Transcript

Dr. Melissa McPheeters

Hello and welcome. I'm Melissa McPheeters for the RECOVER Administrative Coordinating center, and I'm the moderator for today's webinar. Welcome to the RECOVER Research Review or the R3 seminar. The goal of this webinar series is to catalyze a shared understanding of the research of the scientific stakeholder community within the RECOVER consortium. It's important for us to note that this seminar series is not intended to provide any clinical guidance, we are really just focused on the scientific research. I want to start by thanking anyone who submitted questions in advance and remind you that if you do have questions during the seminar, do submit them through the Q&A feature in Zoom. After the presentations, we will try to answer as many questions as we can. Any that we don't get to, we'll try to answer later.

We do post an FAQ on our website, recovercovid.org, along with the recording of this presentation. And that will include the questions that came in advance, as well as the questions that come in during the Q&A, that are related to the scientific topic at hand. You'll also find other general information on that website about the RECOVER consortium and initiative, and we really encourage you to take a look at what we have up there. Questions about other scientific topics are also available there, as well as information about future webinars that are coming up, and we hope you'll join us for those as well. Today, our seminar is going to focus on sex specific differences in COVID and PASC, and we have a really impressive lineup of speakers. I want to tell you a little bit about them before we get started.

Dr. Vanessa Jacoby is a professor ... oops, excuse me. I'm sorry, my computer is having trouble. Dr. Vanessa Jacoby is a Professor and Vice Chair of Research for the Department of Obstetrics Gynecology and Reproductive Sciences at UCSF. She's a clinical researcher with a focus on improving care for people with uterine fibroids. And with the onset of COVID-19, she worked with a multidisciplinary group of collaborators to launch the priority study, which is a nationwide study of pregnant people and their newborns with known or suspected COVID-19. She's going to speak to us today about the impact of COVID on gynecologic health.

Dr. Jim Hotailing is an Associate Professor of Surgery and a urologist at the University of Utah. His work focuses on understanding how genova mutation impact male infertility and its linked to poor somatic health, microfluidic sperm sorting and the genetics of erectile dysfunction. He's the current President of the Society for the Study of Male Reproduction, and he also had the first paper showing that COVID does enter the semen in 2020. He's going to speak to us today about male infertility hormones and sexual dysfunction.

Dr. Andrea Edlow is an Associate Professor of Obstetric Gynecology and Reproductive Biology at Harvard Medical School, and a Maternal Fetal Medicine Specialist at Massachusetts General Hospital. There, she also serves as the Director of Obstetric Research. As the head of the Mass General Brigham COVID-19 Pregnancy Biorepository, a tissue and biofluids bank that contains samples from more than 1,200 pregnant and lactating

individuals, Dr. Edlow has investigated the effects of maternal SARS-CoV-2 on the placenta and cord blood immune profile, and how the placental and fetal effects of SARS-CoV-2 are modified by fetal sex. She'll cover sex differences in the placenta and in trans placental antibody transfer in maternal COVID infection.

And finally, we have a terrific discussant with us today to help tie all of this together. Dr. Torri Metz is the Vice Chair for Research and an Associate Professor of Obstetrics and Gynecology at the University of Utah Health. She's the PI for the Utah Center of the Eunice Kennedy Shriver, NICHD Maternal Field Medicine Units Network, and she leads their study examining the effects of COVID-19 on serious maternal morbidity and mortality. We thank all of our speakers for being with us today, and we hope that you get lots out of this. I'm sure you will. Shane, I'm going to hand it back to you to get us started.

Shane Hamstra

Great. Thank you so much, Melissa. First up, we have Dr. Vanessa Jacoby, talking about COVID-19 and gynecological health. Dr. Jacoby, over to you.

Dr. Vanessa Jacoby

Great, thank you so much. I'm really excited to talk about gynecologic health this morning. There are so many important issues in gynecology to consider, and there have really been three pressure points during the pandemic. First, the impact of COVID-19 infection on these issues. Next, the impact of the COVID-19 vaccines on these gynecologic issues. And third, the widening of existing health inequities that have really led to worse health outcomes for Black, Latinx and Native American people.

There's also been some unique issues in gynecologic health that have unfortunately been a perfect storm of problems during the pandemic. And it really starts with menstrual symptoms often being discounted by both clinicians and researchers, as important health issues. And you can see this because menstruation, fertility and other gynecologic health issues were not included in the vaccine trials and not included in most COVID studies. However, these issues are centrally important and critical to most people, and they tend to really impact health behaviors and health decisions like whether or not someone would accept a vaccine. And this has all been in the setting of a significant amplification of misinformation around gynecologic health and other things during the pandemic. And all of this has been deeply impacted by ongoing and worsening racism in all of its forms, that have heightened disparities in gynecologic care and really excluded populations that are most impacted by COVID from critical COVID studies.

So all of this has led to menstruation and fertility being in the news more than I have ever seen it before. Initially, this really came with many people reporting abnormalities in menstruation during the pandemic, during vaccination, reports of worse disparities in access to reproductive health services among Black and Hispanic women, but then also a huge amount of misinformation that has led to fear about receiving COVID vaccines and its

impact on fertility and menstruation. So I'm discuss a few of these issues today. I'm going to try and do some myth busting, but there certainly is a lot more need for research and discussion around these issues, and I appreciate the opportunity to do it.

So let's start some myth busting just about a incorrect assumption about COVID vaccines that was propagated on social media, that receiving a COVID vaccine could have a negative impact on female fertility. So this came from incorrect information about the Spike Protein and Syncytin-1 protein.

The Spike Protein allows the coronavirus to enter cells, and when we receive mRNA vaccines, we develop antibodies to the spike protein. The Syncytin-1 protein is a placental protein that's involved in implantation and normal pregnancy. There was incorrect information that was propagated widely on social media, that if you received the mRNA vaccines, you could develop antibodies to the Syncytin-1 protein that could lead to infertility. This is not correct. The vaccines do not contain MRA for Syncytin-1, you can therefore not develop antibodies against this protein. And the antibodies that are developed against the Spike Protein do not impact Syncytin-1. So there is no biologic basis that this would impact female fertility, and there have been multiple studies that do not show that the vaccines impact female fertility in any negative way.

The second issue is whether SARS-CoV-2, the coronavirus, could enter the reproductive tract. So this comes from a little bit of a misunderstanding about the receptor ACE-2, which is critical for the coronavirus to enter cells. And the ACE-2 receptor is expressed in the uterus, the ovary and the fallopian tubes, but very, very, very low levels. And actually those tissues don't express the ACE receptor with its critical partner, the proteases, which are required to enter cells. So the SARS-CoV-2 virus has been ... many people have looked for it in the reproductive tract and it is not there. It has not been found in vaginal swabs and in cervical swabs and even endometrial biopsies. So we don't believe it's present, nor would it be persistent in the reproductive tract, which is really critical when we think about long COVID and possible mechanisms.

What is true is that the reproductive tract is a very immunologically active tissue. So the vagina, cervix and uterus have a lot of immune activity, there's a lot of antigen presenting cells in these tissues. The endometrium, the lining of the uterus, produces many prostaglandins and cytokines, which are compounds that are also activated and elevated and heightened during COVID infection, as well as vaccine. So when we think about possible biologic mechanisms, I think the immune activity and the reproductive tract becomes really central and core.

Okay. So we're going to discuss the impact of COVID and the pandemic on menstruation, but first, a brief reminder about the menstrual cycle. The regular timing of the menstrual cycle that occurs approximately every 20 days, comes from a very coordinated, rhythmic, hormonal messaging process between the brain and the ovaries, that's really driven by the secretion in a pulsitile way of this hormone called GnRH. And this is a very robust system that happens hundreds of times in someone's lifetime, but it can be disrupted. And it frequently is, about 30% of females will have some type of disruption in their menstrual cycle by the time they reach menopause.

What are the things that can disrupt the menstrual cycle? Many different stressors, including weight change, changes in exercise, sleep, mental health, and acute and chronic illness. Essentially any stressor to the body will increase stress hormones, and those stress hormones can really disrupt that secretion of GnRH and change the menstrual cycle. So before even thinking about the impact of COVID infection or vaccination on menstruation, we really just need to consider the impact of the pandemic itself because many of these factors have been impacted by changes in our life and lifestyle during the pandemic.

This is where many people started reporting online and through social media, changes in the menstrual cycle during the pandemic. And the most common way this has been studied is through online surveys, where people report changes in their menstrual cycle. But there are some significant limitations in online surveys. One is that they're what we call cross sectional studies, so they're just assessing the menstrual cycle at one point in time. We don't have information about the menstrual cycle before the pandemic started, unless we ask people to recall that, and that can be really challenging and biased when you're asked about your menstrual cycle six months ago, eight months ago, 10 months ago. There also tends to be bias in who answers these online surveys. In most of the studies, you'll see they ask about race ethnicity. For instance, the majority of participants are white, which does not reflect accurately, the diverse population of people with COVID and in particular, the populations that have been most impacted by the pandemic and by COVID infection.

Another way to study menstrual changes though, is to get over some of these limitations of the surveys by using data from menstrual apps. And this is a study that's been often cited, that had 18,000 users of a menstrual app, and compared their menstrual cycle before the pandemic, to their menstrual cycle in the first six or seven months after the pandemic. The people who completed the survey, 80% of them had college education or greater, race ethnicity was not assessed, but again, just thinking about the bias of this sample. But this study, where you had pre COVID menstruation data from apps, did not show a clinical difference in menstruation during the pandemic. Cycle length was the same and the duration of the period was the same. So this highlights the importance of having good pre COVID information when you're studying menstruation.

Okay, what about menses menstruation and the impact of COVID infection? I believe we are very early still in our understanding of this. There are actually very few studies and we need a lot more research in this area. I'm going to show you a couple of studies that are the most commonly cited, and I think they're representative of the literature that's out there, and show you some of the limitations of these studies. So the first one that's commonly cited is this study of 177 women who were hospitalized in Wuhan China with COVID. And they asked them about their menses before they were hospitalized with COVID and compared that to their menses during COVID. And they found that about 25% of them had a change in the volume of their bleeding, primarily with decreased bleeding. This was not impacted by whether they had mild or severe disease. And they found that 28% had changes in their cycle length from before they had COVID to when they had COVID. But of note, almost all of these women had complete return to normal menses within two months.

So when this study came out, people felt that perhaps there were some changes in the menstrual cycle with acute infection, that were really transient and would go back to normal quickly. But then the next study that's also often cited is a study in Indonesia of 158 women also hospitalized with COVID. And here they did the same thing, they asked about their periods before COVID, but then they also contacted people three months after the hospital admission. And in this study, they actually found persistent abnormalities in menstrual irregularity, even three months after COVID infection. So this is little bit different to the thought that many people had, that if you had a change in your menstrual cycle, it would resolve quickly.

The challenges with these studies are that there's very limited data. You could see that these sample sizes are quite small. Most of the studies are in hospitalized patients, where the vast majority of people who've had COVID are outpatients and not hospitalized. Most of the studies are not US based populations, with our diverse population that we want to study. And you can see, they have really short term follow up and this question of persistent symptoms is really important. And then we've talked about this recall bias, where if you're asked about your period six months ago, it can be hard to be as accurate as we would like in terms of assessing changes in menstrual cycle. But what about menses and the vaccine? There's actually a lot more data about menstrual changes after vaccination. And I appreciate the NIH has been really attentive in funding some of these studies.

There have been many survey studies about changes in the menstrual cycle after vaccination. And there was just one that came out 10 days ago that got quite a bit of press coverage. So I'm going to just show you the results of this, it's pretty indicative of what had been in the other surveys. This is 39,000 vaccinated people who completed an online survey, and 56% of them reported a change in their menstruation after receiving the vaccine, primarily with heavier bleeding, but also changes in timing. This study was really helpful in identifying a couple of new things. One is that they considered people on hormonal contraception and it looked like the changes in menstrual bleeding after the vaccine were not different by hormonal contraception. And then they also highlighted the high rates of breakthrough bleeding in people who usually do not menstruate, including people on gender affirming hormones, people on long acting reversible contraception like IUDs, and postmenopausal people, 66% reported bleeding after receiving the vaccine.

But this study has some significant limitations, as we've talked about kind of this bias sample of who these, sorry, who the survey, people who would complete surveys online are, and in this study, 84% of them are white. There's also no pre-vaccine data to compare to what the menstrual cycle is like after the vaccine, and there's also no unvaccinated comparison group. So the next study I'm going to show, tried to tackle some of these limitations. The study actually came out earlier this year. And this is a study that uses the menstrual tracking app Natural Cycles again, the same one we looked at as assessing menstruation during the pandemic. But now the study is assessing about 4,000 people who got vaccinated, compared them to unvaccinated people and looked prospectively then at the menstrual cycle in the three months prior to the vaccine, compared to the three months after the vaccine.

And this study did find a difference in people who've been vaccinated versus unvaccinated and the length between their cycles, but the difference is very small. So in people who got vaccinated, had about a 0.64 days, so less than one day difference compared to people who were unvaccinated, after the first cycle, and similarly after the second cycle. So less than a day difference between unvaccinated and vaccinated people in the cycle life. There was a subgroup of people who got both the vaccine doses for the Moderna and the ... for both the mRNA vaccines, who got both doses within the same month. And those people had a little bit higher difference in their menstrual cycle length of about two days.

The mechanism behind this is thought to be related to the stress induced by receiving the vaccine, which can raise your inflammatory response, your stress hormones, and then impact that pulsitile secretion of GnRH that I referred to earlier. So I think this study has some significant benefits over the one time survey study, but it still has limitations that we've talked about, in regards to who uses these apps. This study did a lot of missing data on race, ethnicity, and also this study of importance only included people who had normal time cycles before they got the vaccine. It's possible that people who have abnormal cycles before the vaccine, might have more abnormalities after receiving the vaccine.

Okay, just going to shift to a few other topics. One is the impact of COVID on early pregnancy. And there is now a fairly large literature on this, and most of the studies have found no increased risk of miscarriage or early pregnancy loss in people who are infected with COVID. This is a study that we did using the priority study data, which is a nationwide study of 1,300 people who were pregnant with COVID. And in our study, we found about 6% of the people had pregnancy loss before 20 weeks, in both the group who had COVID and the group who did not have COVID. So this was reassuring in the sense that it's not higher than the 10% rate of loss that you see in most studies of clinically recognized pregnancies. And this is really consistent with other studies in this area.

And then just briefly, because I know we're going to hear about male fertility later, a lot of people ask about the impact of COVID on female infertility. And I will just summarize to say that there is an existing literature and it is growing month by month, that really is reassuring in terms of female fertility. Studies have found no changes in AMH, FSH, Estradiol, so the function of the ovary and ovarian reserve, there seems to be no impact on the ability to get pregnant naturally or without assistance, and no impact on IVF outcomes. So this seems to be quite reassuring in terms of the lack of impact of COVID infection on female fertility.

Okay, so where should we go from here? What are the key questions we need to focus on in gynecologic health? There's very little information on long term symptoms, which is what we're studying in RECOVER, in terms of long COVID or post-acute sequelae of SARS-CoV-2. And I think we really need to focus on questions that are most important to our patients who are impacted by persistent symptoms. This is a really great study that was developed and implemented by the patient led research collaborative. And in this study they asked about 3,700 people who had persistent symptoms after COVID, what symptoms they were having. And you can see that

menstruation, urinary symptoms, bladder symptoms, and sexual function were really highlighted as critical issues that we need to focus on.

So how will we do this in RECOVER? I'm really excited about RECOVER, I think we're going to be able to answer some really key questions that are important to our patients and to the research community, about the long term impacts of COVID. We'll be able to address whether there are sex and gender differences in the risk of long COVID. There are some early studies that have found that women have much higher rates of persistent symptoms and we'll be able to really dig into this in RECOVER and understand mechanisms behind this. RECOVER participants are asked to answer questions about a wide range of topics in gynecologic health, that have been left out of other studies, including sexual function, menopause symptoms, urinary symptoms, and others that are really critical to help us improve care.

And I think the design of RECOVER has some big strengths that you can see our limitations in the existing research. One is that we have a really large sample, we'll have approximately 10,000 females in RECOVER when we meet our target population, it will be a really diverse US population, and we'll have long-term longitudinal follow up. So we'll really be able to follow people over time to understand changes that occur in their health. And I really think this will have a positive impact on gynecologic care. And I'm very glad that the NIH is supporting this effort. Look forward to hearing your questions. Thanks.

Dr. Jim Hotailing

We published the first paper that showed that essentially COVID does not get into to semen. So I clearly have a strong opinion here. A lot of people have said this is sort of sperm apocalypse, I clearly don't agree with that. Here are my disclosures, none are relevant to this work. So in thinking about this, what other viruses have an impact on infertility in men? Obviously Zika, Ebola, HIV, Mumps, and a lot of this is thought to potentially be due to either orchitis, the virus specifically getting into the testis, which as most of you know, there is the blood testis barrier, it is a privileged environment due to potential antigenicity with sperm. So orchitis is one thing, another potential mechanism is fever, which could also cause issues.

So one way to ask this is to look at the past, what's the closest thing we have to the pandemic? Well it's probably the 1918 flu, so what happened to birth rates during that? Essentially right around the time of influenza, they maybe went down a little bit, but they went up significantly nine months after that. So you could take away that people really like to have sex during quarantine, is one way to view that. And certainly clinically, we've seen our business increase about 350 to 400% since the pandemic started. I think it's just caused people to kind of reevaluate their priorities and put a real emphasis on quality of life.

So what's the impact of SARS-CoV-2 SARS on sexual health? We got to kind of back up and think about what do we need to know about male reproduction before answering this question? And there is a lot unknown, we're probably 20 years behind where my OB GYN colleagues are, in understanding the male side, as compared to

the female side, we're way behind. And what are the limitations really stopping us from answering this? These are the things we need to think about. So what is the impact of SARS-CoV-2 on sexual health? Well, how do you define sexual health and reproduction? There's the long term effects, potential effects on offspring, which most people, including myself, view as the most scary. I don't think we need to be afraid, but that is something that really concerns people. There's fertility, hormones, and then there's erections, and I'm going to kind of try to go through rapidly, the data on each of these.

So first of all, does SARS-CoV-2 cause male infertility? The media have one answer, but I would say science and medicine has a more nuanced one that maybe sometimes we have glossed over a little bit. One thing you need to remember is that correlation does not equal causation, just because there's some correlation doesn't mean it's necessarily causative. And then also that we can't lot all disease together, mild COVID is not the same as severe COVID. There's a huge number of people who are asymptomatic or have very mild disease. My whole family got COVID several weeks ago and I sort of was sneezing a little bit, but was still biking and really wasn't that sick, but there's other people who are in the ICU, severe fevers, multi-organ failure. And I would submit to you that anybody who's in the ICU and gets that critically ill, is going to have a significant impact on their reproductive ability.

Most men are making about a thousand sperm, a second, it's the highest throughput process in the human body. So anytime people get that sick, it can have a significant impact. So one of the keys here is the largest study ever done found no SARS-CoV-2 in semen, that was done in 120 patients. So that would indicate that it's probably not there. Our study, which was the first one that came out in, seems like a million years ago, in the spring of 2020, found, in a cohort of patients from Wuhan, found no evidence of SARS-CoV-2 in the semen. Only two, and I'll review all the literature, but there's really only two studies, which I think are methodologically flawed, showed SARS-CoV-2 in semen, and I'll explain why I do think they're methodologically flawed. So I'll go pretty quickly through this because my colleague covered a lot of this.

And interestingly, there's a lot of parallels on the male and female side. And the take homes are somewhat the same in that we don't think there's a lot of expression of the specific things needed for SARS-CoV-2 to fully get into the reproductive tract on the male side. And the key is you need ACE-2 and TMPRSS2, and the real key on the male side is they have to be co-expressed in the same cells. So you really need both of those to fully unlock the virus getting into things. And we were really fortunate in that we had a bunch of sort of complex single cell RNA sequencing data on human testis from our partnership with categoric organ donors from our transplant program, that allowed us to answer this pretty well.

Well, I don't have to tell this group that COVID is a major problem, huge number of global deaths. I actually looked it up yesterday, there's now over a million documented US cases, 1.03 million and roughly 6.4 million worldwide, and obviously there's a huge number of undocumented cases. And I think you've got to keep in mind that these undocumented cases are out there and are really quite common, in order to think about what the

impact on offspring might be, because we don't have perfect longitudinal data on this. So I think perhaps in viewing this, when you look at COVID, about 80% of disease is mild or moderate, 14% is severe and 5% are critical, and it's estimated, it's not totally known, that 15 to 50% are asymptomatic. And certainly with the advent of home testing, a lot of that isn't even reported.

If you extrapolate this out, there may have been over a billion infections and over 800 million of those were mild to moderate. These numbers are a little bit old, but one in 4.2 are reported to have COVID in the US, and estimated that there have been over 120 million infections. So you would think that if COVID was having a major impact on offspring, you would see that in the data. So when you look at the data, what do you see? This is the CDC infant mortality report. And keeping in mind that maybe 60 to 80% of the US has had COVID, you don't see a significant impact on offspring health, at least early on. It's possible that there will be later effects, but that I don't think that's super likely. So that brings us to another question, does SARS-CoV-2 get into the semen? And to answer this, we really have to kind of stepwise walk through the data.

So first of all, you need to understand the biology of the testis and you have to have access to clinical samples. And this really gets at the issue of causation, of understanding how the virus might get in to the male reproductive tract. Either one of these questions in isolation will not really fully allow you to answer this, because you also need access to clinical samples to assess what's going on. So our research group, this guy, Brad Cairns and Jingtao are both a lot smarter than me, but we've spent about a decade doing this single cell sequencing. We now have over 175 pairs of human testes from age three months to 70, which is never something I thought I would say, but such is the life of a urologist, I guess. We also have this very extensive database with transgender samples and other things and other tissue banks.

And this research program has kind of yielded a lot of insights into germ cell development across the lifespan, infancy, puberty, and we have data spanning the entire lifespan here. So what's the relevance of this single cell RNA sequencing to COVID? Well we have this really big repository, and I think that's critical to understand sexual health and particularly the impact of COVID on it on a cellular level. Our sort of hypothesis was that this data would allow us to determine whether TMPRSS2 or ACE-2 were expressed in the same cells. And the key thing is that you can have expression of both of them in the testis, but if they're not expressed in the same cell, it's difficult or impossible for the virus to really get in there. So when you delve into this to try to look at the question of whether these things are co-expressed, we found no evidence of this. And we looked at the single cell sequencing data, and then we looked at this cohort from Wuhan, which I'll get into in a second.

So the evidence would indicate no. And this is a very fancy complicated plot, but really what the key is here, these are all the different cells sort of computationally reconstructed from spermatogenesis [inaudible 00:36:09] cells, spermatogeny stem cells, and sperm. And what you see is that there are those colored dots showing ACE-2 and TMPRSS2, do show the expression of those different receptors in those cells, based on mRNA

sequencing data. But what you see here is when you look at how many cells co-express those, it's essentially almost zero and nothing that would even be remotely significant.

So this is other data that basically showed the same thing, and this may have been some of the data that was informed by Dr. Jacoby's talk, it was a fertility and sterility paper showing that there was an expression of these in the male or female reproductive tract. So this is more evidence pointing at essentially the same thing. They also looked at BSG and CTSL, which are two other receptors that may mediate viral entry, and essentially found that it's unlikely that these cells have the receptors, which may be the reason that we don't see a big effect on offspring or see the virus getting into the semen.

So speaking of that, this was our paper and we looked at 34 patients from Wuhan, through sort of the unique collaboration. We did not find COVID in any of them. So these are patients with mild to moderate illness and on average, they were 31 days out. Interestingly, we did find about 18% had orchitis, and why exactly that is, we don't know, it's possible that COVID may cause a cytokine inflammatory response that causes some swelling. This was self-reported, so it's not perfect data, but at the time it was kind of the best that we had. So we found no evidence in this pan et al paper. This other paper looked at clinical characteristics and results of semen tests among men with COVID disease in 2019, right when the virus kind of came out. And what you see here is that they looked at 50 patients, up to 12 of them died, so these patients were super sick.

And some of them, from what we can tell, were actually doing the collection in the ICU or in the hospital, which this is sort of the height of the pandemic when it started in Wuhan, so it's certainly possible that there could be contamination there. Six were positive for the virus, and four of those were in the hospital, all of these were much sicker. So it's possible that COVID gets into every single organ system when patients are super, critically ill, but most of those patients aren't going to be trying to have a kid when they're in the ICU. So they found it in six, really of 38 patients because 12 patients died and they couldn't fully assess.

So this is another paper and probably the best data that we have on this. This looked at patients who had mild disease and then moderate disease and also looked at controls. They found no COVID in the semen. 25% of the moderate patients had some testicle pain, again, consistent with orchitis. And really when you look at everything here, when you look at these numbers and this group maybe isn't as commonly looking at semen analysis, but essentially what they found is men with moderate symptoms have worse sperm count concentration, and total motile count right after infection, which feds the question, hey, Dr. Hotailing, you just spent 10 minutes telling us how this isn't a big deal, what's going on?

Well, one thing is is this an effect of the fever? It's well known in male infertility that anyone who gets a fever, their sperm counts go down for 74 days, which is the life cycle of spermatogenesis. And this says nothing about the long term implications. So what's the mechanism of this decreased total motile count? And when you look at it you certainly see here that the total motile count are the numbers of sperm that are moving, are way lower in patients who had a fever. So I would submit that maybe that's what the issue is. This is another paper that

I think really put kind of the nail in the coffin, in whether this is an issue. It was out of Germany, I believe, it's out of Europe, I think it was Germany, I may misremembering there. But these looked at a lot of patients, they looked at a number of patients at different time points after COVID. And this is just sort of your standard demographic table one.

And what they found here, which was quite interesting, is that they really didn't see a long term difference. And they looked at 63 plus days, the percent of patients who had abnormal semen parameters was 2%. So again, very, very low numbers and actually about consistent with what you'd find in the general population. The total sperm count of these patients was 131 million, which [inaudible 00:41:42] semen analysis for my patients, I'd give that like an A minus, it's excellent. The total motile count that was less than 40, was again, only 2.7%, really, really low. The DNA fragmentation, which is another metric of damage to sperm, was low, normal's less than 15%.

But again, only 2% of this cohort was hospitalized, so it's totally possible that in super severe disease, you do see a worse profile, but this data is very reassuring overall. And the key thing is none of these patients had SARS-CoV-2 RNA in their sperm. So when you start putting this all together you see that there is a ... these are really the two studies that showed that some of these men had COVID, the Machado study, some of these samples were shipped to lab, one in 15 had COVID, I think it's certainly that that's from contamination. And then the other one we already talked about in detail, with some of the issues there. So all these other studies showed no COVID in sperm, or semen, excuse me.

So when you sort of add all these up, to me, this is really reassuring and it shows that it's probably not getting into semen, although we haven't done the perfect study to look at that. These studies that showed in four patients of 181, when you put all this data together, could be due to contamination, COVID severity, these also were all from the same study. And when you look at the ones who recovered, it was three or 436, which certainly could indicate contamination. So when we look at other things, you look at orchitis SARS, which is kind of a cousin of the virus we're currently dealing with, it did cause orchitis, and you can see changes in pathology specimens of testis tissue. And it is that some of these patients do have testicular swelling, does this mean that there's long term damage to the testis? I think if there was long-term damage, we would expect some kind of global fertility crisis by now, or global hormone crisis.

Speaking of hormones, when we look at kind of what's going on here, it's really hard to interpret the data, as any systemic illness will kind of attack your male reproductive access temporarily. To really do this, you need to look at pre and post COVID hormone levels in symptomatic and asymptomatic patients, and as far as I'm aware, nobody's done that. The best study we have is looking at men who've had COVID and then controls. And they basically found that testosterone didn't change, FSAs didn't change, LH went up slightly and LH kind of drives testicular function or testosterone production in the sperm, and they found some changes in the T LH ratio and the FSHL LH ratio, but these are very reassuring overall. Back to the question of does COVID impact reproductive health, offspring, infant mortality is sort of decreasing or staying the same, does it get into semen?

The high quality data would say no. Is it in the testis? Possibly in severe disease, but does not appear to have a sustained impact on hormone levels. And in really severe disease, it made [inaudible 00:45:44] everywhere. So we'll close by talking about erectile dysfunction. This paper was all over the media and the media's conclusion was COVID causes erectile dysfunction. This is basically a study out of Miami that looked at penile tissue collected during penile prosthesis surgery and control, and a couple patients who'd had COVID. This is really two patients who had end stage erectile dysfunction at baseline, and two controls, sorry, and they did find potential evidence of COVID here, although I would argue that the analysis of this was suboptimal. But this kind of illustrate some of the issues, would you recommend a definitive treatment to your patients based on an N of two? Another study that looked at this, is this is from the New York Times, can COVID lead to impotence?

This was a registry data by the same author, they found that COVID was associated with an increased risk of ED. The problem with that is that all the risk factors that give you more likely to have COVID, metabolic syndrome, everything else, also they give you erectile dysfunction, and they didn't really list how they did the regression analysis. So if COVID is associated with ED, is it causal? This is a paper we're working on submitting, and essentially what you see here, it looks like COVID may be associated with that. However, in 137,000 men from a claims database that were aged matched, you see that COVID has a risk of ED. However, you look at cardiovascular disease, that should be associated with Ed and you don't see any difference there, which begs the question, is could these analyses be due to confounding?

And finally, I'll end with kind of just a word of caution. There have been 114 papers on Tova that have been retracted. And this was an article we wrote that really just suggested that we need to have high quality research, especially because it's critical to maintain the public's trust in kind of what we're doing. So we need to be cautious with what we're publishing. And then this is just the conclusion I presented before, and I'll stop sharing my screen. And thanks for giving me the opportunity to talk here, hopefully I've convinced you that this is not sperm apocalypse.

Dr. Andrea Edlow

This is maybe a little bit of a different focus, in that this is kind of a deep dive on some collaborative research that our lab did with some of our collaborators, and looking at sex differences in the placental response to maternal SARS-CoV-2 and implications for both fetal development and antibody mediated protection. So I'm a consultant for Mirvie and I also receive funding from Merck Pharmaceuticals, to investigate COVID vaccination in pregnancy, but both of those are outside of the presented work.

A brief overview, I'm going to cover first, the placental interferon stimulated gene response to viruses, and then look at both our results and those of others in sex differences in immunity and placental innate immunity specifically, and then end by talking a little bit about transplacental antibody transfer to the fetus and neonate in SARS-CoV-2 infection, and sex differences in that regard and some future directions for this work. So interferon

signaling is a key aspect of the placental immune response to viral infection. And in other viruses, especially CMV and Zika, type one and type three interferon responses have been demonstrated to play an important role in placental defense against viruses and other pathogens.

But work from Akiko Iwasaki's lab and others, has demonstrated importantly that when these interferon signaling responses become deranged, that this can actually lead to harm. So it's sort of a very fine balance between protecting the placenta against infection, but then when these interferon signaling responses are upregulated to too great of an extent, in this mouse model of Zika virus infection, for example, they showed that excessive type one interferon signaling that was induced by Zika virus infection during pregnancy, led to sort of the mouse equivalent of miscarriage, stillbirth pregnancy loss, and severely disrupted placental function and fetal development. So it's sort of a fine line

As far as what we know about interferon signaling in COVID, a lot of the initial work was really a non-pregnant population, showing that part of COVID pathogenesis involves dysregulated interferon signaling pathways. And we also know that there are sex differences in immunity across the lifespan. This is a figure from one of my favorite reviews by Sabra Klein and her colleague, and just kind of looking at sex differences in both innate and adaptive immunity across the lifespan. And sex differences in the incidence and severity of respiratory viruses have also been well-documented. And so there is sort of a good rationale for thinking that there could be sex differences when mothers are carrying a male, versus a female fetus, in the setting of this new or new at the time that we started this research, virus, SARS-CoV-2. We also rapidly learned that males are more vulnerable to severe COVID-19 infection, with higher morbidity and mortality in men than women.

This also extends down to boys, with boys being demonstrated in meta analyses to have greater odds of ICU admission and severe COVID-19 illness. MIS-C, a very severe form of COVID in the pediatric population, being more common in boys, and even morbidity and mortality in infants, which is already greater than it is in other age groups, kind of being dominated by male, infant morbidity and mortality. And we know that male infants in general are more prone to mortality in the neonatal period, than females, for complex reasons, but whether there's something unique about SARS-CoV-2 in this regard, still remains unclear. And finally, some interesting work by Akiko Iwasaki's lab, also demonstrated differences in innate immune response to COVID by sex, with males having higher plasma levels of innate immune cytokines, and more robust induction of non-classical monocytes, and females having more robust T-cell activation. And finally higher levels of innate immune cytokines were associated with worse disease in females only.

So the rationale for the set of experiments that I'm going to show you today was that placental interferon signaling may play a key role in SARS-CoV-2 viral control at the level of the maternal fetal interface, but also we know that dysregulation in other viruses in these signaling responses, can lead to pathogenicity and adverse pregnancy outcomes. We know that COVID-19 disease is more severe in males across the lifespan, but does this kind of affect extend to mothers carrying a male fetus? And we also looked at sex differences outside of pregnancy

and saw sex differences in interferon signaling in COVID-19 disease. So how does this come to bear on pregnancy? And as a maternal fetal medicine specialist and a physician scientist, that sex differences have been of interest to my lab for several years, even predating COVID and looking at sex differences in the placenta.

So this was the experimental design, we had 68 mother infant dyads, including 38 dyads where the mother was positive for SARS-CoV-2. So an important thing to note that is that in all these dyads, the placenta and the neonates were not infected with SARS-CoV-2, only the mother. And this is typical, and just due to time constraints, I can't show all the data, but it's been well studied that placental infection and vertical transmission are relatively rare, vertical transmission on the order of one to 3% in maternal SARS-CoV-2 infection. So this doesn't represent a sort of anomalous sampling, this is sort of the typical case that the mother will be infected, but the neonate or fetus will not be infected to the best of our ability to tell. So of these 38 dyads where the mother was positive for SARS-CoV-2 in pregnancy, we had 19 mothers carrying a female fetus and 19 carrying a male fetus.

And then we had 30 SARS-CoV-2 negative controls, and these were controls enrolled from early in the pandemic, where we felt confident both by their report, the fact that we were doing universal testing on anyone with symptoms, and the fact that we confirmed this with anti N IgG antibody, that none of these controls had been infected prior with SARS-CoV-2. And again, the controls were 30 dyads, 15 carrying a female fetus, 15 carrying a male fetus, and there were no significant differences between the male and female groups, with respect to the gestational age at COVID-19 diagnosis or the gestational age at delivery. These were largely term deliveries and third trimester infections. There was equal exposure to labor between all groups. And there were no significant differences between groups with respect to maternal comorbidities, such as obesity or hypertensive disorders of pregnancy, chronic hypertension, diabetes, and so on. And there were no significant differences between groups with respect to COVID-19 severity or the time from diagnosis to delivery.

So first of all, I mentioned, no placentas were infected with SARS-CoV-2, and this was an early report of 68 cases from our COVID pregnancy bio repository, and also bolstered by additional cases from a third academic medical center in Boston, and in collaboration with Jonathan Lee at Brigham and Women's Hospital, and Drucilla Roberts, a placental pathologist at MGH, we were able to demonstrate using both viral loads from placental homogenates, as well as placental staining with RNA in situ hybridization, that there were no infected placenta and that the viral loads were limited to detection in maternal respiratory secretions.

So the initial results that I wanted to cover from this sex differences in placental innate immune response, this was work that was led by two postdoctoral fellows in the lab. Lydia Shook and Evan Bordt, and they're now gone on to become independent PIs, which is great, but this work started a couple years ago. So we looked at interferon stimulated genes in the type one and type three interferon signaling pathways primarily, and we observed a sexually dimorphic response of these genes to maternal SARS-CoV-2 infections. So just to orient you to all the figures that follow, the orange will be SARS-CoV-2 positive mothers, and then male or female refers to the sex of the fetus, and therefore also kind of the sex of the placenta, and then the blue is SARS-CoV-2 negative

mothers. And what we saw is that in response to maternal SARS-CoV-2 infection, males were significantly upregulating interferon stimulated genes in response to maternal infection and relative to matched male control placentas, while females were actually down regulating interferon stimulated genes.

And one thing you might notice is first of all, the relative quantity of these interferon stimulated genes was higher in male compared to female placentas that were exposed to maternal SARS-CoV-2 infection in utero. And the second important point, that was very interesting that we want to drill down more in future work, is that we saw that sort of the interferon stimulated gene tenicity or expression in the placenta in general, was higher in female control placenta than in males. And this is part of what really drove the sexual dimorphism, and we think this could have potential interesting implications for understanding more about male versus female vulnerability in utero, especially to various maternal insults that can be inflammatory and understanding more about potentially placental protective factors here. But again, we don't know if interferon stimulated gene upregulation is helpful or harmful, and it's probably a balance.

And this work is not designed to kind of answer that question about what are the longer term outcomes of these offspring, but that is a very important direction for future work and work that we're carrying out now in various ways. So Hofbauer cells, which are fetal placental resident tissue macrophages, are a cell type of interest to our lab for several years, even predating the pandemic, but Hofbauer cells are of particular interest in SARS-CoV-2 infection because they were implicated in the vertical transmission of Zika virus and cytomegalovirus in particular, and so we were interested to see what was happening with Hofbauer cells in maternal SARS-CoV-2 infection. And we found increased density of placental Hofbauer cells in male placentas exposed to maternal SARS-CoV-2 infection on quantitative histology, and this finding was primarily driven by males and the female differences really didn't achieve significance.

So when we broke it out by sex, we saw that this finding was really driven by male placentas. Again, the long term implications are not clear, but we do see that this sort of fetal innate immune response in the placenta is different in male exposed placentas. Interestingly and relevant to some of the work that Dr. Jacoby presented, we didn't actually see differences in classic pro-inflammatory cytokines in the placentas of mothers exposed to SARS-CoV-2 in pregnancy, compared to controls. So sort of the classic actors that you might think of TNF alpha, IL-6, Interlukin-1 beta and so on, we didn't see significant sex differences in this regard, nor did we see a significant impact of maternal COVID infection. So it was sort of these interferon signaling was affected, but not these kind of classic pro-inflammatory cytokines.

So the second part of the story is really looking at trans placental antibody transfer. And these two parts may be more linked than it immediately seems on the face of it. In some ways, interferon stimulated genes and placental inflammation can impact transplacental antibody transfer through the up or down regulation of FC receptors on the placenta, which are responsible for shuttling across maternal IgG. So we know that placental antibody transfer is a very complex function that is dependent on FC receptor mediated transport, as well as

maternal antibody titers. And maternal titers are directly correlated with the level of inflammation in COVID-19, which is something that might be important to think about in some of the results that I'm going to present later about sex differences. But you can see that maternal IgG starts being shuttled across the maternal fetal interface at the level of the syncytiotrophoblast and FC receptors there, which are endocytosed and then kind of spit out into the villi stroma where Hofbauer cells will shuttle them across to the fetal vascular endothelium, where again, the IgG will bind to FC receptors and then be endocytosed and transported to the fetal circulation.

We know from work in chronic HIV cohorts, as well as other maternal viral infections that are chronic, that maternal viral infection does impact transplacental antibody transfer of vaccinatable pathogens and FC receptor expression can be impacted by interferon signaling, and this has been demonstrated in other tissues, not necessarily the placenta. And the last thing to remember in interpreting our results is that typically for vaccinatable pathogens like influenza or pertussis or measles, mumps, rubella, cord, maternal ratios of 1.5 or greater are usual, meaning that the titer of IgG in neonatal circulation or in cord blood is 150% of what it is in maternal blood. And so the placenta very efficiently concentrates these IgGs on the fetal side and that's usual.

So this paper drilled down on an earlier finding from the JAMA Network open paper that I showed before, where we just did kind of simple [inaudible 01:03:00] in a first group of 68 individuals and showed that transplacental transfer of maternal IgG was about half of what it was for influenza directed IgG, and this was in the exact same placenta. So the placentas from COVID infected mothers were transferring HA directed IgG very efficiently, but they were not transferring anti COVID antibodies efficiently. And so we wanted to understand more from a mechanistic level, why was this going on? So we collaborated here with Galit Altars Lab at the Reagan Institute, and she has a platform called system serology, that looks at the transfer of many different antibodies directed against numerous antigens. And here we're showing influenza or hemoglutinin and pertactin PTN, which is anti pertussis antibody.

And so these are dyad plots, so mother is M, cord is C and the COVID negative dyads in here, really refers to the mother's COVID status, are in orange and the COVID positive dyads are in pink. And you can see that whether it's a placenta from a COVID infected mother or an uninfected mother, these placentas are transferring influenza and pertactin and directed antibodies pretty efficiently, either concentrating antibody on the fetal side, as we would expect, or at least at about 100%. But in contrast, the transfer of antibodies directed against COVID, we again saw in a separate cohort, that antibodies directed against various aspects of SARS-CoV-2, whether it's receptor binding, domain spike, or nucleocapsid, all went down on the cord side, relative to maternal. And this is really unexpected and really not what we were seeing for any of the other vaccine directed antibodies.

So in order to understand more about, it doesn't seem like it's the placenta itself that there's a problem with, what is different about these anti COVID antibodies? We did some glycosylation profiling, which is really looking at the sugar attachments on the straight portion or the FC portion of the antibody. And we saw that antibodies generated during acute maternal COVID infection were much more highly glycosylated than the

antibodies that the mothers had in circulation from receiving influenza vaccines, or receiving pertussis vaccines in pregnancy, and this representative substantial difference. And then we also looked at placental receptor expression patterns, and we saw that placentas in the setting of maternal SARS-CoV-2 infection were upregulating non-canonical FC receptors like FC gamma receptor 3A and also co localizing this receptor more with the neonatal FC receptor, FcRn. And this combination of receptor expression and co localization is thought to occur to maximize the transfer of the most effective maternal IgG to the neonate, which is natural killer cell activating antibodies, but FC gamma R3 does not transfer highly glycosylated antibodies well.

So maybe this placental compensatory response, trying to upregulate receptors to transfer the antibody over, was not actually able to accommodate the glycosylation profile of these particular antibodies, which sort of had an inflamed profile. So in the science translational medicine paper, what we then wanted to do was drill down more on sex differences in this regard. And so we wanted to look at, do the maternal antibody titers directed against various aspects of the SARS-CoV-2 virus, do they differ whether the mother's carrying a male or female fetus, and we were kind of surprised to see that the mothers carrying a male fetus themselves had significantly lower anti SARS-CoV-2 directed IgG titers than those carrying a female fetus. And we did not see this difference between carrying a male and female fetus with respect to anti influenza or anti pertussis directed antibodies, whether it was in maternal COVID infection or in controls.

We also wanted to look at FC receptor expression in the placenta, and interestingly, we saw patterns of FC receptor expression at both the gene and protein level. And all of this gene expression was confirmed with Western blotting, I just haven't had space to show it in every slide. But we saw similarly kind of sexually dimorphic patterns of FC receptor placentas in the SARS-CoV-2 exposed placentas, with males upregulating these FC receptors and females down regulating them relative to sex matched controls. And here's some histology kind of demonstrating the co localization of FC gamma receptor three and neonatal FC receptor, which again, you can see here from our quantitative histology, showed this sexually dimorphic pattern where male placentas of mothers with SARS-CoV-2 infection had significantly increased co localization of these two receptor types, which again are co localized to try to transfer these natural killer cell activating antibodies, which arm the fetus with the most protection at birth. But because potentially of the glycosylation profile on these antibodies, this pattern of expression, actually wasn't advantageous to transfer.

And not surprisingly ... I'm just going to look at the time because I want to leave time for questions. Oops, okay, I'll hurry. So not surprisingly because the maternal titers were so much lower, we did see significantly reduced transfer of anti COVID antibodies to the male fetus relative to the female. And again, we didn't see this in SARS-CoV-2 negative or positive dyads for these non COVID specific antibodies. And so again, this was a hint that maybe glycans at least have something to do with it, and we did see sex differences in the glycan profiling. Here, we have the female glycan profiling and I don't want to get into what bisected glycans are versus glycosylated or [inaudible 01:09:18] oscillated antibodies and so on, but you can kind of just look at these different feature plots

that we made with lasso and see that males and females are sort of having different glycan profiles and male profiles were less advantageous to being transferred by the way that the placental receptors were expressed.

So in summary, we found increased interferon stimulated gene expression in males, in the setting of maternal SARS-CoV-2 infection. We don't know if this is potentially partially protective to the male placenta against infection, but also may potentially be harmful, especially as we think of the knock on effects downstream on the developing fetal brain and on fetal organs and inflammation at the maternal fetal interface and what that might do to programming for the long term. And so I think that's going to be an important question to answer. We saw decreased maternal anti COVID antibody titers in pregnancy and carrying a male fetus. And perhaps, we hypothesized, this could be because in order to tolerate a male fetus, potentially maternal inflammation may be down regulated to a greater extent than pregnancies carrying a female fetus. And perhaps this also suppresses the production of antibodies since the amount of inflammation at the time of infection is correlated with the titer of antibody.

We also saw increased baseline expression of interferon stimulated genes and FC receptors, even in female control placentas. And this will be something important to look at, to understand sex differences in antibody transfer at baseline and sex differences in placental defense mechanisms at baseline. And this increased FC receptor expression and co localization in males in maternal SARS-CoV-2. And I think we covered the glycan profile and why this wasn't advantageous. So we know placental antiviral immune defenses do appear to be sexually dimorphic, at least with regard to SARS-CoV-2. It's important to consider fetal and neonatal sex as a biological factor in investigating maternal, placental and neonatal outcomes. So I'm really glad that PASC is doing that, and understanding the implications of placental innate immune response for fetal programming of males versus females will be an important future direction. So I'm going to end there and thank our group of collaborators and our funders, and thanks for having me here today to talk about the importance of sex differences.

Dr. Torri Metz

Thank you so much to all of you for presenting your work today, I really appreciate it. I wanted to cover a couple of things just in summary and just kind of thinking more globally about how these relate to long COVID and the RECOVER initiative, and then maybe answer one or two questions, then I'll turn it over to our moderator. So I think one question that came in the chat and I think is very reasonable to query about and answer, is just how do we see these more acute data that have been presented today, translating to long COVID and translating to the RECOVER initiative? And I would say that, and I'm happy to have the other panelists jump in as well, but we have very little data about long COVID and the reproductive system. We don't have slides that we can put up here to say what is happening long term, because it's just not out there yet. And that's one of the things that the RECOVER initiative is really trying to answer.

Dr. Jacoby shared some shorter term data about sort of saying, yes, we see maybe these disruptions in the very short term, but over a couple months, we see return to normal function. And so really just expanding that data that are available over time to see what's happening as years pass. Same thing with sort of looking at the male fertility aspect of this and Dr. Hotailing's data that he shared, that we do see these changes a very temporarily with acute COVID infection, but the initial data seem to suggest that these changes then subsequently resolve and potentially there isn't day long term impact on male fertility and male sexual function. I think in terms of the pregnancy cohort, which some people did ask about in the Q&A just to address that.

So Dr. Jacoby and I are the two PIs of the pregnancy cohort for RECOVER, we don't have any initial data to share, which was one of the questions. We don't have any initial data to share for anything in the RECOVER initiative yet, but I know that everybody's very interested in that and everybody wants to move that forward quickly, and that's a focus of all of the groups right now. And I don't know if that question is more related to pregnancy or really these longer term, neurodevelopmental effects. Which is one of the reasons that we asked Dr. Edlow to come and speak today because the focus of the pregnancy cohort in terms of long COVID with the offspring, is really what happens to the offspring when they're exposed to SARS-CoV-2 in utero. So when we do get these exposures in utero to SARS-CoV-2 how does that have long term implications?

And that's really what the pregnancy cohort is looking at in terms of the offspring, is does it result in long term neurodevelopmental dysfunction, cardiometabolic dysfunction. And I think what Dr. Edlow's data shows us is that we're going to have to be really careful when we start thinking about that and make sure that we're accounting for sex differences when we're thinking about that. And those, we aren't going to have any data on for probably at least a year to two years. I mean, we have to really wait for these kids that were exposed to these viruses in utero, to have the capacity to do some of the more complex neurodevelopmental testing that's required to really examine that. And just now we're starting to have kids that are reaching the 24 month age range, and we are in our initial sort of testing and the RECOVER initiative is in that 24 month age range. So we hope to, in the future, be able to share more related to that. And I know that Dr. Edlow has some preliminary data about neurodevelopment and exposed fetus., I don't know if you want to share that briefly, Dr. Edlow.

Dr. Andrea Edlow

Sure. Thanks, Dr. Metz. Yeah, I think we had done some insilico work creating electronic health records based cohort, for the reasons that you say, just that it's challenging with this very short turnaround, to have enough directly observed data to really feel confident that a small N can really reflect something. So we actually looked across aid hospitals in New England, within our larger hospital system and looked at more than 7,700 deliveries, including 222 or so where the mother had SARS-CoV-2 in pregnancy. And this was all verified by PCR at the time, we're going to have to develop new methods now that rapid antigen testing and everything is so prevalent. But we were able to do it because we kind of confined our search to the initial six month period of

COVID. And what we saw was a signal toward significantly increased neurodevelopmental diagnoses at age one in these children who were exposed to maternal SARS-CoV-2 in utero.

And this was sort of a ICD 10 code bundle of ICD 10 codes and all the charts that were flagged where the infants had a neurodevelopmental diagnosis at age one, we hand reviewed. And we did find these to be kind of real diagnosises, mostly related to delayed speech, delayed having babbles, words sounds, and also delayed sitting up, delayed grasping, reaching. So motor and speech were the main things that were impacted. Now our ICD 10 code bundle included more ICD 10 codes than we feel that we could carry forward to future cohorts so that we can follow this cohort over time as they get older and may develop more of these codes that are germane, not just to age one but older ages. And obviously we can't look at things like autism and different diagnoses that don't emerge till later in life, at one year of age.

But we did see that early signal and these are unpublished data, but we've also now re queried the data for over 18,000 deliveries in more than 850 cases of maternal COVID, and we have been able to replicate those results and seen again, unfortunately, still an increased risk for neurodevelopmental diagnoses by 18 months of age. So I think what this means long term, it remains to be seen, and it also needs to be replicated in prospective studies, like the ones that you guys are doing. But I do think looking at sex differences is going to be really important. And the other thing that I think will be important is sort of this two hit idea where it could be there's an in utero vulnerability from maternal fetal interface immune signaling, and so on, that's different between males and females, but if also male infants might have somehow increased vulnerability to themselves being infected with COVID in infancy, and what does COVID do as a second hit kind of if the child is infected with COVID.

I think that will be an important thing to look at. Like, are there sex differences in your RECOVER cohorts, in the infant rate of getting COVID and how does that modify the neurodevelopmental outcome in some way, if the mother just had COVID and the child did or didn't get COVID, because there's some data suggest males might be more vulnerable to also getting COVID.

Dr. Torri Metz

Excellent. Thanks so much. There are also a couple of questions that maybe Dr. Jacoby can cover, just related to, you covered a lot about menstrual function, both related to acute COVID, as well as vaccination, but there's a couple of questions in the chat, just about what about cyst formation and fibroids, and sort of other gynecologic issues that can emerge. And are there any data on that or are there any answers to those kind of questions at this point?

Dr. Vanessa Jacoby

Yeah. Thank you. I really appreciate people bringing up these very, very common and critical issues in gynecology. And particularly, I think in the introduction, when they were introducing, one of my main areas of

research is trying to improve care for people with fibroids. So fibroids affect up to 70% of all females by the time they reach menopause. And I can tell you that my review of the literature shows almost no data about COVID and fibroids. The only thing that I've seen is one of the large survey studies that I showed you about menstrual health after vaccination in 39,000 people, they did do a small subgroup look at people who had fibroids or endometriosis or adenomyosis, other common gynecologic conditions, and found that those people were more likely to have menstrual irregularities, particularly heavy bleeding. But again, there's a lot of limitations in that study, as I pointed out, in terms of who answers those online questionnaires, and also not having pre COVID vaccine data. And again, with vaccine studies, we're not talking about just COVID infection.

So I think the key thing, the take home message to me is that these issues have been off the radar for understanding COVID, and we need to put them back as central to all COVID studies going forward, including the RECOVER study. I think one of the chat questions asked about if you're having concerns about your own reproductive health and you've had COVID and you want more information, I'll just say this little pitch to consider joining the RECOVER study, because we are enrolling people who have COVID now, or who have had COVID in the last three years. And if reproductive health issues are of importance to you, we need you, please, please join us, and we hope to be able to answer some of these questions.

Dr. Torri Metz

And Dr. Hotailing, I know that we're all going to need to drop off in a few minutes here, but I don't know if you want to speak at all to do you anticipate that they'll be longer term ramifications of these just generically, when you think about sort of male infertility, male sexual dysfunction, if you see sort of return to baseline in the short term, like you're seeing, would you then expect to see a longer term effect or are those questions relatively answered by the returns that we're seeing in the short term?

Dr. Jim Hotaling

Yeah, that's a great question. Well, the short of it is we don't totally know, but I suspect not. I mean, I can't think of a good pathophysiologic mechanism whereby people would recover in the sort of short-ish term and then have issues long term. I think you may find different things for people who have kind of long COVID and/or get repeat infections. Like I have a nurse practitioner who's had COVID three times, so it would beg the question of, with that cytokine and other load, do you see a long term effect? I think it's unlikely. I think you will see a long term effect from a very broad perspective, just due to the societal and sort of psychosocial and maybe even socioeconomic shifts due to COVID. And those will probably play out over say two to five years, just with people on different emphasis for things.

I mean, we certainly saw a lot of interesting sort of relationship dynamics, is one way to put it, play out when there was lockdown and everything else and couples who were already dealing with sexual dysfunction,

infertility, everything else. So I don't think so, and I can't think of a good reason why you would see something in offspring, like much later, but there certainly is precedent for that. I mean, there's epigenetic changes that happen in sperm from smoking or alcohol use or obesity, that those kids at 18 have different gluco regulatory functions. So we're going to have to see, I mean, I would love to see kind of longitudal big cohorts on the male side, followed for reproduction because we don't really have that, and most of that is database stuff.

Dr. Torri Metz

Excellent. Thanks so much. Melissa, I want to make sure that I'm not skipping things you wanted to make sure that we got the panel to answer in the last minute or two here, or if you kind of wanted just pause there and switch over to online Q&A?

Dr. Vanessa Jacoby

I can address that. Okay. I can address that in RECOVER. So we thank you for that question, we showed some studies have found that women are much more likely to have PASC than men. We are, in RECOVER, collecting samples, where we can run hormonal assays. It's not part of the primary, we're not running those right now as part of the first wave, but we have stored samples and I really hope it's something we can do in the future.

And I just wanted to address one other question in there related to this, just to encourage people to consider enrolling, is that a lot of women following the SCOTUS decision in Dobbs versus Jackson, Mississippi Health, have been reluctant, more reluctant to join studies where they would report pregnancy or menstrual function for concerns, particularly in states where abortion is restricted or prohibited. And I just want to encourage people to let them know that the RECOVER study falls under the NIH certificate of confidentiality, where your information is completely confidential. We're highly attuned to these concerns, and we think it's really important for everyone to feel welcomed into the study. So if you have questions about that, please reach out to RECOVER, we really, really want you to feel comfortable enrolling.

Dr. Melissa McPheeters

Thank you so much for that. What a fantastic set of presentations today. We are at the end of our time. Shane, would you just pull up the slide of the upcoming seminars? I just want to encourage everyone to keep coming back and joining us for these webinars. We have some great topics coming up. And then also just to remind everyone that this webinar and all the webinars will be posted on the website at recovercovid.org, so do visit us there. And thank you very much for joining us. We'll wrap it up.

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