very much for listening to my talk on unexplained infertility, and I hope that you were able to have some meaningful take-home points.

Module 6.3_ ABCs of ART _Recurrent Pregnancy Loss_EN Transcript

Dr. Yasmine Usmani

Slide 1 – Note to Audience slide [00:00:00]

Slide 2 – Title slide [00:00:06] Hello. I'm Yasmine Usmani. I am a reproductive specialist at the Reproductive Care Center in Mississauga, Ontario. I'm very happy to be able to be part of The ABCs of ART and to speak to you about Module six, which is recurrent pregnancy loss.

Slide 3 [00:00:27] So the recurrent pregnancy loss population is a very unique population. We do know that clinically recognized pregnancy loss is common. About 15 to 25 percent of all pregnancies will end in miscarriage. And the majority of these losses will occur less than 10 weeks of gestational age. And this is typically caused by random numeric chromosome errors. These can be trisomy, monosomy or polyploidy. A royal college question is what is the most common, you know, genetic error that contributes to miscarriages and it's actually trisomy 16. Now, recurrent pregnancy loss is a distinct disorder where a woman will experience two or more failed clinical pregnancies. And it's typically less than five percent of women who will experience two consecutive miscarriages. And in fact, only one percent will experience three or more consecutive miscarriages.

Slide 4 [00:01:29] So the objective of this module is to go a little bit further into the definition of what recurrent pregnancy loss is, what pertinent investigations are in terms of diagnosing the condition. What certain etiologies could be. The counseling that's so very important to this population. And then we'll also take a look at some case studies from my patient population as we go through.

Slide 5 [00:01:56] The definition of recurrent pregnancy loss does vary from study to study or institution to institution, so it's made coherent in large scale studies or, you know, analyzing data a little bit difficult. But the two most commonly recognized definitions for recurrent pregnancy loss include two or more failed clinical pregnancies as documented by ultrasonography, which means a heart rate seen on ultrasound, or histopathologic examination, meaning retained products of conception seen on pathologic examination. Another common definition is three consecutive pregnancy losses, and those are not required to be intrauterine. So it could include ectopics as well.

Slide 6 [00:02:41] When you have a patient who presents to your practice with more than one miscarriage, it's typical to offer some form of recurrent pregnancy loss workup after two failed clinical pregnancies. It's often thought to be cruel to wait for a woman to meet the definition of three failed pregnancies in order to offer testing. So we do offer testing when a woman presents with two failed clinical pregnancies.

Slide 7 [00:03:07] When a woman presents, it's very important to take a very detailed history, and this history includes asking many details about her pregnancies. How long it took to conceive them. What was the positive Beta-hCG measured by home tests or did she have a serum hCG drawn? And, you know, what the circumstances around the loss were? Did she have bleeding before an ultrasound, or were there ultrasounds that demonstrated a fetal heart rate? In RPL, if there is a cause that is identified, some consistency can be seen between the histories of the losses, they may occur at a similar time. When meeting a patient face to face, a physical examination is important because you can sometimes discern that there might be some medical conditions that could be optimized like, for example, exophthalmos or eye signs of hypothyroidism. If you can see a acanthosis nigricans, which could be related to insulin resistance, for example. An

elevated BMI. So these are things that you might see just on visual physical examination. Mental health evaluation is a very important part of the recurrent pregnancy loss, support and counseling. It's known that couples with recurrent pregnancy loss have higher stress levels. They also are found to have higher risk of depression and a lot of anxiety around conception and pregnancy in general. So it's very important to check in with your patient right from the beginning to see how they're feeling and what they could really benefit from. Most clinics will have counselors on site that can offer support throughout treatment. And it's important that you make sure that these resources are offered to patients.

Slide 8 [00:04:57] Uterine assessment is an important part of the recurrent pregnancy loss workup. The uterine assessment is a structural evaluation of the uterus and the tubes, and this can be accomplished by sonohysterogram, hysterosalpingogram, hysteroscopy, ultrasound and in some cases, MRI may even be indicated.

Slide 9 [00:05:20] Blood work is also requisitioned as an investigation for this patient population. The blood work typically encompasses a karyotype for both partners to ensure that the chromosome makeup of the couple is normal. So 46 chromosomes then either XX or XY sex chromosomes. Anticardiolipin antibodies, lupus anticoagulant are also drawn as part of a standard workup. Thyroid function is evaluated. There may be some role for inherited thrombophilias to be looked into as well. And also screening for diabetes and monitoring sugar control is also part of the workup.

Slide 10 [00:06:04] When asking about male investigations that may be relevant, DNA sperm fragmentation is one that often comes up and we will talk about this more in detail.

Slide 11 [00:06:17] When going through the literature, you will also see other investigations that are sometimes offered routinely by certain fertility clinics. These can include, you know, a culture and serology of swabs of the cervix or vagina, progesterone levels done serially, as well as endometrial biopsies. But literature reviews have shown that these are less helpful investigations in the diagnosis of recurrent pregnancy loss.

Slide 12 [00:06:46] When the recurrent pregnancy loss workup is complete, certain etiologies may be identified. These could include cytogenic or genetic abnormalities, antiphospholipid syndrome, certain anatomic factors like polyps or fibroids or uterine anomalies. A certain inherited thrombophilia may be identified. Hormonal and metabolic factors that could be optimized can be identified. Infections. Certain male factor contributing to the recurrent pregnancy loss can also be found. Certain psychological factors also may need to be addressed. Alloimmune factors. Certain lifestyle, environmental and occupational factors may come to light. And then there is on its own unexplained recurrent pregnancy loss, where the work up is completely normal and you have to tell your patients that there's no discernable reason why they've had the number of miscarriages that they have.

Slide 13 [00:07:45] So we'll start off with one of my patients. She presented to me as a 27-year-old patient who had conceived twice spontaneously without difficulty and both pregnancies ended in a loss. We got all the details around the losses and we offered her the recurrent pregnancy loss workup. Everything came back normal in terms of her cycle monitoring with an identifiable ovulation. She grew an adequate lining. She had an appropriate ovarian reserve. Her uterine cavity was normal. Her tubes were open. And for her partner, his semen analysis was normal. But what didn't come back as positive was her karyotype. She, in fact, had a balanced translocation between chromosomes 13 and 7, and that is how it was presented there at the bottom in the report that came back. So this came as a bit of a surprise to the couple, and it led to certain other tests being done within the family. But this was a new finding. So, of course, we then referred the couple on to

genetic counseling because counseling is always a big theme throughout the recurrent pregnancy loss population. It's important that when a balanced translocation or some sort of genetic condition is brought to light, that appropriate counseling is provided regarding what this diagnosis means for the patient. Certain translocations can result in ongoing pregnancy more than others, and also the chance of having an ongoing pregnancy once this balanced translocation has been identified. So the couple did go for genetic counseling.

Slide 14 [00:09:26] In terms of these cytogenetic abnormalities, we know that 60 percent of random miscarriage is due to sporadic chromosomal anomalies and, as we discussed, primarily trisomies that do increase with maternal age increasing. But balanced reciprocal translocations and Robertsonian translocations are only observed in two to five percent of couples with recurrent miscarriage. So I've had a few that I've encountered throughout my practice, and this patient by far is a wonderful example of how we then go on to support the couple and manage.

Slide 15 [00:10:04] So in terms of treatment, after genetic counseling, some couples may decide to try on their own, but once there's an identified cause for the recurrent pregnancy loss, there are ways to help optimize conception and ongoing pregnancy. And with today's technologies, this often includes preimplantation genetic diagnosis for the specific identified translocations in conjunction with preimplantation genetic testing for aneuploidy, which are the sporadic abnormalities that affect women of all ages, and you find in the embryos when they're tested. So the goal would be to transfer unaffected by the translocation and euploid, meaning no sporadic abnormality, embryos. If this doesn't appeal to the couple or if they've done IVF, and the genetic testing shows that there's no balanced or unaffected embryos or euploid embryos, then you may consider using donor gametes as a way around the whole translocation and to help optimize pregnancy with genetically normal embryos.

Slide 16 [00:11:16] So with regard to this patient, she and her husband decided to embark on IVF with PGD, preimplantation genetic diagnosis, to identify balanced, unaffected embryos, and then also PGT-A to look for any sporadic abnormalities. After two cycles of IVF and testing 18 embryos, they had banked three euploid, meaning genetically normal, balanced embryos. So shortly thereafter, we transferred one embryo and the family now has a lovely little girl and they've come back to the clinic to transfer another embryo for a sibling for their daughter. So this is, you know, a happy example of how today's technologies can really help with regard to cytogenic abnormalities that may contribute to recurrent pregnancy loss.

Slide 17 [00:12:09] Antiphospholipid syndrome is an etiology that affects 8 to 24 percent of patients with recurrent pregnancy loss, and this would mean blood work that demonstrates a positive lupus anticoagulant or anticardiolipin antibody or anti-beta-2 glycoprotein I. So these are typically requisitioned factors that are looked at in serology for patients when you're trying to investigate causes of recurrent pregnancy loss.

Slide 18 [00:12:41] I had a 32-year-old patient present for assessment after three spontaneous conceptions that all ended in loss early in the first trimester. We performed the recurrent pregnancy loss workup and again everything was normal in terms of the cycle monitoring and ovulation and the structure of the uterus and the patent fallopian tubes and the semen analysis. But we did find that there was lupus anticoagulant that was identified as being positive. In this case, we referred her on to hematology because hematology counseling is important for this particular facet of recurrent pregnancy loss. It's important that they counsel on what antiphospholipid syndrome is, most people haven't heard of it unless you test positive for one of these factors, how it has contributed to the

losses and what can be done about it. So we're very fortunate to have affiliated with certain hematologists who are happy to be part of our patients preconceptual counseling, to help work with the patient to develop a plan and to be part of the ongoing care of the patient once pregnant and after, as well. So in terms of this patient, we have a plan on her chart, which includes starting low dose aspirin and anticoagulation with her next pregnancy.

Slide 19 [00:14:02] So, as mentioned, the standard treatment for antiphospholipid syndrome is low dose aspirin and unfractionated heparin. When 70 patients who had a history of recurrent pregnancy loss and have been diagnosed with antiphospholipid syndrome were started on this regimen, 74.3 percent then had a live-born rate subsequent. It does confer a significant benefit to the pregnancies in couples that have antiphospholipid syndrome and otherwise unexplained recurrent pregnancy loss. When compared to couples who just take aspirin alone in the recurrent pregnancy loss population with antiphospholipid syndrome, you can see there is a marked decrease in pregnancy for the aspirin alone group in the order of 43 percent. So it is the combination of the low dose aspirin and the twice daily unfractionated heparin that confers the higher pregnancy rates.

Slide 20 [00:15:02] Anatomic factors are a part of the recurrent pregnancy loss workup that is important. First, what we look for are congenital mullerian tract anomalies. These would include, you know, a unicornuate uterus, didelphic uterus, a bicornuate uterus, a septate uterus or an arcuate uterus. We know that there are women in the general population who, in the order of about 4.3 percent, have these mullerian anomalies, but they're in a much higher frequency in the recurrent pregnancy loss population. And that's why it's part of the standard workup.

Slide 21 [00:15:41] Top left, you see the structure of a normal uterus, and here are pictorial examples of other mullerian abnormalities. So a unicornuate uterus, the bicornuate uterus, the didelphic uterus and you can see the septate uterus and then the arcuate uterus as well.

Slide 22 [00:16:02] You look internally as well for other anatomic factors, such as intrauterine adhesions or a diagnosis of Asherman syndrome. Uterine fibroids and uterine polyps can also play a role in recurrent pregnancy loss.

Slide 23 [00:16:17] So here is an example of what Asherman syndrome would look like with the intrauterine adhesions or synechiae, and these can result from an infection, uterine manipulation such as perhaps an overly aggressive D&C. And then you can see on the right there that there are fibroids and there's three different types of fibroids. There's the submucosal fibroids that push into the uterine cavity. There's the intramural fibroids that live in the wall of the cavity, and then there's the subserosal fibroids that push out into the body cavity. The type of fibroid that typically affects pregnancy and can cause recurrent pregnancy loss is the submucosal fibroid, the one that pushes into the cavity. Polyps as well take up real estate inside the uterine cavity. And should an embryo implant on a polyp, the polyp may not have the blood supply that can sustain the embryos growth and development, and so can result in subsequent miscarriage.

Slide 24 [00:17:14] For my third patient case study, I had a 35-year-old present for evaluation after three spontaneous miscarriages, all in the early first trimester. Her workup was normalized before, aside from the sonohysterogram that we performed identified a polyp that was a centimeter in its largest dimension, as well as a deep uterine septum that was 2.2 centimeters in depth and it looked much like this. In this case, we did counsel her

regarding, you know, the implications of both the polyp and the septum. And then we offered her surgical management.

Slide 25 [00:17:55] The treatment for these types of anomalies, especially the septum, polyp and fibroid would be, you know, surgical correction of any significant uterine cavity defect. So whether adhesiolysis, meaning taking down any intrauterine adhesions. A myomectomy, which means removing any submucosal fibroid component or minimizing it to the greatest extent. Polypectomy to remove the polyps, as well as septum resection. So I put a little asterisk there because our patient did go for hysteroscopic uterine septum excision and polypectomy. So now that she's post-op and her uterine cavity has been optimized and returned to basically a normal shape, we're working now actively to help her achieve pregnancy.

Slide 26 [00:18:52] Inherited thrombophilias are another aspect of the recurrent pregnancy loss workup that you can read about in the literature. Typically, these are not part of the standard workup, and these tests would include looking for a factor V Leiden, prothrombin gene mutations, protein C, protein S, antithrombin deficiencies. But really, for these to be applicable to your patient you should test if there's a personal history of a venous thromboembolism in the setting of a non-recurrent risk factor, such as, you know, occurring after a surgery or if there's a first-degree relative with a known or suspected high-risk thrombophilia. So again, this is not part of the standard workup, but something to be considered after having taken a detailed history.

Slide 27 [00:19:44] Certain hormonal and metabolic factors are also contributors to recurrent pregnancy loss. So those would include the maternal endocrine disorders of diabetes, thyroid dysfunction and hyperprolactinemia. If a patient presents with very uncontrolled sugars, high hemoglobin A1C, you know this is something that needs to be addressed and optimized. Thyroid dysfunction as well. Thorough investigation for thyroid antibodies to help gauge level of control is important. And hyperprolactinemia does result in impaired folliculogenesis and oocyte maturation and can contribute to a shortened luteal phase. So whenever we have a patient who is found to have diabetes, thyroid dysfunction or hyperprolactinemia that needs optimization, we definitely include endocrinology as part of their care team to follow them longitudinally and to ensure that once pregnancy is achieved, these are all optimized and sugars are within safe levels, thyroids are optimized, prolactin is where it needs to be, but also longitudinally throughout the pregnancy because management of sugars and thyroid management can change as a pregnancy progresses.

Slide 28 [00:21:01] Certain infections are also looked at as a cause of recurrent pregnancy loss. Chlamydia and rubella, cytomegalovirus can be, you know, part of just a basic fertility workup for certain populations. But when certain tests, like ureaplasma, mycoplasma, listeria are requisitioned, you know, they've been identified more frequently in vaginal and cervical cultures and serum from women with just sporadic miscarriages, not necessarily recurrent pregnancy loss only. And there's really no convincing data that infections cause recurrent pregnancy loss. I know that's a little bit counterintuitive, but when the literature reviews have been done, you know, these don't typically have enough evidence to be included as part of just a standard workup for recurrent pregnancy loss.

Slide 29 [00:21:55] In terms of male factors, sperm aneuploidy and DNA fragmentation are discussed. Abnormal DNA fragmentation can be seen in a setting of recurrent pregnancy loss and typically higher rates of fragmentation are seen with advanced paternal age or from certain correctable environmental factors like heat to the groin area, toxic exposures, the presence of varicoceles or increased reactive oxygen species in semen.

Slide 30 [00:22:27] With regard to the DNA fragmentation index, it's reported as a DFI, which is a percentage of sperm cells containing measurable DNA damage. The report typically is returned as either normal, borderline or abnormal, and it's based on the percentage of sperm that demonstrate the damage. So if 15 percent or less exhibit this measurable DNA damage, that's excellent to good fertility potential for the sperm. If the DFI is in the order of 15 to 30 percent, that exhibits a good to fair fertility potential. Abnormal sperm DNA integrity is when DFI is greater than 30 percent, and that's a fair to poor fertility potential. The higher the DFI score is, it correlates with lower success rates in natural or insemination attempts at pregnancy. Abnormal DFI results suggest consideration to the couple to advancing directly to IVF or ICSI treatments that overall lower the DFI value. So we also involve a urologist in this case to help counsel the couple and the male so that they're aware of what, you know, the DFI is if there's any identifiable or reversible environmental causes that can be addressed and optimized and what the expected outcome is if there's no discernible way to improve the DFI. So again, counseling is another, you know, consistent thread throughout supporting recurrent pregnancy loss population.

Slide 31 [00:24:01] When reading the literature on recurrent pregnancy loss, alloimmune factors, such as HLA typing, embryotoxic factors, decidual cytokine profiles, blocking or antipaternal antibody levels, HLA-G polymorphism are all things that are brought up in the literature that could be etiologies for recurrent pregnancy loss. The data is inconsistent and usually lab specific. When certain studies are attempted to be reproduced in different settings, the same findings aren't found. So again, alloimmune factors and these investigations are not part of the basic fertility workup for recurrent pregnancy loss.

Slide 32 [00:24:48] It's also important to counsel patients on identifiable lifestyle, environmental and occupational factors. So cigarette smoking, you know, has an identifiable adverse effect on trophoblastic function and is linked to an increased risk of sporadic pregnancy loss. So it's important that if you have a patient who reports that they're smokers, that you counsel them appropriately and provide them with the supports and resources to embark on smoking cessation. It is difficult and a lot of support can go a long way. Obesity is also shown to be associated with increased risk of recurrent pregnancy loss in women who conceive naturally. So assisting with either endocrinology review, certain weight loss clinics that are medically monitored can be offered, naturopathic services or dietary consultation to help patients embark on lifestyle changes to help normalize BMI are also important factors in the care of patients with elevated BMI. Other lifestyle habits like cocaine or alcohol consumption or increased caffeine consumption has been associated with increased risk of miscarriage. So these are all things that should be counseled on and the patient supported in making these pertinent modifications.

Slide 33 [00:26:15] And then we come to the unexplained recurrent pregnancy loss population. So in 50 to 75 percent of couples with recurrent pregnancy loss, there's no apparent causative factor. And it's important to emphasize to patients with unexplained recurrent pregnancy loss that the chance for future successful pregnancy can exceed 50 to 60 percent, depending on the maternal age and how many conceptions she's had. It's a very difficult diagnosis when you have unexplained recurrent pregnancy loss because the patients will typically say, you know, I don't have any difficulty achieving pregnancy, my problem is keeping the pregnancy, staying pregnant. So if there's nothing that's been identified, where do we go from here?

Slide 34 [00:27:00] So I had a patient present, a 38-year-old woman who had conceived spontaneously three times and again three early first trimester losses. Her recurrent pregnancy loss was normal. Both patient and partner were normal karyotypes. No

coagulation disorder or antiphospholipid syndrome was identified. They were completely managed well from the thyroid perspective. There was no insulin resistance or uncontrolled diabetes. Her hormone panel was normal. So what we decided to do was counsel the patient accordingly, and we'll go into how her story plays out.

Slide 35 [00:27:46] Treatments that you can offer with the unexplained recurrent pregnancy loss can include progesterone, but that is controversial. When you do a literature review, evidence-based medicine states that administration of progesterone to women with sporadic miscarriages is ineffective. But when a patient has three or more consecutive losses, some consideration to starting empiric luteal phase progesterone does have, or there can be some potential benefit. Typically, you let patients know that although controversial, it's not going to hurt you and that there is a potential for help, and most patients are willing to try progesterone.

Slide 36 [00:28:30] There's also another factor that is very important in the unexplained recurrent pregnancy loss population, and that is, you know, the mental stress and anxiety that goes into each pregnancy and wondering if another loss will ensue. So a cohort study of 158 couples with three or more losses were put into two identifiable groups. One of the groups received routine obstetrical care, and the other group received what's called the tender-loving care pathway.

Slide 37 [00:29:07] The tender-loving care protocol or pathway is defined as psychological support, and what this means is weekly ultrasound and doctor appointments so that the patient can see their baby and ask any questions and just basically be reassured on a consistent basis. Questions about, you know, am I able to do this at work? Am I able to travel? Is sexual activity safe? The ability to have the forum to ask these questions as they pop up and to receive adequate counseling is given. So the difference in live birth rates was significant between the two groups. So the group that received the routine obstetrical care had a live birth rate of 36 percent, and the population that was in the TLC protocol group had a live birth rate of 85 percent.

Slide 38 [00:29:59] So with my patient, after counseling, the couple said, you know, we can get pregnant, we are going to try, so no intervention was desired. We made a plan that as soon as a positive pregnancy test was obtained, that she was going to start progesterone, so she had her prescription in hand. And we let her know that as soon as she had her home positive pregnancy test and started the progesterone, we were going to initiate the TLC protocol for her. So in her case, she did conceive. We went through the steps as outlined, and the weekly ultrasounds were of huge benefit to her and her partner, who never missed a call. And I'm happy to report that the couple is well passed the first trimester, all genetic screening has come back normal, and this fourth pregnancy was a charm. And you know, it was just that we started progesterone in the TLC protocol. So, you know, there is some credence to this evidence-based TLC protocol.

Slide 39 [00:31:00] So in conclusion, you know, the evaluation of recurrent pregnancy loss can proceed after two consecutive clinical pregnancy losses. We don't make people wait to have more than two losses to initiate. The assessment will focus on screening for genetic factors, coagulation disorders, uterine anatomy anomalies, hormonal and metabolic factors, as well as lifestyle variables. So we'll always make sure that a karyotype is offered, screening for lupus anticoagulant, anticardiolipin antibodies, anti-beta-2 glycoprotein I, the sonohysterogram or HSG or hysteroscopy, as well as screening for thyroid glycemic control and prolactin abnormalities.

Slide 40 [00:31:49] We know that the majority of miscarriages are sporadic, and are thought to result from just genetic causes, and these are greatly influenced by maternal

age. Up to 50 percent of cases of recurrent pregnancy loss will not have a clearly defined etiology. The unexplained recurrent pregnancy loss population are reported to have future successful pregnancy rates that can exceed 50 to 60 percent, depending on the maternal age and parody. And it's important to know that the evidence-based medicine supports the tender-loving care protocol, which is a supportive approach and encourages future successful, ongoing pregnancy. So thank you very much for listening to my module on recurrent pregnancy loss, and I hope that you were able to gain some valuable information.

Module 6.4_ABCs of ART_Conversation with LGBTQ2S+ patients_EN Transcript

Dr. Marjorie Dixon

Slide 1 – Note to Audience slide [00:00:00]

Slide 2 - Title slide [00:00:05] Good day, everyone. My name is Marjorie Dixon or Dr. Dixon, and I am the founder, CEO and Medical Director at Anova Fertility and Reproductive Health in Toronto, Canada, as well as an Assistant Professor at the University of Toronto in the Department of Obstetrics and Gynecology and in the Division of Reproductive Endocrinology and Infertility. I am a reproductive endocrinologist by trade, and I've been seeing patients for the past 20 years. However, I have a special interest in the services and needs of the LGBTQ2S+ population and the special considerations that need to be taken when caring for our community.

Slide 3 [00:00:56] So I would like to speak to the variety of issues and sensitivities that need to be considered when managing patients in the LGBTQ2S+ population and specifically firstly when dealing with terminology that gender is different than sex.

Slide 4 [00:01:18] So what is biological sex? Biological sex refers to the chromosomes, the genomics, hormonal, and the characteristics anatomically that are used to classify an individual as either female, male, or intersex.

Slide 5 [00:01:34] This is different from gender identity. So biological sex is chromosomal, genomic based. Gender identity is an individual's deeply felt internal sense or individual experience of gender. And that may or may not correspond to the sex assigned at birth, which we know as the sex assigned at birth. This can be either transgender, so for someone whose gender identity doesn't match the assigned sex at birth. Cisgender is someone whose gender identity is consistent with the sex and gender assigned at birth. And then two-spirited is a term used in some indigenous communities, and that encompasses cultural, spiritual, sexual and gender identity, and it transcends in typical roles of men and women and fills a role kind of as a middle center. So gender identity and sex assigned at birth.

Slide 6 [00:02:34] Now, gender expression is the external display of one's own gender. So it's a combination of how you appear, what your mannerisms are, how you behave socially and other factors.

Slide 7 [00:02:48] This is all separate from sexual orientation, and sexual orientation as the emotional or romantic or sexual attraction that's primarily, and not necessarily exclusively, to people of a particular sex and or gender. So we know heterosexual, bisexual, homosexual, pansexual that spans the spectrum, and then asexual.

Slide 8 [00:03:19] So what is the difference or what are the special considerations for fertility care in the LGBTQ2S+ community?

Slide 9 [00:03:29] So what do we know? It's a growing population in Canada, and there are unique health needs for this community. And approximately four percent of Canadians, which encompasses one million people, identify as LGBTQ2S+ and therefore furthermore, 61 percent of the transgender individuals in the population undergo gender-affirming hormone therapy, which we will place is an acronym of GAHT.

Slide 10 [00:04:05] And a survey of trans people in the UK found that the medical transition greatly reduces suicidal ideation and attempts. So trans individuals or people thought more about suicide before transition, whereas only 3 percent thought about it more after their transition. So the body dysmorphia, the feelings of not fitting into your gender assigned at birth are tremendous and can be very difficult for an individual to navigate, particularly in a healthcare system that often is wrought with barriers to care.

Slide 11 [00:04:41] So what are these barriers? They're not always explicit. Sometimes they are implicit, so insidious and quiet and not as overt as we expect them to be, nor is it the experience of individuals in the trans community in particular to feel welcome into our heterosexist-based healthcare system.

Slide 12 [00:05:04] So what does it mean, implicit bias and informed care? So implicit bias is comprised of attitudes and stereotypes and things that are affecting our understanding and actions and decisions, but they're involuntary. They're not purposeful, but they can involve race, health, socioeconomic status and education, gender, and sexual orientation.

Slide 13 [00:05:32] And they can actually serve to complicate and sway treatment decisions which inadvertently cause failures or problems with patient-centred care because the patient can be left feeling is my healthcare provider offering me the best care that they can if they don't see me, for example? Or in our ability as healthcare providers to communicate with patients, so does my healthcare provider properly educate me on my treatment if they're not understanding who I am or where I come from? Trust, do I trust that my healthcare provider has ethical integrity? Do I trust that they have my best interests at hand? And finally, contextual knowledge. Does my healthcare provider understand my beliefs and values? If you feel not seen or heard, do you feel that you're having a good healthcare experience, or can you have the best healthcare experience possible?

Slide 14 [00:06:28] And what we are learning now through the literature and experiences is that these blindspots exist for everyone, and healthcare providers, no matter how empathic we consider ourselves to be, are still at risk of this implicit bias. And there are studies, one of them from Harvard University, which was part of something called Project Implicit, and it was designed to gather information about the hidden biases that influence our perception as healthcare providers. And when it was administered to physicians, to many physicians' surprise, heterosexual healthcare workers favored heterosexual patients over gay and lesbian patients, not intentionally but implicitly. And then race also significantly was biased towards white patients. So much as this is disturbing and we would assume that this wouldn't be happening given the fact that we are aware of those potential biases, it's important to understand that they can work themselves in, they work their way in, and they impact patient care.

Slide 15 [00:07:35] Furthermore, a physician can communicate unconscious biases through their words and body language, so it doesn't necessarily have to be overt. They can be described as microaggressions, little comments. And for a transgender person, one of these kinds of comments that are not so subtle would be, I thought you were a real woman. So healthcare providers are also at risk.

Slide 16 [00:08:06] And how do these implicit biases serve to perpetuate the healthcare disparities? Well, one of those examples is for blood donation and gay men. And since 1997, men who have had sex with men, even once, were unable to donate blood due to lack of testing and technology around HIV. Now, fortunately, this has changed with the advent of science and also with pressure to reconsider for the medical purposes. As of October 2021, the Canadian Blood Services updated their restrictions and a man is now eligible, gay men are eligible, to donate blood if it has been at least three months since he

had sex with a man. Or an individual having sex with men can donate if it's been three months because we understand the opportunity for zero conversion as three months. And then furthermore, in a pediatric study in 2012, a patient's race was found to be statistically significant in affecting physicians prescribing of pain medication. So African American patients were prescribed less narcotic pain medication than their white counterparts. So as disturbing as this is, it's important to understand how these implicit biases can then have an inadvertent negative effect on the provision of care and services to patients.

Slide 17 [00:09:37] It also can influence patient behaviors and decisions. So patients might be less compliant or have lower screening participation or avoid healthcare environments by virtue of the fact that they're expecting that the healthcare providers will not be able to care for them appropriately or will not care for them in a way that they would care for others. And in the LGBTQ2S+ communities, they're less likely to undergo cancer screening, for example, than non-trans heterosexual counterparts and have an elevated rate of cancer diagnosis. So this is a healthcare disparity that occurs by virtue of sexual orientation, gender identity, which we need to do better with. And furthermore, gay men had a 50 percent higher chance of getting a cancer diagnosis, and bisexual women had a 70 percent higher odds of a cancer diagnosis. So we understand what the issue is and now behooves us to understand how to navigate around this or at least deal with it.

Slide 18 [00:10:46] So what can healthcare providers do? What are some of the strategies that we can use to overcome our own implicit biases as we provide care to patients? One tactic or strategy would be to employ counter-stereotypic imaging and stereotype replacement. So what that means is that you imagine the individual as the opposite of the stereotype and consider your responses and reactions. So is there any change to how you're dealing with a patient based on the stereotype? And if so, then you adjust. So much as someone might present as a minority population, for example, you would place into it the majority population and see how you would treat that patient. And if it's differently, you adjust course. And then there's individuation. So you try to learn more about the individual. And then instead of treating them just as a patient or a medical interaction. So what brought them into the office or what are their goals, beliefs and values? Because that validates a patient. And that also makes them feel heard before then you break into your medical discussion.

Slide 19 [00:12:01] Other strategies include perspective taking. So put yourself in another's shoes. And then finally, partnership building, you reframe your mindset in this. There's been a big shift in medicine in the past five years to a more collaborative approach to patient care and sharing the responsibility, as you recognize that the patient has a right to autonomy and self-determination. That doesn't mean that you're not providing the services as the expert, but it does mean that you're validating that the patient has an opportunity to provide insights and participate in what they see as their ideal journey through their fertility care with you.

Slide 20 [00:12:40] And then that is on an individual and potentially clinic-based level, but what can we do on a larger scale, so from a provincial or even from a federal perspective? We can look specifically at social and cultural discrimination and what our implicit biases are. It might be different in Ontario, in a particular province than in another, for whatever jurisdictional reasons. It could be economic. So what are the financial barriers? And are there systems that we can employ or systems that we can build to prevent further economic disparity from preventing patients from accessing care? For the LGBTQ2S+ patients and population, they're more likely not to have insurance. So denials due to gender markers in their patient profile, so they might have transitioned and are using their live name as opposed to their dead name and not be covered through insurance for that. So it's important as healthcare providers to be able to advocate systemically for that to

change and be recognized. And then finally, geographical access. When you live in a metropolitan city, it's easier to find a fertility clinic that understands your specific needs in the LGBTQ2S+ community. Whereas if you are more remote, you might feel more isolated and have difficulty accessing care for those reasons. So we have to see what we can do systemically to employ tools like video calls and telephone conferences for our patients who are geographically removed from our centers.

Slide 21 [00:14:22] So what are some of the unique perspectives in our LGBTQ2S+ community in accessing fertility care?

Slide 22 [00:14:34] So we were just speaking to the disparities in healthcare, especially in sexual and reproductive healthcare, actually. So as a result, patients in the community experienced worsened healthcare compared to the overall population and to the heterosexual, heteronormative traditionals in the way we address fertility care and women's healthcare. Sexual and reproductive healthcare, rather.

Slide 23 [00:15:03] And lesbian, bisexual women are at an increased risk of breast and gynecologic cancer due to fewer screenings. Gay men have an increased risk of prostate, anal and testicular and colon cancer. And actually, with regards to COVID-19, individuals in the community, Canadian individuals, were twice as likely to experience homelessness or housing insecurity during the COVID-19 pandemic. And it was difficult to self-isolate and quarantine, so more at risk of contracting the illness. And then one-third of the LGBTQ2S+ Canadians found it difficult to meet basic needs of housing, food and clothing, and they're especially vulnerable if they lost employment during the pandemic. So it is a very contemporaneous issue, not just in fertility and sexual and reproductive healthcare, but in care overall during the pandemic.

Slide 24 [00:16:03] I guess it brings us to the question of why do these differences occur, and what it breaks down into is there are additional barriers to fertility services for these individuals because of some of the fragmentation of health services. So, you know, primary healthcare and sexual healthcare create sort of a hierarchy. So it screened for sexual health screening as opposed to preventative healthcare. And there is a move in medicine to not just treating illness once it occurs, but in preventing illness, so we need to get into the community to also prevent illness. There's also a lack, or a perceived lack, of cultural competency and discrimination in patients. There's the heteronormativity, which I referred to in the clinic, which I'll discuss a little bit later in further detail. There's lack of long-term research, so some of the research that we have around the community and the needs when accessing fertility care is spare. And then intersecting oppression. So it's almost akin to creating a mountain out of a molehill when there are several barriers to healthcare, so race and potentially religious or perceived religious or ethnic. And then also sexual or gender identity as another barrier. So if you're experiencing multiple layers of oppression or intersecting oppressions, that adds to the difficulty in accessing care, or the lack of a desire to access the healthcare system that is not welcoming or does not recognize the issues of the community in particular.

Slide 25 [00:17:47] So something else to think about is that it's not a monolithic population. Not every lesbian couple will present with the same needs, nor will everyone in the community have the same desire for their family building or fertility care. So, for example, Rachel and Alex are a lesbian couple, and they're both cisgendered women. They're at the clinic to learn more about reciprocal IVF and how that can help them to achieve their family building goals. That is also different and separate from another couple that might be wanting just to proceed with one of the individuals moving forward with donor insemination, for example. Same goal of family building but different approach. And then example number two would be Evan is a transgender man who's interested in undergoing

hormone replacement therapy in the future. But before that, he's there to learn about egg freezing or gamete freezing before hormone replacement therapy or gender affirming hormone therapy to preserve fertility options in the future. So those needs are different.

Slide 26 [00:19:02] It's also important to have emotional and social considerations of dealing with the community because healthcare providers often have a schedule of patients to see and don't necessarily moderate their approach. And it's important to have cognizance or recognize the importance of trauma-informed care in this community in particular. And so the community-based fear of coming out due to discrimination because of their sexual orientation or gender identity or gender expression, and often don't feel included or welcome in clinics. So this can lead to an increased risk of depression, PTSD, substance use or self-destructive behaviors. And fertility care and examination of body parts can be traumatic for some patients, especially for those experiencing dysphoria. So I mentioned dysphoria when transitioning and having to access fertility care and maybe needing to induce a bleed and inducing a bleed in a transmasculine individual or in a trans male can be very traumatic, and the body dysphoria that comes from it. So it's important to have conversations with the patients about one, what they wish you to address the body parts as, and then two, when we're using invasive things, like probes or speculum, would an individual want to place the probe or the speculum in for the examination to proceed on their own terms? Those are things to be considered and understanding that the anatomy, even the reflection of, or referring to gametes instead of eggs and sperm. It's important to have those considerations because those can result in PTSD. And if anyone has experienced sexual trauma in a lifetime, it's important to empower the patients to understand what is going on and to let them participate in their healthcare because you don't want to trigger anything from the past that was uncomfortable or off-putting for patients.

Slide 27 [00:21:13] It's also a culture of consent. You ask first before moving on to a different body part. The transparency, give running play by play. Explain the purpose of the exam. Discuss ways to increase comfort. Ask if there's any discomfort and what you can do to improve the experience during the examination. And finally, support. Ask if they'd like to have a peer in the room during their visit. A peer, a nurse, a support person to provide support in any way through an examination that can be off-putting and triggering.

Slide 28 [00:21:49] And so how do we implement an inclusive how-to process in the clinic? That's a big question.

Slide 29 [00:21:55] So there are several. We can do this on several layers and we have done this in our clinics Anova. But one of them is removing the hetero and cisnormative language in clinic processes. So being aware that our signs, our images, our forms, our consent DocuSigns, all of these are designed for opposite-sex couples, and there are rarely options for pronouns. And so we have included that in forms. We have included it in our signatures, on our email addresses, even when we're video conferencing, we have added pronouns so that people are aware always that pronouns matter, and they can be adjusted. Some patients incorrectly get labeled infertile. My line is always, you're not here because you're infertile, you're here because you're in obligate need of gametes when accessing assisted reproductive technologies. There's a need for education on different parental roles as well, because sometimes staff make assumptions, not by virtue of not wanting to understand the patients, but just not recognizing that the options exist. And it's very important to our patients and the community to feel included and welcome, especially given the fact that they have no choice but to see us, if they didn’t have the need to see a clinic they wouldn't be coming. So it's important for us to understand that our language and our forms make such a difference in the experience.

Slide 30 [00:23:26] Collaborations with the community and other specialists. So we have counselors who are available, and I say you're not being counseled to see if you can be a parent. It's implications counseling. Just how are you going to talk to your child? How are you going to talk to your family? Do you want to talk to your family? Are partners seeing eye to eye? What are the difficulties that might be seen moving forward? And providing some anticipatory guidance for patients to know that they're not alone. And then also to provide specific guidelines for the care of our LGBTQ2S+ patients. So we have a standard workup and sometimes standardized care does not apply. And so you need to be ready to modify and be agile enough to update and change moving forward when things inevitably are pointed out that you could be doing better as a clinic and as clinicians. So one of the studies actually by Johnson and community suggest having a patient decision aid who can act as a patient information resource and offer psychosocial support for families. So we don't quite have a patient decision aid in the clinic, but we do have counselors that work with our patients and establish a relationship early on in their clinic experience, so they don't come in in the end when something happens. Again, it's not reactive, it's preventative.

Slide 31 [00:24:54] So improvement opportunities. Let's now look at some cases and see how it was managed and what are some of the considerations that should be adopted or at least acknowledged to improve the case?

Slide 32 [00:25:14] So case number one. Evan, who identifies as transmasculine, so pronouns he/him, is visiting the fertility clinic with his wife, Amy, who is a cisgendered woman, she/her. The couple is undergoing IVF using donor sperm with Amy carrying the pregnancy. When visiting the clinic to pick up medication for Amy, the receptionist greeted Evan by saying, "Good morning, ma'am. Are you looking to pick up your medication?".

Slide 33 [00:25:51] So these beg these questions, what mistakes did the receptionist make in this interaction? So what could the receptionist, what might she have considered before she did? But what was the mistake she made? It was that she misgendered Evan, so she assumed that he identified as female, as she/her, where he clearly identified as he/him. And she assumed that he was there to pick up medication for himself, whereas it was for his wife. So Evan corrects the receptionist. How should the receptionist react? So center the needs and feelings around the person being misgendered. So acknowledging that it was your error, that there was an error made, don't make excuses or get defensive. There's no offense. It's very clear that Evan is just wanting to correct so that he can affirm who he is and make the receptionist understand how to not make it happen moving forward. But the receptionist then needs to work immediately to regain trust with the patient and then acknowledge a mistake and apologize, correct the use of pronouns, and move on with the topic of conversation so as not to draw unneeded attention to the mistake. But look, I don't know that there is any reason why you should move on with the topic of conversation so that there's no attention drawn. You acknowledge that the mistake was made and that you will make sure that it doesn't happen moving forward again. So it's important for support staff to then bring it to the superiors and say, you know what? This happened by virtue of, there's nowhere on my form that's clear that helps me to understand his pronouns when he comes in or to check. So what's a new process that we can adopt as a clinic to make sure it doesn't happen again? And then how should the receptionist have greeted Evan? "Good morning! What can I help you with?" "I'm here to pick up medication." "Great. Who's the medication for?" So we don't make assumptions. A lot of the biases that come our way is because we make assumptions in circumstances. So let's assume nothing.

Slide 34 [00:28:06] OK. Case number two. Aaron is a cisgendered man, he/him. At his first checkup with a new primary care provider, Aaron begins to discuss his sexual history and

explains that he identifies as pansexual. Later in the appointment, the care provider refers to him as a gay man. When Aaron corrects the doctor, the doctor, they/he/she responds with, "I just can't keep up with all of this diversity stuff."

Slide 35 [00:28:44] So here are our questions. What mistake did the provider make in this situation? “I just can't keep up with this.” It was fairly dismissive. It didn't necessarily validate, but it was the doctor that made the error, and the patient should not be left feeling uncomfortable. So he might have thought that the doctor wasn't taking his sexuality seriously, and furthermore, it is for the community sometimes an outing to come to a healthcare provider and actually tell the truth about sexual orientation. So it's very important not to have a healthcare provider not validate in a way that is important for a patient. And Aaron may feel as though the doctor is rejecting his identity. How should the physician have responded? They should have apologized, acknowledging the error, so apologized for referring to Aaron’s sexual orientation incorrectly. And ask for clarification on what pansexual means to him, particularly if as a physician, this is something new. Physicians don't know everything about everything. So sometimes a primary care physician might not know details and in an open conversation with a patient, would be able to glean more details about what this means to Aaron, the patient. And the doctors should then further educate him or herself moving forward on each term that the patient might use to refer to gender or sexual orientation and gender identity.

Slide 36 [00:30:20] And then the third case. It's the case of Jason. So Jason begins his afternoon shift as a fertility clinic administrator. When the doctor informs him that they are ready for the next patient, Jason opens the next file on the desk and calls out "Robert Jones" as indicated on the insurance form to the busy waiting room, which also has issues, but that's not the point. The point is what happens. So to Jason's surprise, a woman stands up and approaches the counter looking visibly embarrassed.

Slide 37 [00:30:56] The woman corrects Jason, explaining “My name is Robin, not Robert. Robin is the name that I indicated on the intake form. This now has happened numerous times at the clinic and is unacceptable.” Jason, the clerk, confused, checks the rest of the file to find that the intake form does, in fact, state Robin Jones, and that the patient identifies as a transgender woman but does not match the name on the insurance form that he initially checked.

Slide 38 [00:31:30] So here are our questions. How did this mistake happen? So the administrator didn't recognize or realize that Robin’s preferred name was different from the name indicated on the insurance form. And this has actually happened in our clinic. So how can Jason avoid this mistake in the future? So the administrator can ensure that he's checking the intake form, the intake form is that which the patient fills out, as this information is usually more detailed and patient-specific than the insurance forms. These insurance forms are often based on the health card, which made for insurance benefits purposes, have a dead name on it. So the staff needs to receive training on transgender issues to become more sensitive to situations like this and could leave a note on the patient file indicating Robin's preferred name to avoid another negative interaction. And here I will insert what I've mentioned before about escalating it to a manager to implement a system to prevent it from happening so that when we're not dealing with this particular patient, Robin's circumstance, it doesn't happen to anybody else with a similar circumstance. And that's where we talk about systemic and institutional changes.

Slide 39 [00:32:59] So there are also additional resources to consider when we site a clinic and sensitivity training. These would be some helpful resources for you to access. One of them is Not Up for Debate: LGBTQ People Need and Deserve Tailored Sexual and Reproductive Healthcare. And then another resource is Cultural Competence in Fertility

Care for Lesbian, Gay, Bisexual, Transgender, and Queer People: A Systematic Review of Patient and Provider Perspectives. So I understand that it's important for our patients to know that they're not alone and you don't have to reinvent the wheel as a healthcare provider. Recognize the biases, and then you have recourse to resource materials that can help you build a better clinic for your patient.

Module 6.5_ABCs of ART_Oncofertility 101_EN Transcript

Dr. Karen Glass

Slide 1 – Note to Audience slide [00:00:00]

Slide 2 - Title slide [00:00:05] Hi, my name is Dr. Karen Glass. I'm the Director of Fertility Preservation at CReATe Fertility Centre in Toronto, Ontario. I'm also an Assistant Professor at the University of Toronto, and I'm associated with the Sunnybrook Health Sciences Centre.

Slide 3 [00:00:23] I have no conflicts of interest to declare.

Slide 4 [00:00:28] Today, we're going to talk about fertility preservation in young female cancer patients. And our objectives are to describe the ovarian gonadotoxicity in the context of current cancer treatments. We will be able to outline the various fertility preservation strategies that are available to female cancer patients after reviewing this module. And we're also going to discuss the timing issue of fertility preservation options related to various cancers. And I'd also like to discuss today the options of genetic testing in patients who have mutations that cause and increase their risks of cancer, such as BRCA.

Slide 5 [00:01:12] This is important because every year in Canada, approximately 7000 young people aged 20 to 39 are diagnosed with cancer. And fortunately, because of the wonderful oncology team that I work with, only 820 will pass away from their cancer and the majority of these young people will survive their cancer and they will get married and they will want to start a family. And we have wonderful data to show that one of the most distressing things of a young person who's had cancer other than the cancer itself is the potential loss of future fertility.

Slide 6 [00:01:52] So ovarian reserve is something that we talk about a lot in the fertility world. And one of the things that we know from a wonderful study that was mapped out by Dr. Wallace and his staff from the UK is that when a female is in utero and is developing, they have more resting follicles and more resting eggs than they will ever have in their life. And when they're born at age zero at the time of birth, you can see that they're going to have approximately, in terms of an egg population, five, six hundred thousand. And then in the 10- to 15-year-old age where they're going through menarche and starting to have their periods, this number is still very stable. But the number of eggs that a woman will have in their 20s and 30s starts to rapidly decline. The other thing that's very important in this graph is that the population is very different. You can have a 40-year-old who has more eggs than a 30-year-old and still be considered normal in the population. So variation from person to person is huge, and that's really important when I'm meeting a young cancer patient because I have to understand not just how old they are, but who they are as themselves so that we can individualize their counseling and their treatment.

Slide 7 [00:03:24] And this is a famous graph that Dr. Wallace also put in his paper showing what percentage of our eggs do we have left based on age as females are aging? And so at birth we start at 100 percent, and when a young woman goes through menarche, we're already down to around 60, 65 percent. And then I would feel in Canada that the average age that women are starting to think about having a baby is between 30 and 35. And you can see at that point, we're already down to only 10 percent of our eggs. So if you're going to damage this last few 10 percent of the eggs with cancer treatment, you can imagine that you'll be following almost to the zero percent. And when you get to zero eggs left, then, of course, you have no more periods and you're in menopause and you're unable to conceive.

Slide 8 [00:04:19] So what is AMH? AMH is anti-mullerian hormone. It's a biochemical marker of ovarian reserve, and it's expressed by all the little resting early follicles that are in the ovaries. And it's going to decline with age, just like the graph that I just showed you and will be undetectable at the time of menopause. We can measure an AMH at any point in the menstrual cycle, but there are some physiological changes and we know that probably in mid-cycle and after ovulation that AMHs will fall somewhat. We also know that the use of oral contraceptive pills, high BMI, smoking and vitamin D deficiency will also lower AMH levels.

Slide 9 [00:05:02] And so one of the questions we have to ask as fertility doctors are patients who have a BRCA mutation going to already have lower ovarian reserve and thus already potentially be in trouble before they even start trying to conceive? And there's been many, many studies, and I've referenced them at the bottom there. And so when we look at all of these studies and we try and sum them up, we can see that in the very small earlier studies, they didn't seem to find a difference. But as larger studies were done and a little more statistical power to them, we saw that there was lower AMH in women with a BRCA 1 mutation. And then further studies showed that AMH was lower in both BRCA 1 and 2 mutation carriers, if you measured it at the time that a person was actually diagnosed with cancer. So having a BRCA mutation that's not causing a problem may not also cause a problem for your ovarian reserve. But most of the time when I see young women, I see them when they've been diagnosed with breast cancer, and that's how they find out that they have a mutation. And already then, according to these studies, most likely, they have somewhat lower AMH than a person of the same age who doesn't have the mutation.

Slide 10 [00:06:24] So why might that be? You say, well, that seems crazy, that's so mean. These poor women, they have a mutation that causes cancer and they have low ovarian reserve, like why do they have all of these difficulties? So this is a graph by my friend, Dr. Oktay. And what we know about a BRCA mutation is that it does cause problems with double-stranded DNA mutations and breakages. But these breakages are going to lead to damage in the breast tissue and in the ovary and potentially lead to cancer. But the same double-stranded DNA breakages that these mutations cause are also going to cause what we call apoptosis or cell death, and that's potentially going to lead to infertility and earlier menopause.

Slide 11 [00:07:17] So a patient who's going to have cancer treatment, how will that cancer treatment lead to fertility problems? So they may receive chemotherapy, which is going to damage their eggs. They may recede pelvic radiation that is going to damage eggs and potentially damage the uterus. For sure, they're going to have a time delay. So no matter what kind of cancer that you're diagnosed with, you're not going to be able to conceive while you're undergoing treatment. And most oncologists are going to tell their patients that they should wait one year, two years, three years, and in some cases, five years until they feel that they're out of the woods and they go ahead and try to conceive. So then, they have an aging that's a natural process in the ovaries, if you remember that graph, I showed you at the very beginning. And so they'll have older ovaries and a harder time to conceive just based on age. Some of the cancer treatments that they do will be gynecologic, so they may have an actual removal of an ovary if it has a tumor or removal of the uterus if it has a tumor. So obviously, if you don't have an ovary or you don't have a uterus, you will have infertility and you will need help. And many of the pelvic type cancers are going to have potential damage to fallopian tubes and scarring related to surgery from the cancer treatment.

Slide 12 [00:08:46] So how does chemotherapy cause damage to the eggs? Well, we know that there is destruction of the growing follicles. We know that there's a loss of the primordial follicles. And we know that there's also ovarian atrophy. So there are three mechanisms there.

Slide 13 [00:09:05] And so chemotherapy can affect both dividing cells and resting cells, so some of our eggs are developing and those are the eggs that are in the dividing cells, and some of them are in the resting stage and those are also going to be sensitive to the chemotherapy. And so basically, it's going to cause breakages in the DNA, and the breakages and the amount of damage is going to be drug and dose and age-dependent. So the older you are, the more damage and the more effects you can see.

Slide 14 [00:09:38] And so one of the first questions that I ask when I see a young woman who's going to have a cancer treatment is what kind of chemotherapy are they going to have? So we know any of the alkylating agents, the most common one that I see would be cyclophosphamide because it's used generally in all breast cancer treatments and are going to be of the highest risk, the most chance of amenorrhea, and the most chance of permanent damage to the resting oocyte. The carboplatin/cisplatin medications are often used in cervical cancer and also in ovarian cancer, and those would be an intermediate risk. And some of the antimetabolite drugs, which are commonly used in things like Hodgkin's lymphoma, are a little bit lower risk. So it's less likely that a patient will be in premature ovarian failure after that type of medication and the damage that will be done will be less permanent. There are many new drugs that are coming out, many chemotherapy agents that are not even on this graph because we're still trying to figure out what they do to eggs and what they do to sperm. And that would be many of the new immunotherapy type medications, especially the ones being used for melanoma, and melanoma as one of the most common cancers in younger people.

Slide 15 [00:11:04] And then you may say, OK, well, how do these chemotherapies cause damage to the ovaries and the resting follicles? And so many of my colleagues have done some really good research and they've looked, OK, where is the damage, and exactly where in the ovary does it cause the damage, and how does that make you lose your eggs? And so this is more for information purposes, this graph, just to sort of see that it's extremely variable from one type of chemo to another to what kind of damage is done.

Slide 16 [00:11:43] Cyclophosphamide is one of the chemotherapy agents that we're always the most worried about, and this was a study just to show that the dosing of it is going to be very important. So this was a study that was done in mice looking at how many follicles they had based on the dose of cyclophosphamide. So they had lots of eggs if they didn't get any cyclophosphamide, and if they had a high dose of cyclophosphamide, they really had very few eggs after the treatment. And so the different regimens for chemotherapy may have the same drug in it, but the total accumulative dose might be higher or lower in one chemotherapy regimen than another. And so it's very important to know exactly what regimen the patient is getting to understand what their potential for fertility in the future will be.

Slide 17 [00:12:36] And this is a nice and bright picture to show you that eggs can be damaged when they're in the preantral phase, so they're resting and they're not really dividing, and this is going to be FSH-independent. And then they also can be damaged when they are FSH-dependent, when they are in the follicular growing phase. And so the reason that understanding the difference here is important is understanding why when we use GnRH agonists to try and prevent damage to the ovary, that it can prevent some of the damage, but not all of the damage. And that's because the ones that are FSH-dependent,

these ones here can be protected, but these ones here that are FSH-independent cannot be protected from the GnRH agonists, such as Lupron or Zoladex.

Slide 18 [00:13:34] So if we had the perfect world, we would have a way of preventing the damage. So just like police officers wear a bulletproof vest, we could give the ovaries a chemo-proof vest. And so one of the research areas that is looking at this is looking at a little protein called PUMA. And if we could block PUMA, then we could prevent the cells from dying. So if you see this graph over here, you will see that PUMA is a protein that modulates the cell death, and if we can block it, then the cell won't die. And so we are doing different research studies to try and see if we can figure out an inhibitor of PUMA that we could give to the ovary to protect it, but not give to the cancer. So you have to think if someone had breast cancer, we do want the chemo to go to the breast, and so it's in the IV and it's flying around, but how could we put PUMA only in the ovary to protect the ovary and not protect the breast cancer because we want that to be killed by the chemo? And so this is a research area that really needs some work to be able to figure out how this could help.

Slide 19 [00:15:01] And so the next question we have to ask is, how much damage does the chemotherapy cause? And so if you look at the pink circle and you look at the green triangle, the person having the eggs with the pink circle didn't get any chemotherapy and the person who had chemotherapy has a green triangle. So if you look for a person that has, on this line, primordial follicle count and you compare it, you can see that it's about 10 years difference. So the person in the control group looks like they're sitting at approximately 33, 34 years old, and the person who got the chemotherapy is only 23 or 24. So you can see that the chemotherapy brought the actual number of primordial follicles down in an equivalent of 10 years of aging. And so when I'm talking to my patients and I'm counseling them about the chemotherapy that they're going to get, I'm going to say it seems like depending on what kind of chemotherapy they're going to get, that your ovaries will be damaged and it will age you approximately 5 to 10 years. And I think that's a good way of explaining it because saying you have an 80 percent chance that you'll be in menopause five years after your chemotherapy, it's hard to understand that, and I feel that for them, it doesn't really help them understand the damage that's being done. But if you say your ovaries will be aged 5 to 10 years, I think that makes it very, very clear for them. So if they are 38, they go, oh, my ovaries will be like a 48-year-old, I understand 48-year-olds have a really hard time getting pregnant, and I don't think I'd be able to get pregnant. And so I try and keep it simple, and that's how this graph by Dr. Oktem helps us explain it.

Slide 20 [00:16:58] Ovarian radiation that's going to the pelvis is completely damaging. And we know that patients who are going to have pelvic radiation are going to go into menopause, and that's going to be age- and dose of the radiation-dependent. The other thing that's important is where is the aim of the radiation and will it go to the uterus as well? So patients who are having cervical cancer treatment have full-on radiation to the cervix and the uterus, and so we know that they will not be able to carry a baby because of the damage. In patients that are going to have local radiation but not specifically to the uterus, then we've noted in post-radiation pregnancies that there's an increased risk of a miscarriage, preterm birth, low birth weight, IUGR, and placental problems, and even stillbirth. So many of those patients will choose to have a surrogate for their future pregnancy.

Slide 21 [00:17:59] And this is showing what are the chances of ovarian failure or premature ovarian failure and early menopause based on what dose of radiation? So the most important thing here, which is very obvious probably to any fertility person, is the younger you are, the more radiation you would need to completely knock out your ovaries. And so these doses of radiation that are in 20- and 30-year-olds are actually lower than

the standard radiation that a patient would receive if they needed pelvic radiation for, like rectal cancer or cervical cancer. So that's why these patients are almost 100 percent of the time going to be in premature ovarian failure.

Slide 22 [00:18:45] So now I'd like to talk about fertility preservation options for females. So they have five general categories that we like to talk about. One is to do nothing and figure it out later. Two is to do IVF and cryopreserve oocytes or embryos. Three is to do IVM and cryopreserved oocytes or embryos. Four is to protect the ovaries from treatment. And five is to freeze ovarian tissue.

Slide 23 [00:19:19] So one of the things I always talk to my patients about when I'm counseling them is you're here for me to explain it all to you, but let me tell you that if you don't do anything right now, there's always a Plan B. So Plan A was that they hoped they would get pregnant naturally, and then they were diagnosed with cancer, so we have to sometimes move to Plan B. And so what could be that do nothing now and figure it out later option? So maybe for a patient that's going to receive less damaging chemo, such as a Hodgkin's lymphoma patient receiving an ABVD, they may choose to just get through their cancer treatment, and assuming that all is well post-cancer, then they could proceed to do an IVF cycle to freeze eggs or embryos. However, in the provinces such as Ontario, where IVF is funded, IVF is only funded if you have an urgent medical emergency, so they would have a funded cycle to do IVF before they started their chemotherapy, but if they come back a year after chemotherapy and they're cured of their cancer, they no longer have a funded cycle. Also, one of the situations that I've seen is a patient who has milder chemo, such as Hodgkin's, and they feel like I'm going to be fine and I don't really have to worry about it, and then three months into their treatment, they see that the treatment is failing and the plan has changed. So now, instead of receiving a milder chemo, they're going to receive stronger chemo or even a bone marrow transplant, and that's going to render them in premature ovarian failure. But at that point, three months into their chemo, they have chemotherapy on board. And at that point, you cannot do IVF because the oocytes that are currently in the ovary are already damaged and there would be no point going through an IVF cycle because those are damaged oocytes that will not fertilize. So if a patient feels overwhelmed and they decide to do nothing at the beginning when they have their counseling, then if they were in menopause and they come back in two or three years and they want a child and they're unable to conceive with their eggs, then they obviously could use a known or anonymous donor and their oocytes to be able to conceive. And in patients who are going to have pelvic radiation, of course, in the future, they're going to need a gestational surrogate. And that would also apply, of course, to a patient having a hysterectomy.

Slide 24 [00:21:53] I also would like to mention male fertility preservation just in the point when we're starting to talk about the options as freezing of sperm is covered by OHIP in the province of Ontario for one sample for any men that are going to undergo medical therapy that will damage their sperm. They can also choose to freeze more than one sample if they wish, of course, in different clinics it will be different in pricing. At the clinic that I work at, we charge 150 dollars for any additional samples. Also, there is a Canada-wide charity called Fertile Future, which will cover up to 350 dollars for sperm cryopreservation and that will work in the provinces that don't have funding. And all of these provinces that do have funding, usually the storage fees, which will run in different clinics anywhere from three to four hundred dollars per year are not covered. Patients can be seen very quickly and should be easy to fit in time to do sperm freezing prior to them proceeding with their medical therapy. Obviously, for a gentleman, it's much easier to get their sample into a freezer faster than it is to get eggs out of a female.

Slide 25 [00:23:15] So option number two is to do IVF, so in Ontario and in Quebec, the provincial health plans will cover freezing oocytes for one cycle of IVF. In our clinic, we charge approximately 1500 dollars to fertilize the eggs, make an embryo, and then put the embryo in the freezer in a patient that has a partner. So let's say they have one child together and then she's diagnosed with cancer, and they would like to freeze their embryos, they feel their relationship is very stable, then, of course, they can go ahead and do that if they would like. Unfortunately, the provincial government, though, does not cover the fees to freeze an embryo. And the storage fees are 300 dollars a year at our clinic and generally they would be about the same in other fertility clinics across Canada. It takes about 10 to 12 days to stimulate the growth of oocytes on an ovary. And then once the eggs have grown, we do our trigger and the egg retrieval is going to be about two days later. So altogether, if you add that up, it's going to be 12 to 14 days until a patient can start their chemotherapy. And fortunately, because based on a paper that came out of California, we know now that we can start treatment anywhere in the menstrual cycle. We call that random start IVF. It takes a little longer if you start after or at the time of ovulation, but we can do it and we got to do what we got to do. So we have to get those patients started and we get them going so they can proceed to their cancer treatment without any delay. Obviously, if you're going to do IVF, the patient must be post-puberty. So this isn't something that we can do for a nine-year-old who's about to have chemotherapy.

Slide 26 [00:25:13] Now, for some provinces, there is no funding, and so what could the patients do if they can't afford IVF? And so some of the clinics may have a reduction in their fees for oncology patients, I believe that most do. And there's also the Fertile Future charity that has their Power of Hope program. And based on the Power of Hope program, they will look at the patient’s status so the patient has to be landed or a Canadian citizen, and the patient has to be less than 40 years old and they can help them out with some funding.

Slide 27 [00:25:53] And so currently, the grants are for anywhere from about 1000 to 2000 dollars. As well, the pharmaceutical companies have been very generous if we complete a form asking for compassionate medications for oncology patients. Most of the time, they will help us out with compassionate medications if the patient doesn't have a drug plan.

Slide 28 [00:26:19] So this is a very busy chart showing all the conventional versus luteal phase starts. Basically, what to learn from this is conventionally the patients come in and they're on day two to three of their period and we start our gonadotropins and then we would start the antagonist. As the eggs were starting to grow and the estrogen was starting to rise, we would trigger and we would do a retrieval two days later. So that's traditional IVF that we've all seen and we all know and love. But maybe the patient is in the middle of the follicular phase and they're going to ovulate in a few days. Well, that's OK. We just start the gonadotropins. They will ovulate partway through the IVF, and what happens is they release the one big follicle that's ahead of the rest and then the rest of the follicles will continue to grow. And then we do our trigger and do our retrieval just like we would do, and we start the GnRH antagonist when the smaller follicles are growing. So we let that big one go. It's like the sacrificial lamb so that we can get all those second-line follicles that we got growing. And similarly, with a luteal phase start, what we see is you have two options. So you can help them ovulate so that they pop out their lead egg and get things going. Or we can see that they've already ovulated and once they've already ovulated, so here's the spontaneous LH surge and we're like a week past it, start our medications and we just start their eggs growing again so that ovary is busy. It's causing two batches of eggs to grow in one cycle. Very often when you do these luteal starts, the patient will have a period in the middle of the IVF, and so I always warn them not to be concerned if they have bleeding in the middle of the IVF, that it's not damaging any eggs

or any part of the IVF. It's just that their uterus and their ovaries are in a different place. So they are not coordinated, they're uncoordinated at that point.

Slide 29 [00:28:36] So how do we take the eggs out? We call it an OPU, ovum pick up, and we do it in an office-based procedure. We use conscious sedation. In our office, we use fentanyl and Versed. Procedure will be anywhere from five to 20 minutes. The more eggs you have, the longer it takes. It's very low risk for complications, and once the eggs come out, the patient can choose to either freeze them as an oocyte or fertilize them and freeze an embryo, depending on if they have a partner.

Slide 30 [00:29:08] So here's a nice picture of how we do an egg retrieval. So basically, it's a transvaginal ultrasound. It has a needle guide on the ultrasound, and the needle will go through the wall of the vagina and into the ovary. And as you can see, the ovary is all blown out with these beautiful follicles. Once the needle goes into the follicles, it's attached to a suction machine, and the fluid that's in the follicles will have the cells and the oocyte, and that will be aspirated with a little suction path and then go into the test tubes. We then pass that test tube through to our embryology staff who will look under the microscope, and when they look under the microscope, they'll find the eggs. So while we're doing the retrieval, right away we hear from our team. You have one egg, you have four eggs, you have 10 eggs. And so we're getting feedback right away, which is wonderful for the patient to know that it's going well and they're going to have some eggs to work with.

Slide 31 [00:30:13] And so what can we do when those eggs come out and once we've decided to fertilize them? We can make our embryos. So here is a beautiful human oocyte. Here's a day three embryo that has eight cells. And here is a blastocyst. So usually the embryos will get to the blastocyst stage in five or six days, and an average blastocyst has about 150 cells.

Slide 32 [00:30:44] So how are we going to fertilize those eggs? So we have two choices. In traditional IVF, the sperm and the eggs are just placed in the little dish together and the sperm have to swim naturally around the egg and fertilize the egg. But more and more now, probably at least 80 to 90 percent of cases in the Canadian IVF labs, we're doing what's called ICSI, or intracytoplasmic sperm injection, where we take one sperm and we've picked the strongest, best looking, fastest swimming sperm, and we're going to place that with a little needle directly inside the cytoplasm at that oocyte. And then we'll place it in the incubator and watch that embryo growing for the five or six days.

Slide 33 [00:31:40] Oocyte freezing originally was thought to be somewhat experimental, but for a while now, the American Society of Reproductive Medicine has considered it no longer experimental and in basic studies, especially for patients who are under 35, we know that the success rates will be quite equal whether you freeze an egg or an embryo. And it's very important because there are many patients that will have ethical, moral or religious reasons for not freezing an embryo.

Slide 34 [00:32:10] And this is a copy of the ASRM guidelines, and as you can see, these came out in 2012. So I feel like this is sort of obvious and kind of old news now.

Slide 35 [00:32:25] Now, how about patients with breast cancer? They have a very different situation than most cancer patients in that they have a few stages to their treatment. So if they're going to have their breast cancer surgery first, then they can have IVF and have a fertility preservation treatment before the surgery. So obviously, this is important that we've received the referral early. So in one of the studies that we did with the team at RUBY, which was a Canada wide program for young women with breast cancer, we showed that we can make sure that breast cancer surgeons are

knowledgeable about how important fertility preservation early referral is. And now we most of the time do receive the referrals before their surgery if they're going to have surgery first. And in some of our patients, they may have BRCA mutation. They may have a lower ovarian reserve. They may be a little bit older, like 38 years old. And so they may even have time to do two cycles if the referral comes early and if they don't have enough eggs and if they wished. So we may be able to do a cycle before surgery and then they have their surgery. And then there's usually a six- to eight-week time period after surgery and before chemo starts, which is plenty of time for us to get a second IVF cycle in. But many breast cancer patients do receive chemotherapy first. That's called neoadjuvant chemotherapy, and therefore they will only have time to do one IVF cycle, and that has to be before the chemotherapy because after obviously there's been damage to the ovaries already.

Slide 36 [00:34:09] So one of the questions that I'm often asked by patients is, is it safe for me to do IVF before my surgery because I have breast cancer and my tumor is estrogen receptor positive, so won't the estrogen from the IVF cause my breast cancer to spread and grow? And so the majority of tumors are estrogen receptor positive, probably about 80 percent, and we do know that patients' estrogen levels go quite high during an IVF cycle. And we know that there is a protein called TFF-1 that actually stimulates the migration of human breast cancer cells, and it's regulated by estrogen. So if you look at all of this, you could say, yeah, Dr. Glass, you shouldn't be doing any IVF in these patients until after their surgery because you could be causing their cancer to spread.

Slide 37 [00:35:00] But we can look at the rebuttal of that, and that is that we are going to use the aromatase inhibitors. We are going to keep the estrogen level at physiological levels all through the IVF. And so their estrogen levels may be only above the natural level that they would have if they were ovulating for a few days at the end of the cycle. Also, there's lots of data now that's showing that pregnancy after breast cancer is safe, and that's for nine months. So a couple of days of estrogen during IVF is very small compared to nine months. Nine months is a lot longer of having a high estrogen. The other thing is, the most important point here, is that we've done research and we've looked at it and we've compared the patients who decided to do IVF before their chemotherapy in breast cancer treatment, and from the data, there is no decrease in the disease-free survival in the patients who opted for fertility preservation. Now, obviously, we could not do a randomized controlled trial because you can't force patients to do IVF or not.

Slide 38 [00:36:13] So let's look at these stimulation protocols. So in patients with estrogen dependent tumors, so this could apply also, for instance, to patients who have endometrial cancer, we can use breast cancer drugs during the 10 to 12 days of stimulation, and we'll keep the estrogen in a very physiological level during the stimulation. Also, we're going to use a GnRH agonist trigger and that's going to help prevent OHSS, and it's also going to decrease the estradiol level after the retrieval. Now, this is really important because our patients need to move on and get their cancer treatments done. So I never want my patients to finish their IVF cycle with OHSS. It's going to delay things and that is going to frustrate the oncologist and frustrate the patient. And so both of these drugs have been used as fertility drugs in the past. Tamoxifen was actually originally invented to be a fertility drug, and then they found that it didn't do great things to the endometrium. And for that reason, it was discontinued as a fertility drug and discovered that it was a great drug for breast cancer.

Slide 39[00:37:26] So let's look at this study by Azim et al. It's a small study with 79 women, and these women all had IVF with letrozole and recombinant FSH. And they compared them to 136 women with breast cancer who decided not to have IVF prior to their chemotherapy. And there was a huge range in the follow-up of these patients, but the

median amount of follow up was about two to three years. And when they look at the hazard ratio, the patients who received the letrozole, which is a breast cancer drug and did their IVF, had a lower risk of having recurrence than the patients that did not do the IVF. Now, obviously, we have to be concerned about who chose to go ahead and who didn't. Again, not randomized, but at least it makes us feel a little

Slide 40 [00:38:19] bit reassured that the numbers weren't even close, never mind being even similar. So the letrozole group, as you can see, has a much lower disease-free survival compared to the control group, especially when you look and start to get four or five years out, you can start to see the difference. (*Correction 'The relapse rate is lower for the IVF patients).

Slide 41 [00:38:46] One more important thing that I counsel my patients about when they're undergoing IVF and they have a genetic mutation such as BRCA, is that we have the ability to stop the transmission of the genetic mutation to their offspring. So we call that PGT-M or preimplantation genetic testing for a monogenetic disorder. And so they would see a genetic counselor to explain the process. We would make a probe using either their blood test or a cheek swab of their DNA. And then we can actually test these few little cells that we've biopsied from the embryo for their specific mutation. So not only can we test to make sure that the embryo is genetically normal, ruling out things like Down syndrome, trisomy 18, trisomy 13, but we can also find out which embryos have their mutation and which don't, and then only do an embryo transfer of the embryos that do not have the mutation. Now you can imagine you need a lot of embryos to end up with a good outcome when you do this, because depending on how old the patient is, anywhere from 50 to 80 percent of their embryos may not be genetically normal or will be aneuploid. And then if you randomly flip a coin, 50 percent of the embryos should have the mutation and 50 percent should be their good gene that doesn't have a mutation. But again, these are all sort of random events. In one of my worst-case scenarios, I had a patient who had eight embryos, so I thought we were going to be fabulous and it was going to be good to go, and seven of her eight embryos contained her BRCA mutation. So randomly speaking, you just never know how that's all going to work out. The other thing about genetic testing for a single gene disorder is that it's quite expensive. The biopsy fee and the testing to make sure that embryos are genetically normal with regards to the chromosomes is 4000 dollars, and then the additional testing for PGT-M is another 4000 dollars. That's the pricing in our clinic. So altogether, that would be about 8000 dollars. And in Canada, many of the clinics do not have their own lab, and they would send the biopsy specimens to the U.S. and then the patients would be paying for this testing in U.S. dollars. And so it could easily be much more because of the strength of the Canadian dollar currently.

Slide 42 [00:41:25] So if a patient comes to me and they're doing fertility preservation and it's an emergency situation because they currently do have cancer and they have a partner, usually what we do is we go ahead and do the IVF and we make embryos using ICSI. We don't want any extra sperm stuck on the outside of that embryo when we're doing the biopsy. And at the time of creating the embryos, we do the biopsy and then we freeze them. And then usually at that moment in time, their life is upside down. They're off work. They don't have a job. And I explained to them, once you've biopsied the embryos, you don't have to worry. It's in the freezer, we have the DNA amplified, and when you're ready, we can go ahead and run the testing. So many of the patients will hold off and actually pay for and do the testing six months later or a year later. And so they do have some time there to get through the difficulty of the cancer treatment and the initial shock of what's going on before they proceed. Also, in many of the patients, they may have a very strong family history for cancer, and we feel like they may have a mutation, but the mutation testing is just being started. And the genetic mutation from the cancer centers, if they're in a regular program, is a two-to-three-month waitlist for that result to come back. So many

times at the time that we're doing the IVF, we don't know yet if they have a mutation and we find that out after anyway, so we have to wait to run that testing. If a patient comes to me and she's single and she does know that she has a mutation, I explain to her that we don't test an oocyte, we only test an embryo. And so in the future, when she had a partner and was ready to use her eggs and fertilize them, then we could do the genetic testing if they wanted.

Slide 43 [00:43:29] So in terms of research, what we're hoping for the future is that we won't have to actually biopsy an embryo to get the genetic mutation. So in the first days of preimplantation genetics, we would actually take polar body biopsies. And then it was the day three embryos that had about eight cells that we would take one of the blastomeres of the eight cells. But in modern times, it's always the trophectoderm that we biopsy, taking off anywhere from two to five of the 150 cells. So wouldn't it be better if we didn't have to do anything to the embryo itself? So embryos sit in a little culture dish with their media, and so we know that the bathwater, not the baby, figuratively and literally, will have some genetic material in there. And so there's already research that's been done and testing that's being done, not for PGT-M, but for PGT-A, showing that you can test and do noninvasive PGT-A, essentially. And so without causing any harm to that embryo, you can find out if that embryo is normal. The other less invasive testing that people have thought about is taking some of the fluid from the blastocyst. So when that blastocyst expands, it has a little bit of fluid in there and that's called a blastocentesis, and that also is another way of testing without actually biopsying the cells off of the embryo.

Slide 44 [00:45:14] So one of the questions that patients and I spent a lot of time talking about is, is pregnancy after breast cancer safe? Is it safe if you have a BRCA mutation? Is it safe if you don't have a BRCA mutation? And so Lambertini and his team just came out with this paper in 2020. They had a huge population of 1252 BRCA mutations, most of them being BRCA1, 430 were BRCA2, and poor ladies, 11 who had two mutations, one of each. And they followed them for a long time, so 8.3 years post breast cancer. So that is a really long, long follow-up. Interesting to me, only 195, which is 19 percent of those patients, went on to have a baby. And of those, only 150, which is 77 percent of the 195, went on to deliver because not surprisingly, there was a miscarriage rate that was 10 percent and even eight percent of them chose to terminate the pregnancy. And most importantly was they looked at these patients and they compared them to patients who had breast cancer and did not have a BRCA mutation, and there was no difference in the disease-free survival if you had a pregnancy or you didn't, and there was no difference in the overall survival.

Slide 45 [00:46:55] So here are some of these graphs that oncologists love to use, looking at how many years go by and what percentage of the population remains disease free. So up at the top is 100 percent, meaning you have no disease and you're healthy. And as the numbers go down, more people are starting to have relapses and recurrence. Interesting in this graph is the pink line is the pregnancy cohort. And you can see if you look, the pink line stays on top of the non-pregnancy blue line, meaning more people were disease free at the 10-year mark in the pregnancy cohort than the non-pregnant cohort. And here you can break it out in terms of who had BRCA and had a pregnancy, and who didn't have BRCA and had a pregnancy. This population was quite small, but the pregnant BRCA2 population, you can see it does seem to have more disease that was recurrent in that particular group, but it was a very, very small group of patients. So maybe we needed more patients to weigh that out.

Slide 46 [00:48:11] So if we look at this slide and first we look at graph A, we can see four lines. The most important lines to look at here are the top line and bottom line, which are pink and red. In the top line, we have the patients who were hormone receptor negative,

so their tumors are not responsive to estrogen, who became pregnant. And you can see that they had the highest disease-free survival of all the cohorts. The green line and the blue line are the patients who did not become pregnant, and some were hormone receptor negative, some were hormone receptor positive, and their disease-free survival was very similar. Unfortunately, the bottom line where there was the lowest disease-free survival, where the patients actually had some recurrences of the cancer were the patients who were hormone receptor positive, who did become pregnant. So it's not an extreme difference, but we have to worry that was it the estrogen of the pregnancy, which would be responsive because the tumor had estrogen receptor in it, that potentially years later, they seem to have started to have some recurrences. So these numbers of these patients when you look at how many were pregnant and hormone receptor positive was only 60 patients, and the amount who were still in the study years later wasn't very many patients that were still being followed, but we still worry was there something going on there? And this will be something that will come out when the positive trial is published. In graph B, we have overall survival, so we are looking at patients who were actually alive, and it does not account for if their tumor had recurred or not recurred. And when you look at the two lines, you can see both nonpregnant and pregnant patients. When you look at this 10-year mark, overall, the survival looks exactly identical, which is very reassuring.

Slide 47 [00:50:33] So option number three is to do IVM or in vitro maturation. So what if we don't have enough time to go through a whole IVF cycle? What we can do is have a collection of immature oocytes from unstimulated ovaries, so they don't need expensive fertility medications, and they don't need two weeks to get this done. We could just do a retrieval of their unstimulated ovaries at any point in the menstrual cycle. And then any eggs that we get out, we could mature them in a culture dish and then we could freeze the mature eggs, or we could fertilize immature eggs. And then if they grow to a blastocyst, go ahead and freeze them. So some people like the idea of this because it's faster and it's less costly. But we do know that it does have about half the success rate of traditional IVF. And also, some people are looking at maybe trying to take these eggs out and mature them if we've taken out like, for instance, an ovarian tumor. So if a patient had ovarian cancer, we would never want to take that ovary and put it back in their pelvis once they were cured of their cancer because there may be microscopic cancer there. But we could take the eggs that are immature out of the ovaries, mature them in the culture dish and then freeze eggs or embryos, depending on their marital status.

Slide 48 [00:52:00] So here's a picture of some immature eggs that are removed. And then here's a picture of the mature eggs that they become after being in the incubator with the correct media for a couple of days.

Slide 49 [00:52:16] So our fourth option is ovarian protection, and as I mentioned before, when we are at the best point of fertility preservation research, we will be able to protect the ovaries without having to do anything. We'll have that chemo-proof vest for the ovaries. But currently, we have a couple of options that we're looking at. We can do ovarian transposition of an ovary for a patient who's going to receive pelvic radiation, we can use GnRH agonists such as Lupron or Zoladex to offer some protection, as I mentioned earlier in the talk, and there's some research looking at the S1P agonist. This is currently just in a research mode. It's been used on monkeys. Again, going to have to figure out a way to protect the ovaries without protecting the cancer.

Slide 50 [00:53:11] So here's a picture of an ovary that has been cut off at its base at the uterus and flipped up to the pelvic sidewall. We can do this laparoscopically and then we put a few stitches to hold the ovary on the pelvic sidewall. Usually, it's about the same height as the belly button. So if they're going to receive pelvic radiation, this ovary is out of the way of the ovarian field and should not be damaged by the radiation.

Slide 51 [00:53:48] So ovarian transposition does require a patient to have a general anesthetic and have a surgery and requires OR time, and so certainly during COVID, that was very, very challenging and it's still sort of a backlog in terms of surgery to be able to do this. Fortunately, these patients are considered high priority and if we need to, we've been able to try and work that out. When I first started practicing, patients who were getting pelvic radiation would receive only radiation, so if you move the ovary, it would be out of the field and no damage could come to it. But for about the last 10 years, the radiation oncologists often use chemotherapy along with the radiation, so it is sensitizing the radiation. And usually they use platinum-based chemotherapy, which is intermediate in terms of its damage. So even though I've moved the ovary away from the radiation, the chemo will find the ovary no matter where it is. Obviously, if I'd moved an ovary up to the level of the belly button, we've cut off the fallopian tube from the uterus and there will be no natural ability for the patient to conceive after. We do do IVF on transposed ovaries, that's a transabdominal retrieval, and it's tricky, but we do them if we need to. And I find those ovaries lose some of their blood supply and are not going to work just as well when they're transposed as prior to them being transposed. Most of the patients who are having an ovarian transposition are having pelvic radiation, and so they're going to need a surrogate anyway when they go ahead and they're ready to conceive.

Slide 52 [00:55:29] So does ovarian transposition work? So Dr. Hoekman and his team in 2019 looked at all of the studies that were published and all together, it was a systematic review with 765 patients, and all of these patients were going to receive external beam radiation. And it seemed that the patients who had chemotherapy with their radiation had the worse survival of the ovaries, but the patients who just had radiation had the best survival. Also, in the patients who received external beam versus the brachytherapy, the brachytherapy is the local therapy for cervical cancer, the external beam goes higher, and so it isn't surprising that the success rates would be intermediate. Also, to note that depending on who did the surgery and different clinics and different protocols, the complication rates could be anywhere up to 28.6 percent. So that's quite a high complication rate, perhaps with the patient getting a hematoma or needing a blood transfusion. And so whenever we're doing surgery, we have to weigh the risk-benefit profile and discuss it with the patient so they can decide what's best for them.

Slide 53 [00:56:52] And so one more protection mechanism, as we talked about, was the GnRH agonist, and so those injections are usually given monthly but can also be given every three months. They should be covered by a drug plan if the patient has a drug plan because it's not a fertility drug, per se, and so the cost depends on the pharmacy, but approximately 400 dollars per month for an injection. In patients who are receiving chemotherapy, if we're giving the agonist during the chemotherapy, we want to make sure that their platelet counts have recovered from the chemotherapy because the injection is given intramuscular and we don't want them to get a hematoma. And when we're counseling them about the side effects of the Lupron and the Zoladex and the agonists in general, I always explain that it's the same side effects of a patient going through menopause, including hot flashes, perhaps vaginal dryness, difficulty sleeping. And so that in some kinds of cancer, like, for instance, if they have a non-Hodgkin's lymphoma and they’re going to have the agonist during their chemotherapy to help protect them, that we may be able to give them estrogen add-back as their tumor is not an estrogen receptor tumor. And so we always ask, well, how do these medications work? And we feel that having the agonists on board is obviously going to suppress the pituitary-ovarian axis, and then there's going to be reduced blood flow and hopefully, therefore, reduced perfusion and less chemotherapy gets to the ovary to cause the damage. Also, these medications are known to sort of inhibit cellular apoptosis, so cell death. And so they cause cell death to not happen. Then you've protected the ovaries.

Slide 54 [00:58:42] And so in terms of the studies being done, they've done studies of the studies and meta-analyses of the meta-analyses, and this has been looked at and looked at and turned upside down and backwards to decide did these drugs actually protect the ovaries? And when you look at the overall results of all of these meta-analyses, we know that we feel confident that the GnRH agonists are going to be effective in preserving ovarian function and reducing premature ovarian failure or amenorrhea post-chemo. But what's controversial is do they truly, therefore, help with fertility and future pregnancy? And most of the studies, unfortunately, were cut off soon after the chemotherapy, and they didn't follow the patients for five to 10 years to see, well, what percentage of these patients tried to conceive and what percentage were able to conceive? And so we would need more data to be better able to answer that question.

Slide 55 [00:59:44] So if you look at them in terms of during breast cancer patients. So this soft trial was presented at the big breast cancer meeting in San Antonio, and they took more than 3000 patients, and they compared the Tamoxifen all by itself to Tamoxifen with a GnRH agonist versus Exemestane, which is an aromatase inhibitor and a GnRH agonist. And the largest advantage was in the group of patients that were less than 35. And that's probably because these patients will ovulate. And when you're on Tamoxifen, if you measure the estrogen levels at the time of ovulation, they're actually super physiologic. Just like when we used to use clomiphene citrate for stimulations during an IUI, we would see these super, super high estrogen levels. So even though Tamoxifen was blocking the cancer and works for the cancer in the breast, the super high amount of estrogen, you have to wonder what it was doing.

Slide 56 [01:00:41] And so if we compare the less than 35-year-old age group, we can see that the disease-free survival with Tamoxifen alone was 67.7, with tamoxifen and a GnRH agonist it was 78.9, and with an aromatase inhibitor and a GnRH agonist it was 83.4. So if patients can handle the side effects, because clearly there is a lot of side effects of these medications put together, then there is an obvious survival benefit to using more than one hormonal blocking treatment after the chemo and the surgery.

Slide 57 [01:01:22] Freezing ovarian tissue is something that is considered pretty new in Canada or even in all of North America, but has been done much more readily for years and years over in Europe. The nice thing about it is that it's available for both pre- and post-puberty patients. We know that we could not just freeze 10 or 20 eggs, but we may be able to put hundreds or thousands of primordial follicles in the freezer. And except for the need for an OR day to be able to take out a piece of the ovary, there's no medical delay for going ahead. It's less costly, assuming that wherever they live, a laparoscopic surgery is covered by their health plan, and no ovarian stimulation medications are required. Obviously, it does not require that the patient has a male partner or a sperm at that point in their life. But we do know that they're going to end up having at least two surgical procedures. Maybe IVF will be needed as well once the ovarian tissue is transplanted back into the pelvis. So the patients will have the risk associated with two anesthetics and the risk associated with two laparoscopic procedures. There is also a theoretical risk of there being some cancer left in that small piece of ovarian tissue that you're putting back in. So you have to wonder, could we cause a recurrence in a patient who is in remission by transplanting the tissue? So the European groups that are doing this more readily generally are doing PCR testing on these small pieces of tissue to ensure that there is no cancer in them before they're transplanted back.

Slide 58 [01:03:09] And what about in a breast cancer patient? So if 80 percent of breast cancer patients are estrogen receptor positive and they're currently in menopause, but I'm going to put back their ovarian tissue, I'm going to take them out of menopause, so I'm

going to be essentially giving them back the hormones that we thought we should be taking away, according to other trials, such as the soft trial. And in a BRCA1 or 2 mutation carrier, I would never want to take an ovary out and put it back in. That's just ridiculous because we know that they have an increased risk of developing ovarian cancer. And so it doesn't really make any sense to take out an ovary, freeze it, and then go ahead and put it back in.

Slide 59 [01:03:56] And so do we consider ovarian tissue freezing experimental? So officially in Canada and the United States, it's still newer. And so I feel like this is going to be clinic-dependent on how many cases they have done. And so most of the places in Canada have only done a handful of these cases of ovarian tissue freezing and many of the clinics may not have transplanted any tissue back. So obviously, in our hands, this should be considered experimental. Although, some of the agencies, such as American Society for Reproductive Medicine and the European Society for Human Reproduction, are saying, oh no, we know how to do this, and it's no longer experimental in our locations.

Slide 60 [01:04:47] So we know that when you transplant these grafts of ovarian tissue back, that generally they will last two to four years. So if you have a 30-year-old and you put the tissue back when she's 32, clearly it's not going to work until the natural age of menopause, which would average out at about 50 or 51 in Canada. And so there is at least 130 pregnancies and live births that have been reported now, mostly from Europe and Israel and some from Asia, and most of the pregnancy rates are much higher in the women who are under 35 years of age. And most of these clinics are using the slow freeze technique, whereas many clinics in Canada no longer have their slow freezers. We've all switched over to vitrification. So in Asia, and I feel like in most of the clinics that are doing ovarian tissue freezing in Canada, were using the vitrification method.

Slide 61 [01:05:43] So these are some great photographs of laparoscopic surgery. And this is showing some different methods that you can put the tissue back in when you go to do it. So this is the pelvic sidewall, and they're just opening up the peritoneum and putting the little pieces of tissue under a pocket in the peritoneum. And you can see when you go back in to look what's going on, that here is that tissue that is kicked in and started to work again, and here's a nice follicle that's developing under the peritoneum in that patient. And in some cases, the patient had one ovary removed or part of one ovary removed, so you could put the pieces back in the other ovary. So that one that received the chemo damage and maybe it's not doing great and they're in borderline menopause, so you can open up this ovary, and sew the pieces into the ovary, because you can see that ovary has great blood supply still, and then that ovary can hopefully continue to develop and have its follicles grow. And so some of what the Europeans are still figuring out is once you transplant those tissues back, we know it takes a few months for them to get blood supply and thaw out and kick in and start functioning. But should you just wait for patients to conceive naturally? You can see here's the fallopian tube hanging over the ovary. Maybe they can just, you know, get pregnant the old-fashioned way. Or as soon as the ovary starts working, should we take an advantage that it's ready to go and go ahead and start doing IVF and trying to make embryos? And that's a research question still to be answered.

Slide 62 [01:07:29] Let's talk a little bit about endometrial cancer because it's a very specific cancer with specific issues. So what we've shown is for grade one, stage one endometrial cancer that they can be given progesterone treatment such as Provera or Megace, which are pills or a progesterone IUD, such as a Mirena. So these treatments do not cause harm to the ovaries. It's just that the patient will have a delay until getting pregnant until they can reverse their endometrial cancer, and they will obviously be getting older as they have this delay. But what we do know is that you can go ahead and do IVF if

you have a Mirena inside your uterus. And so while we're protecting the uterus from endometrial cancer, we can do the IVF, and then we can freeze the embryos, repeat the endometrial biopsy, make sure the cancer is gone, remove the Mirena and then go ahead and start doing embryo transfers. And this is a protocol that I use routinely in the numerous endometrial cancer patients that I've seen in my office. So if the patient comes to me and they're already on Provera or Megace, then I cannot do IVF. So patients are on very high doses that suppress them, and they won't respond to fertility drugs on those high doses. And these patients can be complicated medically to look after, as many of them have type 2 diabetes. Many of them have high BMI and obesity. So you have to understand what is the cutoff for your clinic, if you have a cutoff, in terms of weight or BMI to be able to proceed with a retrieval. Many of them have PCOS and even if you remove the Mirena, they're not going to start ovulating. So you can't just take out a Mirena and say, oh, come on in on day two when you get a period. They may never get a period. They probably never got a period before. Many of these patients may have hypertension, and it's very common for them to have thyroid disease as well.

Slide 63 [01:09:29] So if you look at a meta-analysis of fertility preservation in grade one, stage 1A endometrial cancer patients, this paper from 2018 had 619 patients and some had oral progesterone, some had the Mirena IUD, and some had a hysteroscopic resection followed by progesterone. And so in terms of the remission rate and the relapse rate, it seemed that they all seemed to work quite well. Although, the best remission rate was with the hysteroscopy with the progesterones, probably because we got out the cancer because we could see it with our eyes, with the hysteroscope. Rather than just waiting for the treatment to melt it away, we just physically took it out, so that tends to work better. And the pregnancy rate seemed to be the highest in the patients that had a Mirena, and in this study, some of them had a GnRH agonist or progesterones to go along with that Mirena.

Slide 64 [01:10:36] So this chart may be helpful to understand that. In the patients who had the combination of the Mirena and the progesterone, the remission rate was about 73 percent, the relapse rate was about 11 percent, and the pregnancy rate was about 56 percent. So that's amazing to know patients who were told they could have a hysterectomy, and that's the correct treatment of their cancer, 56 percent of them went on to have a baby. And so really, fertility preservation is quite minimal in this endometrial cancer population. In terms of a hysteroscopy and progestins, you can see very, very high remission rate. The relapse rates are very high in all of this group. And what that's telling you is that you cannot just fix the endometrial cancer and say goodbye, get pregnant, good luck. These patients have to be associated with a fertility clinic, and as soon as they relapse, at that moment, they have to go ahead and actively try to conceive with a fertility clinic. (*Correction ‘As soon as they are in remission, they should get pregnant’).Telling them to naturally try to conceive puts them at a very high risk, especially the patients who are just on Megace and Provera to have that cancer come back. And then whenever they have cancer in the endometrium, they're not going to get pregnant. So if they're not being followed and having recurrent biopsies to make sure everything's OK, unfortunately, their cancer could come back and could even progress. But no matter which treatment you can use, you can see that the pregnancy rates are really, really great, considering this is a population that was told they should have a hysterectomy initially.

Slide 65 [01:12:16] So let's summarize everything that we talked about today. First of all, we noted about the radiation and chemotherapy can have very significant gonadotoxic effects, that embryo cryopreservation is the most well-established fertility preservation strategy, but oocyte cryopreservation is obviously, these days, a very commonly effective option. And other options such as in vitro maturation or ovarian tissue cryopreservation are promising areas of research. Prevention of apoptosis is the key to long-term fertility and is

the key of any research that I hope to do to prevent the damage of the cancer treatments for the future. And our patients with estrogen-sensitive tumors and BRCA type mutations have very specific needs and issues during fertility preservation consultation that we need to address as practitioners.

Slide 66 [01:13:16] So thank you so much for listening. I feel that fertility preservation is the pot of gold at the end of the rainbow, so the patients have gone through this very challenging, shocking thing to be young and be diagnosed with cancer right when they're hoping to have children. But the oncology teams are amazing, and hopefully they're going to cure their cancer so that we can go ahead as the fertility team and give them that baby that they've always desired and always wanted. Thank you so much for listening.

Module 6.6_ ABCs of ART_Fertility Preservation_EN Transcript

Alex Lagunov

Slide 1 – Note to Audience slide [00:00:00]

Slide 2 - Title slide [00:00:08] Thank you for dialing in. This is Module 6 of ABCs of ART. And today we're going to talk about fertility preservation. My name is Alex Lagunov. I'm the Lab Director of CCRM Toronto/Hannam Fertility. I'm also a Director of Lab Technology and Training for the CCRM IVF Network. We're a network of 11 clinics now throughout North America and a member of the Board of Directors of CFAS.

Slide 3 [00:00:36] So to start off, I just want to give you a quick overview of what I'm planning on talking about today. Starting off, we're going to do a little bit of a discussion and conversation about cryopreservation, evolution of cryopreservation techniques over time. We're then going to focus on fertility preservation, specifically talking about indications, available procedures used to date, oncology versus non-oncology fertility preservation. We're going to then specifically talk about oocyte and embryo freezing and ovarian cortical tissue cryopreservation as well. So that's the map for this talk.

Slide 4 [00:01:17] Starting off from the bare basics definition of cryopreservation is: cryopreservation involves the cooling of living cells and tissues from 37 degrees Celsius, temperature of our body, down to negative 196 degrees Celsius, in order to arrest all biological activities and preserve them for future use. Negative 196 is a temperature of liquid nitrogen. That is what we store gametes and embryos in inside the labs. And these cryotanks you could see here on the left, we call them cryotanks, whatever you call it.

Slide 5 [00:01:56] Just a little bit of a background for you because I'm an embryologist by trade, I want you to understand a little bit of a behind the scenes of what it takes to freeze gametes and embryos. And alluding to that, I want you to understand the challenges that are involved. And cryopreservation really is a three-pronged approach. The purpose of preserving fertility, the purpose of cryopreservation is preserving fertility through the freezing and storage of sperm oocytes or embryos, and now today we're going to talk about ovarian tissue as well. And the challenges of those are, as I said, three prongs, is ice crystal formation, osmotic shock and toxicity. Toxicity of the media used that we preserve these gametes and embryos are. So mechanical, thermal and chemical are the three important things to consider when you're building out standard operating procedures for a cryopreservation technique inside the lab. So mechanical being manipulating the gametes and embryos and how they're handled. Thermal is the temperature stability of the environment that they're in right at the moment, while they're being cryopreserved. And chemical is the exposure to various cryoprotectants during the cryopreservation technique.

Slide 6 [00:03:19] So how is it done? Well, I tried to create a pretty straightforward diagram here for everyone to understand. So there's four steps really involved to cryopreserve a living cell, whatever that cell may be, in this case, it's oocytes or embryos. And what it entails is, in order to cryopreserve the cell successfully, you need to get rid of as much of the water from those cells as possible because crystal ice formation is something that could very quickly lead to embryo degeneration and cell death. So what we're trying to do during the entire process is to get rid of that water as quickly and as safely as possible. So step two involves exposing the cell to permeating cryoprotectants. And if you remember, from first year undergrad or biology or high school biology, hypo and hyper osmolarity, where depending on the environment of the cells, the cells are bathing in, they can either retain or leak water out of the cells. And in this case, permeating cryoprotectants will enter inside the cell. They can be glycerol or other things outlined here in the diagram. And through that, they will then cause the cell to shrivel up and the water

will continue to rush out. And in order to maximize that process in step three, we expose eggs and embryos to non-permeating cryoprotectants, such as sucrose or trehalose. There are various other non-permeating cryoprotectants available on the market as well today. And so those will then help further facilitate that rush of water outside of the egg and the embryo. So they almost kind of shrivel up for that process. And then after a certain amount of time of exposure, we then place them in liquid nitrogen and preserve that biological content. So the important factors to consider, as I said, time during that protocol is very important to understand. Temperature and rate of cooling is very important. The cryoprotectant used is important to select the right cryoprotectant, and that's what standard operating procedures are for in the lab. Concentration is very important as well, that cryoprotectant inside the media. The size of the cell and the amount of cytoplasm that will vary depending on the type of cellular freezing versus eggs versus embryos would be different. Surface area and the permeability of those cells is also very important to consider. So all in all, I just want everyone to understand that this is a very complicated process that we've all streamlined and it's very readily available in most IVF labs to date. But there are a lot of steps that these gametes and embryos have to go through in order to successfully freeze and then thaw.

Slide 7 [00:06:15] And thawing is a very important factor to also think through because it's not just about the freezing. The thawing protocol has to be adhered to in order for this to be a successful outcome for the patients. And the things to look out for for us as embryologists in the lab is to prevent ice crystal formation, as I mentioned, when we're thawing. So to make sure that the right temperature and the temperature and rate of warming is adhered to. We need to make sure we prevent osmotic damage as well. So osmotic damage could occur if the rate is too high and the osmolarity of those cells changes really quickly, and that could lead to cell death as well, or damage. And a step-wise dilution of these permeable cryoprotectants that is also very important to adhere to when we're thawing or, as we call it, warming those gametes or embryos. And that could lead to, if it's adhered to correctly, a very happy oocyte, as we have here on the right or embryo.

Slide 8 [00:07:11] All right. So evolution of techniques. I mentioned that in my first slide. I'm going to discuss that and I'm not going to go into too much detail on it. Slow freezing is kind of a thing of the past. It's a protocol that was available to labs, and that's how cryopreservation really began of gametes and embryos through slow freezing. They require low concentration of cryoprotectants. They require a programmable freezer that needs to be purchased by the lab, and it's done at a slow rate of cooling. So it's over time very slowly that could take several hours that it requires a slow dehydration of cells to minimize the ice crystal formation. On the other hand, vitrification is sort of a newer technique. It's been around for about 20 years now, so it's not so new anymore, but what it does is it exposes the cells to a high concentration of cryoprotectant for a short period of time. No mechanical equipment is needed. It's a very rapid rate of cooling, so you expose it to cryoprotectants and then you plunge it a liquid nitrogen, versus slow step by step in a controlled rate freezer that goes from 37 down to -196.

Slide 9 [00:08:29] The evidence behind that is all favoring vitrification. So this is, again, the paper from Michelle Lane and David Gardner, one of the seminal papers on this showing that vitrification should be the favoured choice for labs. It's 95 percent survival versus 80 percent favoring vitrification for embryo survival. As you can see here in the diagram, percentages of survival, fertilization rates, blastocyst formation and blastocyst cell number all favoring the vitrification protocol over slow freezing with statistical significance on all the bars of those diagrams. So again, vitrification for oocytes and embryos should be the favoured choice, as it has been over the past 20 years for many, many clinics.

Slide 10 [00:09:17] Let's talk a little bit more about fertility preservation now. So we'll take our focus away from the lab and then talk about patients here. So fertility preservation is a fundamental issue for individuals of reproductive age or prepubescent children whose fertility may be compromised. The individuals capacity to reproduce may be depleted by age and various conditions, such as genetic syndromes as well as treatment with various gonadotoxic substances.

Slide 11 [00:09:47] Indications for fertility preservation. It could be a variety of different things. It could be due to delayed childbearing so called for social reasons. So women of reproductive age delaying their fertility, freezing their oocytes. It could be cancer related. If someone is diagnosed with cancer during reproductive age, they would go through freezing of embryos or oocytes or ovarian tissue. Or non-oncological medical indications as well. It could be, you know, various immunological conditions that could lead to that. Dr. Glass can allude more to the medical side of indications for fertility preservations. The variables that could have a factor in outcomes are definitely age, maternal age, diagnosis, whether it's cancer or what type of treatment they're going through, and personal situations. It could be financial, it could be marital status or absence or presence of partners that could lead to either oocyte or embryo banking, in that case.

Slide 12 [00:10:44] And so here we are. The three approaches to FP or fertility preservation are embryo cryopreservation, oocyte cryopreservation or ovarian tissue cryopreservation, or as we call it, OTC. And I'm going to talk a little bit about those three in further detail here and then we're going to specifically narrow in on every one of those further in the slides. So specifically on embryo cryopreservation, what's required here is obviously a sperm specimen source is required for embryo cryopreservation. A patient does have to go through controlled ovarian stimulation, so the ovaries have to be stimulated with hormones in order to yield a certain amount of eggs, of course, that could be created into embryos. You definitely need access to an IVF center, so somewhere where there is a center in proximity that these patients can drive to or go to, and it's not experimental, it's not an experimental technique. It yields about 95+ percent survival rates of embryos that are then thawed and transferred. Cost is a factor. It's costly because you have to go through controlled ovarian stimulation and a full IVF cycle leading after it. Oocyte cryopreservation on the other hand. Of course, no sperm source is required for this because you could freeze the oocytes. Controlled ovarian stimulation is necessary, so the ovaries have to be stimulated by hormones through various injections. And access to an IVF center is a prerequisite as well in this case. Survival is about 80+ percent, not as high as embryo survival, but still pretty good. And it's non-experimental, so now it's routine clinical practice. Cost is usually less than it is for a full IVF cycle because there is no embryo culture or fertilization involved. OTC, ovarian tissue cryopreservation, no sperm specimen is required of course, no COS is required. Access to hospital is a must because the surgery has to happen inside the hospital. It is experimental still. Tissue implantation potential is a variable, as this is still being studied to date because it's experimental. Only about 87 live births documented to date with this technique after transplanting the tissue. And cost is a variable because it's still experimental. The cost is not well defined, so it will vary from hospital to hospital.

Slide 13 [00:13:07] A little bit of a deeper dive into every one of these. Embryo cryopreservation entails controlled ovarian stimulation, egg retrieval inside the lab, ICSI or IVF to fertilize those eggs with the sperm. We then culture embryos in the lab for six to seven days, after which embryos that are viable on day five, six or seven are cryopreserved. Then they can be stored in the cryobank indefinitely until patients need them to go ahead with their next steps of their fertility journey.

Slide 14 [00:13:38] On the oocyte cryopreservation side, there are fewer steps involved. So COS is required for this step. Certainly next step will be egg retrieval, so these eggs will be surgically retrieved and placed inside the lab. The lab will then go ahead and vitrify or freeze those oocytes, and then they will be stored in the cryobank of that IVF lab indefinitely until the patients are ready to go through with their next steps of their fertility journey.

Slide 15 [00:14:09] A little bit of data to support oocyte cryopreservation. First successful pregnancies dating back to the 1980s. Vitrification has become the predominant choice of freezing given how the recovery rates of survival and then further blastocyst formation are after vitrifying oocytes compared to slow freezing. And this is a good paper published in JAMA looking at live birth rates per embryo transfer for four thousand patients. They compared the two, fresh donor oocytes versus frozen donor oocytes. The reason why they chose the donor population is because generally the donor oocyte patients are of young reproductive age with very good oocyte quality. So that's kind of like the gold standard for that study. And so they found that the rates were different, but 56 percent with fresh versus 47 percent with frozen donor oocytes. Favouring the fresh, but nonetheless, the donor oocyte pregnancy rates are still very good. And therefore, it is no longer an experimental technique. It is clinically available for patients to go through if they are willing to preserve their fertility this way.

Slide 16 [00:15:27] Next up, we're going to talk about ovarian tissue cryopreservation, or as we call it, OTC. There are two different types of options that you could undergo with regards to ovarian tissue cryopreservation. It could be whole ovary or ovarian cortical tissue cryopreservation. The whole ovary is a pretty complicated technique. As it says the whole ovary is excised via laparoscopic surgical techniques. The disadvantages of this one, it's a long surgical time. It's difficult to freeze ovaries this way for the lab. And of course, there's been no live births documented to date with this technique. Now, on the other side, cortical tissue cryopreservation is a very different story here. It's a dissection of a part of the ovary, so the section that has the primordial follicles located on it. It's also done laparoscopically, very minimally invasive. Tissue is then transferred into the IVF lab on ice. It's dissected in the lab into small pieces that I'm going to show you further in a couple of slides ahead. And it's then cryopreserved, again, either via slow freezing or through vitrification. And this debate is still being studied on the OTC side, although we know vitrification is favored on the oocytes and embryos. On the OTC side, the protocol in the lab is still being studied.

Slide 17 [00:16:54] Here's a quick overview for everyone for the next part of the talk. We're going to talk about indications for OTC, protocols involving OTC and the data that supports ovarian tissue cryopreservation option for patients.

Slide 18 [00:17:11] So indications for OTC could be a variety of different things. So really two main reasons for going through with OTC treatment is women with high risk of ovarian failure and ovarian digenesis or degeneration, so women that are in urgent need of preserving their ovaries because of that. And women in need of immediate gonadotoxic treatment. So women that are not able to go through controlled ovarian stimulation and be injected with hormones will be recommended this OTC option. And this could include prepuberal girls, hormone sensitive malignancies and obviously, like I said, insufficient time to go through with controlled ovarian stimulation.

Slide 19 [00:17:59] Medical indications for OTC. As you could tell by this heavy data diagram, it could be a variety of different reasons. But generally, what we see is about 35 percent, the bigger chunk of this pie graph, is due to hematologic malignancies and certain lymphomas and other myelodysplasias, et cetera. So that's the biggest piece of that pie.

And then there's many other reasons, you know, gynecological malignancies and non-malignant diseases as well that would be referred to go through with OTC.

Slide 20 [00:18:39] So then on the lab side, as I mentioned, slow freezing versus vitrification, it still hasn't been fully concluded which one is the better of the two with respect to OTC because we're seeing similar outcomes in terms of integrity of tissue after thawing and survival of primordial follicles between the two techniques. So this is an area that still needs to be further investigated to understand which protocol is favoured between the two.

Slide 21 [00:19:13] In the lab, what was required once this tissue makes it to an IVF lab is several steps like I outlined here. There are eight things that we would need to make sure we have stock of in the lab. And this can include modified HTF medium that we use for embryo culture, various types of dishes, scissors, syringes and tweezers in order to dissect the tissue, and I'll show you some images to understand what that means.

Slide 22 [00:19:44] Once the tissue makes it to the lab, it has to be further cut and dissected into very small pieces, as you can see here in the bottom left. These pieces are placed inside vials. They're called storage vials, cryopreservation vials. And then are either plunged directly into liquid nitrogen after exposure to cryoprotectants as we've talked about in the earlier slides. And then depending on whether it's slow freezing or vitrification, they will then go through exposure to cryoprotectants and a timely approach to vitrification after that. And again, the two techniques.

Slide 23 [00:20:27] So the data to support this. First live birth from an OTC treatment was recorded in 2004. That's published in The Lancet. Ten years later, an expert opinion publication published by the American Society of Reproductive Medicine, ASRM, they have deemed it as an experimental technique. They're mentioning that patient consent is required in order to make sure they go through this and understand that it is experimental, that there are, you know, outcomes and expectations to be set through an experimental technique. And you know, they do mention that it can be an option for patients with various types of cancer and other non-cancer disorders that would lead them to preserve ovarian tissue where the ovary is not affected in those treatments.

Slide 24 [00:21:20] The committee opinion that was published again in the ASRM did have some questions that they are guiding the field to study and investigate further. Things like ideal cryopreservation technique, as I mentioned, maximum time for storage of those ovarian tissue. How long can we store them for? Is it a year? Is it five or 10 years? Is there any compromise to the long-term storage of this tissue? We don't see it on the oocyte and the embryo side. You can store them indefinitely without compromise, as far as we know to date. But is it the same for ovarian tissue? We don't yet know. Optimal site for transplantation. And this is something that Dr. Glass can allude more to in her presentation. Expected survival of cryopreserved tissues, so what are the expectations of survival? Chance of successful hormonal function of that. Can you then go through hormonal treatment for COS and stimulate that tissue to produce follicles and oocytes from that? And of course, chance of pregnancy. At the bottom line is what are their chances of achieving a live birth after going through something like this? So still to be studied.

Slide 25 [00:22:27] 2014, again, there was a successful fertility preservation following ovarian tissue. It was a great publication that went into more specifics about the technique. And you could see in a live birth, these are the little tissue pieces that are cut out from the cortical tissue, that are then put on these cassettes and inserted into the vials and then cryopreserved inside those vials in liquid nitrogen.

Slide 26 [00:23:00] So another slide on comparing slow freezing and vitrification. There was a large study published that tried to do a meta-analysis of 229 studies. After filtering it, they only came down with six studies that were eligible for the meta-analysis, according to criteria. And they did not find any statistical differences in terms of impact on primordial follicles between slow freezing and vitrification.

Slide 27 [00:23:27] So it's still an open conversation. The current status of it. So where are we at with it? These are all the publications that have been published as of 2015. Looking at various outcomes here, live births in the end is quite low, as you could see, 6, 4, 2, 6. So very few patients because very few patients do come back to have this transplanted and stimulated and restored. Nonetheless, as of 2015, they report 93 percent restoration of ovarian activity. Pretty good. 23 percent pregnancy rate. And by 2015, there were 37 live births worldwide after this technique.

Slide 28 [00:24:09] By 2018, the number goes up to 87 live births, and that's the last we have to date, and you could see the breakdown of frequency of methods used to obtain pregnancy. This is various methods, so spontaneous conception seems to be the higher of all of them between live births on the outcome side. And so that's great to see as well. And you could see here on the right that this number of transplantations done, and in pregnancies related to that as well. So 87 births, which is great.

Slide 29 [00:24:51] So to conclude, sum it all together. Vitrification creates a successful model for cryopreserving gametes and embryos, with over 90 percent survival varying across different types of cells that we’re freezing, as I mentioned. OTC is still an experimental technique, so we have to approach it with caution. Being aware of that, as I mentioned, patient consenting and delivery of that information is very important. No definite protocol and agreement across the labs for OTC, vitrification versus slow freezing. And so few live births and lack of data on endocrine function of the transplanted tissue is still being studied. And so we're hoping that the field continues to generate more and more data that can then help guide these patients towards making this a good path that yields good pregnancy rates for patients that not only have OTC as an option for preserving their fertility. Thank you very much, and I hope you all learned something new today.

Module 6.7_ABCs of ART_Genetic Testing_ EN Transcript

Alex Lagunov

Slide 1 – Note to Audience slide [00:00:00]

Slide 2 – Title slide [00:00:04] This is Module 6 Genetic Testing, as you see here. And just a little bit about myself to start off. My name is Alex Lagunov. I'm currently the Lab Director of CCRM Toronto. We've built this place about seven years ago in 2014, and we have been here since. I've recently taken on the role of Director of Lab Technology and Training with the CCRM IVF Networks. We have 11 clinics currently throughout North America. And I also have been part of the Canadian Fertility and Andrology Society over the past seven to eight years in various roles and currently was elected to be one of the members on the Board of Directors. So that's basically a little bit about myself and moving into our talk.

Slide 3 [00:00:50] We're going to start off with some real basics, hopefully for everyone. And you would've learned this in nursing school or whichever background educationally that you come from. As you know, human somatic cells contain 46 individual chromosomes, as you see here on the right side, and comprising to 22 pairs of chromosomes plus your X and Y, the sex chromosomes. So this lays the foundation of what we're about to talk about here and one of the most critical steps.

Slide 4 [00:01:26] Preimplantation genetic testing, or as we call it now PGT as the abbreviated form, by definition of the International Glossary on Infertility and Fertility Care published in Human Reproduction, it is a test performed to analyze the DNA from oocytes, polar bodies of the oocytes, or embryos, they can be cleavage or blastocyst, for HLA-typing or for determining genetic abnormalities. And I highlight the key items that we're going to focus in our presentation today being blastocyst stage embryos and determining genetic abnormalities, because that is what PGT is geared to do at this point. It's a clinically available tool that's been evolving since 1989, when some of the first pioneering work was done by researchers in the US. And it's really paved the way for some amazing techniques that have evolved in our field that have allowed to perform genetic testing on embryos.

Slide 5 [00:02:39] So what we're going to focus on today, this is basically an overview here for everyone. And essentially there are four key subdivisions of PGT-A. So meaning that there are, within the same umbrella, we can break it down into PGT-A, PGT-M, PGT-SR, and niPGT. That is for now, as I mentioned. And the key part of that is for now, because the field is growing so much and there's constantly new tools being added to the toolbox that allow for us to expand on these abbreviated terms and offer new optimized care for our patients. And that really is the main driving force behind all of these new technologies. So we're going to talk a little bit about the four of these in very brief terms, comparing apples to apples. There's a few categories that I've outlined here for all of you to be aware, and then we're going to zoom in on every one of them in the slides to come to break down the specifics of each and every one of these tests. So PGT-A, as you could see here, stands for preimplantation genetic testing for aneuploidy. 'A' stands for aneuploidy. It can be referred to as CCS, comprehensive chromosome screening, or PGT-S, as people used to call it, preimplantation genetic screening. It's a test that will compare whole chromosome abnormalities, so as referring to the slide previously, it will look at the entire chromosome and determine whether there is a gain or loss of that chromosome in the biopsy part of that embryo. Biopsy is certainly required. We're going to talk about that in further detail. It is definitely available for all IVF patients. Whoever wishes to proceed with it as per doctor's orders and consultation with the couple, and it does not require any personalization or prep. It also is geared towards reducing miscarriage rates after

subsequent transfers. PGT-M stands for preimplantation genetic testing for monogenic diseases. The ‘M’ stands for monogenic. It also used to be referred to as PGD, preimplantation genetic diagnosis. It's a test for inherited or specific gene disorders. So disorders that can get passed on from the parents to the child. The biopsy is certainly required of an embryo. It is available to couples at risk of having a child with a specific genetic disease, and there is a workup required, unlike with PGT-A, no patient personalization prep is needed. With PGT-M there is a workup that's required where maternal and paternal swabs of DNA are requested by the genetics lab in order to proceed with the embryo screening. And again, here it also reduces the risk of genetic disease, of course, after subsequent transfers. PGT-SR stands for preimplantation genetic testing for structural rearrangements, and it also used to be referred to as PGD in the past. It's been rebranded as PGT-SR. It is a test for inherited chromosome rearrangements, and we're going to talk about that in further detail. The biopsy of embryos is certainly required for this step. It is available to all couples carrying translocations, inversions or deletions. We're going to talk about that in detail as well. There are seldom cases where patient prep and gene prep is required, not for all. So it could be similar to PGT-A, where patients can just go through the cycle without any delays. Or it could be similar at somewhere along the lines of PGT-M, where patients would need to send their DNA swabs prior to starting their IVF cycle in order to get the proper preparation done in the genetics laboratory. And so this type of test, once the results come back, it's geared to reducing the risk of pregnancy loss and, of course, developmental disorders that some of these rearrangements may carry. Last but not least, niPGT is somewhat more of a new technology that's emerging onto the market of PGT. It stands for non-invasive preimplantation genetic testing. There are some companies that are just starting to introduce it into their clinical realm of technologies. Not all of them are comfortable with offering it, and it's a test for whole chromosomal abnormalities, so very similar to PGT-A. The only main difference in this compared to the PGT-A is that the embryo biopsy is not required, and I'll explain to you what that means. It is available to all patients and no prep required. And again, it's geared to reduce miscarriage rate for the embryos. So that covers the four that we're going to discuss today.

Slide 6 [00:07:56] And right now in the next several slides, we're going to focus more specifically on PGT-A.

Slide 7 [00:08:04] So aneuploidy has, as some of you may already know, a significant association with maternal age. And just want to draw your attention to this graph that's put together by Mandy Katz-Jaffe Group at CCRM. The 38-year mark is kind of like that pivotal point where blue is your euploid blastocysts and green is the percentage of aneuploid blastocysts. And so aneuploidy, as you could see, increases with age and therefore the number of available euploid, genetically balanced euploid embryos that would be available for transfer, decreases subsequently with age at the breaking point of 38 years old. So if you're a patient that's going through PGT-A testing and you're 36 years old, 55 percent of your embryos would likely be euploid, whereas your chances of having a euploid embryo when you are 43 could be quite a bit lower.

Slide 8 [00:09:07] So this sets the stage for real benefits of PGT-A. So after seeing that graph, it’s important to note that chromosome aneuploidies cause infertility and are a major cause of maternal age-related decline in fertility. Also, 50 to 70 percent of spontaneous miscarriages are known to be due to chromosome abnormalities. And that's a known fact, and also, a reduction in miscarriages through PGT-A will further improve or further optimize the time spent for patients in treatment because it could reduce the amount of time that it takes for the patients to achieve a successful pregnancy. It could help with subsidizing some of the costs, given that they may have to transfer fewer embryos because they are transferring embryos of known genetic status. And of course,

emotional trauma with the reduction of miscarriages would be reduced as well. So the key thing here again, as I mention the last step here, reduce time to pregnancy, through all of these this is what it would lead to is the reduced time to pregnancy, so a shorter time to be able to conceive a healthy baby.

Slide 9 [00:10:30] This is a slide from our Canadian registry, it's called CARTR Plus, and it's available to all members of CFAS, Canadian Fertility and Andrology Society. So in the slide deck shows that euploid transfers result in higher pregnancy rates, independent of maternal age. So as you can see the line above, starting with 53.4 percent, is the clinical pregnancy rates for patients in certain age groups. And you can see this line rides well above the line below, which is a line that does not require PGT-A. So that is the real benefit of this, backed up by Canadian data.

Slide 10 [00:11:15] So a couple of key things to note for PGT-A/M/SR frankly is all of these genetic tests require a technically demanding procedure called embryo biopsy. As I mentioned in the table above that all of these tests do require the biopsy. What I am referring to is a technique where an embryologist with assisted technologies that we have in the lab is able to use a holder on one side on the micromanipulator with a biopsy pipette on the other side, where they can pull out several blastomere or trophectoderm cells of a blastocyst and cut them with a very fine laser away from the embryo, cryopreserving the embryo afterwards and sending the cells that you've taken off the embryo into a genetics lab for testing. So it's a very technically demanding technique. It's probably, in the realm of the skill set of an embryologist, definitely up there as the most technically demanding procedure that an embryologist can do, so only the most skilled embryologist are able to do this biopsy successfully. There have been several approaches to embryo biopsies over the years, so as I mentioned this is not a new technique. It's been around for over 20 years. And initially it started off as a biopsy where groups would be able to take a polar body from an embryo, from an oocyte, and assess the genetic component of a polar body under the impression that is obviously a representation of the genetic component of the oocyte. However, that would only take account of the genetic component of the egg on the maternal side and not the sperm. Further down the road, clinicians were able to successfully biopsy cleavage stage embryos, so here and in the image below, where you can take a blastomere as we call it, which is one cell out of an eight-cell or a 10-cell embryo, and we'll take that one cell and test it for an aneuploidy as well. So the fraction of cells that you are taking out of here, so let's say it's an eight-cell embryo, so it's 1/8 of the embryo that you take out for assessment and talk about safety and its concordance with the representation of the entire embryo as well, is not very high. When we move on to the blastocyst stage, this embryo is anywhere from 150+ cells. And so when you take about three to five to fracture themselves of that embryo, you're taking a very small fraction of those trophectoderm cells. And you pull them out and you cut them with the laser, as I mentioned, and one side goes for testing, the embryo gets cryopreserved and stays in the lab. So this is the technique in a nutshell.

Slide 11 [00:14:20] Like every technology, there are always pros and cons. So the limitation of biopsy techniques is important to note in this presentation as well. So from an embryologist, which is who I am, the limitations are, first of all, the laboratory has to be comfortable and be successfully set up with a freeze all method where they are comfortable with freezing all blastocysts and they have a good rate of recovery or giving them a good rate of recovery and success further on after warming them and of course, subsequent transfers. So the lab has to be set up for proceeding with a freeze all approach, and that requires the entire team, the REIs, the clinicians, the nurses, the patients, of course, and then the lab backed up by their own QA and QC systems that support the freeze all technique. Certainly, patients as I mentioned in the few graphs below that aneuploidy increases with age, there would be some patients that have very few or

sometimes not any euploid embryos for transfer after going through a freeze all cycle with biopsy screening. So that's something that I wouldn't say is a true limitation, but it's something to be very aware of when you're proceeding with biopsy. It's very important for that counseling on the nursing side and in the REI side to occur where the patients are aware of these potential outcomes, where you may have your embryos tested and you may get none of them come back as euploid, so therefore not available for transfer because aneuploid embryos are not available for transfer because they're genetically abnormal. And the other thing is called no result rates, it's important to be aware of as well. The no result rates, what they mean is, and I have a little table here for you to explain it to you a little better. So no result could occur for a variety number of reasons. And it happens when the cells are sent for testing to the genetics lab and in turn, when the results are received, let's say some would be aneuploid, some would be euploids or genetically balanced, genetically unbalanced, and then some may come back as no result. So about 1.2 to 5.7 on average of those results could come back with a no signal result. And a lot of people would wonder, you know, embryologists or physicians or patients, frankly, what happened there? Why did we not get a result? How is this possible? Well, there's a variety of reasons starting from number of cells that were biopsied by the lab, the embryo quality and the quality of the DNA of that embryo, which is something that could be completely out of our control. The transport part of the cells. So of course, as I said, after the biopsy is completed, the cells are then sent for testing to the genetics lab. And these genetics labs are often not around the corner. They have to get packaged in a box and sent to sometimes across the border, sometimes across other sites in Canada, where you know, they have to stay refrigerated, ideally on dry ice. And if there's ever any issues during transport and the temperature increases, DNA is only stable when it's frozen. By the time it's thawed, it can be degenerating very quickly. So cell transport is a variable that could affect the no result rate. Cell lysis through that is certainly a direct outcome of cell transport issues and DNA quality. So cells could just be lysing during either transport or analysis tubing, meaning that embryologists have to place the cells inside the tube during that process. It's another sensitive step, and the genetics lab will then receive the cells and they have to go through their protocols and steps to ensure that they can deliver results. And if there's any issues on their side that are also out of their control with regards to the DNA, it could result to a no result signal. So again, important to be aware of those three things, ability to do a freeze all successfully, being aware of the reduced number of available embryos. So let's say you freeze 10, you will likely not have all 10 of those embryos available after PGT-A screening. And of course, no result rates that could further complicate the outcomes once the patients receive the results. Now, for those no results as well, there are some options for patients. Oftentimes, it's once they receive them, the recommendation is to proceed with transferring the euploid embryos first, if there are euploid embryos remaining, and if there aren't any, then they can have the option of either re-biobsying those no result embryos with hopes that they can then get the result on the second biopsy, or potentially transferring those embryos with a no result rate as you would if you were transferring an embryo that did not go through PGT-A testing.

Slide 12 [00:19:42] So interestingly enough, the rate of PGT-A, or the intent to perform PGT-A as this slide states, has been increasing quite steadily over the past seven years. And this is again another image from our Canadian CARTR Plus registry. So as you see, 2013, about one percent of clinics, or one percent of cycles, in Canada were PGT-A cycles. And if we look at 2020, over a quarter of all cycles were using PGT-A technology for screening. So it's definitely going up. We know this is the overall rate of 12 percent over the past seven years. But if we look at 2021, I'm certain that number is going to continue to increase because of the power of this technology.

Slide 13 [00:20:32] So just quickly, I've mentioned the process and how it all works, but just so you understand the little image here. So as I mentioned, day three biopsy is still an

option for some clinics, although there are quite a few publications highlighting the fact that it could be pretty traumatic for the embryo because you're taking one out of those eight cells versus three to seven cells of about a 150 or 200 cell embryo. And also correlation embryos can actually self-correct themselves from day three to day five and onwards or towards blastocyst. So some of those day three embryos could be aneuploid at day three, but then they can actually self-correct themselves and become euploid as they reach blastocyst stage. Because that is the most rapid stage of embryo development. Between day three and day five, six and seven is the most rapid stage of cell cleavage. Just to put it in perspective, from day one, from the day of fertilization, to day three, the embryo is usually about eight cells, so it goes through about three to four replication cycles. After day three to day five is another three to four days of development. That embryo goes from about an eight cell to about 150 to 200 cell embryo. So a very rapid stage of development, a lot of things changing, cells differentiating into trophectoderm, which becomes the placenta and into the inner cell mass, which becomes the baby. And so taking a biopsy on the blastocyst stage is a lot more well-represented of that embryo further down the line as it gets transferred, as it would be on day three. OK, so after the biopsy is completed, the cells are placed in a tube in an embryology lab. They are then packaged up, they are shipped to your reference lab. Reference lab conducts the PGT-A chromosome analysis using their technology, which has also been evolving rapidly. The genetic report is put together by that team and is sent to the clinic, and then the clinic along with the nursing team and the REI team then further decides, has a consult with the patient, and they decide which embryo to go through first. They set them up for a frozen embryo transfer protocol, and then they go through with their steps. So again, as I mentioned, all of these genetic tests are only able to be offered in programs that are able to freeze all their embryos and not go through with the fresh embryo transfer. Embryos during all this time, it's also important to note that the embryos themselves stay frozen in the clinic and the cells, only those three to seven cells of each embryo that are put in these tiny little tubes, they then travel to the reference labs for genetic testing.

Slide 14 [00:23:15] All right. Another little curveball to add to the mix of PGT-A is chromosomal mosaicism. And this is somewhat of a new topic that's emerged over the past several years. And what it really means is, without getting too worried with it, as we're just going to read the first point here, is mosaicism is the presence of cells with more than one genotype within a single embryo. OK, so what that means is, this picture kind of tells it all. So here's your trophectoderm that becomes the placenta. Here's your inner cell mass that becomes the baby. The blue are the euploid cells. The yellow are the aneuploid cells. And, as you can see, taken this biopsy of these one, two, three, four, five, six cells, you can have some of them that are euploid and some of them that are aneuploid. So when you get that result, you're going to get, not a clean blood result, and I will show you what that really means on a graph with an output level. But you're going to get some results that are not 100 percent one way or another. So this could be a result of a couple of various issues that could happen in embryo such as mitotic nondisjunction of sister chromatids, back to the basics of biology 101, to a phase when the sister chromatids are separating, the mitotic nondisjunction could occur and the separation of chromosomes does not line up to occur properly. Anaphase lagging is also related to that process and chromosome breakages as well. So those three things could lead to a higher level of mosaicism. Estimates of embryonic chromosomal mosaicism in the publications have been inconsistent. It's been pretty variable, which could be really frustrating for clinicians to talk about this technology because it hasn't really been well-defined. Although we now understand that anywhere from two to 30 percent mosaicism rates are not uncommon, and they are influenced by clinical procedures, certainly lab protocols, and patient variability. So they do differ from lab to lab, from patient to patient, and of course, from clinic to clinic. So the problem is, in order to tell the true level of mosaicism in embryo, you have to take the whole embryo, as you can see in this picture. And then you will really know what the

level of mosaicism is. But if we biopsy up here and took all those euploid cells, levels of mosaicism would be zero. So the nature of the beast of mosaicism is there, we have to be aware of it, and we have to understand how to deal with it when we receive our results.

Slide 15 [00:26:07] So this is what the output looks like in a genetics lab. So just so you understand, the visual is that the genetics lab will perceive and then based on these images, their algorithms will tell them whether an embryo is euploid or aneuploid. So here, this is a euploid female embryo, these are chromosomes. So five, seven, nine, 21, and you could see them in little columns right here. All these little dots are signals received from the digested DNA. Once those biopsy cells, as you've seen, entered the lab, they've been amplified because it's only three to five cells. So they amplify the DNA, so they grow it out and remultiply the sequence and then they put it on a microchip, and the microchip gives them all these reads. So all these little dots are the tiny little reads of DNA for that embryo, and then their software will then line up a curve and compare it to a euploid reference manual where the embryo is genetically balanced, and it will tell how far below or above, if whether there's a gain or a loss of chromosomes, compared to their controls. OK, so for example, here's a trisomy 16 and 19, and this is a male. So you could see, here's a gain trisomy. So there's three sets of that chromosome. There's also a trisomy of 19. There's also another set of chromosome 19, versus here, where everything is just balanced. So what does mosaicism look like? So mosaicism as you could see here, there is a true gain where it comes up to that green line and the red line. So it's a true whole chromosome gain here and here. Over here, you can see that this mosaicism only goes halfway down, meaning that it's a mosaic embryo. So not all the cells in that embryo had a loss of chromosome two. So it's a monosomy of two. Perhaps only some of them did. So based on this, the lab will then determine the level of mosaicism, so that percentage, and then the physicians with the nurses and the lab will determine whether this is an embryo that is recommended for transfer or not.

Slide 16 [00:28:29] All right, so that concludes PGT-A. Moving on to the next part of the slides, we're going to talk a little bit about PGT-M. It's a little shorter on this side.

Slide 17 [00:28:38] So PGT-M is geared towards helping at-risk couples, genetic risks couples, to have children free of their familial genetic disease. It's a PGT-M test for specific conditions in biopsied embryos. These conditions are outcomes of the pre-workup for genetic testing done by the clinical team for the couple. Sometimes the patients will come forward and say, hey we'd like to look for a certain disorder that has been in our family that they're aware of. And then they will be tested for this on the genetic level by the genetics lab that the clinic decides to work with. Most often, again, these labs are not locally situated in the individual practices. They are independent of IVF. They have their own entity set up and the cells have to be shipped to them as well. This includes autosomal dominant, recessive, X-linked, de novo conditions. Some of the examples of PGT-M, or things that you could search in the embryos for or screen embryos for, are things like sickle cell disease, spinal muscular atrophy, BRCA mutations, Huntington disease, et cetera. So you could see how the power of this technology is so immense that you're able to change the genetic component and the risk for these diseases in children and their offspring onwards. Because you can actually, if you look at these genetic trees, by controlling and only transferring the embryos that are not affected, you are then helping the offspring of those children become free of those higher risks that they would potentially carry. The labs are able to offer screening for over 400 single gene disorders. So really, any disorder, as far as I'm aware, that is identified that carries a genetic link is able to be screened for in a genetics lab as long as it's evident to the patients through either a carrier type test that's often offered by the clinical team, or just patients' self-awareness of certain things and then coming forward to the clinical team to discuss that. So again, and the other important thing to note is the accuracy once the embryos come back as unaffected is over

95 percent, is above 95 percent accuracy for those results. And of course, these patients will then be followed with, similar to PGT-A and other patients, they can continue to do NIPT testing after they're transferred to confirm if they'd like to. But this is on the embryo side.

Slide 18 [00:31:30] A little bit about the process of how this works. So oftentimes I get questions from our nursing team about what kind of expectations they can set for the patients. And it's a great question to ask because every reference lab generally has their own protocol for how to set up the patients and the type of protocols that they have to go through. But generally speaking, this is how it's done. So the couple will have a counseling appointment with the nurses and their primary MD., where they will discuss their concerns and the reasons for potentially proceeding with PGT-M. They will be offered to speak to the reference lab that offers this type of testing. The reference lab will then ask them to sign a series of consents, patient informed consents, making them aware of all the pros and cons and all the details and inner workings of offering PGT-M to them. They will then be asked to provide their DNA samples from both partners to the lab so that they can work on prepping these things called primers, where they pick up the specifics of these genetic carried disease or any links because every case, every patient, is different. And that process itself could take up anywhere from three to six weeks with preparation. So it's important to know about that the patients should not start an IVF cycle until they have gotten the full set up and all the consents and all the details signed and ironed out with the genetics lab. So normally then, once everything is completed, the genetics lab will notify the clinical team that the patient is all set up. We have them ready to go. Let us know when you're ready to proceed with IVF and we'll be expecting that biopsy when the embryos are ready. OK, so quite a bit of a workup for PGT-M. You don't need quite the same workup for PGT-A because, as I said, it's offered to any patient and there's really no workup needed. The workup is essentially all done in the IVF lab. So after that, the patient will go through controlled ovarian stimulation, COS. Of course, they'll undergo IVF and embryo culture and towards blastocysts ideally. And at that point, embryos that are blastocyst quality will be biopsied for genetic screening. And the cells will be sent to the genetics lab. PGT testing will occur. The results will then be combined to and sent to the clinic. Now something new that's been offered to the patients more recently is a combination of PGT-M and PGT-A. The reason being is, you know, if you do the PGT-M and you're only looking for a specific gene of interest, let's say you're looking for the BRCA gene, for example, and you are trying to find unaffected embryos. Well out of 10 blastocysts, if you have 10 blastocysts and you biopsy all of them and you may have three or four of them that are non-affected or non-carriers of BRCA. However, you don't really know whether those are genetically balanced according to the chromosome numbers. You don't know if every one of those has a pair of chromosomes, only one pair. So what some labs are able to offer is a combined PGT-A and M process where you will know that it's euploid, so meaning that there's a set of chromosomes for each, and that it's either affected or not. So you'll have sort of a lower chance of miscarriage, so a higher chance of implantation. And certainly, it will be free of that gene of interest that they're looking for.

Slide 19 [00:35:15] This is what a little map kind of looks like, where you have an example where you have a father that's a carrier. You can see this little yellow spot on their gene on their chromosome. And the mother, that's a carrier as well for a certain thing. And they get their embryos and they have four blastocysts biopsied. Embryo number one is unaffected, embryo number two is a carrier. So that's a carrier from the paternal side. This is a carrier from the maternal side. And this is an affected embryo that represents both genes and therefore is an affected embryo. So what to do with those embryos? Well, first of all, the unaffected embryo definitely is the first choice. Hopefully, that embryo is genetically balanced, so euploid. The other two, number two and number three, question mark. That's what I put, because it's a conversation that has to occur with the patient and the clinical

team in order to decide whether they're aware of the risks or understand, not the risks, but the details and they're consenting to go through with the transfer of an embryo that is a carrier for kind of like what they are, essentially. And of course, this is actually a pretty good outcome. You may get outcomes where you're they're either all carriers, or they're all affected. So it could go many different ways. And this is why counseling, especially in the nursing side and in the physician side, is very, very important to set the right expectations with the patients prior to proceeding with these options.

Slide 20 [00:36:55] All right. So closing that chapter and moving on to PGT-SR.

Slide 21 [00:37:00] PGT-SR stands for small rearrangements. So chromosome rearranges may occur via several inherited or spontaneous mutations. Somewhere about three to four percent of recurrent pregnancy loss is what they comprise. So patients that undergo recurrent pregnancy loss about three to four percent of those will have some sort of small rearrangement abnormalities and that could lead to miscarriages. PGT-SR is offered to patients with known carrier status of a genetic translocation, deletion or an inversion. I'll explain to you in a little bit what they mean. And again, it is determined by a carrier type of an intended parent. So they'll have to go through a carrier type, if they either have any reasons for it because of their familial history or they're generally concerned, or the REI just orders carrier type to know whether or not they do have any translocation, deletion or inversions within their chromosomes. So it's kind of like a zoomed-in view of going a little bit further beyond the whole chromosome level. You're narrowing in on chromosomes and you're trying to break them down a little bit more specifically and weeding out any abnormalities within that chromosome, those individual chromosomes. This is sort of a new technology that is able to proceed with the technology that we have today on the genetic side used for screening purposes. Balanced translocation may pass through to their children, so these children may carry on some of those translocations if they're not screened for. PGT-SR may increase chances of a successful pregnancy by allowing to transfer unaffected embryos with the correct amount of chromosomal material.

Slide 22 [00:38:38] So as I mentioned, the duplications, deletions, inversions, or translocations. So duplications are repeating part of chromosomes. Deletions are missing part of a chromosome. So as I mentioned, not a whole chromosome like PGT-A, but a part of a chromosome. Inversions is a reversed orientation of chromosomes. And translocations are changes of segments between the chromosomes, so same segment switching between chromosomes. OK, so all these things could lead to pregnancy loss and therefore are used to screen for PGT-SR.

Slide 23 [00:39:16] Clinical example, specifically narrowing in on deletions. So deletions are syndromes defined as clinically recognizable genetic disorders characterized by small loss of a part of a chromosome, as you can see here in the image below. The imbalance or the cumulative effect of the imbalance, on these independent disease genes within the deletion region is what determines the overall phenotype or the outcome on the baby, on the child, including intellectual disability, developmental delay, or certain dysmorphic features that carry with that specific deletion phenotypic features.

Slide 24 [00:40:02] So some of the examples of deletions are Cri-du-chat syndrome, partial chromosome six deletion. Images are here from left to right in the same order. Prader-Willi syndrome, partial chromosome 15 deletion. And Wolf-Hirschhorn Syndrome is a partial chromosome four deletion as well. So all these things are able to be picked up and screened for through PGT-SR testing.

Slide 25 [00:40:34] Deletion syndromes have a frequency of about 0.5 percent in prenatal testing and they account for about one in 700 live births. And they also can be observed

on any chromosome and they can be of various sizes and locations, so it's completely at random.

Slide 26 [00:40:56] So now finishing off with niPGT, we're going to talk a little bit about that and there's another little glimpse of even newer technologies I'm going to introduce after that called polygenic scoring, but we're going to get into it in a couple of slides here.

Slide 27 [00:41:10] So niPGT is, as I mentioned, non-invasive PGT screening. And the beauty of this and the exciting part of this to us as embryologists is that word on its own, non-invasive. Patients often will ask, well you know, you're biopsying my embryo, is it going to damage the embryo? Is it dangerous and is it invasive? And to that question, the answer is, you know, it is an extra procedure that your embryo has to undergo. Therefore, to say that it's non-invasive would not be true. So it is a minimally invasive tool. It is very clinically well validated, especially in the labs that do a high number of PGT-A. So it's important for patients to be aware of, you know, labs of choice or clinics of choice and maybe the amount of PGT-A that they conduct. And so recently, there's been an emerging amount of technologies being able to pick up a little bit more sensible, a little bit more fine-tuned DNA signals from collection of spent culture medium from cleaved embryos or blastocysts. So as you can see here, invasive PGT-A is blastomeres or blastocysts, you take those cells, you cut them with the laser, and you test them. And then you do all this, this is the different platforms of genetic analysis that are used. There is PCR, NGS, aCGH and then SNP. And for non-invasive, you have one embryo and one drop, you culture that embryo in the same drop from day one to whatever day you choose to according to your lab protocols, and then instead of taking the biopsy of that embryo, you take the spent culture media that the embryos are bathing in and that culture media is rich in nutrients and supplements. There are many, many different companies to present day that offer various types of culture media and is for every lab to pick and choose their own recipe, according to the standard operating procedures in which culture media they choose to culture in. And independent of that, this non-invasive PGT is able to, when you send them those five, 10 microliters, tiny amount of media in that same test tube. So instead of the cells you send them this spent media, they're able to then correlate DNA that represents that embryo in that culture media and provides you with a result that well represents that embryo. Now the question is how well is it represented in the culture media? Why would there be DNA in the culture media? Why do embryos shed DNA into culture media? So this is something very new. It is something that's available within for sure one very large PGT-A or niPGT lab.

Slide 28 [00:44:00] And so here's the answer to that question that I just alluded to. Concordance with trophectoderm biopsy is the big question. So a lot of embryologists and clinicians are now wondering, okay, well, if we offer this test to our patients, let's say the accuracy with PGT-A is 95-98 percent concordant with the whole embryo. And here, what does that mean? How certain are we that that DNA that's free floating in the media, how well does that represent the trophectoderm cells and the baby? Well, as you can see here on the left, it actually varies quite a bit. So I'd say the technology, it's on its way. It's not quite there yet, but it's on its way. And you know, there's papers like Kuznyetsov, actually a group from U of T, finding the highest concordance up to 90 percent, compared to trophectoderm cells. But it is variable. It's about up and around 80 percent, and why? Why does it vary so much? Well, because there's actually quite a few variables. As I mentioned, there's a chance for maternal cell contamination and polar bodies or cumulus cells or cells that surround the egg. So if you leave that egg and embryo in the same culture media, those cumulus cells that feed the oocyte during maturation could then further go on and contaminate your culture media and present another source of DNA, which wouldn't be embryonic. Embryos could self-correct, as I mentioned earlier in the slides, because there is a self-correction mechanism happening in the embryos where the embryos shed

aneuploid cells outside of their embryo. So those shedded cells could be a part of the mix, they may or may not be. Those are the things that we're still learning. And of course, culture media contamination. As I mentioned, there are various culture media available on the market and you know, that could be a variable as well. So again, something that I think definitely will have a much larger footprint in clinical practice in the years to come. But we're 2021 here, and this is what the data shows. I think in the next several years, we will move further away, likely, we'll probably move further away from biopsying the embryos and towards more of a non-invasive approach of performing biopsy, where we're able to with more certainty, without biopsy, determine the genetic stability and component of those embryos.

Slide 29 [00:46:26] PGT-P, very last topic. Just wanted to kind of give you a quick glimpse of this. It's again, also very emerging, very interesting.

Slide 30 [00:46:36] It's called polygenic scoring, PGT-P. Polygenic disorders are diseases caused by genetic changes in multiple genes. PGT-P is taken from biopsy trophectoderm cells. So the same biopsy cells travel to the lab. There's actually a laboratory available in the U.S. that's offering this where they will then take the biopsy and provide an output with respect to relative risk of those embryos being at high risk of certain diseases, such as breast cancer, prostate cancer, coronary artery disease, heart attacks, diabetes. And then you can see here, this is an example of an output where it would say, embryo number one has a 0.8 percent developing type one diabetes versus 1.1, so a 33 percent reduction in the relative risk of developing type one diabetes. Again, you could talk about nature versus nurture, or genetics and et cetera. So a very interesting topic. It's emerging. I think it's interesting to know about it. I don't think it's going to necessarily affect you right away with respect to counseling patients, because it's a very new technology, but definitely becoming more mainstream as the years will go by. Polygenic scoring is a topic to be aware of.

Slide 31 [00:48:02] Now, we've talking about aneuploidy. We talked about how labs can influence the rates of aneuploidy and how it's very important to be aware that the quality of the lab has a factor in producing certain aneuploidy ratios in every age group. So when we set up a laboratory at an IVF lab and when we operate a lab as embryologists, the aim is always how to mimic the uterine environment to its fullest. So what does that mean? Well, we have to go by what's been published in the literature and some of these important seminal papers and peer reviewed papers that talk about the pH that is the most ideal pH for in vivo metabolomics and processes to occur. So let's say it's 7.3 is the critical pH. Controlling for osmolarity, so having the right osmolarity parameters in culture media is very important. Controlling for chemical stress. Exposure to light. Certainly, there's no light inside the uterus, and so that minimum light. Certain oxidants that can affect the embryos are important to be aware of. Mechanical stress, so moving the embryos is important, how dynamic or static the embryos are. Temperature, hugely important of course. Temperature of a human body does fluctuate, but it's a very narrow, as you know, scale. Exposure to toxins, certain pollutants is another one that's important to be aware. So all these little factors have to be heavily weighted and controlled for within the IVF lab in order to give the patient's best chances of maximizing the yield of their genetically balanced or euploid embryos.

Slide 32 [00:49:57] These are just not my words, I just wanted to show you there has been a few publications and this is a big one where cellular stress is associated with aneuploidy. It's a review that talks about various stressors, mitotic stress, replication stress, metabolomic stress and how those can affect the stability of genes or affect rate on aneuploidy.

Slide 33 [00:50:23] This is a paper by Santiago Munné, who founded one of the largest genetics labs in the world called CooperGenomics, where they looked at donor egg cycles. So these are embryos generated from donor eggs. So it's kind of like the calibrated group, where generally donor egg patients are much younger than the average population of infertility patients, and therefore they have very high and very similar outcomes all across the board. And that's the standard. And then the variables are different labs, so different fertility centers. So these are 42 different fertility centers that took on a variety of different donor cycles. And you can see the mean incidence of embryo ploidy or euploidy actually differed quite a bit for the same population of patients, whereas you would probably want to see somewhere around 70 percent euploidy rates. So generally, these patients are 30 to 35, even younger patients than that. And as you saw in my first few slides, their percentage of euploidy is 70+ percent. It doesn't turn out to be the same. So lab is another factor that drives that stability across the board as well.

Slide 34 [00:51:38] And a few other papers, Jason Swain, one of our CCRM colleagues, published a good paper on IVF lab influencing aneuploidy rates worth looking at. And there's quite a few other publications if you're interested you can look into if you are wondering about the lab influences on outcomes of aneuploidy.

Slide 35 [00:51:59] So finishing all off, I'm not going to get into reading these out loud, but just wanted to leave you with the slide. Actually, I do have one more case study to discuss, I almost forgot. But this is the conclusion. These are all the tests that we discussed today. Hopefully, it was all clear.

Slide 36 [00:52:12] Patients seeking fertility care. She's 38 years old, presents a healthy lifestyle, nothing concerning. Her family history supports fertility. Her karyotype, as we discussed, karyotype is normal, no issues identified. Her partner is 40 years old, normal sperm parameters. So a clinical team would categorize them as unexplained infertility. Because of that, everything comes back normal. They're recommended to try intrauterine insemination. So they go through three IUIs over the span of four years, which is a long time. And you know, they achieve no pregnancy. And they proceed with PGT-A, where first cycle, she’s 38 years old. They get 12 eggs, 10 of those are mature, eight become fertilized with ICSI. They actually get four blastocysts frozen on day six of culture. So embryos can be frozen on day five, six, and some labs on day seven as well. And they do four transfers, frozen blast transfers. The first three embryos, single embryo transfers, resulted in no pregnancy. Fourth transfer develops into a biochemical pregnancy loss, meaning that it implants but then it shortly after miscarries at 20 days. So then they decide to go ahead with another cycle. At this point, after all the transfers and IUI and then the cycle without PGT-A, they are 40 years old, so the chances of having euploid is not as high. They retrieved 11 eggs. Eight are mature, six fertilized. They end up with four, the same amount of embryos as they did before, four good quality blastocysts. And they go through PGT-A testing. They freeze two on day six and two on day seven of culture. And the two blastocysts frozen on day seven come back as euploid, which generally would not be the first in order to be transferred if they did not do PGT-A. Generally, the labs will choose to transfer day five over the six and then day six over day seven because they ideally should be at the blastocyst stage by the day five mark. So if you didn't do PGT-A, the first ones to get transferred usually would be the day sixes. Those two day six blastocysts actually came back as aneuploid. And so when they go through their first single blastocyst transfer with a euploid embryo, they end up having a live birth at 38 weeks old. 38 weeks of gestation. So that's all I have. Thank you very much.

Module 6.8_ ABCs of ART_Preimplentation Genetic Testing_EN Transcript

Diane Reid

Slide 1 – Note to Audience slide [00:00:00]

Slide 2 [00:00:04] Welcome to the ABCs of ART module six. To introduce myself, I am Diane Myles Reid, a certified genetic counselor specializing in reproductive genetics. For this model, I've been asked to share a genetic counselor's perspective on genetic testing in the fertility clinic.

Slide 3 [00:00:25] Today, I would like to describe pre-implantation technologies, PGT-A, PGT-SR, and PGT-M, and use case examples to illustrate key considerations when counseling about PGT.

Slide 4 [00:00:39] To provide an overview, genetic counselors can work in many clinical settings, such as prenatal genetics clinics, pediatrics clinics, cancer genetics, and more recently, genetic counselors may be part of the clinical team in a fertility clinic. In general, genetic counselors are providing patients and families with information about the risk for genetic conditions and genetic testing options. Genetics and genetic testing technology is complex and ever evolving. And our goal as genetic counselors is to communicate complex information in a way that patients can understand, taking time to answer their questions and support them as they make inform decisions for themselves and their families. In the fertility clinic, genetic counselors are helping patients to understand genetic testing that is offered to them as part of their fertility treatment.

Slide 5 [00:01:38] Before we get started talking about genetic testing options, let's review some basic information about genetics. As you know, DNA is the molecule that makes up our genes and it encodes the blueprint or all of the instructions that our body needs to grow, develop, and function from day to day. Our genes and DNA are packaged into structures called chromosomes. Chromosomes are found in every cell of our body, and we should have 46 chromosomes in every cell of our body.

Slide 6 [00:02:15] In the fertility clinic, genetic counselors help patients who are considering pre-implantation testing as part of their IVF cycle. Ideally, patients have the opportunity to meet with a genetic counselor who can help them to understand why the test has been ordered and how it might be helpful for them. As a general overview, the pre-test genetic counseling discussion is important to explain that pre-implementation testing is completed on the embryo before embryo transfer and implantation. It serves as an opportunity to discuss potential risks, benefits, and limitations of the testing. And as well, we can discuss the associated costs and logistics. As part of this discussion, we will review the patient's reproductive, medical, and family histories. Gathering this information allows us to determine if any additional investigations are required. It also allows the patient to share their fertility journey and the genetic counselor can begin to establish a relationship with the patient. It also offers an opportunity to provide patients with as much information as possible to clarify goals and expectations and to elicit any underlying emotions or motivating factors the couple may have relevant to their decision making.

Slide 7 [00:03:41] In order to do any pre-implantation genetic testing, embryos must be biopsied and cryopreserved. This image is illustrating the biopsy of an embryo at blastocyst stage, when an embryo is made up of one to 200 cells. The embryologist removes five to six cells from the trophectoderm which is the outer layer of cells. The

trophectoderm is destined to become the placenta and tissues supporting the pregnancy while the inner cell mass, the pink clumping of cells that you see in this photo, will become the fetus and the baby. It's important that patients are aware of the potential risks associated with biopsy and cryopreservation. There is a small chance that the biopsy may cause the embryo to arrest or that the embryo will not survive the cryopreservation and thawing process.

Slide 8 [00:04:44] Pre-implantation genetic testing for aneuploidy or PGT-A is a tool to aid in selecting euploid embryos for transfer. Next generation sequencing is the genetic testing technology used to screen or assess the chromosomal DNA and the cells of the embryo. An embryo that is euploid is an embryo with a normal chromosome complement. The normal chromosome complement is 46 chromosomes as typically at fertilization, 23 chromosomes are passed on in the egg, 23 in the sperm. An aneuploid embryo is an embryo where there is an abnormality in the chromosome complement. Aneuploidy refers to a missing or extra chromosome or any gain or loss of chromosomal DNA. The goal with pre-implantation genetic testing for aneuploidy is to select euploid or chromosomally normal embryos for transfer in order to improve chances of implantation and ongoing pregnancy.

Slide 9 [00:05:59] This slide is a visual of the normal or euploid chromosome complement. You can see that there are 23 pairs altogether or 46 chromosomes in total. PGT-A is a comprehensive screen of all of the chromosomal DNA. PGT-A provides information about all of the chromosomes.

Slide 10 [00:06:26] PGT-A may be offered to many patients who are having IVF, including patients who are of advanced reproductive age, those who have a history of recurrent implantation failure, or recurrent unexplained pregnancy loss. PGT-A may be offered to patients and couples who have had previous pregnancy or a child with a chromosomal abnormality. And in some clinics, PGT-A may be offered to all patients or couples who are considering IVF.

Slide 11 [00:07:02] This slide is showing the possible results with PGT-A testing. A euploid embryo has the normal chromosome complement, and these embryos have the highest chance for implantation and ongoing pregnancy. The goal with PGT-A is to identify and transfer euploid embryos. An aneuploid embryo is an embryo that has a chromosomal abnormality detected in the cells that are biopsied from that embryo and an aneuploid embryo most likely will not result in a successful implantation or ongoing pregnancy. One of the goals of PGT-A testing is to avoid transferring aneuploid embryos. We can also find an embryo that is mosaic, which has a mixture of cells with a normal chromosome complement as well as cells with an abnormal chromosome complement. Another possibility is that there is an inconclusive result or no result. With PGT-A, two to three percent of embryos result with an inconclusive result.

Slide 12 [00:08:17] Aneuploidy or chromosome abnormalities in embryos is associated with the age of the individual providing the eggs, often referred to as maternal age. In this slide, the blue bars are showing the chance at each age for the embryo to have a euploid result and the green is indicating the chance for the embryo to have an aneuploid result. You can see that as maternal age increases, there are lower chances for an embryo to be reported as euploid and higher chances for an embryo to be reported as aneuploid. You will note that even for younger patients, women in their early thirties, for example, each embryo has a 30 to 40 percent chance to be aneuploid.

Slide 13[00:09:25] What are the potential benefits of PGT-A? With PGT-A, the goal is to select a euploid embryo for transfer, which is associated with a higher success rate per embryo transfer and allows for more confidence with single embryo transfer. PGT-A can minimize the chance of miscarriage by avoiding transfer of aneuploid embryos, PGT-A can minimize the number of embryo transfers and time to achieve an ongoing pregnancy, and it may also minimize the emotional trauma related to unsuccessful transfers or pregnancy loss. Some couples find that PGT-A may assist with decision making about next steps. Having information about the chromosome status of their frozen embryos can allow them to decide whether they might do another IVF cycle or whether they have enough euploid embryos frozen that they're comfortable to move forward with an embryo transfer. Another potential benefit of PGT-A is that it can allow perhaps lower costs associated with fertility treatment as a result of having fewer embryo transfers.

Slide 14 [00:10:55] As with any genetic test, there are limitations. What are the limitations associated with PGT-A? Well, PGT-A is highly accurate, greater than 97%, but there is always a small chance of misdiagnosis. As well, PGT-A cannot detect all chromosomal abnormalities and it is not able to screen for single gene conditions or birth defects. As mentioned with PGT-A, there is a chance, two to three percent chance, for each embryo to have an inconclusive or no call result.

Slide 15 [00:11:43] Further, it's important that patients are aware that with PGT-A there very well may be fewer embryos available for transfer. And with PGT-A, there is no guarantee. Transfer of a euploid or normal embryo does not guarantee a successful pregnancy or a healthy baby. There is also the possibility of undesirable or unexpected results such as no euploid embryos available for transfer, no embryos to biopsy, or a mosaic result.

Slide 16 [00:12:24] What is a mosaic embryo? Well, mosaicism means that within the embryo biopsy, there is a mixture of cells with normal chromosomes and cells with abnormal chromosomes. Mosaicism is detected in approximately 10 to 20 percent of embryos, and it is not related to the age of the patient. It's important for patients to be aware of the expected frequency of a mosaic result and this can be specific to the fertility clinic or the PGT lab. It's also important that patients are aware of the clinic policy regarding transfer of mosaic embryos as some clinics will not transfer mosaic embryos or may only transfer low level mosaic embryos. The decisions about transfer of mosaic embryos can be very challenging for patients and clinicians.

Slide 17 [00:13:30] In summary, the goals of PGT-A pre-test counseling are to allow patients the opportunity to make an informed choice. We discuss a lot of information about PGT-A, and we are hoping that patients can prepare themselves to receive abnormal, unclear, or mosaic results. This discussion also allows patients to opt out of PGT-A testing if they feel they are not comfortable with the limitations for the potential for uncertain or mosaic embryo results. It also allows an opportunity to make a plan for results disclosure and to dispel any misconceptions that patients may have. For example, patients commonly think that because we are looking at the DNA and testing all of the chromosomes, that this can guarantee a pregnancy or a healthy baby, and this is not true. Another misconception is that PGT-A can test for genetic conditions, for autism, birth defects. Again, we want patients to be aware that this testing is not able to test for everything and cannot guarantee a healthy baby.

Slide 18 [00:14:56] Genetic counselors are usually disclosing PGT-A results in the fertility clinic. If a euploid embryo is available for transfer, most couples elect to move forward with

transfer of the euploid embryo, although some couples may choose to do another IVF cycle to create more embryos. As part of the disclosure of results, we will review the limitations associated with PGT-A, explaining that while these results are highly accurate, there is always a small chance of error or misdiagnosis and that couples do have the option to consider prenatal screening or testing during pregnancy. It can be a difficult discussion if there are no euploid embryos available for transfer.

Slide 19 [00:15:46] Let's review some case examples. In case study one, the patient is 40 years old, healthy, and has a normal karyotype. Her partner is 42 years old, healthy, and also has a normal karyotype and normal sperm parameters. They have a history of unexplained infertility. They had three intrauterine inseminations, which were all unsuccessful. Their first IVF cycle was when the patient was 38 years old. There were 12 eggs retrieved, 10 of those were mature and eight fertilized. There were four blastocysts that were frozen on day six. The couple went on to have four frozen embryo transfers. With the first three transfers, there was no pregnancy. The fourth transfer resulted in a biochemical pregnancy.

Slide 20 [00:16:42] The couple then went on to do a second IVF cycle with PGT-A. At the time of their IVF cycle number two, the patient was 40 years old, there were 11 eggs retrieved, eight of those were mature and six fertilized. Four blastocysts were biopsied and frozen, two on day six and two on day seven. The two day-seven blastocysts were euploid or normal and the two day-six embryos had aneuploid PGT-A results. The first frozen embryo transfer resulted in a successful pregnancy and live birth at 38 weeks gestation. This case illustrates how PGT-A can be helpful to identify a euploid embryo for transfer. Euploid embryos have a higher chance of leading to implantation and ongoing pregnancy, although pregnancy is not a guarantee.

Slide 21 [00:17:57] In case study two, the patient is 39 years old, healthy, has a normal karyotype, 46 XX. Her partner is 39 years old, healthy, normal karyotype, 46 XY, and normal sperm parameters. Review of their family history was not concerning. The couple has a history of unexplained infertility. They've had three intrauterine inseminations. The first two were unsuccessful and the third IUI resulted in pregnancy, although miscarriage occurred at six weeks gestation. The couple then decided to do IVF with PGT-A. The patient was 39 years old at the time of retrieval. There were 15 eggs retrieved, 12 were mature and eight fertilized. Three blastocysts were biopsied and frozen. Two were biopsied on day five and one on day six. All three of the embryos that were biopsied for PGT-A were aneuploid. This case illustrates that sometimes with PGT-A, all of the embryos are aneuploid and there are no embryos suitable for transfer. It's important that patients are aware that this is a possible outcome when doing PGT-A testing.

Slide 22 [00:19:38] Even if couples know that there's a chance that all of the embryos may be aneuploid, they are still disappointed, shocked, frustrated, or maybe feel hopeless. They ask questions, like, why did this happen? Will this happen if I do another IVF cycle? What can I do to change the outcome? These are difficult conversations and challenging. Aneuploidy occurs by chance. It's a chance occurrence. We know it's related to the age of the patient. However, it occurs by chance. Couples who are considering another IVF cycle must know that while we hope for a better outcome in another cycle, it's not a guarantee. It's very difficult to know what the outcome would be in another cycle and whether they would have a euploid embryo to transfer.

Slide 23 [00:20:41] Let's talk about mosaic embryos. With PGT-A testing, there is always the possibility of finding a mosaic embryo, and mosaic results pose ongoing challenges

and counseling dilemmas. Genetic counselors are tasked with assessing and explaining mosaic embryo results and also discussing potential prenatal, neonatal, and long-term outcomes following mosaic embryo transfer. Some of the challenges associated with mosaic embryo results include the fact that our understanding of mosaicism and embryos is constantly evolving. And it's very difficult to predict what the impact of the mosaic embryo result may have on a resulting pregnancy or live birth. There has been a lack of evidence-based guidelines for recommendations for prenatal testing.

Slide 24 [00:21:49] When we meet with couples to review the mosaic embryo results, we want to give specific details about the result, whether the mosaicism involves a whole chromosome or part of the chromosome, whether there are more than one chromosome seen in a mosaic embryo. It's very difficult to predict the outcome of a mosaic embryo transfer because a few trophectoderm cells that have been biopsied from the embryo may not really reflect the entire trophectoderm or the embryo. We know that it's the inner cell mass that will become the fetus and the baby, and we have no way to biopsy the inner cell mass and therefore we have no way to know what the chromosomes are in the cells of the inner cell mass. We know that in general, there are lower rates of implantation and higher rates of miscarriage associated with transfer of mosaic embryos. However, we also know that some mosaic embryos do implant and lead to ongoing pregnancy and live birth. Recent studies, in fact, do not show evidence of increased risk in ongoing pregnancies or live births following mosaic embryo transfer.

Slide 25 [00:23:34] It's really hard for couples to know what to do with their mosaic embryos. It's hard for them to make a decision. In helping couples make these decisions, we can review all of the options. They may choose to transfer their mosaic embryo, knowing that it's associated with lower rates of implantation and a higher chance of miscarriage. They may wish to continue to store their mosaic embryos while proceeding with another IVF cycle in an attempt to find a euploid embryo. They may wish to discard their embryos and perhaps proceed with another IVF cycle. Couples think about again, the lower chances of success with a mosaic embryo and a higher chance of miscarriage. For some couples, they are not comfortable to transfer a mosaic embryo, knowing that there is lower chances of success as compared to a euploid embryo transfer. Every couple has to decide for themselves if they're comfortable to take that chance to transfer that mosaic embryo, and sometimes couples have personal values that dictate what their decision will be. I have certainly had some couples that have indicated that they feel that they need to transfer any embryo that has any chance to lead to a successful pregnancy. While on the other hand, perhaps couples who have had miscarriages, they may not be willing to transfer a mosaic embryo. So these are difficult decisions and I think genetic counselors play an important role in helping couples decide what they will do with their mosaic embryos.

Slide 26 [00:25:38] Pre-implantation genetic testing for structural chromosomal rearrangement is called PGT-SR, and this test is offered when one of the intended parents has a chromosomal rearrangement, like a balanced translocation or an inversion. When there is a structural change with their chromosomes, the individual has all of the chromosomal material present, but it may be rearranged or moved around within their cells. An individual with a structural chromosomal rearrangement is usually healthy, although having this chromosome rearrangement means that they have an increased risk for infertility, miscarriage or stillbirth, and a higher chance to have a child with a chromosomal abnormality involving a gain or loss of chromosomal material. The goal of PGT-SR is to identify embryos that are euploid or have a balanced chromosome complement for transfer.

Slide 27 [00:25:38] Couples who are having IVF with PGT-SR may or may not have a history of infertility. Some couples can conceive quite easily but have a history of recurrent miscarriage or stillbirth and come to the fertility clinic to have IVF with PGT-SR to avoid a miscarriage or stillbirth and to optimize their chances for a healthy pregnancy. There is an increased chance for aneuploid or abnormal results with PGT-SR. Some studies have shown that the chance for an embryo to be euploid or balanced is approximately 10 to 15 percent. And therefore, when couples are having IVF with PGT-SR, it's important that they're aware that each embryo has a higher chance to be abnormal than normal. And in fact, it is possible that they may not have any embryos that are suitable for transfer. As part of our discussions for PGT-SR, we also have to think about age-related risks for aneuploidy and therefore, the age of the individual providing the eggs is important. It's also important that couples are aware that they may need multiple IVF cycles in order to find one or more embryos that are suitable for transfer.

Slide 28 [00:28:29] Case study three, the patient is 35 years old and healthy. Her partner is 36 years old and healthy, and they have a history of unexplained primary infertility, so they've come to the fertility clinic. As part of their fertility workup, chromosome testing has revealed that the female partner has a balanced translocation between chromosomes one and five. So this individual has 46 chromosomes in all of her cells as she should, but there is a rearrangement between chromosomes one and five, meaning that part of chromosome one has switched places with part of chromosome number five. The male partner has a normal chromosome karyotype, which is 46 XY. The couple have opted to proceed with IVF and PGT-SR.

Slide 29 [00:29:30] In their first IVF cycle, they had 12 eggs that were retrieved. 10 were mature and 10 fertilized. There were five blastocysts available for biopsy on day five. These are the results with PGT-SR. Embryo 1AP is aneuploid with a gain of chromosome 12. Embryo 2AP is unbalanced. There's a gain of chromosome one and a loss on chromosome five. So this is an abnormality related to the translocation in the female partner. Embryo 3AP had an inconclusive result. Unfortunately, there was not sufficient DNA, or the quality of DNA was poor, and the lab could not provide a conclusive PGT-SR result. Embryo 4AP was aneuploid with a gain of chromosome 21. This chromosome abnormality is unrelated to the translocation in the female partner. Embryo 5AP was unbalanced and aneuploid. So, there was a loss of chromosome one, a gain of chromosome five, and in addition, a gain of chromosome 16. There were no embryos recommended for transfer and the couple went on to have another IVF cycle.

Slide 30 [00:31:12] In their second IVF cycle, there were 20 eggs retrieved, 18 were mature and 17 fertilized. There were 12 embryos that were available for biopsy at blastocyst stage, and one embryo from their previous cycle was thawed and rebiopsied. This was the embryo that had an inconclusive result. So, in total 13 embryos were tested with PGT-SR. In this cycle, three of those 13 embryos were euploid or balanced and 10 were unbalanced or had aneuploid results. If we consider both cycles, there were three out of 17 embryos that were euploid or balanced and suitable for transfer. This couple went on to transfer euploid embryos and had two successful pregnancies. I think this case very well illustrates that it may take more than one cycle to have a successful pregnancy. And it also illustrates that many embryos will not be euploid or balanced and suitable for transfer.

Slide 31 [00:32:36] Let's move on to preimplantation genetic testing for single gene conditions. We call this PGT-M. The M stands for a monogenic or single gene condition. PGT-M is offered to couples who are at risk to pass on a specific known genetic condition. These are couples where one member of the couple may have a genetic condition and

genetic testing has identified a very specific genetic change in the gene related to that condition. Or couples may be carriers, which means that they are healthy, but they have a genetic change that can be passed on to their offspring. With PGT-M, we can select embryos that have not inherited the familial genetic variant or change and in doing so can lower the risk of the genetic condition in their offspring. Many couples who come to the fertility clinic for PGT-M are not experiencing infertility and may only be having IVF for the purpose of PGT-M.

Slide 32 [00:34:03] PGT-M can also be used for HLA matching. Stem cell transplant or SCT is the only cure available for certain rare blood diseases and finding a stem cell match within a family is not always easy. Some couples who have a child with a rare blood disease may opt to have IVF with PGT-M in order to find an embryo that is a stem cell match for the affected sibling. If a stem cell matched embryo is identified and the embryo transfer results in a successful pregnancy, the stem cells can be collected from the umbilical cord at the time of delivery.

Slide 33 [00:35:00] For couples who are considering PGT-M in their IVF cycle, there is a lot of information that must be discussed ahead of time and pre-test counseling will review the single gene condition that is present in the family and the chance for the familial gene variant or mutations to be transmitted to the embryo. Some genetic conditions are autosomal dominant and, in this case, 50 percent of the embryos would be affected with the genetic condition. Some genetic conditions are autosomal recessive, in which case, the intended parents are generally healthy, but are carriers of a genetic change and the chance that they would each pass on the genetic change to an embryo is 25 percent chance. Our discussion will focus on the chance for each embryo to be affected with the single gene condition in the family. And as well, we want to review age-related risks for aneuploidy and the option to do PGT-A testing as part of their IVF cycle in addition to PGT-M, so that they may ultimately choose an embryo that is unaffected with the genetic condition and also euploid.

Slide 34 [00:36:40] Also, with PGT-M, there is a process of test development. With PGT-M, the test is unique to the genetic variance in the family, and it is necessary to develop a PGT test that will be suitable for their embryos. It takes time to develop a test for the embryos. It generally takes between four to six weeks for the reference PGT lab to build a test for the PGT-M cycle. The PGT lab will require samples from the affected family members or both members of the couple if they are carriers. They may require samples from an affected child or other family members. With PGT-M, there are higher costs and generally the cost of PTG-M for one IVF cycle is anywhere between 5000 to 7000 dollars. It's important that couples are aware that they may require multiple cycles of IVF with PGT in order to be successful finding embryos that are unaffected with the genetic condition and euploid, and that ultimately there will be fewer embryos suitable for transfer.

Slide 35 [00:38:16] In case study four, patient is a 34-year-old G1P1L1 woman, she's healthy. Her partner is 37 years old and healthy, and their first pregnancy resulted in a daughter affected with cystic fibrosis, which is an autosomal recessive condition. Genetic testing has identified genetic mutations in the child and the mom and the dad are both carriers. So, mom and dad are healthy, but each carry one copy of the genetic change. There is a 25 percent chance recurrence for their future children to be affected with cystic fibrosis. The couple would like to have PGT-M to select embryos that are unaffected with cystic fibrosis.

Slide 36 [00:39:19] This slide is illustrating autosomal, recessive inheritance, showing that each parent is a carrier of the genetic condition. They have one copy of the gene variant, and they have a second copy of the gene that does not have the gene variant. There is a 25 percent chance for each embryo to inherit two copies of the gene variant and be affected with cystic fibrosis.

Slide 37 [00:39:47] These are the results of their IVF cycle. They had IVF with ICSI, PGT-M for cystic fibrosis, and PGT-A. There were five blastocysts that were biopsied. Embryo one was unaffected with CF but was chromosomally abnormal or aneuploid. Embryo two was unaffected with CF, was found to be a carrier, and was euploid. This is an embryo that would be recommended for transfer. Embryo three was an unaffected carrier for cystic fibrosis but was chromosomally abnormal. Embryo four was affected with cystic fibrosis and chromosomally normal. Embryo five was affected with cystic fibrosis and chromosomally abnormal. In total, only embryo two is an unaffected carrier and euploid. So, when couples are doing PGT-M for a single gene condition and PGT-A, it means that they will have fewer embryos that will be available for transfer.

Slide 38 [00:41:12] Case study five. In this example, the patient is a 31-year-old G1P1L1 woman. She, herself is healthy. Her partner is also 31 years old and healthy. The couple have had one pregnancy together, and that resulted in a son who is affected with a rare disorder called Hemophagocytic Lymphohistiocytosis or HLH for short. This is an autosomal recessive condition. And again, genetic testing has identified genetic variants or mutations in the child that are causing the HLH. Mom and dad are both healthy but are carriers of the gene change for HLH and there is a 25 percent chance for each of their future children or embryos to have HLH. The couple have chosen to have PGT-M to select embryos that are unaffected with HLH, as well as to select embryos that are an HLA match for their son. So, the condition HLH is one of those conditions that can be treated by using an HLA match.

Slide 39 [00:42:40] The couple had IVF with ICSI, PGT-M for HLH and HLA matching, and PGT-A. In their first cycle, there were four blastocysts that were biopsied, and these are the results. All of the four embryos were unaffected with the HLH. The first embryo was unaffected. Embryos two, three, and four were unaffected carriers, meaning that they had one copy of the genetic change for HLH but still had a working copy of that gene and thus unaffected. Embryo one was found to be an HLA match as well, but that embryo had an aneuploid chromosomal result and was not recommended for transfer. The remaining three embryos were all euploid but were an HLA non-match. This couple chose to proceed with another IVF cycle to create more embryos with the hopes of finding an HLA match.

Slide 40 [00:44:00] These are the results from their second IVF cycle. In this cycle, there were six blastocysts that were biopsied. Embryo one was again unaffected with HLH but was an HLA match. However, the embryo was aneuploid. Embryo two was unaffected with HLH, was an HLA non-match, and aneuploid. Embryo three was unaffected with HLH, an HLA non-match, and euploid. Embryo four was HLH affected but was an HLA match. Embryo five was HLH unaffected carrier but was a non-match and euploid. Embryo six, HLH affected HLA non-match. I think these results illustrate how difficult it is to find embryos that meet criteria for transfer when testing for a single gene condition and trying to identify an HLA match. From both of their cycles, there are embryos that are unaffected with HLH and euploid. However, none of those embryos was an HLA match. And unfortunately, the embryos that were found to be an HLA match were either aneuploid or affected with the single gene condition.

Slide 41 [00:45:54] In general, genetic technology and testing options are ever evolving. Counseling is complex and challenging, and each case is unique.

Module 6.9_Optimizing Fertility Through Lifestyle Changes_EN Transcript

Dr. Basim Abu Rafea

Slide 1 – Note to Audience slide [00:00:00]

Slide 2 – Title Slide [00:00:07] So welcome to today's talk. My name is Basim Abu Rafea. I'm one of the fertility specialists at London Health Sciences Centre fertility Clinic. And today I'm given the privilege to talk about optimizing fertility through lifestyle changes.

Slide 3 [00:00:24] We're going to be going over a bunch of items today. And what I'm going to talk about is what is present from an evidence-based perspective. So, please bear with me. Each of these topics can be a whole talk on its own. So, I'm going to try to give summaries of everything and I hope you find it useful. So, to start off, I'm just going to go briefly over the definition of infertility. And I'm sure you probably heard this before with other talks in this series. It is defined as 12 months of trying with unprotected intercourse, regular, without any success. The American Society does tweak it a little bit and say if you're 35 years or older, then it's only six months because time is of the essence. And I think one of the items we're going to talk about is implications of age on fertility.

Slide 4 [00:01:18] So, the items we'll be talking about is the fertile window, coital practices, fertility-awareness methods, diet, body weight, exercise, and of course, everybody's latest favorite, exposure to environmental toxins.

Slide 5 [00:01:35] So, from lifestyle changes to fertile window,

Slide 6 [00:01:39] there's a lot of research that was done on this, and most of the research on this was actually done in the seventies. And I had the pleasure to work with some of these people in the past before they retired. We know that it's better for the sperm to be waiting for the egg rather than the egg waiting for the sperm. The study that's quoted here says that the best success rate happens when intercourse occurs two days before ovulation and that's because the sperm is waiting for the egg when it comes out. The recommendations for the longest time, if you go online, will always say every other day. However, the American Society has tweaked that to daily, if possible, around the time of ovulation. So, frequency of intercourse is another thing, but it's always best to have the sperm waiting for the egg rather than the other way around.

Slide 7 [00:02:31] The coital

Slide 8 [00:02:32] practices is a huge topic, and this is where it comes down to when to have intercourse, when to do it and how frequent. And what a lot of patients are concerned about when they do this is that they worry about if they have daily or every other day intercourse, that their sperm counts will go down and it won't be as effective. And you can easily reassure them that that's not the case. They still retain good sperm production as sperm production is always ongoing. And, as I said, daily unprotected intercourse is optimal. Social aspects may interfere with this, work. Those are different variables that are difficult to account to, so at least every other day. We try to target peak times of ovulation around that time, and we're going to talk about ways of predicting ovulation.

Slide 9 [00:03:27] One of the things that comes up lately with lifestyle modifications, there's a lot of stress these days. A lot of patients who are anxious about the fact of going through the fertility journey and trying to achieve pregnancy. There's also a lot of information floating around, whether it be online or other sources.

Slide 10 [00:03:49] And these were reviewed and looked at scientifically, and this is an example of what's called a Cochran review. So, a Cochran review is kind of the ultimate reference for us from an evidence-based perspective as healthcare workers. And this one looked at psychosocial and educational interventions. So, if we were to sit down and educate people and try to help them reduce their stress and do all those interventions on whether this makes a difference with regards to anxiety or depression or on live birth outcomes**,** actual evidence shows it's very low. So, in the randomized control trials that have been out there, they have not been found to improve live birth rate, which is our end result. So, at the end of the day, we don't aim to belittle anybody's stress or anxiety but if a patient is wondering whether their anxiety or stress is their cause of infertility, then that is not the case and the evidence so far does not suggest that at all. Interventions, they may help them emotionally or psychologically but not necessarily in their achieving their objective of going through having a live birth.

Slide 11 [00:04:58] So, existing trials so far were generally poorly designed and executed. In the future with better designed trials, there may be a role for certain interventions, but they've got to demonstrate efficacy.

Slide 12 [00:05:12] Fertility awareness methods. I'm sure for anyone out there who has searched for any form of fertility awareness method, they are plentiful.

Slide 13 [00:05:25] Ovulation prediction kits are most common. They're sold everywhere. They've been around for a long time. They have become very sensitive and very accurate and now digital in how they present their data. They can range from cheap in costs if you get them from through Amazon to very expensive. Not only that, there are now companies that produce complex gadgets that help predict fertility. And I believe in the latest release of the Apple Watch software and their product, they actually have cycle tracking software incorporated into their operating system or iOS because they've seen the potential for that and the value of it. So, this market is growing. The evidence for it is not very strong but it definitely helps couples if they're trying to do things on their own and they want to try to minimize interventions. Usually I guide them towards a cheaper method rather than more expensive ones because they're all more or less the same efficacy. They have not been studied head-to-head against each other. Cervical mucus listed here. I actually had the pleasure of working with a gentleman who developed a post-coital test, which looks at cervical mucus, and then he retracted his own studies. So, it's very difficult to prove whether it causes a problem or not, but it may help patients predict their ovulatory time but has not been scientifically proven to be of great value. Basal body temperature charting, which is something we used to do, 15, 20 years ago, prior to the abundant availability of all the ovulation prediction kits through urine. And this would help the patients have a retrospective view of when they most likely ovulate. So, they chart their temperatures and this helps them in the next cycle predict when they ovulated because the temperature goes up actually after ovulation, so they've usually missed the window by the time it goes up. Use of lubricants in fertile. This is something very important and a lot of patients ask about it. We get a lot of patients who have experienced some discomfort or dryness, especially when they have to have frequent intercourse while trying to achieve a pregnancy. A lot of people are aware that a lot of the lubricants used may be harmful for sperm and therefore counterproductive. So, it's always important to counsel them that

whatever lubricant they choose, it must be safe for sperm. Examples for these are something called Pre-Seed or Conceive Plus, which they can find at their local pharmacies.

Slide 14 [00:08:03] So, diet. This is a huge topic.

Slide 15 [00:08:07] So, we know that obesity impacts fertility outcomes. We know that being extremes of weight, either direction, causes issues with pregnancy. We know that certain foods can be harmful, such as if you eat a lot of seafood, mercury levels can be high in them. And there has been studies done specifically with regards to fertility diet and these studies have shown that adherence to fertility diet was associated with the lower risk of infertility and ovulatory dysfunction. Now, in general, it is a healthy diet that you're looking for and it is very difficult in this day and age to try to pinpoint one exact dietary supplement. But following the Health Canada recommendation is always a good one and keeping track of your calorie counts is another approach. At the end of the day, all these studies have not demonstrated changes in live birth rates because pregnancies occur in the people who eat the worst diets and in the people who eat the best diets. So, although there has been a trend towards reducing the chance of infertility, there is no solid proof for evidence based on the studies done.

Slide 16 [00:09:27] Important things when it comes to diet. The most important piece of evidence we have is the value of folic acid. So folic acid, especially being taken at a dose of 400 micrograms daily at least two to three months before pregnancy and two months into pregnancy, reduces the risk of neural tube defects in the baby by 70 percent. And this is of tremendous value and is very well established. And if there's a history of previous neural tube defects, or if the patient has a high risk of developing spina bifida, then they should be on five milligrams a day. And that's something usually their physician would prescribe for them. But, folic acid is the most important thing and it's always surprising how many patients come to our fertility clinic and they have not been using any prenatal vitamins.They're on every kind of other vitamin except the most important one. You'll see a lot of patients on vitamin B12. And vitamin D, now, almost everybody is on it because of our large series of patients that we've looked at, almost 90 percent were deficient when we've screened them. So, there's no harm but it hasn't been correlated yet solidly with live birth rates.

Slide 17 [00:10:37] These are generally a list of various levels of vitamins in different prenatal vitamin supplements. So Preg Vit, Materna, and Jameson Prenatal. Now, keep in mind most of the studies done on prenatal vitamins with regards to miscarriages, live birth rate, preterm labor, all that was on Materna. So that's usually the one that’s generally recommended, but all of them are of value, so there isn't one that's less effective than the other as long as the patient is taking a prenatal vitamin. That is very important, and it must include folic acid.

Slide 18 [00:11:10] So this is just a brief description about the importance of folic acid and how it works, and you can find more resources through the SOGC, The Society of Obstetricians and Gynaecologists of Canada, through their journal and about the levels of supplementation and their importance.

Slide 19 [00:11:26] Other nutrients. There's always a lot, so going through the omegas, the iodine, the herbs, all that. Again, a balanced, healthy diet will supplement all your needs. It is very difficult to track specific vitamins than just to take vitamins as your

supplements. These are hard and there's no solid evidence that this makes a difference in improving live birth rate. So, eating a healthy diet in general should be sufficient.

Slide 20 [00:11:55] Antioxidants and their benefits. And this is a huge topic because, as you can see, since the large study that looked at vitamin improvement in longevity and cardiac health and all that, and nurses have demonstrated that there hasn't been a benefit from vitamins in that perspective. You'll see a shift in the marketing towards all the antioxidants and things like omega three and CoQ10. And if you ever go to Costco, you'll notice that that section has changed into this kind of merchandise. And the evidence is still in the works, and I'll go over some detailed examples. But at this point in time, you'll see some evidence about Coenzyme Q10 being maybe beneficial for patients with poor ovarian reserve, but it hasn't demonstrated solid or robust evidence when it comes to live birth rate in general population. So, the evidence is always very conflicting when it comes to these.

Slide 21 [00:13:00] So when all this evidence was reviewed, there was low to very low quality evidence. So it's very difficult to make sure you recommend something of antioxidants without solid evidence behind it. At this point in time, no one's been able to demonstrate robust evidence for value.

Slide 22 [00:13:18] So in this slide, I'm going to discuss specifically inositol as an example of the antioxidants. And this is a very popular supplement that is gaining popularity, specifically in the PCOS patient population. It was very popular with the naturopathic doctors and prescribed regularly, and it actually has been studied medically. And at this point in time, the evidence for it is not very robust, but it is still undergoing a lot of trials. It's actually listed in the current guidelines for PCOS but the quality is still not strong enough to recommend its routine use.

Slide 23 [00:14:03] No pooled evidence is available for the use of inositol versus placebo and other antioxidants such as insulin-sensitizing agents, ovulation induction agents, or other types of inositol for women with PCOS undergoing pre-treatment for IVF or fertility treatment. So you'll see this statement coming up in most studies that are reviewed and published on this, including the Cochran database, which reviewed all the published randomized trials.

Slide 24 [00:14:29] Androgens. This is something that's been around for a few years and you might see patients asking about supplements called DHEAS or dehydroepiandrosterone. And although in Canada this is restricted, they can easily buy it from any supplement store in the United States or online. And there are some studies in poor responders or patients with low ovarian reserve that it may help before they go through IVF. The evidence is not that strong but there may be a role for this. But we're waiting. There are more trials ongoing at this point in time. So, if you're ever asked about it, this is one of the things that can be used in poor responders. You'll see a lot of the new prenatal vitamins come with DHEAS, which is very interesting. And that is not supported by evidence and I'm not sure where the safety is for younger populations to take this medication in the context of absence of poor response, poor ovarian reserve, and not going through IVF.

Slide 25 [00:15:36] Aspirin. And that's again because it's readily available, although it's not a supplement, but people can buy it anywhere. And baby aspirin is what we're talking about. All the studies so far have not demonstrated any value for aspirin supplementation. It is given sometimes in certain cases and recurrent miscarriages, but again, the evidence

is very weak for this substance. It does have a role, specifically in certain medical conditions, and it's usually combined with heparin and that's a whole different story. So, it's not used as something that patients should take as a supplement.

Slide 26 [00:16:11] So Chinese herbal medicine and it's use in subfertile women. And I'm sure you've driven by a fertility practice with Chinese acupuncture and Chinese herbal medicine, and they're becoming more popular. Our patients are frequenting them. They also have been studied scientifically and at this point in time, the review of all the published literature and this systemic review came out last year and did not show support for the use of Chinese herbal medicine in improving outcomes, specifically for PCOS**,** and those are the main patient populations that target this type of treatment. So, just to keep that in mind, there's no shown harm either at this point in time. So, I tell patients, if you want to do it, you can, but the evidence doesn't support it.

Slide 27 [00:17:03] Body weight. This is a very sensitive topic

Slide 28 [00:17:09] because nobody wants to tell a patient they're overweight but we know that it can have implications. We as a clinic in London, because we are hospital-based, we get sent patients from all across the province to do IVF because we're the only ones who don't have a BMI limit. That's because we have a hospital backup and we're not privately located outside the hospital. But increased weight does make it more challenging for patients to achieve pregnancy. It carries higher risks and I'm going to go over these.

Slide 29 [00:17:52] So there's reduced fertility and there's a lot of hypotheses as to why the reduced fertility, but mainly it does impact ovulation. It can impact intercourse itself. It can impact implantation. And we have a research project on the goal, specifically looking at that, at the level of the lining of the uterus. It can also impact rate of miscarriages

Slide 30 [00:18:15] and pregnancy outcomes, per se. So fertility rates in patients with obesity are known to be reduced and they can definitely be improved by weight loss. However, the evidence again is not very strong. It is specific and it is strong in certain populations such as PCOS patients who have ovulatory problems, and if they lose weight they can resume spontaneous ovulation and improve their outcomes. We know if we do IVF, and we've published on this, they are harder to do procedures on just because of technical difficulties. And as I said, it has impacts on implantation and clinical pregnancy and live births and possible higher rate of loss.

Slide 31 [00:19:03] There's also maternal risks with high BMI when trying to get pregnant. If you are pregnant, there's higher risk of gestational diabetes, preeclampsia, and peri-partum risks**,** and of course, a higher rate of what's called operative deliveries, such as caesarean section. And babies may require admission to NICU if there's diabetes, and so on. So, prevention is the best medicine. Usually we'll counsel patients on weight reduction. However, sometimes these patients may struggle and it's difficult in a clinical scenario when the patient is older and time is not on their side to achieve both objectives simultaneously, so we take that into consideration when counseling. Other than

Slide 32 [00:19:46] ovulatory dysfunction, so for patients who are obese, we usually tell them they might need higher medication dosing if they're going to be treated. And we talk about impacts on egg quality, endometrial function, we talked about miscarriage rate and maternal-fetal environment. These are all things, I'm just repeating them again, because they're very important. One item that is

Slide 33 [00:20:07] neglected when it comes to fertility is men and the impact on semen parameters in the context of obesity. And we know that obese men may have a higher rate of lower sperm counts and it may have impacts on their sperm motility. So, there may be male factor-related issues that correlate with high body mass index and that's something to keep in mind as well. So there's a lot of studies that have

Slide 34 [00:20:36] demonstrated that obese men have lower sperm counts, higher abnormal forms. They also have alter sperm functions, so motility is affected. They also have endocrine alterations, so usually their hormones are a little off. There is a higher correlation with sexual dysfunction, so erectile mainly and ejaculatory dysfunction. And if they become diabetic, then we talk about the risk of retrograde ejaculation. That creates a whole different problem. So as I said, medical issues, including diabetes, which develops from that. These are things to keep in mind, so it's not only the female patient that gets affected with this, it's also males.

Slide 35 [00:21:19] The Canadian Fertility & Andrology Society recommendation for obesity is before starting fertility treatment, patients should really try to look at managing appropriate comorbidities such as diabetes, hypertension, dyslipidemia. And these are things that the clinician will look at. These are things that are usually screened for in the clinic when the patient starts their fertility investigation journey. We do counsel patients with regards to mainly the most important thing is lifestyle modification because with diet and exercise**,** it's not only their journey to fertility, but we want them to modify these items, their diet and their excise, for their healthy benefit for longevity. And this impacts their health in general. So, this is what we counsel first choice, and if we're unable to do so, then we talk about alternatives such as medical or surgical interventions if possible or if needed. Unfortunately these are sometimes not very available or may be expensive.

Slide 36 [00:22:20] Exercise and fertility. It’s very hard to say

Slide 37 [00:22:25] exercise is not good at any point of time. Exercise is always good. So, the recommendation is 30 to 45 minutes a day of moderate exercise and there's a recent paper that just came out a few weeks ago. And I'm not sure when this talk is going to be given, but you'll see it where actually they say there's a correlation now with longer life expectancy and better quality of life with moderate to severe exercise of up to an hour a day on a daily basis. So, so far everything we know about exercise is the more you do it, the better things are. And so definitely everybody will benefit from it, whether you're overweight or not. So, this is something to keep in mind and I would generally not recommend someone stopping exercising. There are certain points of time when a patient is extremely athletic and that's a different story altogether. Those patients may represent with their own endocrine problems because of their extremes of involvement in sports.

Slide 38 [00:23:35] Lifestyle advice for people with infertility. This was a huge study from a review of a Cochrane database that was published last year. They looked at approximately 5,400 records. You can see that up in the top corner. And they filtered them down because the Cochrane requires good quality, evidence-based papers. So they look at generally randomization and good methodology for the research. And after reviewing all of these and weeding them out, they only came down to four studies that were included in their meta-analysis. So, the reason I put this slide up is just to highlight the importance of making sure you select the right level of evidence when counseling because there's something published for everything, but the level of evidence makes a difference. So as an academic faculty and a teacher for medical students at residents, we always remind them evidence-based medicine is key. And so this is just to highlight how difficult it is sometimes

to weed out what is of value and what is not. This is just the perfect example of one topic, which is lifestyle advice. So pharmacological and non-pharmacological

Slide 39 [00:25:02] strategies for patients who are obese. There's a lot of new medications that can help with weight loss. And at this point in time, there's no data available for comparison that would look at live birth rate. So, it may help with weight reduction, but we haven't seen things that are solid evidence to say this changes outcomes from live birth rate. But eventually, if the patient becomes healthier, then that's of significant value. Environmental toxins

Slide 40 [00:25:35] Those are not difficult to touch on, but I don't think we see anymore or know much of what we're breathing or eating or drinking. There's so much modification in a lot of what happens around us and I think this is what led to the organic market and all that we see around us.

Slide 41 [00:26:00] But we'll talk about common things. So, caffeine. A lot of people are worried about coffee intake and can they drink coffee if they're trying to get pregnant, is that going to cause a problem? So usually, the current recommendation, this has actually been looked at quite extensively, and the American Society says one to two cups of coffee a day, and I'm not talking about the Tim Horton's extra large cup, I'm talking about the regular cup of coffee, is fine. It's acceptable. Alcohol, there's no safe limit if you're pregnant and in pre-pregnancy. At this point in time, alcohol has not been shown to be good. So, not to exceed two drinks a day, and that's what the American Society of Reproductive Medicine currently recommends. But definitely if you're trying to get pregnant, I would generally ask patients to avoid it, even the men, because it also has negative implications on sperm.

Slide 42 [00:26:55] Smoking. Everyone's aware of its negative impacts on everything. And so there's no point in time where smoking is acceptable. It also affects fertility and that's been shown in multiple studies to correlate with infertility. So, it definitely is something that we heavily counsel patients on quitting. And this includes e-cigarettes.

Slide 43 [00:27:20] So, smoking reduces ability to achieve pregnancy, delays in conception, increase in risk of miscarriages, and that's been shown as well**,** and early onset of menopause. It does damage ovaries. We also see it's outcomes in IVF cycles, less eggs when we do IVF cycles. So, e-cigarettes is definitely something

Slide 44 [00:27:39] we also counsel patients against because it does have a lot of chemicals and we try to say please stay away. So, it's not an acceptable alternative**.** Anything inhaled is not very good. It's all chemicals that are bad for you and probably bad for your reproductive potential and baby. This is a paper published in 2018

Slide 45 [00:27:57] looking at reproductive consequences of smoking. And you can see here what we talked about, delayed conception, ovarian follicular depletion, so less follicles, sperm parameters get effected**.** Mutagenic potential, so that's another issue to keep in mind. Early pregnancy effects. So basically small progestational age, so the babies are always smaller. Effects on maternal smoking and what happens to their males in the future, it's what's called a Barker hypothesis. So, things that the mother is exposed to while she's pregnant can impact their child long term in the future. And of course, as I mentioned earlier, impacts on fertility outcomes for treatments. So, it's never a good thing

and the patients are extremely heavily counseled on quitting. So, abstain from smoking cannabis.

Slide 46 [00:28:49] So far our research at Western was done on rats and has shown developmental impacts on fetuses born to mother rats who were exposed to cannabis. So, with the hypothesis it may affect and probably does, so we say avoid it and abstain from it while trying to achieve a pregnancy. And so this is something again, we talk to patients about and we do ask. And it's interesting that our patient population that come to our clinic looking at fertility treatments, most of them know that they should stop this and have stopped this before they even get to us. So, in case they do, we counsel them to stop. And that's for both men and women.

Slide 47 [00:29:34] It’s very difficult, we try to tell patients to avoid them and look at avoiding products that may be heavy with pesticides, such as berries, looking at organic, and you have to make sure there's no additives as well. So, these are very, very difficult to weed. I suppose proper washing and trying to cut back on substances that may have high pesticide doses. But again, there's no correlation yet with potential outcomes. There's nothing in the evidence that has been solid but there is a trend, and it's a very difficult one to prove at this point in time. Cosmetics and things.

Slide 48 [00:30:19] Again, this is another example of what's in your shampoo or what's in your bar of soap or in your toothpaste and then you have all these microplastics. And so, it's definitely a new world. Try to just be cognizant, tell patients to be cognizant of what you're using. Try to look into what's in your products and do a little search and see, if you're using the internet search, do useful searches and see if this has any impacts on what you're trying to achieve in regards to pregnancy objective. But it's very, very difficult to avoid becuase these are daily products that are available to us all the time.

Slide 49 [00:30:59] So when it comes to summary, with regards to fertility and fecundity, diet, exercise, and environmental factors do have a role to play. The evidence is not very strong, but it is definitely developing and there are certain things that make common sense and must be followed. So, we always try to counsel patients in that regard. And we try to coach them with regards to fertile window and what kind of supplementation they actually need while trying to pursue their fertility journey and achieve their objective. And we counsel them with regards to their weight and watching their weight and treatment methods or approach to a healthier lifestyle, which can impact their chance of getting pregnant and the health of their baby and child. Certain items that can be harmful or are known to be harmful, they need to be highlighted. Definitely smoking and cannabis. These are items that need to be highlighted. Important things to keep in mind as well when we're talking to patients is what their line of work is. Some patients have exposures to certain chemicals in their work environment, so that's something they should, if suspected, bring up to their physician to discuss further.

Slide 50 [00:32:17] This is a list of resources of some of the stuff that we talked about. I hope you find this useful and helpful. It was a pleasure giving this talk.