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From the campus of Harvard Medical School, this is ThinkResearch, a podcast devoted to the stories behind clinical research. I'm Oby.

And I'm Brendan. And we are your hosts. ThinkResearch is brought to you by Harvard Catalyst, Harvard University's clinical and translational science center.

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For many cases of infertility in women, current research doesn't tell us everything. There are several known causes of infertility, but also many unknown reasons why a pregnancy would be unsuccessful. It's commonly known that advanced age leads to difficulty becoming pregnant, but we don't understand why. When it comes to IVF treatment, less than half of all cycles result in pregnancy.

Doctor Caroline Mitchell wants to understand the mechanisms behind causes of infertility and how much of it is due to the makeup of the vaginal microbiome. Dr. Mitchell is an Investigator at the Vincent Center for Reproductive Biology at Massachusetts General Hospital, and an Associate Professor of Obstetrics, Gynecology, and Reproductive Biology at Harvard Medical School. Dr. Mitchell, thank you very much for joining us.

Thanks for having me.

You received a pilot grant to study how the vaginal microbiome affects fertility. What are the aims of your study?

So our first aim is to compare the vaginal-- and actually also intrauterine and gut microbiome-- between couples with two types of infertility-- idiopathic infertility, where we really don't know the cause of infertility, and then male factor infertility, where we think the female partner is as normal as we're going to get in a fertility clinic. The second aim of our project is to compare those three different microbial communities-- so vaginal, intrauterine, and gut-- between women who achieve pregnancy with an IVF cycle and those who do not achieve pregnancy with that IVF cycle.

You're looking at infertility. How much do we know about the causes of infertility?

So we know a great deal about some causes of infertility. So things that are easy to measure would be sperm number and function, if people don't ovulate or produce eggs at all. That's a pretty easy thing to measure and diagnose.

But then there is a good proportion of women-- or couples, I should say-- who aren't able to get pregnant and we're really not sure why. And often, those are the folks who do in vitro fertilization because that's probably the best shot. But even in those cases, pregnancy is achieved in only 30% to 50% of cycles. Age is sort of an intangible. We know that age decreases people's chances of achieving pregnancy, but exactly why and how is not totally clear.

And is there-- is it a very-- it's sort of like an ambiguous cutoff? Is there-- you know, some people can get pregnant into their 40s, some people can't, and it's just-- so there's a lot of unknowns there?

Right, correct. And much of that is likely due to genetic problems with an egg or an embryo that lead to very early miscarriage or not achieving pregnancy at all. And so I think often these are things we don't quite have the tools to measure. And one of the things we wonder is if some of these cases where people just don't achieve pregnancy is due to the microbiome.

So there's been some work looking at microbiome and infertility. How is your project different from the previous work that's been done?

So there are a couple groups or types of studies that have been done about microbiome and infertility. There's the older work that was based on culturing the microbes, which is sort of all we used to be able to do. And those studies looking at culture did show some associations between what we think of as the beneficial vaginal bacteria-- the lactobacillus-- and live birth after an in vitro fertilization cycle.

With the advent of molecular sequencing, there have been a couple studies that characterize the microbial communities by molecular sequencing. A couple of these look at vaginal swabs and catheters that are used to deliver the embryo into the uterus, but neither of those studies characterize the microbial community down to the species level, so you get broad strokes. But we know that different species of lactobacillus specifically have some very different functional effects. So the data out there don't characterize species down to that level, which our study will do.

And then one of the bigger studies that's been done was done by a group in Italy which used endometrial biopsies and characterized the microbial community quite well. But endometrial biopsies in folks undergoing IVF are not something that is sort of clinically practiced in the US, and so we'll be looking really at vaginal swabs, the catheters that deliver the embryo, and then also at the gut microbial community.

And so you're looking at patients in in vitro fertilization clinic and IVF clinic. Why did you choose to look at IVF patients?

Two reasons. One is that in Massachusetts, this is something that's covered by many insurance providers, but around the country, this is something people pay for out of pocket. And when you're paying out of pocket for something that is successful 30% to 50% of the time, that can be devastating. And so if we could figure out something that would improve those results, that would be a huge service to patients and to couples.

So you mean the people that are paying out of pocket, that 30% to 50% would go up to 70%, 80%, whatever?

That would be great.

Yeah.

That would be our hope so that you didn't have to pay for cycle after cycle.

And these are expensive procedures.

Correct. Between \$15,000 to \$20,000, depending on what medications you're using and where you are.

The second reason to study an IVF population is that you know when pregnancy is expected. The time horizon is quite short, whereas folks who are trying to achieve pregnancy outside of a clinic-- certainly there is a fertile period, but you don't have the exact day, the exact sort of timing that you do with an IVF population. And so for a research study, it's actually kind of nice.

Mhm. To have that controlled environment?

Correct.

You know exactly when the egg has been implanted and--

Correct, exactly.

So let's talk about some of the nuts and bolts of the study. How are you enrolling patients, and what kind of screening are they undergoing?

So we're relying on our clinical colleagues to help us identify our two groups of patients. So folks who are characterized as male factor, where it's really a sperm issue, and folks who are idiopathic, where all of the testing that's been done by the clinical team really hasn't revealed a cause for the infertility. And so the screening is really just the clinical workup. And our research coordinator talks with the clinical teams each day about the patients who are coming to clinic and who might be a good candidate.

The second step is then asking the patients if they're at all interested in participating. And really that's what it is. It's based on the clinical profile and people's willingness to provide the samples.

The data collection is really also, after the cycle, we'll look at what medications did people get to stimulate the ovaries? What was the quality of the eggs and the embryos? Did the team have any trouble during any of the procedures? So that's all data we'll pull from the clinical chart.

And then the real study work and analyses is once we have all the samples, is then extracting DNA, characterizing the microbiome with the molecular sequencing, and then doing the analytic comparisons.

OK. So the samples is the stool sample, the catheter sample--

And vaginal swabs.

--and vaginal swabs. OK, great. Is there anything else around the kind of process of the study that you think would be important for people to know?

The great thing about vaginal swabs is they're pretty easy to do. And what we've learned is that it doesn't matter if a clinical provider does them or a patient self collects the swab, they actually sample the vaginal microbiome equally well. So that's lovely. That's a very easy sample. For me, this is the first time I'm asking people to collect stool samples, which honestly I think is the harder thing to do.

Harder in terms of you having to tell people to do this or them--

Yes.

--going home and doing it.

All of those.

All of-- yeah, yeah.

And also the sample processing is super different, and I'm not sure-- I'm a gynecologist, so I think the vagina is fine and I think poop is kind of gross.

[LAUGHTER]

Otherwise you would have gone into a different specialty.

Exactly. So that to me is the biggest barrier to our study is the stool collection, honestly. Because everything else is an extra swab at the time of procedures that are already happening, so it's very easy for the participants. But the stool collection is not an easy thing, for anyone I think.

Do you process the stool samples in your lab or you send him out?

Yes, we do. And we essentially-- one of my collaborators, my co-investigators on the project, Doug Quan, is someone who's done a lot of microbiome research and does this regularly, and what they found is that turning the stool sample into kind of a slurry and freezing it in little pellets with liquid nitrogen works best. Again, this is a new frontier for me, and thank goodness I have a wonderful research coordinator who has agreed to do this and he finds no problem with that.

Wow, stool slurry.

Exactly.

I think that's all we need to say.

I guess the one other thing I should say and one of the challenges of looking at intrauterine microbial communities is that especially the embryo transfer catheter is kind of designed not to pick up bacteria. And so characterizing the bacteria in the intrauterine space is really a high risk proposition because I'm not sure how much DNA we're going to find. And when you have samples that have a low amount of DNA, you are as likely to find DNA from your DNA extraction kit, from the environment, from the water, as you are to find real bacteria that were there in the person.

And so one of the things that's very critical in studies like this is having adequate controls so you know which microbes you're finding from the water you're using, from your DNA extraction kit, and which are really there in the samples.

Mm. Yeah. So how do you ensure that you have those controls? How do you differentiate?

Right. So one thing we do is we run every reagent through our DNA extraction. Because often the swabs or the transfer catheter we'll be in a fluid, and so we take some of that fluid that never touched a person and we run that through our DNA extraction.

Other people have advocated putting in a defined group of microbes through your DNA extraction so you know I found all those, good. My process works good. And I found like three other things with those guys that I totally did not put in there. Those aren't real, and if I see them in my samples then those aren't real either.

And so you'll see a lot of studies of the upper reproductive tract that say, we find a ton of pseudomonas in the upper reproductive tract, which might be true, but there's pseudomonas in the water and they don't report their controls. And so I don't know if I believe those results or not. And part of our plan is to really have aggressive and appropriate controls to be sure that if we find something, we know it's real.

So I wanted to go back. We talked about the previous work looking at the microbiome, and I don't remember if you stated this, but I was curious what findings those previous studies had shown and was there anything that led you to think, OK, maybe this is an area worth further exploration?

Right. So the first thing to know going into this is that humans are the only species on the planet that we've looked at with the lactobacillus dominant vaginal microbial community. Not monkeys, not chimpanzees, not our closest relatives. We are it, which suggests to me that it is evolutionarily linked to reproduction because otherwise, why would we have selected for that?

And the studies that have been done before-- so the one culture based study which was done in an IVF clinic, when they cultured streptococcus from either the vagina or from the embryo transfer catheter, the live birth rate in those cycles was 6%. When they cultured lactobacillus from the transfer catheter, the live birth rate was closer to 80%, which is a really dramatic difference. That was a study of 90 people in Seattle, so limited and possibly not generalizable, but very provocative.

And then the Italian study which did this endometrial biopsy in the cycle before the IVF cycle-- so it's a little bit temporally different than at the time you put in the embryo. But when they found a lactobacillus-dominant community in the endometrium, those women were more likely to go on to have pregnancy implantation than the women with no lactobacillus dominance.

And so this combined with the fact that when people have a lactobacillus-dominant vaginal community, we know there's less inflammation. There's better pregnancy outcomes. It suggests that lactobacillus may be the key to not just do you deliver at term or pre-term, but do you get pregnant at all.

So you mentioned a couple of the challenges of just the study-- how you're conducting it. But what are some specific challenges of conducting this study in an IVF clinic?

So the first challenge to being in an IVF clinic is that patients are overwhelmed. It's a lot of new information to get, especially when you think about going into IVF. You're going to have to give yourself injections, there's this whole procedure. It's incredibly overwhelming. So adding on additional procedures or information I think is challenging, and especially because we're asking people to do a stool collection, which to me is, as I said, a barrier on top of what they're already doing. So that's one.

Number two is that as in vitro fertilization techniques have evolved, there's a little more nuance, and so not every cycle is the same. And so when we're comparing people, I think trying to get as similar cycles as possible is our goal, but what we've found as we've started enrolling people is it's really limiting our pool of participants, and so we're trying to think about whether we should broaden our inclusion criteria.

So we initially planned to just do fresh cycles, which is where you retrieved the ovaries, and then you create the embryos, and you put that embryo back five days later. It's like all in real time. That is not always possible, and certainly for people who are doing genetic diagnosis or who have any other kind of issues, often those eggs will be retrieved, the embryos created, and then they're all frozen. And then--

Right. So--

--a couple months later, you prepare the endometrium and put the embryo back.

Yeah. So you have that difference, like some eggs are frozen, some are--

Correct.

And so that could possibly affect the--

Correct. And so initially, we were not planning to include frozen cycles, but people are more and more going to more frozen cycles because they're getting better success rates with those. And so that is one of the challenges. Just the IVF technology is evolving even under our feet as we're doing this study.

I think you mentioned a few of the issues with looking at the microbiome, like differentiating between bacteria that are just there and what's actually coming out of the samples. Are there any other things you want to say about that?

One challenge, looking at the microbiome in these cases, is that idiopathic infertility is a bucket of things we don't know, and it may not be all the same thing that we don't know for all the people. And so some of those folks might have a microbial problem and some might have something totally different.

And so although we calculated our sample size based on the power to detect differences in microbes, if only 10 of the people in our idiopathic infertility group really have a microbial problem, we might not be able to detect that. It might take a much larger number of people. But in the absence of any data, we're sort of starting small.

The second problem, especially for a gut microbiome-- and we're including this stool sample to get a sense for are people with infertility-- or certain types of infertility-- or people who go on to achieve pregnancy, yes, no? Do they have broad differences in the microbiome? Is everything about their microbiome different, or is it just the reproductive tract?

But the gut is a very diverse community. A healthy gut is diverse and wonderful, but that's a lot of different things to compare. And so with a small sample size, we may not be able to see a difference even if it's there, again, just because there's so much going on, especially in the gut microbiome.

So with the-- like you said, the diversity of microbes in the gut, how do you overcome that? Is it a sample size question? You just get 1,000 people, 10,000 people? Or how are you working on overcoming that in your study?

So in our study, again, we're trying to have what we hope are two very clear phenotypic groups. And we'll be looking both at sort of broad community diversity in the gut microbiome, but also to find individual microbes that might be different between these two groups. And if we don't see a difference, I think it's very plausible to say that it is a reproductive tract issue.

That said, Dr. Quan, my collaborator, is also involved in studies comparing rectal and vaginal microbiome and looking at associations. So I think we'll be able to pull data from those studies and say, oh, possibly this gut colonizer predicts this phenotype in the vagina. And so other data will help us understand what we should be doing in fertility studies.

The two goals of your study are to improve IVF success rates and identify how the microbiome affects fertility. How do you see these two questions informing one another?

IVF success rates certainly get at one piece of achieving pregnancy, which is pregnancy implantation-- for the most part. Because you get an embryo-- I mean, everything else happens in a dish. Creating the embryo, you pick the best egg, the best sperm. And so pregnancy implantation is what we're looking at with IVF.

And for many people, either those who have recurrent miscarriage or folks who can't get pregnant on their own, the same microbial pathways may inform folks who have normal cycles and just can't get pregnant. So we think they're ovulating, we think everything is working fine, but just something isn't connecting. And so I think IVF is sort of a microcosm and allows you to focus on one particular piece of the achieving pregnancy cycle, and probably the piece that we understand the least well.

Is there anything else that you think is important for people to understand about this study?

It is astonishing to understand how little we know about how people become pregnant, this very fundamental, biologic thing. And the fact that, again, humans are the only ones with these lactobacillus-dominant vaginal communities I think suggests this very important influence on reproduction that we really don't understand.

And one of the big questions in the whole microbiome field is are microbes a marker of what's going on, or an effector of what's going on? Are they just showing us the people who are lucky, or are they creating that luck for the people? And if they're the creators of good outcomes, then microbial interventions are the wave of the future. And if not, then we should be looking for the underlying cause. So I think that's really the crux of what we're trying to get at, is should we be focusing on the microbiome or should we be focusing on the host? And that really defines the future research agenda for this field.

Great. Well Dr. Mitchell, thank you very much. It was a pleasure to have this conversation with you.

Thanks so much for having me.

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Next time on ThinkResearch--

On the one hand, we are lucky in the US because we have this amazingly bountiful food system. On the other, the food in the US is cheap, it's very accessible, it's very unhealthy, and it's strongly marketed to us. And that combination really negatively impacts our health because it causes us to eat too much. And eating too much and poor diet in general is responsible for hundreds of thousands of deaths in the US each year from things like heart disease.

Dr. Sara Bleich talks with us about the food industry and health in America.

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