DEVELOPING A NEW CHOICE: THE UNRAVELING OF REPRODUCTIVE MYSTERIES

INTERVIEW WITH DR. POLINA LISHKO

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Polina Lishko, PhD, is an Associate Professor of Cell and Developmental Biology in the Department of Molecular & Cell Biology at the University of California, Berkeley. Dr. Lishko is the principal investigator of the Lishko Lab, which studies the steroid regulation of various tissues, from the brain to the reproductive organs, to improve reproductive health and contribute to treatments targeting neurodegenerative disease. She was recently awarded the 2020 MacArthur "Genius Grant" for her work on mammalian fertilization, contraception, and infertility treatment. Dr. Lishko is also an adjunct associate professor at the Buck Institute's Center for Reproductive Longevity and Equality, where she performs research on ovarian aging with the goal of delaying reproductive aging and its associated age-related dysfunctions in women of child-bearing age. In this interview, we discuss Dr. Lishko's research on factors that can influence sperm fertility as well as her recent work on the effect of steroid hormones on neurodegenerative disease.

BSJ: What sparked your interest in women's reproductive health and contraception?

PL: My colleagues and I were mainly focused on male fertility in the first four years of our research, but male fertility and female reproductive health are two sides of the same coin. Our initial focus was to fundamentally understand how sperm cells are able to find an egg. Basically, we wanted to know what makes sperm cells fertile. We found several key molecules that sperm cells express that are required for their motility and ability to find an egg. Naturally, we began to wonder, "Can we utilize those targets to make sperm cells less fertile?" This led us to the realization that our findings could help in developing a potential contraceptive tool.

As my team and I began reading about contraception, it was shocking for us to realize how unsatisfactory female contraception currently is. Essentially, only a few options are available, and what we have on the market is mostly hormonal contraception. There are a few exceptions, like copper intrauterine devices (IUDs), but most contraception is hormonal and was introduced in the 1950s. They, of course, do what they are supposed to do, but they come with many side effects. We noticed that some of the contraceptive options used in the past were based on traditional folk medicine, so we began looking into what components of these herbs and natural products could affect sperm cells. We identified certain compounds and had a scientific eureka moment when we realized that particular sperm cell components could be targeted by key molecules that we were working with. And that is roughly a short story on how we started. We did not initially have the goal of developing a contraceptive. It all stemmed from scientific research and basic curiosity, which is actually a common trend seen in many research directions.

BSJ: What are the disadvantages or risks associated with hormonal contraception, and how does your research on non-hormonal birth control aim to address these issues?

PL: If the pills from the 1950s and 60s had to go through FDA approval now, I speculate that they would have a very hard time getting approved, since hormonal contraceptives come with a plethora of side effects. When we talk about side effects, we are talking about not only acne, but also blood clots, depression, suicidal thoughts, and weight gain. Some contraceptives come with a black box warning [the most stringent FDA warning indicating serious

safety risks], particularly because women who start taking these contraceptives early have an increased rate of osteoporosis. Several common contraceptives can also increase the risk of glaucoma [a condition that damages the optic nerve]. Additionally, women who smoke are highly advised against taking certain hormonal contraceptives because the combination of smoke, tobacco, and hormonal contraceptives increases the risk of cardiovascular issues, including blood clot formation. And this is just the beginning of the list. Of course, some researchers claim that the invention of the pill is one of the 50 greatest inventions of our civilization since it was a big game-changer. However, while it improved the economy and helped women lead better lives, it also came with some drawbacks. I think it is time to introduce better alternatives.

BSJ: In your opinion, how does society's current attitude towards male contraception differ from what you have seen in the past?

"It all stemmed from scientific research and basic curiosity."

PL: It is getting better. I would say that many men are now very much in support of male contraceptives. They understand how important male contraception is, and they want to help their partners and take their share of the contraceptive burden, so to speak. There are now also several non-profit organizations, such as the Male Contraceptive Initiative (MCI, https://www.malecontraceptive.org), that spearhead this development. They have various educational tools and receive support, though still not as much support as one would like them to have. In the past, contraception was perceived as mainly a female problem to solve because men do not get pregnant. However, times have changed, and a lot of men are now aware that they have to take their share of the responsibility.

BSJ: In one of your papers, you study TRPV4, an ion channel capable of triggering sperm hyperactivation. What is sperm hyperactivated motility, and how is it triggered?

PL: Sperm cells are like amazing nano-machines that have different motility patterns and are equipped with multiple tools in order to reach their goal. They have a simplified motility form, which looks like a snake wiggling around, that helps them get from point A to point B. They can also rotate along their axis in order to penetrate through a viscous environment. However, when they encounter particularly hard objects like the zona pellucida [the thick protective shield of the egg], they need a high-power motility mode called hyperactivation. For many years, scientists have known that the key to hyperactivated motility is calcium. Calcium ions enter the sperm tail, and this influx of ions triggers an internal mechanism that allows sperm cells to change their motility pattern to a more asymmetric bending pattern of the tail. In a viscous environment, this translates to a powerful forward push.

BSJ: What is the role of the calcium ion (Ca^{2+}) channel CatSper in this pathway?

PL: Ion channels are molecules embedded in cells' plasma membranes that essentially serve as pores through which ions can enter or leave the cell. Some ion channels in sperm cells are unique to sperm cells alone, while some are also expressed in other cell types. CatSper, the calcium channel of sperm, allows calcium to

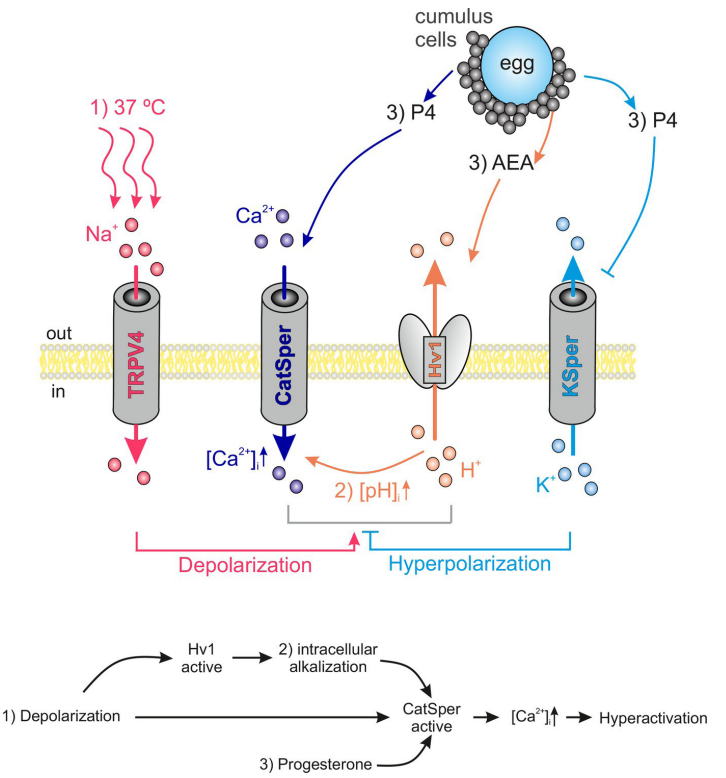

Figure 1: Model of sperm hyperactivation pathway. *At 37°C, TRPV4 embedded in the sperm cell membrane is activated, allowing for an influx of sodium (Na^+) ions that induces membrane depolarization. This depolarization activates CatSper and Hv1, the latter of which expels protons out of the sperm. The resulting intracellular alkalization further activates CatSper, leading to a calcium ion (Ca^{2+}) influx that triggers sperm hyperactivation. Image licensed under CC BY 4.0.*

enter the sperm cell and trigger hyperactivation. It is a channel that is only expressed in sperm cells and nowhere else in the body. This makes the channel very attractive as a potential contraceptive target, because a drug acting against this target would not affect anything else in the body. This makes the potential drug not only effective, but also safe.

BSJ: What are the main mechanisms that interact to affect CatSper function?

PL: In human sperm, the CatSper channel requires a few things in order to become activated. Firstly, it requires progesterone, a steroid which is released after ovulation that eggs use to tell the sperm, "Hey, I'm here." It also requires that a certain pH be achieved within the sperm cell; in other words, there must be a particular concentration of protons inside the sperm tail, and a lower concentration is preferred. CatSper activation also relies on temperature and voltage, which is a measure of how positively the inside of the sperm cell is charged. For human CatSper to get fully activated, the voltage inside the sperm cell needs to be about +30 millivolts. Progesterone will be delivered to the sperm by the egg, and the pH inside the sperm cell is regulated by transporters and other ion channels. But what regulates the voltage? What regulates the depolarization—the positive charge—on the sperm plasma membrane?

This is where the temperature sensitive channel, TRPV4, comes forward. You need the temperature of the sperm cell to be between 34–37°C in order for this channel to be active in conducting sodium and calcium. We believe this temperature is the initial trigger activated when the sperm cell encounters a warmer area. TRPV4 gets activated and brings positive ions inside the tail to create an initial depolarization, and this creates a condition under which CatSper can get fully activated, assuming progesterone is also nearby. In essence, the synchronous action of several key elements is required to engage hyperactivation.

BSJ: What is the significance of your discovery of TRPV4 as a temperature-sensitive depolarizing channel in sperm?

PL: TRPV4 is important from a basic scientific perspective because if you want to know how a sperm cell works, you need to know all of its critical elements. From a contraceptive standpoint, TRPV4 might not be an ideal target because it is not only present in sperm, but also present in other cells such as choroid plexus cells in the brain, which produce cerebrospinal fluid. TRPV4 is highly expressed in these cells and regulates normal brain function. For example, mutations in TRPV4 are associated with hydrocephaly. TRPV4 is also found in osteoclasts, which maintain bone density, so it is not an ideal target unless a potential contraceptive targeting TRPV4 could be applied locally in the female reproductive tract without any way of getting inside the rest of the body. In that case, you would not need to worry about side effects because if it stays in the female reproductive tract, it would only affect sperm cells. However, if the drug has to go through the bloodstream, I would not target TRPV4.

BSJ: Aside from your research regarding the sperm hyperactivation pathway, you have also conducted research into the effects of external factors like endocrine-disrupting chemicals (EDCs) on sperm fertility. What are some examples of EDCs, and how can we be exposed to them in our daily lives?

PL: The fact that bisphenol A (BPA), phthalates, and other plasticizers affect our physiology has been known for a long time, but surprisingly, these compounds are still around us. They

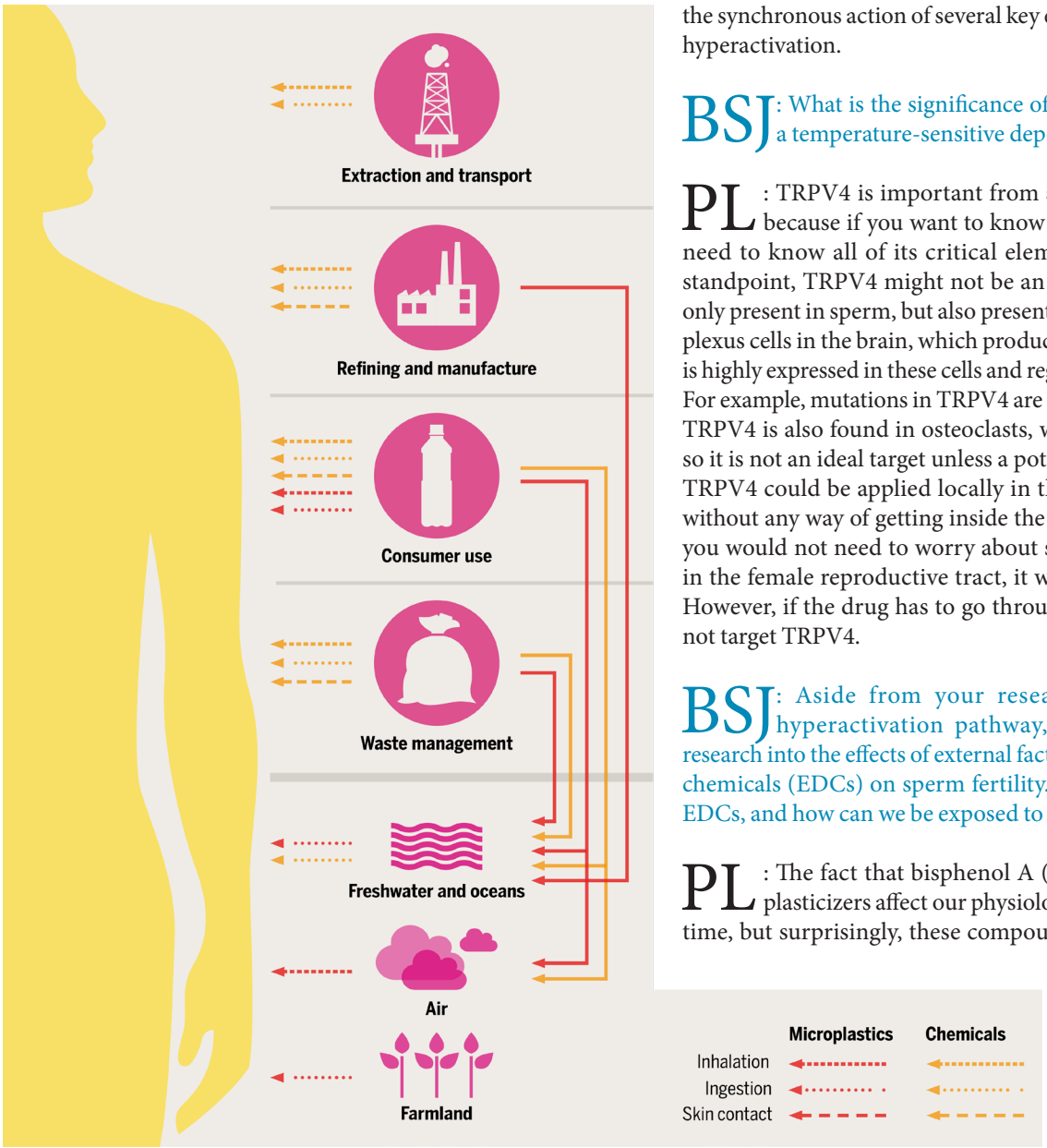

Figure 2: Examples of routes of exposure to endocrine-disrupting chemicals (EDCs). *Image licensed under CC BY 4.0.*

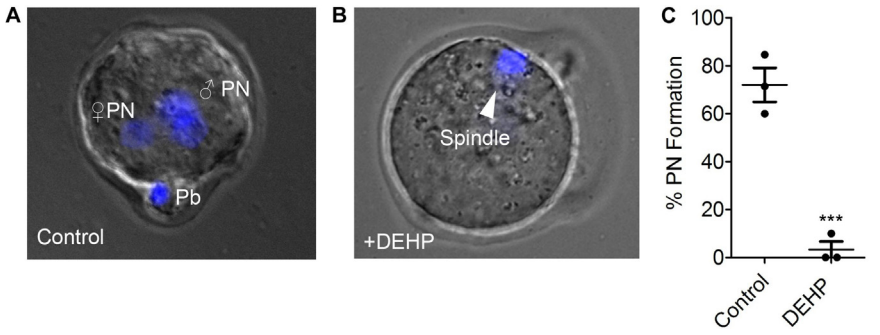

Figure 3: Images of eggs nine hours after in vitro fertilization (IVF) by control versus DEHP-treated sperm. *Figure 3A depicts a successfully fertilized egg with two pronuclei (PN) and one polar body (Pb). Figure 3B shows an unfertilized egg after IVF with DEHP-treated sperm. Note the significantly lower formation of pronuclei in DEHP-treated sperm in Figure 3C. Image licensed under CC BY 4.0.*

are included in our plastic products because they are a very cheap and efficient way for manufacturers to make plastic more bendable. Unfortunately, these compounds are not well attached to the products, so they leach into whatever environment they encounter, including water, food, and cosmetic products. These plasticizers are also components of paper receipts and could eventually end up in your digestive system if you do not wash your hands before eating.

Why are these compounds called endocrine disruptors? BPA, for example, has the ability to mimic the effects that estrogen has on the body—even though BPA is quite different from estrogen structurally—which can lead to negative side effects, including tumor formation. In the United States, only one manufacturer provides the tubes for dialysis; those tubes are made of Tygon and plastics that contain plasticizers. Women who are undergoing blood dialysis treatment are advised against getting pregnant, and if they do get pregnant during treatment, they are advised to consider an abortion because there could be extreme teratogenic effects on the developing fetus that lead to severe birth defects. Biological males can develop feminizing features due to plasticizers mimicking female sex hormones. Additionally, infertility is a possible side effect of dialysis, but not many comprehensive studies have been done, so it is unclear whether this infertility is because of plasticizers or because of other chemicals that patients are exposed to during the course of treatment.

BSJ: Why did you choose to focus on the effect of acute exposure to phthalates in your study on EDCs?

PL: We knew that long-term exposure to phthalates is negatively associated with male fertility. Phthalates are hydrophobic and tend to accumulate in our fat deposits, and the research has shown that the fat deposits around reproductive organs can accumulate and release toxic molecules as fat levels fluctuate in the body. However, the effect of acute exposure to phthalates on fertility is less clear, and we were interested in understanding what would happen if sperm cells were briefly exposed to these chemicals.

In our research, we took mouse sperm cells, exposed them to phthalates, and then introduced them to fresh, unexposed eggs. We found that DEHP is one of the most toxic and widespread phthalates that really messes with sperm fertility. The sperm move more or less the same, but for whatever reason, they cannot fertilize the egg. Further investigation revealed that phthalates do not mess with ion channels as much as one would expect. What they do instead is damage sperm membrane integrity by increasing production of reactive oxygen species (ROS) in sperm cells. The following is all speculation, but given that prostate cancer is on the rise and certain types of cancer cells are also known to be affected by ROS production, this is something people might want to look at—that endocrine-disrupting chemicals could cause additional harmful effects, which we have not yet researched in depth.

BSJ: In 2018, you co-founded YourChoice Therapeutics with the goal of developing safe and effective non-hormonal birth control. Although you have since left the company, could you tell us a bit about your time there and the experience of developing a marketable contraceptive?

PL: It was a very interesting experience because I was not trained to be an entrepreneur—I was trained to be an academic scientist. If you do not have prior experience as an entrepreneur, you face a steep learning curve when going through the entire process of understanding how startups function and how drug development operates. However, it is entirely possible to create a new company. You do not have to be a field "expert" to become an entrepreneur. Anyone can do it, whether you are an undergraduate student, graduate student, faculty, or individual who is not associated with academia. Of course, you do need to have an intellectual property (IP) and a marketable product, something that people would like to buy and use.

In our case, novel contraceptives were our product. The research for these contraceptives initially started in our lab. This project was actually spearheaded by two graduate students, one of whom graduated just last year and started her own company. They came upon this interesting compound, the mitochondrial uncoupling protein niclosamide, which had been approved in 1982 to treat tapeworm infections. The compound has poor bioavailability (so it does not get absorbed into the bloodstream by our gut), but it effectively messes with tapeworms' mitochondria, the energy-producing organelle. People infected with tapeworms can take up to 1 gram of this drug and nothing will happen to the individuals, but the drug will drain the worms of their energy, leading them to detach from the gut and be expelled.

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We began exploring this mitochondrial uncoupling mechanism under the question, "If we uncouple these sperm mitochondria and drain them of energy, can they still fertilize an egg?" The idea was that the compound niclosamide could be contained in a capsule or something that could be applied locally, and then it could drain the energy of sperm cells similar to how it drains that of tapeworms. Furthermore, it would not be absorbed by the female reproductive tract because of its low bioavailability.

Overall, the experience was fun and somewhat rewarding because I was not only developing knowledge or participating in teaching a new generation, but we were actually developing something people could use—a real product that can improve their lives.

BSJ: What do you think needs to be done to make birth control more affordable and accessible?

PL: Contraception or any other medicine must be affordable. It is ridiculous how expensive certain drugs and life-saving medicine are in our country. The whole system needs to be modified to make sure that these products are available to anyone who wants to use them. The government must step in and make certain firm policies to ensure that people can afford products. The pharmaceutical industry is unlikely to initiate these changes due to the ways those drugs are developed. The drug development process, and everything in it, requires a lot of money. Large corporations can afford to fund this process, but startups may have a hard time financially. Even large companies require a lot of resources to put drugs on the market, and that money must come from somewhere. If the government has certain policies regulating pharmaceutical sales, it can lower the prices of products. The pharmaceutical companies also have to be a little bit less greedy in certain areas. We have affordable aspirin right now. The chemical synthesis for compounds that could serve as contraceptives should not be more difficult or expensive, on average, than the synthesis of aspirin. It is all possible.

BSJ: Finally, we understand that you are doing research into the link between sex steroid hormones and neurodegenerative diseases like Alzheimer's disease. Could you tell us a bit about this project?

PL: Patients with Alzheimer's disease have an impaired ability to clear cerebrospinal fluid (CSF), which is why the CSF ends up accumulating a lot of "junk": amyloid proteins, excessive neurotransmitters, misfolded proteins, and so on. These contribute to the development of the disease in the patient. Women who have a late onset of menarche [the first occurrence of menstruation] and/or early onset of menopause are exposed to steroid sex hormones for a shorter-than-average period of time. These women, consequently, tend to develop Alzheimer's disease at a higher rate, which is significant considering that two-thirds of Alzheimer's patients are women.

There is a direct connection between these hormones and the systems in our brain that participate in CSF clearing, and we want to understand what is going on. What is interesting is that the choroid plexus, the part of the brain that produces CSF, does not express any classic progesterone receptors. Instead, it expresses TRPV4, the receptor that we found in sperm cells. We are currently conducting research into the molecular mechanism behind how steroid hormones participate in CSF clearing and, in turn, how this could lead to the development of age-linked neurodegenerative diseases like Alzheimer's disease.

"The chemical synthesis for compounds that could serve as contraceptives should not be more difficult or expensive, on average, than the synthesis of aspirin."

Since my actual background is in neuroscience, and not in reproductive biology, this new research direction feels somewhat like a homecoming for me.

In late May, Professor Lishko quoted Golda Meir, the fourth prime minister of Israel, on Twitter when expressing her stance on recent events in the Israeli-Palestinian conflict. Her comment was widely criticized, particularly by members of the UC Berkeley community, with several individuals claiming that her statements conveyed racist and discriminatory implications. Additionally, during this time, several older tweets from Professor Lishko were also critiqued as being transphobic. In acknowledgement of the current situation and out of respect for all parties involved, the Berkeley Scientific Journal requested that Professor Lishko address her critics and clarify her intentions. Here are her responses.

BSJ: You have previously written on Twitter, "And yes, biological sex is binary for most of the species, including humans….All the rest is unscientific manipulation of terminology and demagogy." Some trans scientists critiqued this comment, and others that you have made, stating that they seemed to question and undermine the identities of trans and non-binary people.

If you believe your comments were misinterpreted, how did you mean for your comments to be understood by the public?

PL: This comment and the discussion pertinent to it referred specifically to the biology of sex, the binary form of gametes, and definitely not to gender identities, of which there are many. The biology of sex refers to the specifics of sex determination as it is known in reproductive biology on the genetic, cellular, and organismal levels. What this sentence actually meant in the context of the discussion is that for most species that reproduce sexually, nature evolved a binary form of sex determination and sexual reproduction: the female and the male genotype/phenotype. One form produces female gametes (eggs), and another form produces male gametes (sperm cells). It is possible that during billions of years of evolution, many other forms emerged, and yet the most successful form of sexual reproduction for most species is through the binary forms

of the phenotypically and physiologically different gametes. In my opinion, conflating the basics of reproductive biology with gender identities is a manipulation.

BSJ: You have also quoted on Twitter, "Golda Meir: 'Peace will come when the Arabs love their children more than they hate us.'" Some individuals have critiqued this comment, claiming that this statement felt like an inappropriate generalization to an entire community. If misinterpreted, how did you intend for the above quote to be interpreted?

PL: By no means was this quote directed to all people of Arab heritage. From my understanding, the historic meaning of this quote referred to terrorist organizations and definitely not to all people of Arab heritage. One has to know the context, the history of the old quotes, before jumping to conclusions or accusations. The simple message that I was trying to send via this Tweet is that more love is needed to end the hate. Unfortunately, this message was misinterpreted.

BSJ: In your opinion, what is the intersection between a scientist's social views and their research endeavors? Do a scientist's personal views influence the quality or outcome of their research?

PL: It is a recipe for disaster when ideology or close-mindedness cloud the mind of a scientist. Here is a simple example. True scientists observe nature and its laws with an impartial mind and build hypotheses and conclusions based on their unbiased observations. Many scientists are also quite gifted people and not only excel in their particular discipline, but also show talents in art, music, crafts, etc. However, when it comes to scientific observations and conclusions, it is imperative that their scientific side—the side with an impartial and calm mind—always prevails. Let us consider what happens if there is an intrapersonal conflict within an individual between their scientific and artistic sides. Imagine two sets of scientific data obtained from the same experiment—one is artistically perfect and appealing, but scientifically incorrect, and another is not so artistically pretty but scientifically correct. If in this intrapersonal conflict one's artistic side is victorious, one could end up with data manipulation and scientific misconduct, which might even put the lives of others in danger.

BSJ: As a researcher working toward developing new drugs in the field of reproductive biology, it is important to include people of different identities in your research. How do you ensure that your research, where possible, is inclusive of individuals of all identities?

PL: Our research activities require that we enroll participants to take part in our IRB-approved studies to provide sperm cells for our research. The nature of this research, the strict confidentiality of our participants, and the IRB-specific requirements mean that we cannot collect any personal data or identifiers and we cannot ask our participants any questions related to their health, identities, or other personal information. Any individual who can produce sperm cells is welcome to participate in our studies during the period of active enrollment.

BSJ: How are you actively working to promote a culture of equity and inclusion within your lab, regardless of an individual's racial background or gender identity?

PL: From the very beginning, our lab has welcomed anyone who is enthusiastic about the research we are doing and is eager to become a responsible member of the team, regardless of their age, beliefs, religions, political affiliations, racial backgrounds, gender identities, etc. Our only limitations are the physical space to accommodate our team and the funding to support the work we are excited about. True diversity makes the team stronger, and this is what we strongly believe in and always have.

This interview, which consists of one conversation and one email communication, has been edited for clarity and brevity.

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