

Exploring the world of human development and reproduction

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ABSTRACT Susan Fisher has spent her career studying human development, proteomics, and the intersection between the two. When she began studying human placentation, there had been extensive descriptive studies of this fascinating organ that intertwines with the mother's vasculature during pregnancy. Susan can be credited with numerous major findings on the mechanisms that regulate placental cytotrophoblast invasion. These include the discovery that cytotrophoblasts undergo vascular mimicry to insert themselves into uterine arteries, the finding that oxygen tension greatly affects placentation, and identifying how these responses go awry in pregnancy complications such as preeclampsia. Other important work has focused on the effect of post-translational modifications such as glycosylation on bacterial adhesion and reproduction. Susan has also forayed into the world of proteomics to identify cancer biomarkers. Because her work is truly groundbreaking, many of these findings inspire research in other laboratories around the world resulting in numerous follow up papers. Likewise, her mentoring and support inspires young scientists to go on and make their own important discoveries. In this interview, Susan shares what drove her science, how she continued to do important research while balancing other aspects of life, and provides insights for the next generation.

KEY WORDS: *placenta, angiogenesis, implantation, pre-eclampsia, VEGF*

KR: How did you first get interested in science?

SF: I have been interested in science for as long as I can remember. Since I grew up in the country, I was surrounded by nature. I was particularly interested in development. I would make the rounds of all the trees in the neighborhood and pick up eggs that had fallen out of the nest and preserve them in vials of methanol in order to study developmental series. I really wanted to be a cool kid so it took me decades to admit that I had been doing this. But that was my first foray into development. I was also interested when calves were born. At that time I did not know about the placenta. But I thought it was a very cool process, and I knew that something came out after the baby. I just didn't know what. So I was interested in biology and my father bought me a chemistry set that he set up in the basement. This was very popular with my friends. So unlike the eggs, I shared the chemistry set and my experiments with everyone. Spilling alcohol on the cement floor and lighting it was a particular amount of fun!

PD: Susan, probably many people don't know that you have an astounding voice and were a singer when you were young.

Can you talk about that?

SF: Early on everyone pushed me to pursue a musical career. It was obvious very early on that I had a very large mezzo soprano voice. Since I was in a rural area, there were many opportunities for me to sing in churches and in musical theater. I also was extremely fortunate to have a music teacher who knew about statewide activities and took me to audition for an Illinois music educator's chorus that went with the band and orchestra to Paris, France during the Vietnam War. Composers wrote music for us because they were very interested in hearing young American adults perform music about the Vietnam War. That was a tremendous opportunity and I got to experience what it was like to perform for thousands of people on a very large stage. All of my musical experiences have served me very well in science. What people may not realize if you have never been in that world is that it can be very hard on the ego, as it's perfectly possible that someone more talented than you will walk in the door and replace you at the drop of a hat. I think

Abbreviations used in this paper: UCSE, University of California, San Francisco.

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that's where the classic musical person's ego comes from—this sense of insecurity and imminent potential loss. Science is very much like this in that there are probably many people working on the same problem that you are and it's very competitive. Every time you open a journal or go to a meeting the possibility exists that someone will have scooped your biggest finding. So I think that's a lesson that I learned how to deal with early on through my singing career. I think that my stage experience also has helped me in learning how to deliver a seminar without putting everyone to sleep, although I think that topic could probably be debated.

PD: As I reflect on Susan's comments here, it strikes me that she transmitted these lessons to us in many ways. Science is a field filled with big egos, but Susan trained us to put the work, not our pride, first. She told us not to worry about looking smart or dumb in front of people, but to ask questions and find answers. The ego wasn't the important thing, it was the work and the pursuit of scientific truths. I think that this approach fosters collaboration, networking, and ultimately leads to faster results and more fun along the way.

KR: How did you get interested in the placenta?

SF: When I was a graduate school student at the University of Michigan, we all had to take a techniques course. As with any laboratory experience there was a lot of standing around. The professor who was teaching the section on electron microscopy was very interactive and liked to tell us about his career and research projects. At the time he was working on cardiac pathology. He told us about these experiments, but he also said that this was not his first love. If he could get money to work on the placenta, that would be what he really wanted to do. I had absolutely no idea why anyone would be interested in the placenta. So I asked him what made them placenta such a fascinating organ. He immediately launched into this very long and enthusiastic explanation of how this organ was able to interface with the mother even though half of its genes belonged to the father. He explained how this defied every rule of transplantation. He went on to describe in some detail how the trophoblast cells invade the uterus. By the end of the class I was completely hooked. When we had to pick a topic for our embryology paper, I chose the placenta. By the time I finished that paper, I was as enthusiastic about the placenta as he was. I knew that at some point in my life I would pick up this topic again and study it, which has certainly been the case. However, I didn't begin working on the placenta until a few years after I joined University of California, San Francisco (UCSF), when I did break the cardinal rule of not working on more than one project as an assistant professor.

PD: You studied saliva before the placenta. Why?

SF: The saliva project was definitely more fundable. Even then, Child Health was super hard to get funded from so the Dental Institute was very appealing. I think it speaks volumes if I tell you that my first R01 submission on the placenta was site visited by a team of three people because they could not believe that I could do what I had proposed.

PD: Because of access to tissue?

SF: Yes, part of it was access to tissue and part of it was that my CV didn't really line up that well with the grant I had written. I think they wanted to see if I was a real person.

PD: Your laboratory has always been involved in more than one field. Currently, you have subgroups that study the placenta, embryonic stem cells, and mass spectrometry. Why did you set your group up this way?

SF: Well I've always been interested in looking at science from many different perspectives. I studied plant physiology and pea seedling development in college. I knew that I really loved development, and I thought that plants were such a tractable system. At some level they were because you could do bucket biochemistry on them. But it was in the time before there were any genetic methods, so it was really a dead end. For that reason, I went to graduate school in human anatomy. I decided that if I couldn't work on plants, I was going to work on humans. After that I was quite interested in going back to something that was more so-called hard science, and I vacillated between x-ray crystallography and mass spectrometry. At the time, a postdoctoral fellow's career in x-ray crystallography depended on whether or not you could crystallize your protein of interest. I decided that that was way too technical and chancy, and that I would do mass spectrometry instead. That was also no cakewalk because at the time the instruments did not run very well, and we would spend a lot of time making samples and you would pray as you injected them into the mass spectrometer that nothing would go wrong with either the instrument or the data system. So when I was training, I had the luxury of being able to obtain a very wide scientific education before I started my own lab and needed to focus. As a result, we have carried all the various themes, except for plant physiology, through the work that we have done over the years.

KR: What are the pros and cons of having such a diverse group?

SF: It obviously takes a lot of work to stay credible in more than one scientific area. It's always funny to me that my mass spectrometry colleagues do not acknowledge that our lab does developmental biology, and most of my developmental biology colleagues don't know about the mass spectrometry portion. But I have always enjoyed both of these fields so much. It has given me a wonderful chance to get to know two different scientific communities in a very



Fig. 1. Aka Prakobphol in the lab circa 1998.



Fig. 2. Susan Fisher and long-time collaborator, Caroline Damsky, with whom she worked on trophoblast cell adhesion molecules (circa 1995).

deep and meaningful way. I always find it interesting the ways that they are alike and the ways that they are different. The differences are obvious. However, the many likenesses would surprise a lot of people as it takes a great deal of creativity to master a technique as complicated and varied as mass spectrometry. The good side about having two facets to a career is that if one part is going badly you can always work on the other. This has been the case many times. Instead of fretting about some disaster in the placenta world, I would work on mass spectrometry data for awhile and forget the trophoblast troubles. Conversely, if our mass spectrometry work was going badly, it always seemed like our developmental experiments were going gangbusters. So, there have been many more upsides to working in two different areas than downsides.

PD: Was having long-term employees in addition to graduate students and postdocs helpful in maintaining progress in diverse fields?

SF: Our lab has an unusual organizational structure as compared to most. Since we work in different areas, I thought it would be important to have a senior scientist who could lead each. Early on I was lucky to recruit Aka Prakobphol (Fig. 1) who was a DDS PHD and could very independently lead our work on the carbohydrate structures in human saliva that mediate bacterial and leukocyte adhesion. Later on, we were equally lucky to have Dr. Olga Genbacev join our group. She had headed her own group in Yugoslavia before she had to move due to the political unrest that preceded the war. She had originally worked with Dr. Rich Miller at the University of Rochester. Cannon here in Silicon Valley recruited her husband; therefore, she was looking for a position in the bay area. She visited me, and we hit it off immediately. Ever since she has been a critical component of everything that we have done, first in placental biology and later in human embryos and human embryonic stem cells. Finally, about a dozen years ago, it was apparent that UCSF needed more mass spectrometry capabilities. I was given the resources to start a mass spec facility and again used the same organizational model to hire senior people who could direct the day-to-day efforts. By now I'm convinced that this is the way to go for labs with diverse interests such as ours.

KR: Can you talk about a few of your favorite discoveries and the excitement that you felt during the eureka moments?

SF: One of my favorite moments, which I think is something that everybody has in common, was the first time I got something to work in the lab. I can remember when my first assay for an enzyme activity in pea seedlings worked, I was absolutely astounded. I still get that same sort of bubbly high when someone shows me a piece of data in the lab.

We have had so many high points but a few come to mind as being particularly special. For many years we worked on adhesion molecules with the laboratory of Carolyn Damsky (Fig. 2). We were very excited to observe that as trophoblast cells invaded the uterus they entirely switched their adhesion molecule repertoire (Damsky *et al.*, 1992; Damsky *et al.*, 1994). We thought that this was important because it might be a model for what cancer cells do over protracted time as they undergo changes that lead to malignancy and metastasis. But we couldn't really make any sense of what they were trying to change into. We knew that they were turning off their original ectodermal or epithelial phenotype, but we could not figure out what they were trying to become. Finally, out of more desperation than anything else, we decided to test a few molecules that were thought at the time to be unique to vascular cells or endothelial cells and bingo! That was it. Every vascular-type adhesion molecule that we tried, the invasive trophoblast expressed (Damsky and Fisher, 1998; Zhou *et al.*, 1997b). So that was very exciting, to understand that the trophoblast cells were actually undergoing a new type of epithelial to endothelial transition. We figured at the time that this must be a critical component of normal pregnancy, and that failures in this process might be associated with the pregnancy complication preeclampsia, which is associated with abnormal trophoblast invasion and failure to remodel the maternal vasculature. This was indeed the case and was our first real entrée into the molecular placental defects that underlie this syndrome (Fig. 3; Zhou *et al.*, 1997a).

Another really exciting time was when we began to get the sense that oxygen concentrations were essentially trophoblast developmental regulators (Genbacev *et al.*, 1996; Genbacev *et al.*, 1997). At the time we didn't really have any specialized equipment to study this phenomenon, so we decided to put our cultures in an anaerobic incubator. We thought that this would be a cheap



Fig. 3. Yan Zhou when she first joined the lab in 1990.



Fig. 4. Olga Genbacev and the Fisher Stem Cell Group (2010). From left to right, Matt Donne, Tamara Marsh, Tristan Juhan and Nick Laroque, with Olga Genbacev seated.

and easy way to determine if physiological hypoxia played any role in trophoblast biology. This experiment was tried by Dr. Olga Genbacev (Fig. 4), the only person who is usually as crazy as I am. So we came back the next morning and Olga came flying into my office and said “look, look, look”! And we did not even need the microscope to see that there were many, many, many more trophoblasts in the cultures that had been placed in the anaerobic incubator. That was the beginning of our work on oxygen effects and HIF pathways, which has continued to this day.

Another really interesting time was a collaboration with Dr. Bob DeMars at the University of Wisconsin. His graduate student, Susan Kovats, had worked out a 2-D gel electrophoretic method for mapping human major histocompatibility class I antigens. Through another colleague, Bob contacted me to ask if we had succeeded in purifying trophoblast cells from human placentas, and I said that we had. He asked us to send them several preparations. When they did the immunoprecipitation 2-D gel electrophoresis experiments, it was immediately crystal clear that trophoblast cells expressed HLA-G, rather than conventional class I molecules (Kovats *et al.*, 1990). At that time we felt that we had the major explanation for why the mother does not reject the placenta. This is a very humbling

lesson, because more than 20 years later we still have not identified the NK receptors that might recognize HLA-G, despite trying very hard with many of my wonderful colleagues in immunology at UCSF. Despite this fact, I’m still very enthusiastic about HLA-G, and hope that we will eventually solve its mysteries.

PD: Can you explain how you came to study embryonic stem cells?

SF: Our work in human embryonic stem cells came about quite accidentally. My colleague Roger Pedersen decided very quickly and abruptly to move to Cambridge in 2001. He showed up in my office early one morning and asked if my group would like to take over the human embryonic stem cell program that he had started. He knew that we knew a lot about human development and had human embryologists in our group. Therefore, we were uniquely placed to be able to derive and characterize human stem cells. I convened a quorum of the people in my group who this would affect and asked if they would be interested. Everyone was enormously enthusiastic about the possibilities, so I told Roger that we would give it a try and UCSF made that possible (Fig. 4). So that was how we entered the human embryonic stem cell field. I have to



Fig. 5. Penny Drake and Susan Fisher around the time that she received her PhD in 2001. Sometimes what you see depends on who is wearing the glasses!

say that working on human trophoblast also made this possible as we had freezers and freezers of human-specific reagents, such as antibodies and probes, that would allow us to interrogate human cells. This was a major asset. I also had taken human embryology at the University of Michigan, and so I knew how important the principles of embryology were to stem cell biology, something that is often ignored. Therefore, we were in a good position to apply developmental principles to stem cell biology, particularly early fate decisions.

KR: Do you have any advice for young investigators trying to establish their own labs?

SF: I don't know if I have a very good answer for that. I know that it's a particularly difficult time for young people to start their careers. This is the third major downturn in funding that I have experienced in my career, so I definitely understand the problems that tight funding creates. One major fallout is how difficult it is to publish papers in good journals. I think that people are in general in a very bad frame of mind and not predisposed to be open to new ideas and concepts. There is also a certain amount of competition as people know that high profile papers will lead to grants and so forth. This makes it a tough environment for everyone, but especially for young people who are getting started. The NIH has realized this and instituted a few rules that help beginning academics get grant funding, but in my experience this only delays reality setting in as competitive renewals can be the first tangle with a study section on a level playing field. I think that the best way to succeed in this environment is to trust your instincts. After all, you have made thousands of good decisions about experiments or projects up to now. If you had not you would never have been hired. Yes, we all make mistakes and they can be painful. But to be offered an assistant professor position your successes have to far outweigh your mistakes. This is the lesson that you should never forget.



Fig. 6. Yan Zhou (left), Kristy Red-Horse (middle), and Aka Prakobphol (right) celebrating Kristy's Ph.D. thesis defense.

PD: Having you as a mentor demonstrated that it was possible to be both a serious scientist and a devoted parent. Thank you for that. I vividly remember how shocked I was, and what a breath of fresh air it seemed, the first time I heard you cooing to your then 4-year old daughter over the phone in your office. At UCSF, which was full of people trying to impress each other by how much they worked, it seemed so brave and somehow irreverent when we would be at a meeting in your office with people who seemed to me to be quite important, and you would stand up without ceremony and announce that you had to leave for your daughter's



Fig. 7. The Fisher group in 2005. Fisher Lab holiday party circa 1997. Also pictured are members of Diane Barber's lab, with whom Susan shared space for several years. The "Placenta is the Center of the Universe" Fisher lab t-shirt from that year is featured.

basketball game. Can you talk about that?

SF: I always really wanted to have a very strong family. And I never really wavered in that desire. It was always the most important thing to me. People who know me professionally would be very surprised to hear me say that, but I am more committed to my family than I am to my scientific career, so it was never really a struggle for me to choose between the family or work. Obviously I am gifted with the kind of stamina it takes to do both very intensely and I have a tremendous love and respect for science, but my family has always come first.

PD: You mentioned stamina. Can you talk a little about what you had to do to make it work?

SF: I found out I was pregnant with Molly as we were driving across the country and I was taking an assistant professor position at UCSF. I had not been feeling well, and my husband Carl kept saying how unusual this was because he had never seen me nervous about anything and he could not believe it was happening now. I was so sick to my stomach and had all these very weird symptoms. Finally, by the time we reached San Francisco we both put two and two together and bought a pregnancy test and it was positive. At the very same time, within less than 48 hours, he received a letter that he'd been accepted to medical school on the East Coast. So we were separated for the first 2 1/2 years. That meant that Molly and I had to function very independently, and it also meant that we had the chance to be very close. But we also had some pretty harrowing experiences and things did not run perfectly on a day-to-day basis, that was for sure. But I got used to working when she would sleep and trying to get by on smaller and smaller amounts of sleep, and I never had any problem with this. So in many ways I was lucky. I don't have any magic solutions because it definitely takes a huge amount of hard work. I was able to burn the candle at both ends and actually enjoy it. So that habit of working hard in the lab, and playing hard with my family has continued and I feel like I benefited enormously from it. Having quality time away from science gives you the time and distance you need to have new perspective on your work. And I think it really benefited my daughters to have always taken for granted the fact that they would have meaningful careers.

KR: You received the Outstanding Mentorship Award from UCSF several years ago. We both wrote enthusiastic letters of support for that award and both benefitted greatly from your guidance, example, and training. Can you talk a little bit about the role of a mentor as you have experienced it?

SF: One of the greatest pleasures has been the opportunity to train talented students. You two are prime examples. It's such an amazing experience to watch someone enter a research lab as a relative novice and emerge as a very experienced and sophisticated scientist. This is obviously quite a journey and it's such a privilege to be able to take this with students. You both were so enthusiastic and committed to science and talented at both doing experiments and writing up your work. When you see young people like this you know that the next generation of scientists is in good hands.

PD: One of the most rewarding and fun parts for me was learning to write and edit scientific documents, literally by sitting next to you with a computer on my knee while we went over every word together. Through that process, I learned scientific**writing by apprenticeship.****KR: I do that with my people now too. It is so much fun, and they all have great ideas themselves. That is one of the things that I didn't expect, how fun it is to see other people have successes.**

SF: Oh that's wonderful Kristy, I'm so glad to hear it because I think it is too. The incredible benefit you get as the lab head is that you get to know people in a very close way that would be impossible to have happen any other way.

PD: Can you talk about that a little bit?

SF: I think that if you give people a lot of freedom you get to see how people think and how they operate and how they solve problems. And I think it works if your students know that you will jump in and save them if they need saving. During that process you really get to know someone from the inside out. You know how brave they are about trying things, you know how hard they will work, you know how resourceful they are. These are the kinds of things that I often feel that parents don't ever even get a chance to appreciate. And then when you go through the process of writing a piece of work up together the whole relationship goes to a completely new level because now you're putting together stories and pictures that go along with a story and then interpreting results, and sometimes you find out that everyone has been looking at this from quite different perspectives. It's a particular thrill when students see ramifications in data or possibilities and results that are new and exciting and that are their own creative efforts.

As a summary to this interview, Kristy and I wanted to share a few of the major insights and habits that we learned as graduate students with Susan. This is difficult to do, because the most important lessons that we learned, we probably do not remember learning. We simply internalized them as young graduate students and have taken them for granted since then. However, looking back and trying to summarize from the distance of more than a decade, I come up with this list:

Be brave; Ask interesting questions; Write, then edit, edit, edit, edit; Trust your instincts; Be inclusive and open with your work; Avoid politics as much as possible; Celebrate data and achievements; Laugh openly and joyfully; Work hard; Be true to yourself and your family.

And, if we turn back the clock a bit, to remember what it was like as a student in her lab, a glance at the Acknowledgements section of my thesis yields this:

"...if I picked the winning lotto numbers every day for twelve months straight, I still wouldn't be as lucky as I was the day Susan Fisher became my mentor. Like many other faculty members at UCSF, Susan is a top-notch scientist. Studying with her has provided me with incredible opportunities to learn about all aspects of being an academic researcher, and I feel very well-grounded as a result of her guidance. However, Susan's non-scientific attributes are what really distinguish her as a fabulous mentor. Her sense of humor has been a godsend, and her seemingly perfect balance of family, science, and sanity have served as an inspiration to me."

The Acknowledgement section goes on a bit longer, and then ends, "Thank you, Susan, thank you, thank you, thank you for the whole shebang." That seems in some ways a fitting end to this piece as well. My father once told me that the best way to honor

people who have done good work for the benefit of others was to remember them, and to say thank you. Susan Fisher, we honor your work and your humanity. Thank you for sharing them with us (Figs. 5-8).

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