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March of Dimes Prematurity Research Center

STANFORD UNIVERSITY

What's inside?

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Q & A WITH KARL SYLVESTER, MD

ADDING METABOLOMICS TO OUR INTEGRATIVE APPROACH TO PREMATURITY RESEARCH

Karl Sylvester, MD, a member of our Prematurity Research Center (PRC) is leading a new effort at Stanford focused on metabolism.

With an official launch in 2018, the Stanford Metabolic Health Center aims to serve as a purpose-driven biobank for moms and babies who are patients at Lucile Packard Children's Hospital Stanford. By analyzing human samples, including blood, urine, and stool, the Center hopes to provide all patients with a blueprint of their molecular health that can be merged with their electronic health record to create a comprehensive health profile.

It's an ambitious goal. While clinical coordinators are working to consent new patients at the hospital, Dr. Sylvester and his team are busy analyzing existing samples. Thanks to a partnership with our PRC, they have been able to examine the Stanford cohort from a metabolic perspective and ask: What does it mean to have a normal progression of pregnancy versus one that leads to preterm birth? (Helping to guide this inquiry is our PRC Principal Investigator David Stevenson, MD, who serves as a Co-Director of the Metabolic Health Center.)

Dr. Sylvester is particularly suited to lead this new effort centered on metabolism. He has focused his career on studying acquired diseases of prematurity and is an expert on necrotizing enterocolitis (NEC), a serious intestinal illness in newborns. He is specifically interested in the first 30 days of life—a critical period that affects a baby's health trajectory for decades to come.

Dr. Sylvester says it's gratifying to be in the business of promoting health," particularly in newborns where the stakes are high and the potential for altering the life-course is so great.

The following has been excerpted from our longer conversation together.

Karl Sylvester, MD
Professor of Pediatric Surgery
Co-Director of Stanford Metabolic Health Center

Laura Hedli (LH): How do your efforts with the Metabolic Health Center interface with those of the PRC?

Karl Sylvester (KS): I think they're very complimentary. Because of my membership in the PRC and also serving as a Co-Director of the Metabolic Health Center, I see really tight synergy. For example: One of the fundamental questions that we are pursuing is: Could you describe the risk of preterm birth from a metabolic perspective?

There's a multi-omics focus to a lot of what takes place in the PRC. One of those technologies is metabolomics, which we are utilizing to profile the normal trajectory of a growing, developing fetus and for determining when that pattern is abnormal so that we can estimate a potential risk of preterm birth or growth restriction. Using the same blood draw, we feel it will be possible to simultaneously create diagnostic and prognostic tests for pregnancy while simultaneously assessing a developing fetus for risk of acquired diseases of the newborn. Early on, we have derived very compelling data models using a metabolomics platform. This suggests these techniques will be powerful in helping us describe pregnancy in terms of metabolite shifts as well as growth and development of the fetus.

The PRC has correctly said the solution to the problem of premature birth is to prevent preterm birth altogether. Effectively achieving this end necessitates making novel observations during pregnancy, and prior to any clinical signs of impending preterm birth. That said, it is also plausible there are cases of preterm birth that are appropriate or indicated from a biologic perspective, to either protect the mother or prevent further detriment to the developing fetus. We anticipate that metabolomics may also help to uncover the signs of those "indicated" cases. If we can make progress toward reducing preterm birth to an irreducible minimum,

the question becomes: What can we say about the preterm or growth restricted newborn's health risk after birth?

We think there's a common theme that's emerging: In a deliberate way, size matters. The smaller the newborn baby in terms of birth weight, the more biologic fragility, the more clinical risk—that has been the norm. And now we're beginning to quantify those size-based risks through the lens of altered metabolism, and possibly provide the biologic mechanism for size-based risk. It will get really exciting when we come back to the strategy that the PRC has taken around prematurity and we start pulling in other omics and other molecular data pieces to solve the puzzle of prematurity and low-birth-weight newborns.

LH: How can you use metabolomics to assess a newborn's risk?

KS: It is important to understand that metabolomics is the measure of a large number of small molecules that represent phenotypes. Phenotypes describe the interaction between our genes, proteins, and cellular functions that can tell us about the sum total of the biology of a given patient at any given time. We believe that health and disease states can all be described in terms of metabolites, and metabolites can be significantly influenced by drugs and nutrition. Thus, the contribution of metabolomics to risk assessment in the newborn is substantial. Metabolomics may allow us to:

1) Quantify the degree of metabolic alterations in premature and low-birth-weight newborns at birth.
2) Understand the impact of microbial exposures and the required colonization of the newborn gut at birth. The microbiome of the gut is a whole area of focus for prematurity.
3) Assess the impact of type of birth, whether C-section or vaginal.
4) Understand how the NICU environment impacts for better or worse the metabolic machinery contributing to health or disease in the newborn.
5) Determine the impact of what and how much a newborn is fed on that newborn's health and disease risk profile.

We are very interested in developing the tools to determine a newborn's comprehensive health and disease risk profile. Our anticipation is that these tools and the understanding they bring will enable us to develop strategies that will impact not only the newborn's health trajectory but will persist across the lifespan.

LH: I know you're particularly excited about a certain probiotic that is in use in newborns, Bifidobacterium infantis (B. infantis). Tell me more about how B. infantis may help alter a baby's metabolism for the better.

KS: One of the fundamental changes that occurs during the transition from fetal to newborn life is colonization of the gastrointestinal tract by symbiotic bacteria. Gut colonization is required to help humans digest, process, and metabolize the food that we ingest. This process is profoundly critical in the newborn wherein the gut at birth is blank slate whose microbial ecology must be assembled from scratch. This represents a tremendous opportunity or threat depending on whether the process occurs in a health-benefitting or disease-promoting manner.

"DO WE HAVE THE TOOLS WITH ENOUGH SENSITIVITY TO UNDERSTAND: ARE WE HAVING A NET BENEFIT, OR A NET DETRIMENT, IN THE WAYS THAT WE CURRENTLY FEED BABIES AND THE MICROBES THAT THEY'RE EXPOSED TO IN THE NICU?"

Giving a baby a specific probiotic, we now know that you can change up to 60 percent of all the metabolites that are being produced in the gut. This represents a really profound treatment effect size at a molecular level. Because of the manner by which B. infantis metabolizes mother's breast milk, the metabolic by-products of this relationship have a significant health-benefitting effect due to their metabolic capacity. One of the many additional benefits of B. infantis is that it prevents its potentially trouble-causing cousins from being able to move in and colonize, thus eliminating metabolic contributions from bad actors.

I think the example of B. infantis highlights the types of insight into intervention based upon the biology of metabolism that we'd like to provide using metabolomics. Since growth and nutrition are so critical to a baby's earliest weeks and months, this represents a tremendous opportunity that metabolomics is helping us to understand and quantify.

LH: What do you think is underappreciated about metabolism in relation to prematurity?

KS: It's not been an area that has been deeply investigated.

Do we have the tools with enough sensitivity to understand: Are we having a net benefit, or a net detriment, in the ways that we currently feed babies and the microbes that they're exposed to in the NICU?

The current metrics of healthy growth are based upon population norms that are unlikely to be universally applicable. Measurements of length, weight, and head circumference have been used to describe "normal," but what constitutes normal is different depending on the standard and the specifics of the population to which they are applied. We have some early data to suggest those measurements are not as sensitive as perhaps molecular or metabolic indicators are when we think in terms of understanding healthy growth and risk of disease. We are beginning to re-think some long-held clinical strategies, and develop a finer way to interpret how newborns are thriving from a metabolic perspective in the critical first weeks of life.

LH: So more of a precision health approach?

KS: This is exactly precision health for the newborn using metabolism to understand and possibly change behaviors and clinical practice accordingly.

DON'T FORGET ABOUT THE FATHER

MICHAEL EISENBERG, MD, ENCOURAGES US TO CONSIDER HOW DADS INFLUENCE PREGNANCY AND BIRTH OUTCOMES

"Don't forget about the father."

Michael Eisenberg, MD, says it's his mantra. Dr. Eisenberg is a urologist working with our Prematurity Research Center (PRC) to better understand how a father's age impacts pregnancy and birth outcomes.

"Most of the focus in perinatal health has been on women," Dr. Eisenberg says. "We should start to look at the father at this point because I think there's going to be a fairly sizeable contribution that we may be able to intervene upon."

Two recent retrospective, population-based cohort studies showed advanced paternal age is associated with negative outcomes for both infants and mothers. Older fathers had an increased risk of having a baby born stillborn, preterm, at a lower birth weight, and lower Apgar score. Mothers with older partners were more at risk for developing gestational diabetes.

MEAN AGE OF FATHERS ON THE RISE

Dr. Eisenberg had been noticing men coming into his clinic who were becoming fathers later in life. He wanted better data. He saw the Centers for Disease Control and Prevention (CDC) published a brief charting the rise in maternal age. What about dads, he wondered.

Because birth certificate data collected through the CDC's National Vital Statistics System is publicly available, Dr. Eisenberg and his team decided to analyze all live births in the U.S. from early the 1970s (when data was first collected) to 2015. They found the age at which men are fathering children to be increasing among all races/ethnicities and education levels and across all regions of the country.

Overall, dads are on average about 3.5 years older than they were in the 1970s.

In the 1970s, about 4 percent of children in the U.S. were born to fathers over 40. Today, it's about 9 percent. Similarly, 0.5 percent of all children in the U.S. were born to fathers over 50 in the 1970s. Today, it's about 1 percent.

It's reasonable to assume more middle-aged dads benefit offspring in certain ways. They likely have more money and may create a more stable home environment for their child. Yet, risks may begin to add up too when fathers are over 40.

OLDER DADS ASSOCIATED WITH NEGATIVE OUTCOMES FOR KIDS AND MOMS

A 2018 paper published by Dr. Eisenberg and colleagues in the *British Medical Journal* estimated that advanced paternal age (≥ 45 years) is responsible for 13.2 percent of premature births and 18.2 percent of gestational diabetes in births associated with older fathers. The results are based on a cohort of all documented live births in the U.S. from 2007 to 2016 (over 40 million). Dr. Eisenberg was interviewed about the study's findings by prominent media outlets including the *New York Times* and the *BBC*.

Notably, advanced paternal age showed significant associations with adverse birth events including NICU admission, assisted ventilation, postpartum antibiotics, or a seizure after birth. Associations were present in all stratifications of maternal age (mothers aged <25, 25-34, and >34) and persisted after adjustment for maternal and paternal covariates.

The impact on maternal outcomes is a particularly novel finding as well. Not only can a father's age affect his child's health, it can also impact his partner's. (The mechanism for this may be through the placenta.) For mothers partnered with fathers aged > 45 years, their odds of developing gestational diabetes were 28 percent higher than compared to mothers partnered with fathers aged 25-34 years.

One hypothesis is that these negative associations may be the result of genetics. Because men continually make new sperm, there is a chance for errors during DNA replication. It's estimated that a man accumulates two mutations in his germline each year, and outside studies have shown mutations increase the risk for preterm birth.

The problem could also be epigenetic. A older man has been exposed to the environment for a longer period of time, which could lead to histone modifications or methylation changes that get passed on to his offspring.

Mean Age of Dads is on the Rise in the U.S.

Years	Mean Age
1972-1975	27.6
1976-1980	27.9
1981-1985	28.6
1986-1990	29.4
1991-1995	29.8
1996-2000	30.2
2001-2005	30.4
2006-2010	30.6
2011-2015	31.1

Stillbirth, too, is associated with advanced paternal age. Published in *Annals of Epidemiology* last year, a study of nearly 10 million California births from 1991 to 2011 showed a J-shaped association between paternal age and stillbirth. Older fathers (>30 years mean age) had increased rates of stillbirth deliveries; on the other hand, so too did younger dads (< 30 years mean age). This association persisted after adjusting for covariates, though the risk was slightly lowered for older dads.

Why both ends of the age spectrum would be at risk of having a child delivered stillborn is not clear. Future research is needed to figure out whether this risk is biologic.

The stillbirth study also measured the association between maternal age and stillbirth, finding the hazard ratios for older mothers (aged ≥ 40) to be 2-5 times greater than that for older fathers.

"That paper nicely demonstrates that most risk lies on the female's side, but I think the father shouldn't be discounted," Dr. Eisenberg emphasizes.

The effects older dads have on their kids will likely persist throughout the lifecourse. Dr. Eisenberg is senior author on a recent study published in *Andrology* that reviews data which shows advanced paternal age is estimated to be associated with a substantial increase in early-onset cancer and neuropsychiatric disease in offspring. (The March of Dimes did not fund this particular research.)

Dr. Eisenberg is now turning his attention to examine associations between paternal health and birth outcomes. Early research suggests fathers today are less healthy than they were 10 years ago.

"I think age is very easy. When you begin to examine health it's more challenging both because definitions of healthy versus not are less precise because of a dearth of data around that question," he says.

Birth certificate data only records the age, race and education level for fathers. The father isn't listed about 10 percent of the time, and it's estimated that the wrong father is listed on 4-5 percent of all birth certificates.

Michael Eisenberg, MD, appears on BBC World News.

> "WE SHOULD START TO LOOK AT THE FATHER AT THIS POINT BECAUSE I THINK THERE'S GOING TO BE A FAIRLY SIZEABLE CONTRIBUTION THAT WE MAY BE ABLE TO INTERVENE UPON."

Meanwhile, we know much more about the mother from the birth certificate—the specifics of her birth and details of her pregnancy also.

SHIFTING TRENDS IN MODERN BIRTH

The business of birth undoubtedly skews maternal. A woman receives health guidance from her provider during a preconception checkup, which may include a physical exam, pelvic exam, and in some cases genetic tests. She is monitored closely during pregnancy and screened for a variety of health conditions during prenatal consultations. If she is 35 or older, she is flagged as having "advanced maternal age" and often undergoes even more testing.

An expectant father, on the contrary, is under much less scrutiny. We don't talk about "sell-by dates" for sperm as we do for eggs. Their biological potential persists. In fact, Ramjit Raghav reportedly became the oldest father on record when his second son was born in 2012 when Raghav was 96 years old.

Still, we could start to learn more about the father during prenatal visits or even earlier if he's present and willing. Equipped with findings from these studies and others, we could educate men about paternal contributions to pregnancy.

Principal Investigator for our PRC David Stevenson, MD, trusts scientific investigation will reveal more about what dads add to the mix.

"Although it mainly remains mysterious as to how various attributes of the father might translate into the dispositions of the fetus and mother beyond genetic or epigenetic mechanisms, it is just a matter of time before we make such causal connections," says Dr. Stevenson. "In particular, examining the epigenome of sperm from donors with known exposures or perhaps even of the embryos conceived with the use of sperm from donors with various attributes and histories might provide some insights."

Of course, age is just one factor. "Age is not modifiable. But as couples are thinking about when to have a child, I think it's another piece of information they should take into account," Dr. Eisenberg says. "Most children born to a father over 40 are going to do well, but there is some risk."

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