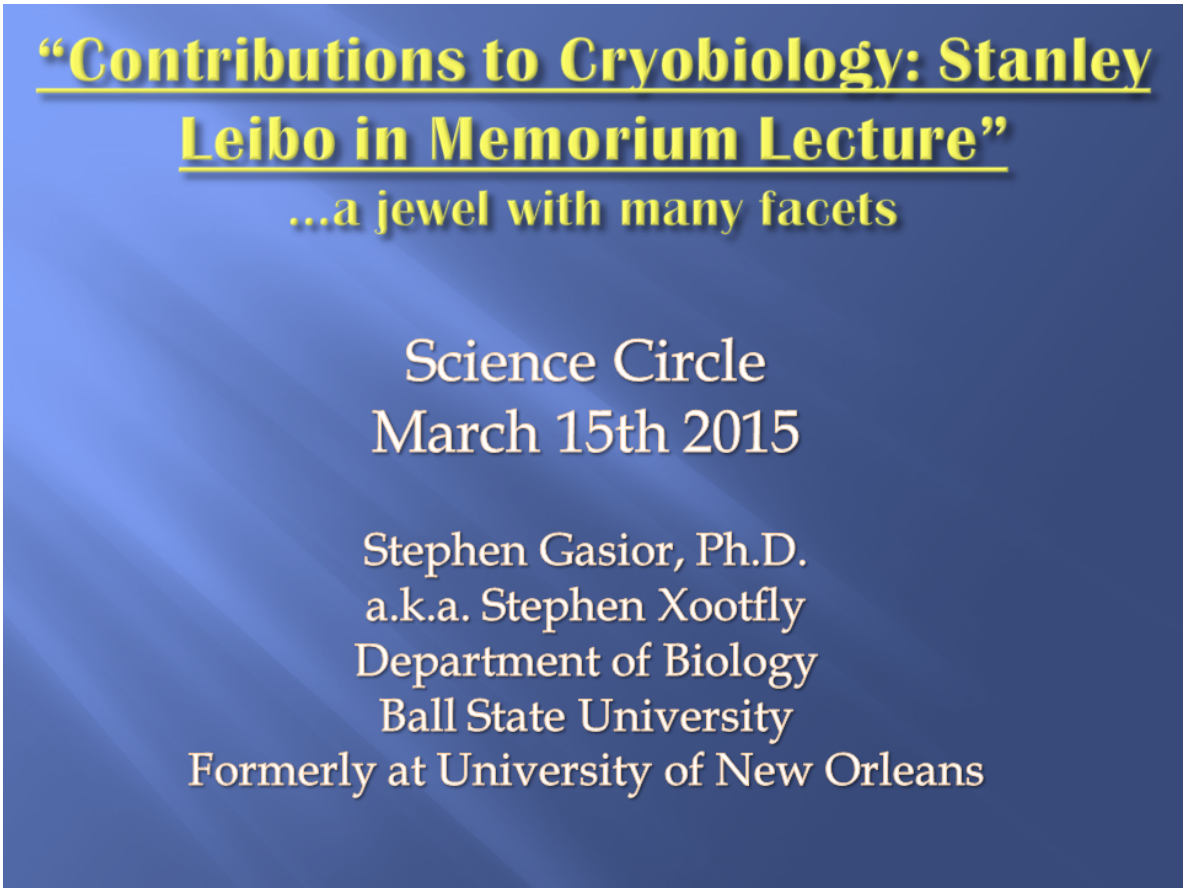
I'd like to start off and thank everyone for coming. This is a combination of learning about some interesting science that is very applicable, in many ways, they biotechnology and cultural events. But also is, in many ways, a very personal conversation. We are going to talk about Contributions to Cryobiology by the late Stanley Leibo, and he was someone that I knew as another faculty at the University of New Orleans.



He passed away almost a year ago from cancer. And I knew him largely as the older guy who did some research with freezing stuff, at least for the first 3 to 4 years I was at UNO. And being busy, I didn't spend a lot of time interacting with him because he was very busy traveling and conducting a research program. About the last 3 years I was there, he started actually teaching some of the basic biology classes. And that's the opportunity where he said "I hear you know a little bit about technology so maybe you can help me get some stuff set up." And from then, really, started interacting more. And what was always really amazing about him was his enthusiasm for science and his enthusiasm for really interacting with people and talking about stuff. So, he always had a bunch of great stories of some of the classical big figures in biology. Not just in his field of cryobiology.

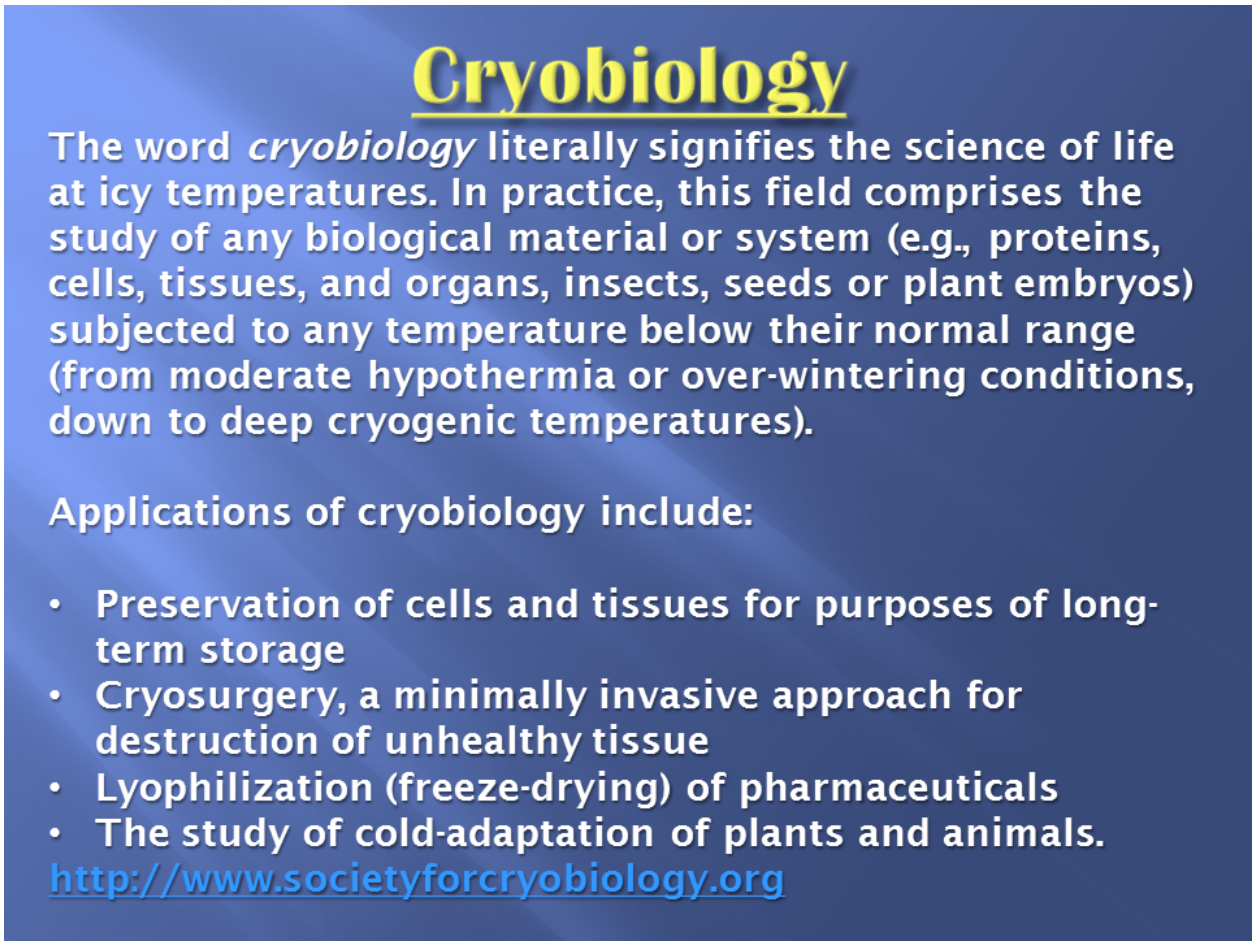
So, I felt that the best way I could honor him in passing would be [1] to learn a little bit more about his field and his research. And that is something, again, is something I've started doing, and I'll share a decent amount of that. But then also [2] honor him by conveying his work and what that field does to a more general audience. So, thank you for helping me with that second goal. And, I hope you enjoy it.

He did his graduate work at Princeton and then moved on to Oak Ridge. This is a picture of him from the obituary that was on the International Society for Embryo Transfer. Showing him hard at work in a training program that he constantly put on during his lifetime. Now what I would like to, oh sorry, ... And during the last part of his professional career, in terms of appointment, was at University of New Orleans from 1999 to 2014. Where he mentored graduate students, taught classes, and was also involved with the Audubon Institute involved in some research looking at species conservation.

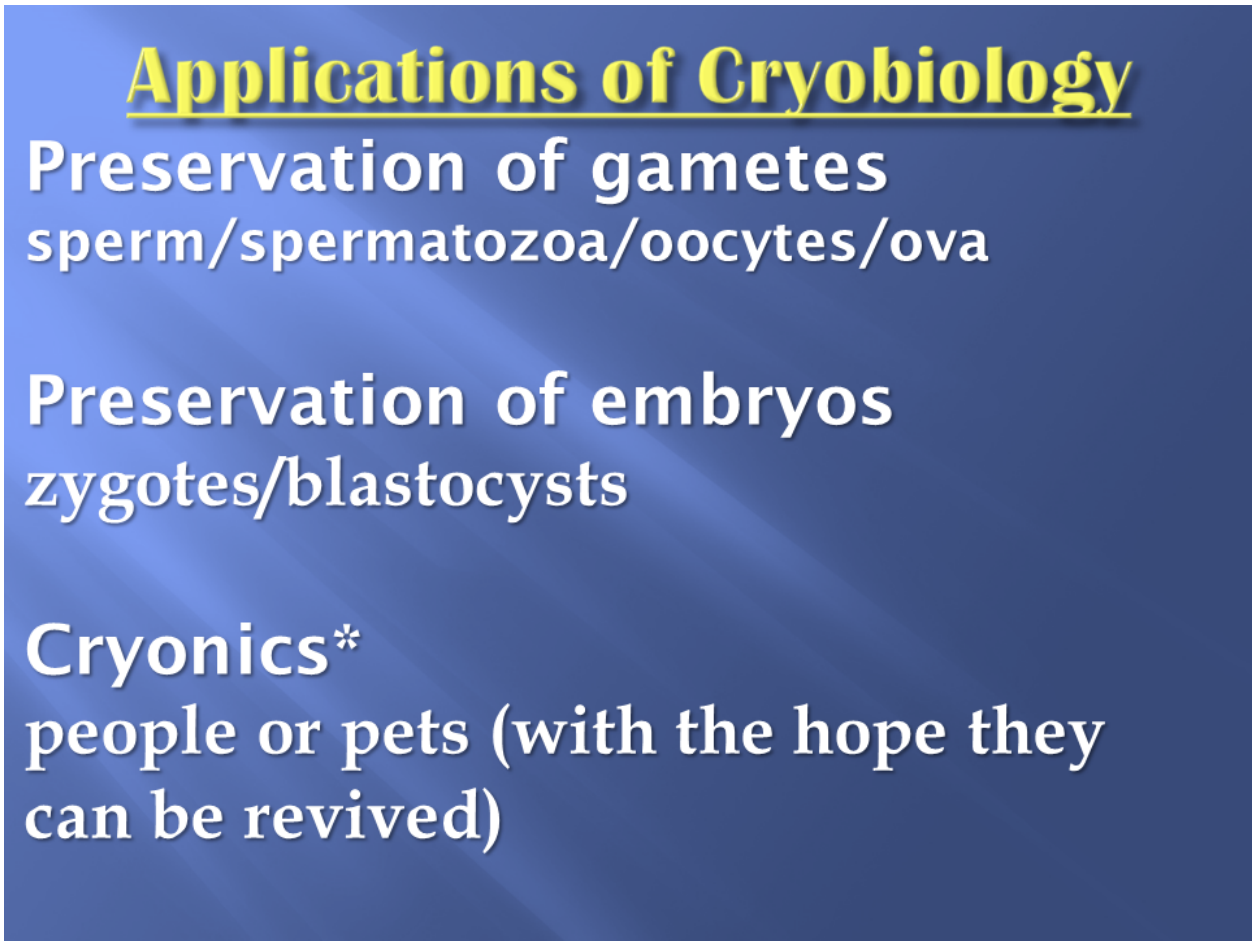
I'm going to start and mostly talk about cryobiology, and again, this is a definition coming from the Society for Cryobiology. The definition being “cryo” meaning something cold and “biology” related to biological organisms. The professional aspects of the field really revolves around trying to preserve biological specimens with the integrity of keeping them viable and using them for some biological purpose. Some of these examples that they mention are:

- long-term storage
- cryosurgery
- stabilization of pharmaceuticals
- study of cold adaptations of plants and animals.

You've probably heard of these biological organisms that live in the Arctic or Antarctic oceans. But the large majority of the focus of Dr. Leibo's career actually had to do with preserving cellular components of organisms in order to then revive them. A somewhat specialized within that [larger field].

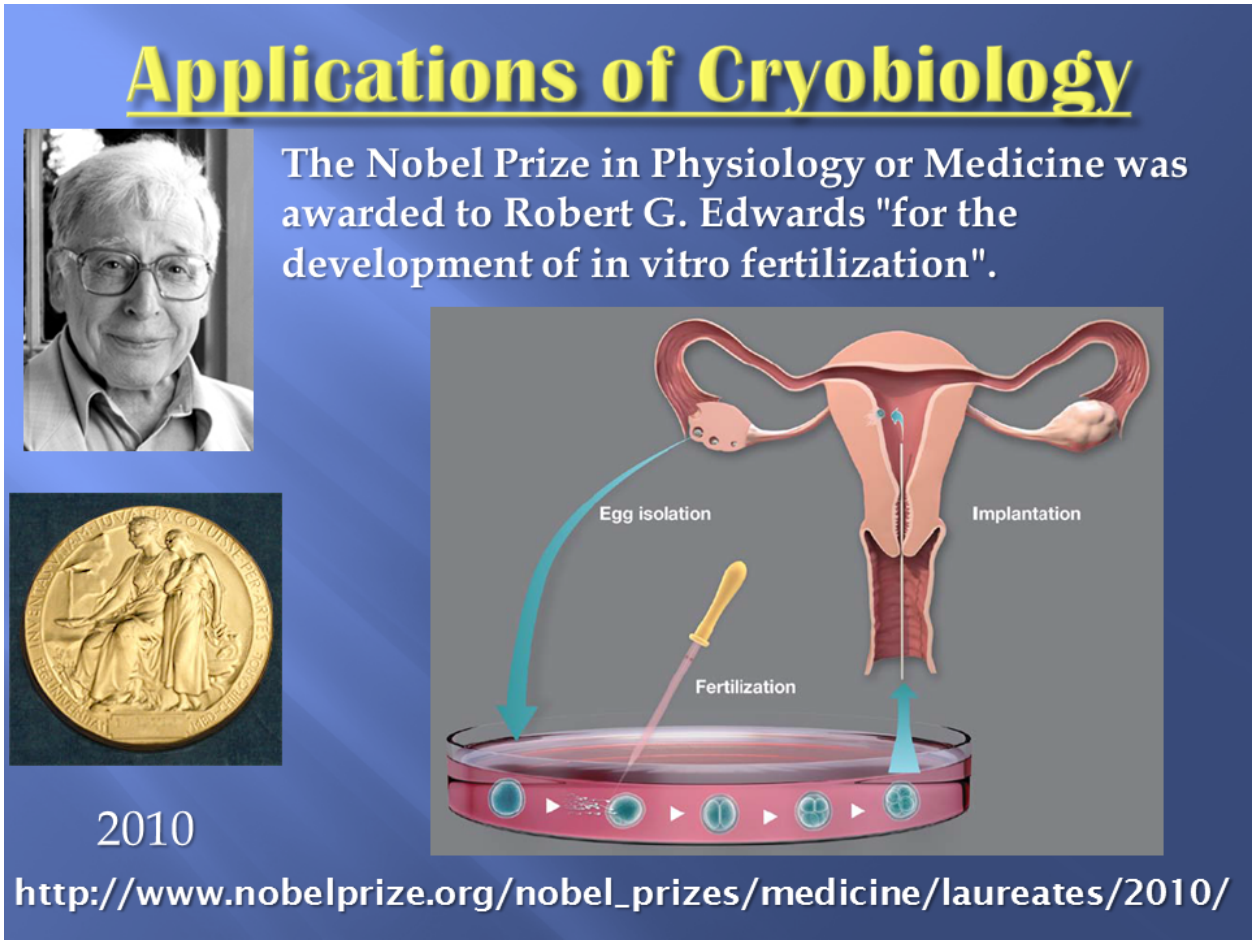


And some of these applications would involve the preservation of gametes: sperm, spermatozoa, oocytes, ova. Preservation of embryos. So after fertilization, either the 1 cell to the 8 cell to the blastoff stage where there are maybe several hundred cells involved. And then, and I have an * here also the study of cryonics, where people or their pets preserve their bodies through freezing at very cold temperatures. And again, this is something that I put an * because many hard core cryobiologists would not include cryonics in this type of [research] field because we don't know, just hope, that those people can be revived to them become a viable intact organism.



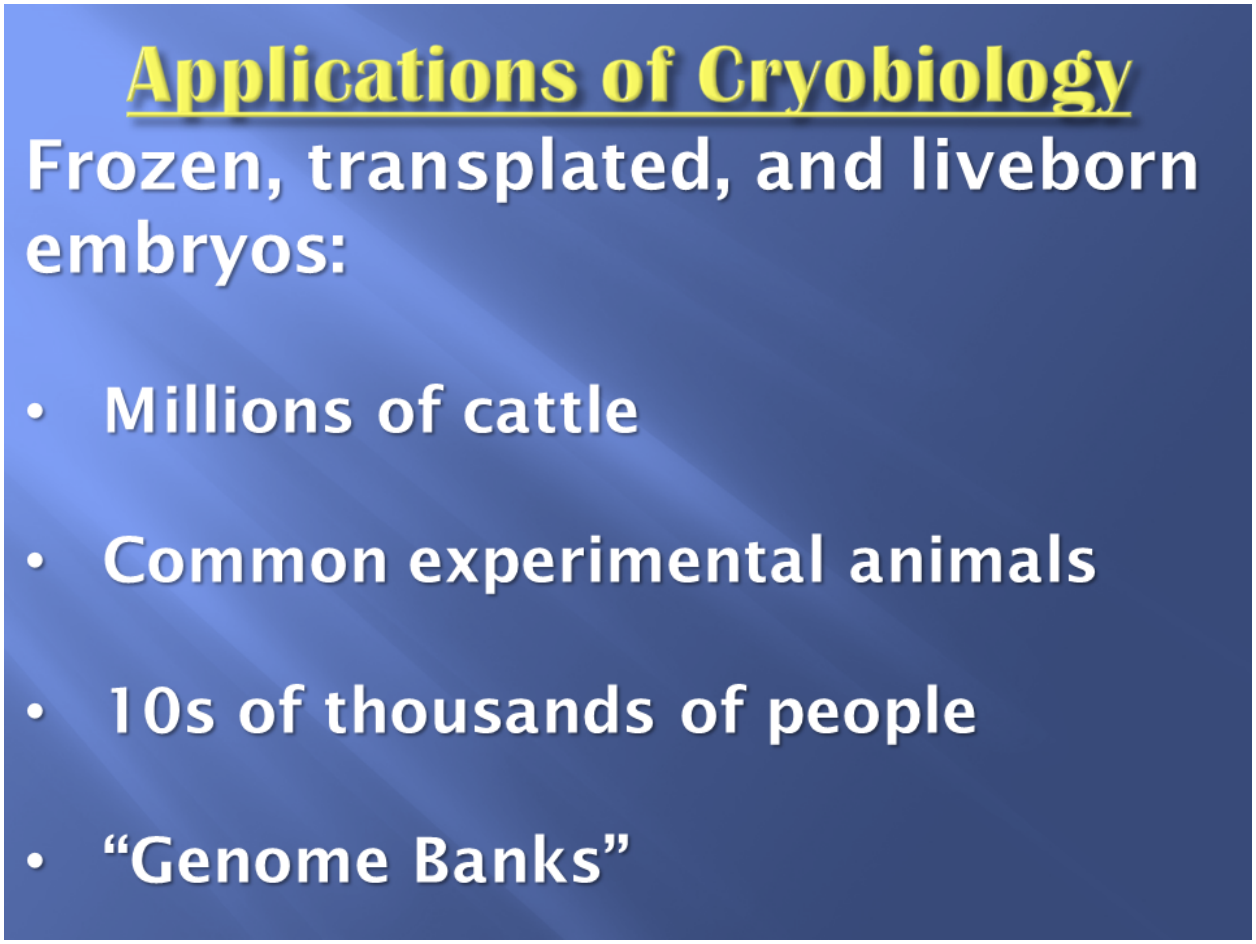
One of the most well-known applications of cryobiology is related to, is one component of, what's known as in vitro fertilization. And so, The Nobel Prize was awarded to Dr. Edwards in 2010 for the development of in vitro fertilization. And this is a process by which gametes, eggs, can be extracted from a female. Put in vitro, inside of a little dish, which is what in vitro means (outside of a cellular system), in a test tube. And then exposed to sperm one of which would fertilize it begin the embryogenesis process and then be reimplated into that woman or another surrogate uterus for then ultimate development into a new person. Or, again, when applied to other mammals, amphibians*, lizards* would lead to a new individual. So one aspect that makes in vitro fertilization most powerful, you can do this without freezing

anything, it can be very direct. But in terms of its wide scale applicability in culture and livestock, then it's something where being able to freeze stuff so that it can be more conveniently be implanted and have surrogates, that is what makes it a more feasible technology.
[*so amphibians and lizards don't have uteruses. I crossed synapses here.]



Now in terms of the number of situations in which this has occurred, there have been millions of cattle that have had embryos frozen and reimplanted. And this is one of these things where if you have cattle-stock, it's much easier to transport large number of very specialized breeding strains or transgenic by airplane or across continents by being able to transport frozen eggs as compared to the whole animal. And in other cases, you just don't know how well a particular breed or an animal is so you can have more copies of it later. Pretty much all the more common laboratory research animals that you find in biomedical research, the mouse, macaque, hamsters. These have all repeatedly and consistently been shown to be something that you can preserve. And again, for science, it's very useful if you've developed breeds or transgenic strains to be able to freeze them away so that you can ship them to other researchers or to go back and look at them in a new way or a new type of experiments after you've had the wisdom of time and other experiments to say "Oh, I wish we'd done that.

There have been tens of thousands of people of people born of IVF many along with cryopreservation of the embryos.
And, of course, one of the things that I think is going to be and one of the contributions Dr. Leibo made, was making, during the end of his career was Genome Banks. For a lot of species where wild populations are disappearing from the planet that if we have the opportunity if we can get ourselves around to find a way to preserve the habitat of these animals, if we have them frozen away as embryos (or their sperm and eggs) that something that we can then revive these species that are endangered.
And there is a question in the audience about the freezing of maybe other types of cells. I was trying to focus here on those that are involved with Leibo's work. But, yes, the preservation of many cell types is something ongoing, very important, for biotechnology including things like umbilical stem cells. Depending on how you view them those are very useful for potentially replacement cells for that individual or potentially to be use for purposes like stem cell differentiation.



Now this is one thing that does hit the popular culture in many cases. Probably all of you that are stateside, and maybe some of you in other countries as well, have heard about Nadya Suleman, also known as Octomom, and again, she is someone who received a certain about

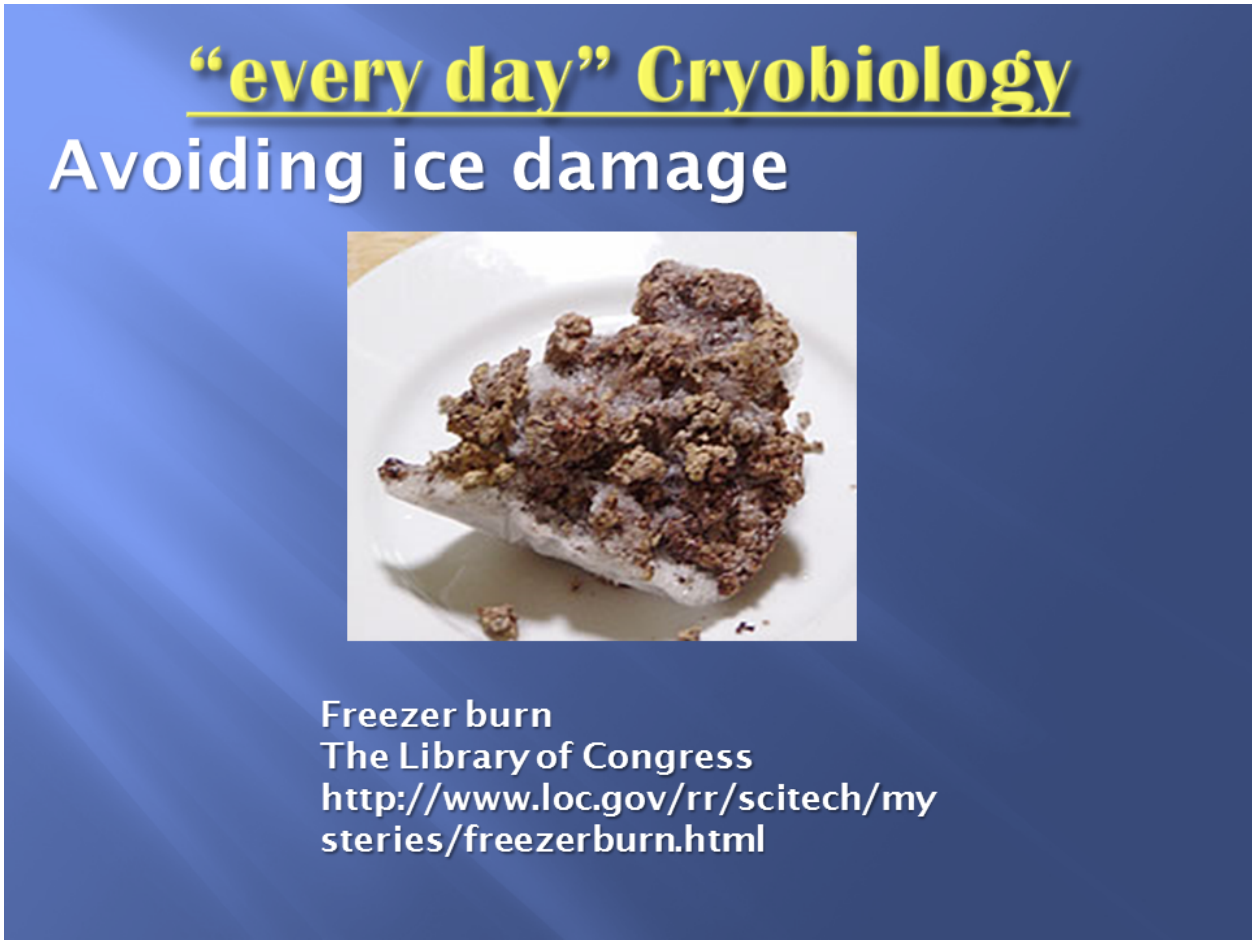
of celebrity slash what I would also call /notoriety for being a woman who was really only the second person at the time to have given birth to 8 human offspring at one time.
There have been others since that time and what was very interesting about this and something that permeates the popular culture is that many people felt she, well; there was a lot of moral judgment involved about how people perceived and discussed this particular situation. One thing to keep in mind is that while she made the request to use the remainder of the embryos she'd had preserved earlier in her life the ultimate decision of how many to implant was made by the doctor. And, he ultimately, from what I read, lost his medical license for having implanted so many at one time.
Info and Image from:
http://en.wikipedia.org/wiki/Nadya_Suleman and…
http://img.metro.co.uk/i/pix/2010/01/26/article-1264505502852-06FF95AE000005DC-853361_636x300.jpg

So, in terms of the scientific literature. Cryobiology is a growing field. Cryobiology is something that really hasn't reached 100 years in terms of age. In terms of the first early publications. But, it's a growing field in terms of basic research. I performed a PubMed search

using the key terms you see here and these are the number of papers that were published, which could include review articles, in the year 2014.
There's a video of a talk that Leibo gave at Cyro2013 in which he shows a graph from the beginning that in essence there is an exponential growth in the number of publications related to cryobiology. So, I think it's a field that is growing and has a very rosy future.

Now, let me introduce you to a little more about what cryobiology is really about. Is that I think on way to sum it up, and what I've seen from many people in the field, is that cryobiology is really about trying to avoid ice damaging tissues. And so this is an image of freezer burn hamburger. Where if you put meats or any number of items in your freezer, and they are allowed to sit there for a pretty long period of time, and they also probably go through some freeze-thaw cycles (again, not a freezer that just stays frozen but these ones that are more common that don't accumulate ice) and you also don't wrap them up and preserve them, then this is the type of hamburger meat you get out of them. This is something that can affect the palatability, the flavor and in many ways the texture of. Has anyone ever done this where you are maybe really really hungry and you look back in that freezer and say “maybe but maybe NOT!” This is just in terms of food that you might eat. Again, think of the potential implications

of intact cells. And if you are preserving embryos to be a future child, this is not the type of thing you'd like to have happen.
Image from: http://www.loc.gov/rr/scitech/mysteries/freezerburn.html

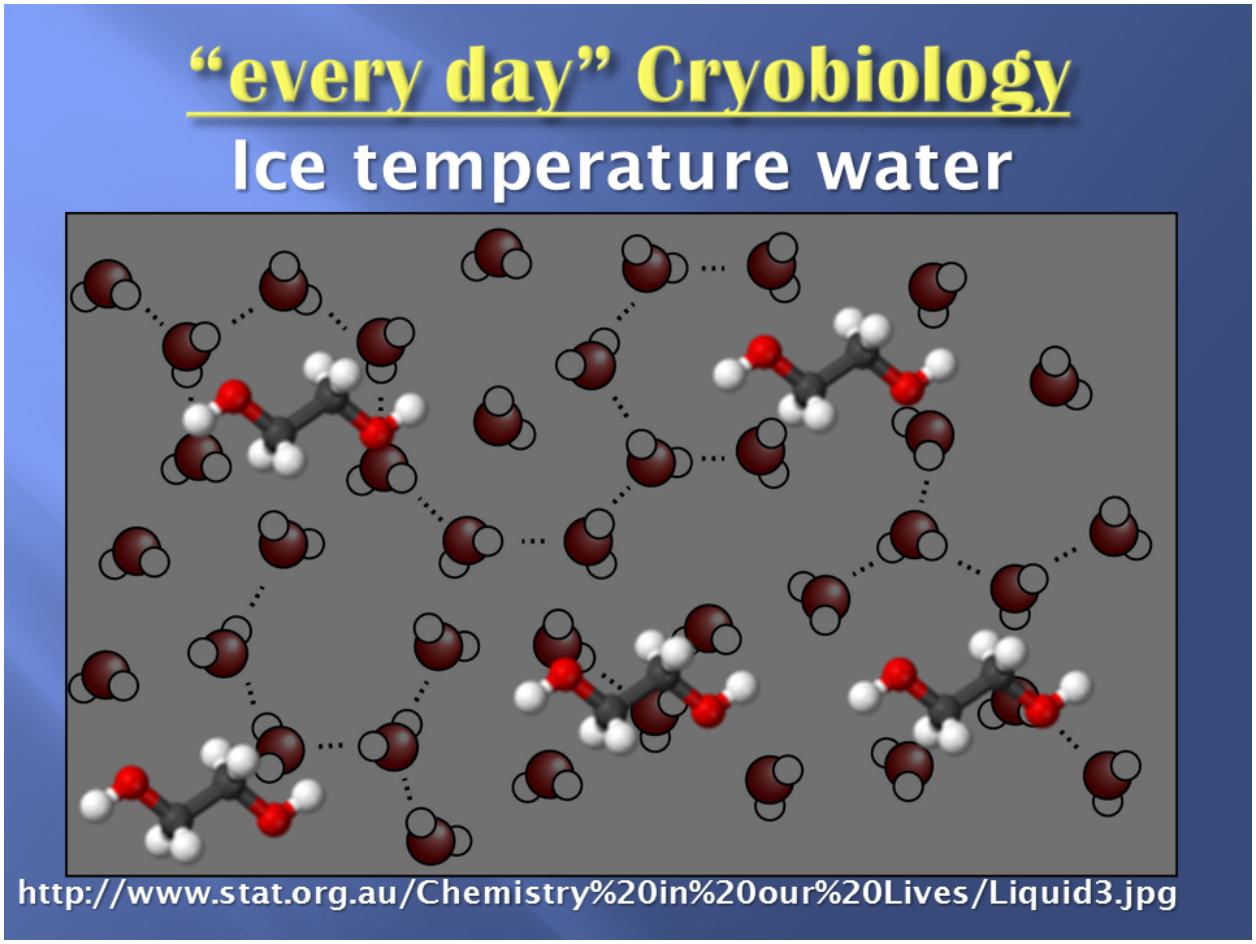


So what is the chemistry involved. In terms of understanding ice one of the really interesting features of ice, and I don't want to get too much into in the chemistry, is that when you get it really cold, the molecules like to line up with each other to form crystals. This is an image from an online biology textbook, Boundless.com, and it shows water molecules forming a kind of crystal lattice and you'll notice the spacing of them, is very regular. And one of the consequences of that spacing is that ice is less dense than liquid water. And that is why the ice-floe in the adjacent picture, again when you think about ice it's something that is always floating above water. It's important to recognize that, again, that the lower density, but also, if you have water inside a cell then as those crystal form during the freezing process, they will also expand the tissue, interact with the membranes to take up more space. So, if you've ever tried to cram up a suitcase too full or ever tried to fill a water balloon with too much water, this is basically what kills cells and tissues when you freeze them.

Many of you are probably familiar with one of the ways that we overcome this problem. This is a problem with cars. In order to maintain engines from getting too hot. We have radiator fluid in them. And that radiator fluid helps distribute the heat and not just in the metal. But if you just had water running around your car that is just plain water which would distribute heat nicely, if your car were to get to freezing temperatures than any ice in the tubes in the pipes would expand then crack the pipes or break the rubber and that would leak and be no good to anybody. So everyone is familiar with this idea of anti-freeze. And so, the molecules that you'll find, propylene glycol, the molecular structure of that is on the bottom left, a couple of carbons, a couple of oxygens, a couple hydroxyls, is what you find in most modern anti-freezes. It's less lethal than the molecule you'll find to the right, ethylene glycol, which is something that if you were to drink it has some very bad crystal formation in your kidneys and that will shut those down and that will cause you to die from not being able to filter the waste. And then things like is ethanol and methanol and glycerol that are all common biological molecules also work to keep ice from becoming a crystal as you go below 0 degrees Celsius.

Another molecule that I have there on the bottom right, it's not used as an antifreeze, it's somewhat toxic to biological organisms at high doses, is dimethyl sulfoxide. That's not something you'll not find in consumer products, but it is something that is used in cryopreservation technique. So, I mention it here.
Images from: http://en.wikipedia.org/wiki/Antifreeze and appropriate linked out chemical descriptions.

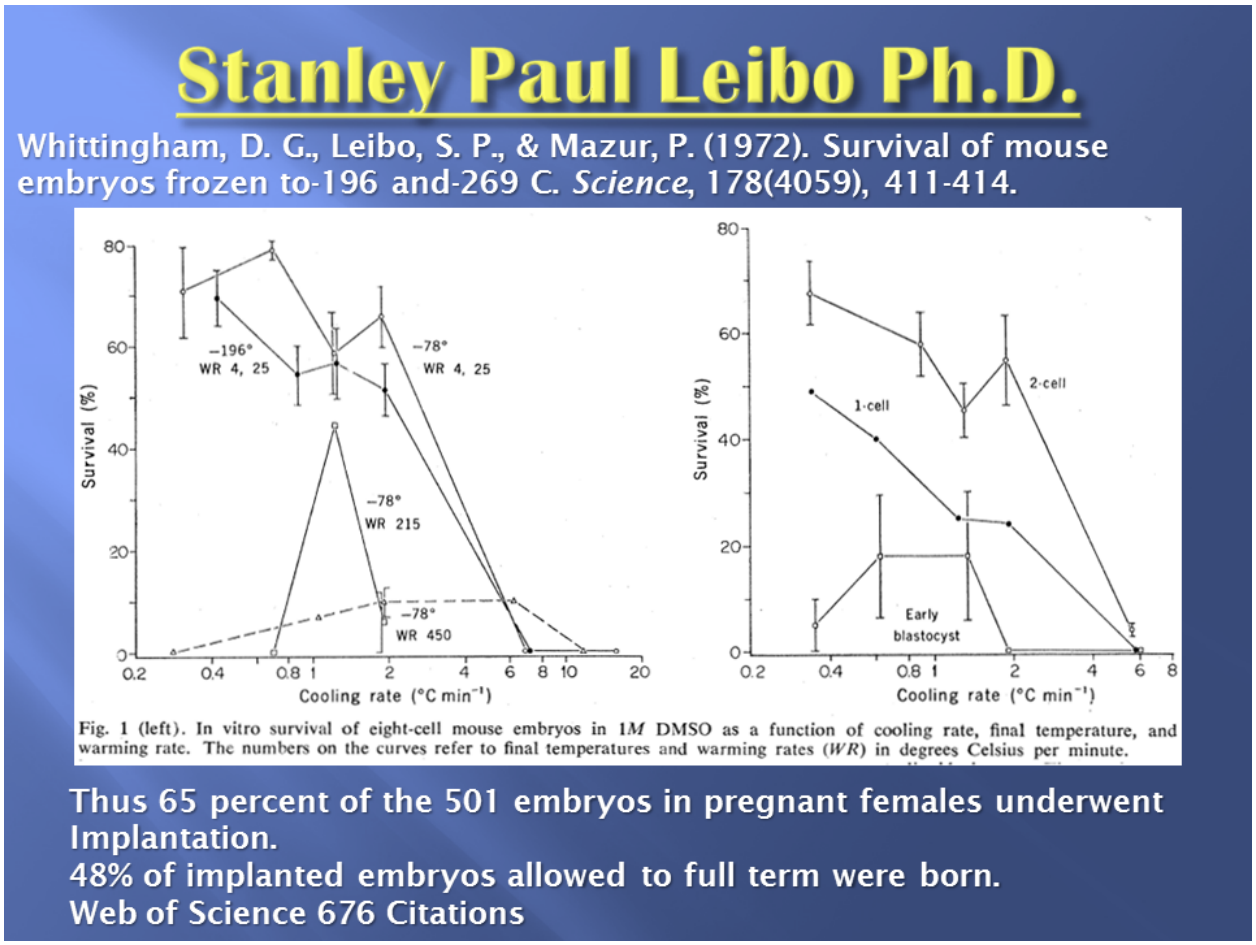
The way these types of molecules work is by including them in a solution of water molecules, then they interfere with those bonds between the water molecules between the hydrogen and oxygens in in a way that you can never get a proper nucleation of crystals and thus the process is less likely to damage cells. But nonetheless, one thing that is important to recognize is that alone, having solutes in solution, is not enough to keep the cells from being damaged.
Image from: http://www.stat.org.au/Chemistry%20in%20our%20Lives/Liquid3.jpg



One thing that everyone is pretty familiar with is that overexposure to very cold temperatures can lead to frostbite. And that's something that can lead to gangrene, and the ultimate amputation of limbs. Once you interrupt that blood flow, you can't get oxygen to the tissues and they will die. And it's also very painful. This is just an example of Jack Nicholson in The Shining. This process that even though the scientific community had these types of antifreeze, they were still finding that in most cases for these embryos in particular they were still sensitive to the freezing process that just mixing the two together was not a guarantee that you could then thaw the cells and get them to be viable, implantable, and that will lead to new organisms.
Image from: http://eandt.theiet.org/magazine/2008/19/science-without-deadline.cfm

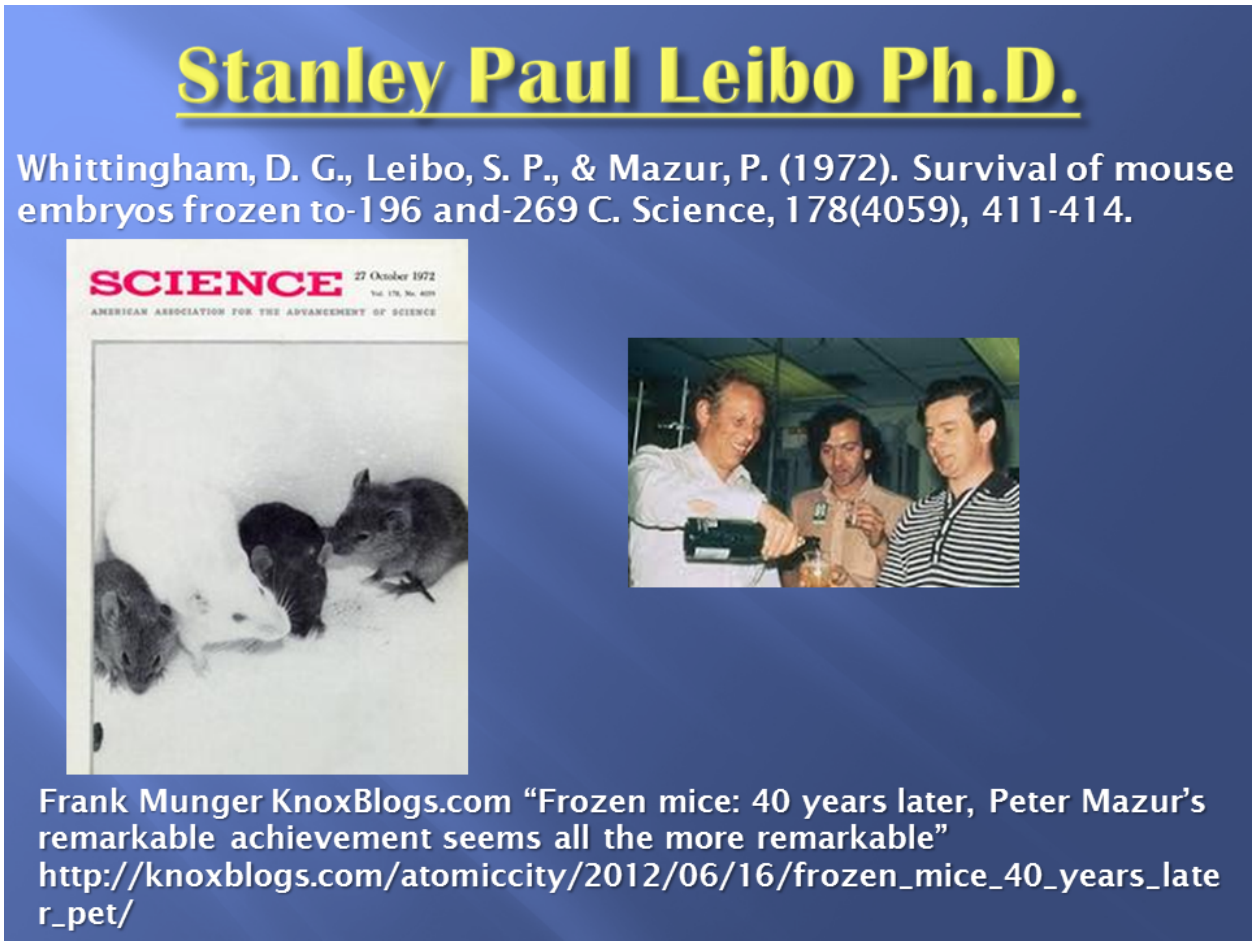
This was particularly true in mammals. And so this is the main scientific contribution that Dr. Leibo made to the field is that, in combination with the visiting Dr. Whittingham and his graduate adviser Peter Mazur, in 1972, they published the ability, for the very first time ever, gotten in the mouse to freeze the embryos and lead to successful pregnancies in multiple surrogate mice. I'm going to walk slowly through this graph. This is really the only graph I wish to show you. That what you see on the x axis (on the bottom) is the cooling rate. Going from left to right, the rate of cooling, the number of degrees per minute, at which the embryos were frozen, going from left to right, slowest to highest. And the Y axis, you have the % survival of embryos and in this case, % survival is defined as going from 1-2 cell stage under the microscope going to the blastocyst stage. And that they progressed to the next stages of embryogenesis. And what you'll see in both graphs is that the slower the cooling rate then the higher the viability. NO matter what cell type you have, if you cool them too quickly, then nothing survives. I've read up on quite a bit of literature at this point, and Peter Mazur give a very nice summary of this at his Cryo2013 talk. Those just because you have added antifreeze molecules to your solution there are two things a slow cool rate helps you with.
1) it's not just that you have water not becoming crystals. It's also the rate you are dehydrating the cells. If you are slowly dehydrating the cell, then whatever osmotic pressure you have or

whatever sort of cell structure or function you have the slower you pull the water away the better.
2) More importantly as you freeze stuff you are still forming microcrystals. And so those microcrystals have the capacity to create cellular damage.
So if you are doing it more slowing in the presence of antifreeze, it inhibits those microcrystals from forming and thus causing damage to the cell. And what I have here at the bottom, if I have interpreted the table correctly, That 65% of the 501 embryos actually successfully went through progression in the mice and that 48% of the mice allowed to go to full term, there were some animals sacrificed in the middle in order to look, 40% of mice were bored of those allowed to go to full term.



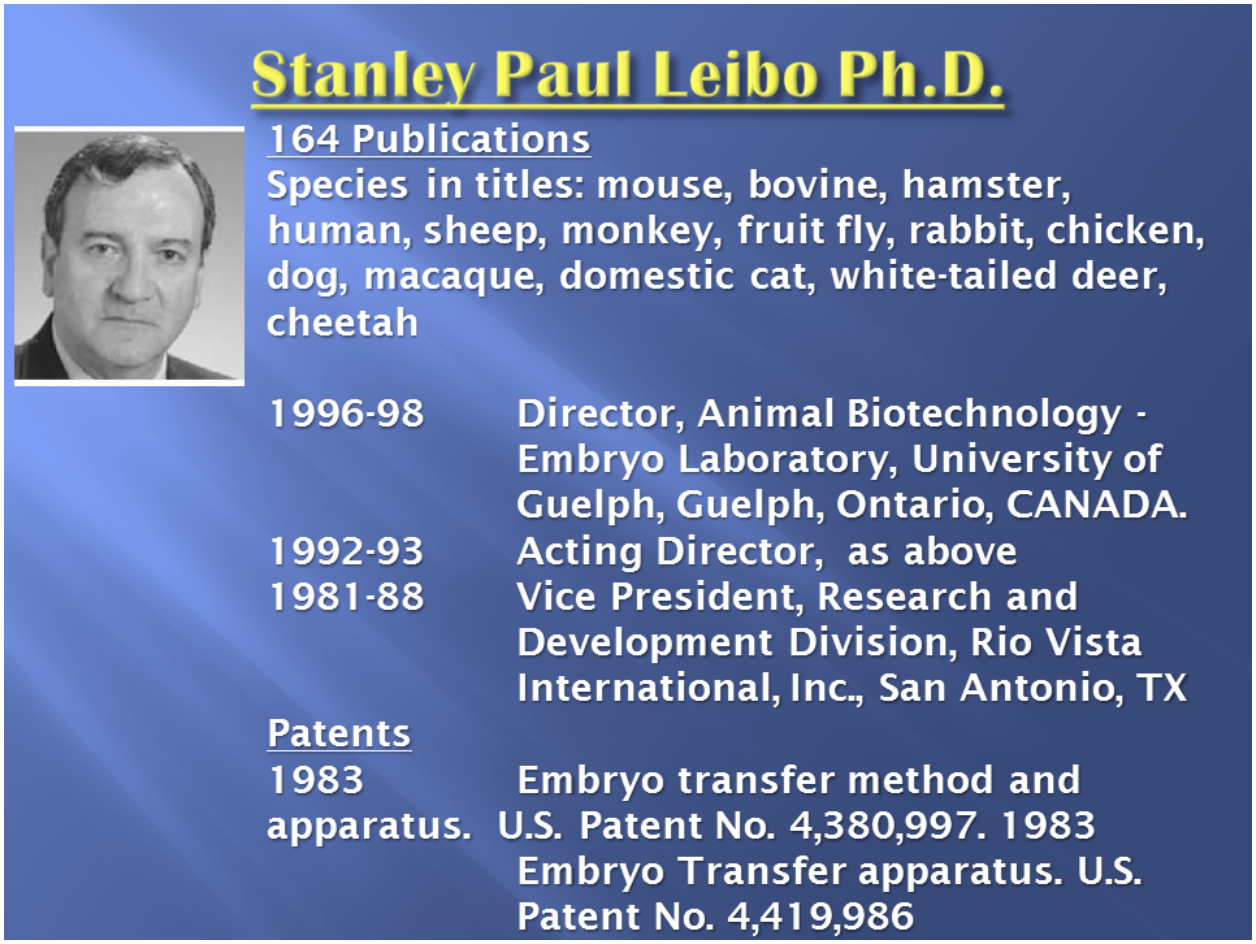
That ended up making the cover of Science Magazine for that publication. And in addition was certainly a great cause for celebration. This methodology of what's called slow cooling is still the primary and most popular method for the vast majority of mammalian embryo freezing. And Dr. Leibo has a history and a large number of publications that talk about this methodology for a variety of cells used including spermatozoa spermatocytes, ova, oocytes as well as other species' embryos.

There is another technology called vitrification. I didn't really find any particularly helpful images to explain this process, vitrification is a process by which you can use a variety of chemicals were you can rapidly cool cells down in the presence of antifreezes and you get a glass-like, amorphous, phase structure of water such that, at least in human embryos, you get a higher survival rate than you do with slow freezing. But, it's not a technique that is being abandoned because it's a lot easier to hand a large number of samples and it's a little bit less sensitive to how specifically you are doing the protocol. This paper is basically credited with the explosion of frozen embryo transfer that you find very widespread in livestock as well as in scientific labs. They overcame something that was a very specific technical challenge; even though they'd had these antifreeze technologies the ability to get a high rate of survival of the embryos was a milestone in the field.



To summarize Dr. Leibo's contributions from that point on, looking in World [meant to say Web] of Science see a total of 164 publications. Many of those are reviews. But, if you look at the variety of science that he performed over his lifetime there are a large number of species involved including mice, fruit flies, rabbit, rhesus monkey, the macaque,. And the cheetah. The cheetah publication I saw a graduate student present sometime related to that. So, the

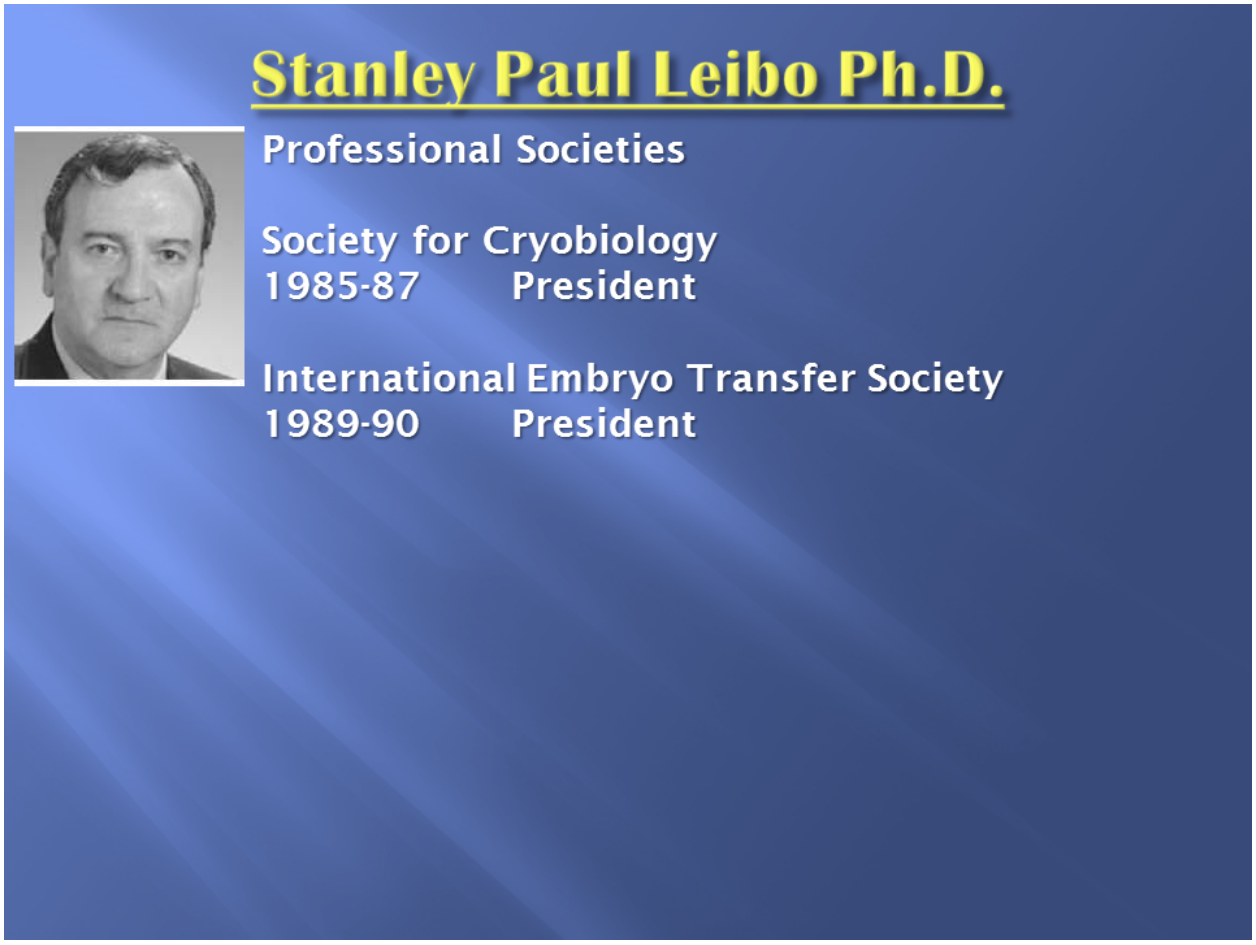
ability to sperm, and embryos, and eggs from a wide variety of species is actually very challenging.
One of the features of species is how their gametes interact. And that is something that can evolve very rapidly. And, that, they all develop very specialized interactions which have the consequence for these freezing technologies: one protocol that works for one organism may or may not work for another.
One of the challenges that is seen for cats is that the sperm have a very low survival rate with whatever sort of freezing technique you have. That was the work of the graduate student's where I saw the talk was trying to find ways to modify the protocol to allow a higher survival rate. That is something people are very interested in. when it comes to the cryopreservation of rare or threatened species making sure you work through the technologies, finding ways this is reliable, and viable to actually preserve their genomes for maybe decades until we can deal with habitat loss. Fine tuning these techniques is very important.
During his time, he was not just an academic; he actually was a director of animal laboratories both at the University of Guleph and a company, Rio Vista International down in San Antonio, Texas. He does have two patents to his name related to embryo transfer methods.



Later he was also awarded outstanding professional awards. This is just a highlight of a few: The Pioneer award from the International Embryo Transfer Society. He was also awarded and given honorary membership as the Spallanzani Lecturer for, I think, the Society for Spermatology.
He was inducted as a fellow of the society for Cryobiology “recognizing his recognize exemplary contributions to the discipline of cryobiology and service to the Society.” And I think that's something I want to talk about and will show something about his contribution to that.
As well as being an honorary lifetime member of the American embryo transfer association.

I'm going to highlight a couple of his society appointments. He was the president in the 1980s of both the Society for Cryobiology and the international embryo transfer society. One of the interesting dilemmas that came during this time was whether cryobiology should accept or recognize cryonics. So I mentioned that cryonics is the attempt to preserve humans and sometimes pets in order to hope that future technologies will allow those to be revived. You see some degree of ridicule in the press or entertainment. If you've ever seen Futurama with the little head in the floating jars. I hate to spoil this but if you haven’t seen it by now you probably aren’t going to, the movie Vanilla Skye. Both the original as well as the Tom cruise, the main plot twist there is that the main character has been cryogenically frozen and revived.

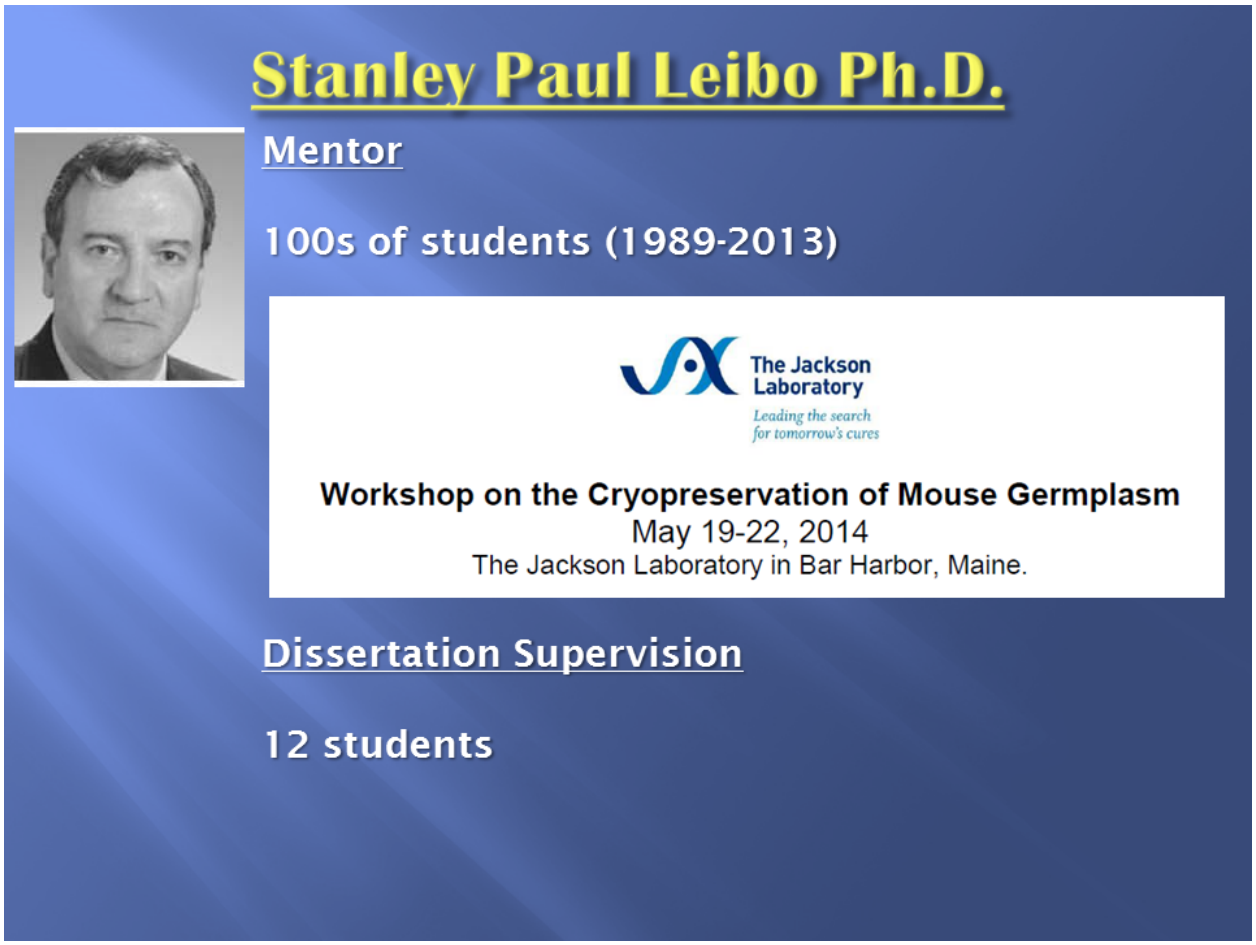
And a lot of people will credit him [Leibo], even the cryonics, a lot of people were very much against this along with a lot of heated voiced, but it's generally regarded that Dr. Leibo guided that argument and was a cooler head in the overall conversation.



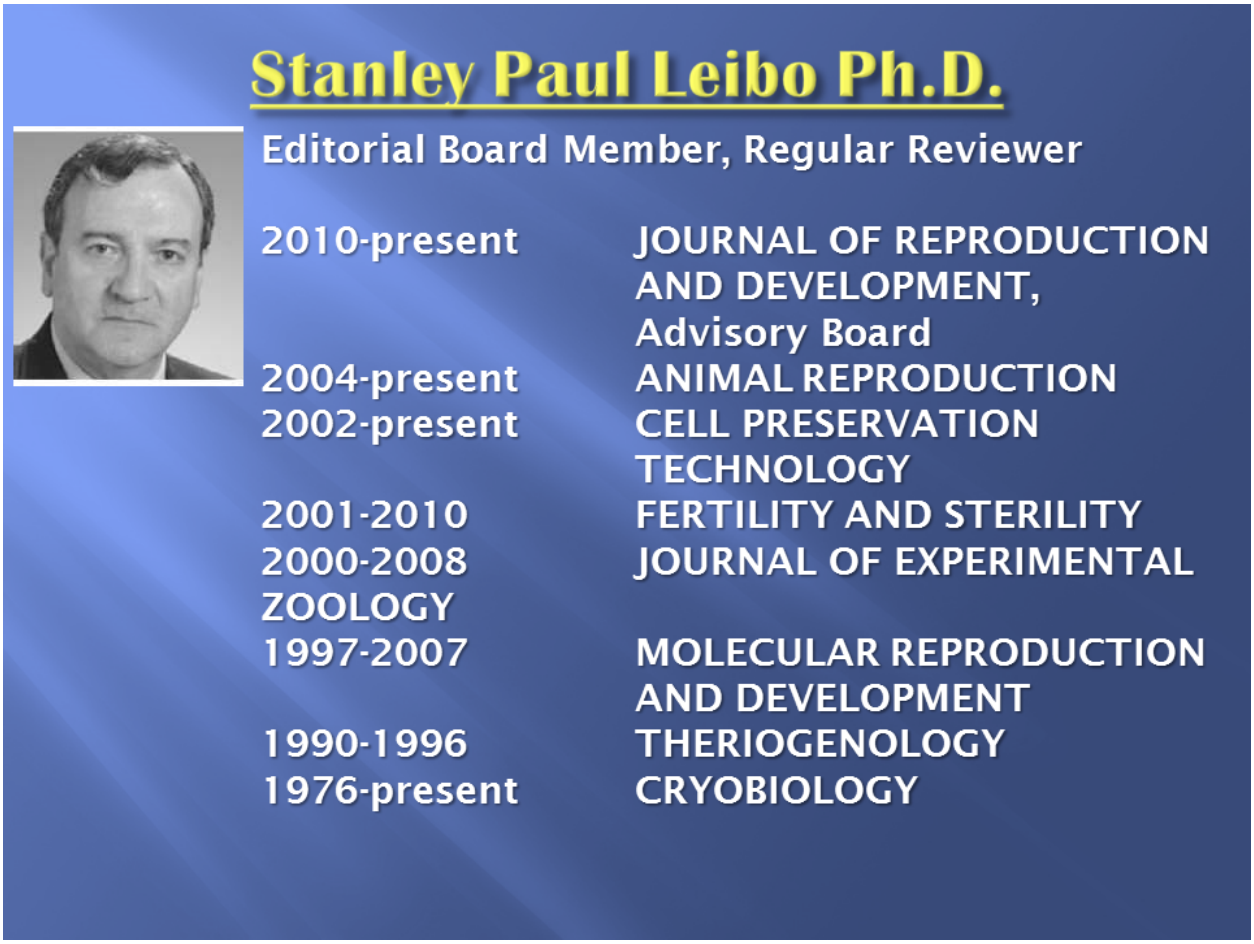
IN terms of being a mentor to the field, every year from the beginning and the founding there is workshop on the cryopreservation of mouse germplasm which is sponsored by the Jackson Labs in Maine. For those of you unfamiliar with the mouse research field, the Jackson Labs is an invaluable resource for people doing research in mice. They maintain a variety of mouse strains they maintain specialized transgenic versions. It's a repository that allows to not have to have multiple sites and multiple people try and maintain and create mouse stocks. And he would, every year, provide a workshop related to mouse cryopreservation. From what I've read, there are probably hundreds of students that have gone through and there are probably lots of hands out there than have been guided by Dr. Liebo's experience. He was also the dissertation supervisor for 12 students. This is information I got from his curriculum Vitae which let me mention was kindly provided by the University of New Orleans Biology department after his passing. That's one thing that's kind of interesting about Dr. Leibo is that because he was such since he's been around the field for so long, it's pretty hard to track down non publication resources about him and his life.

One of his former students who is now a research faculty put together a memorial video for him. I'll put that link in at the end.

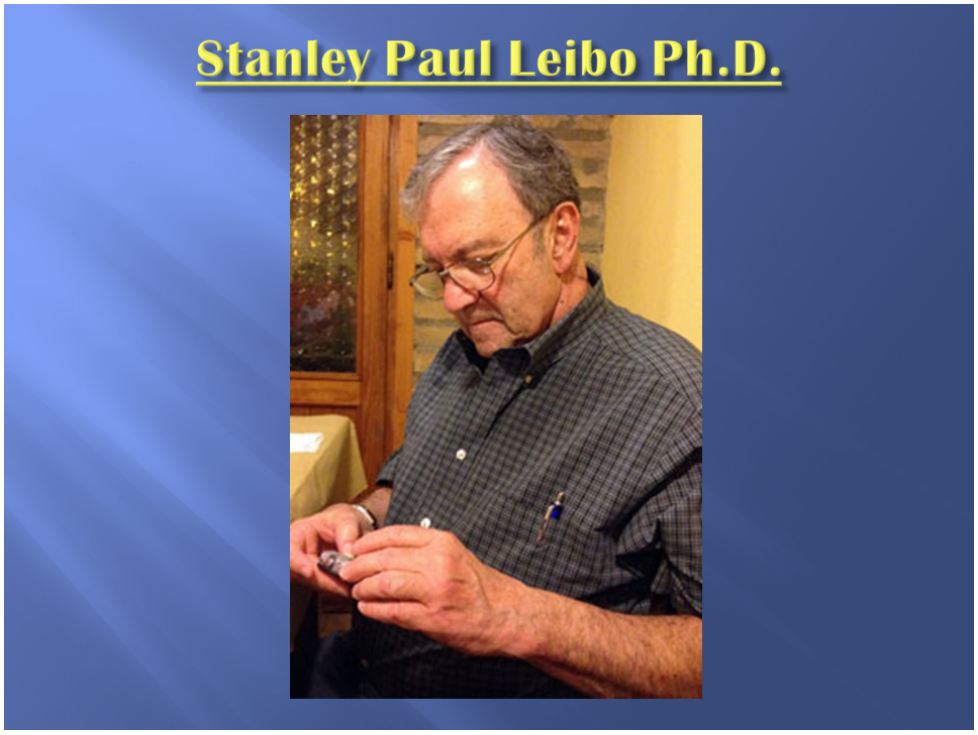
Image from:
http://www.slideshare.net/mdconferencefinder/workshop-on-the-cryopreservation-of-mouse-germplasm-33538295



His contributions did not end with the research. One of the primary roles of academic faculty in the research area is to maintain the communication and to both nurture journals and to be a member of editorial boards but also to review lots of manuscripts. He was heavily engaged and involved in the editorial boards and reviewer for pretty much all of the major journals in cryobiology. And one thing you'll notice here is that a lot of these are “to the present” and so I think one thing about Dr. Leibo is that when he made a commitment to doing something he was really going to stick to it.



I would like to close here with a personal reflection. One of the motivating reasons for wanting to honor him and give a presentation is that not a lot of people know about his contributions to cryobiology. Is that there are A lot of people that are colleagues of yours that when you are busy you don’t' necessarily focus on trying to get to know them personally and to spend that time trying to very specifically understand what just have they have done in the field or what they are but also the stories they can share about what has gone on in the field. And their perspective on what science use to be like. What's interesting is that the reason I started interacting with him more was that he was not very familiar with clickers in the classroom and getting Blackboard to behave. And those types of things. So one thing I have to say that I'm ultimately very thankful for in terms of being one of his go-to people, is that it gave me the opportunity to meet a luminary and a pioneer in his field. And also someone who was just an extremely, extremely nice man and very generous with his time and expertise.



Tribute to Dr. Stanley Leibo https://www.youtube.com/watch?v=2kjSv130F-c
Other resources and reference material:
Stanley Leibo presents at Cryo2013: https://www.youtube.com/watch?v=0VSZEnR5fL8
In memoriam: Stanley Leibo: http://transtechsociety.org/blog/?tag=stanley-leibo
In Memorium: UNO Biology Professor Stanley Leibo:
http://www.uno.edu/news/2014/InMemoriamUNOProfessorStanleyLeibo.aspx
In Memoriam:
http://www.societyforcryobiology.org/index.php?option=com_content&view=article&id=93:stanley-leibo&catid=19:site-content
Frozen mice: Celebrating a 40-year success story by Frank Munger:
http://www.knoxnews.com/news/local-news/frozen-mice-celebrating-a-40-year-success-story
Mazur, P., Leibo, S. P., & Seidel, G. E. (2008). Cryopreservation of the germplasm of animals used in biological and medical research: importance, impact, status, and future directions. Biology of reproduction, 78(1), 2-12. : http://www.biolreprod.org/content/78/1/2.full

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