Title	Commentary on the Achievements of the Laureate: Ryuzo Yanagimachi by two experts closely associated with him
Author(s)	Hidenori Akutsu / Atsuo Ogura
Language	English
Event title	The 2023 Kyoto Prize Commemorative Lecture
Publisher	Inamori Foundation
Issue Date	10/30/2024
Start page	1
End page	20
URL	https://www.kyotoprize.org/wp-content/uploads/2024/10/2023_yanagimachi_en.pdf

URL for Japanese translation: https://www.kyotoprize.org/wp-content/uploads/2024/10/2023_yanagimachi_jp.pdf

The 2023 Kyoto Prize Commemorative Lecture

Commentary on the Achievements of the Laureate: Ryuzo Yanagimachi by two experts closely associated with him

Hidenori Akutsu

Thank you. My name is Hidenori Akutsu. I work at the National Center for Child Health and Development. Originally it was intended to have Dr. Yanagimachi himself here, sharing the story of his career as an inquisitive researcher of fertilization studies with that wonderful warm smile on his face (Fig. 1).

Fig. 1

I can easily imagine him humorously looking back on the life of research that he and his wife, Hiroko-sensei, led together.

Many people studied under Dr. Yanagimachi, were attracted by his character, and were profoundly influenced by him. I don't know if I am the right person to be here, but I wish to share what little I know about his exceptional personality. Please bear with me. As noted in the video we have just watched, when Dr. Yanagimachi was a student at Hokkaido University, he read a textbook that contained Weismann's germ-plasm theory, which had a profound impact on him.

While researching fish fertilization, he realized that little was known about the fertilization of mammals, including humans. Because fertilization is the very first step in the life cycle of all mammals, including us, it occurred to him that research into mammalian fertilization would be very important.

He was lucky enough to study in the United States under Dr. M.C. Chang, a great pioneer in mammalian fertilization studies at the time. In the fall of the year he turned 32, Dr. Yanagimachi left for the United States with his wife, Hiroko-sensei. He was to study at the Worcester Foundation for Experimental Biology (WFEB) in Massachusetts. There, he finally got the chance to do what he had long desired: study mammalian fertilization. Feelings of expectation and anxiety about life in the United States must have been mixed in ways that we can't even imagine.

This is the only suitcase that the couple took to the United States, which must have been full of their hopes and dreams (Fig. 2). The sight of Dr. Yanagimachi standing by this carefully preserved suitcase evokes in our imaginations the various feelings he must have had then.

Fig. 2

This is a snapshot taken in Dr. Chang's lab (Fig. 3). When I was studying at the University of Hawai'i at Mānoa (UH Mānoa), Dr. Yanagimachi used to invite members of his lab over to his house for a New Year's party, as I assume he had done every year. On that occasion, he showed us some slides of photos taken while he was at WFEB. He was so passionate about photography that he showed us a lot of shots of the town, as well as his lab.

Fig. 3

While researching under Dr. Chang, he was given the research topic of leukocytes and sperm capacitation. Dr. Yanagimachi once said that he was told by Dr. Chang, "This is your bread and butter, and I expect you to do it for three days a week. But you can pursue whatever topic you choose for the remaining two days." I assume that Dr. Chang had faith in him, thus giving him space to research freely.

As it happened, the work he did on those "free" days accomplished historic achievements. Dr. Yanagimachi once told this very intriguing story in a review article in a professional journal. One day, on a whim, he put a golden hamster's sperm and egg that he was using for another experiment onto a watch glass filled with medium, covered them with paraffin oil, and incubated them in a small laboratory oven at 37°C. On the right of this slide is a diagrammatic representation of how he did the experiment (Fig. 4). Several hours later, without much expectation, he was surprised to see through a microscope that some of the eggs had been penetrated by sperm in culture on the watch glass. In that moment, in vitro mammalian fertilization was discovered.

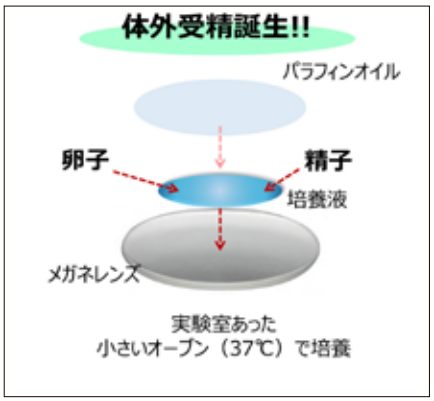

Fig. 4

So, why did Dr. Yanagimachi bother to reproduce fertilization in vitro in the first place, when it naturally occurs in vivo? I believe it was because he wanted to deepen his understanding of the fertilization phenomenon to the furthest possible point. If he could reproduce in front of his eyes the phenomenon that occurs in vivo, or in the oviduct, to be exact, his research would advance significantly. I think this was his reasoning. As a side benefit, he made great contributions to the development of in vitro fertilization (IVF) for medical purposes.

I love this photo of Dr. Yanagimachi smiling gently and looking full of confidence (Fig. 5), and I used to gaze at it for a long time. In doing so, something in the photo captured my attention: a small oven in the background. Could this be that small oven in the lab? Is this the small oven in which in vitro fertilization succeeded for the first time? That idea went through my mind. This could be merely my imagination. I must admit, my assumptions have often led me astray. Anyway, this photo gave me such a thrill that I wanted to take this opportunity to share with you my possibly mistaken notion.

Fig. 5

At UH Mānoa's John A. Burns School of Medicine, Dr. Yanagimachi set up his own lab (Fig. 6).

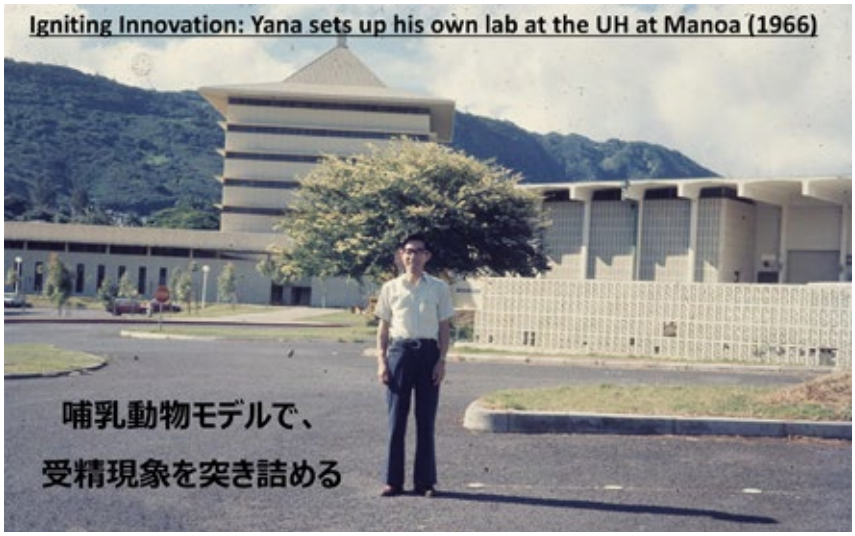

Fig. 6

He was 38 at the time. This is where he made a series of achievements that delved into the fertilization phenomenon. He knew how important observation is. He was among the first to introduce an electron microscope to his lab and published numerous papers using a huge collection of photos taken with this apparatus. This red book titled The Physiology of Reproduction is an essential textbook for students of reproductive biology. In one chapter on mammalian fertilization written by Dr. Yanagimachi, he cited as many as 1,735 papers (Fig. 7). His wife supported him by taking images with an electron microscope (Fig. 8). The many wonderful electron microscope photos that captured the fertilization phenomenon were in fact the fruits of such joint efforts by the couple.

Fig. 7

Fig. 8

Dr. Yanagimachi often conveyed several messages to younger people in his numerous lectures and reviews. First, he said, think of a subject of importance and great promise. Keep asking what is fundamentally important and what is essential. Second, have a clear vision and conceive a well-planned approach. And third, gather as much necessary information as possible. Don't hesitate to use other researchers' help if you need to. Dr. Yanagimachi certainly knew the importance of joint research that transcends disciplines.

This slide shows the present status of assisted reproductive technology (ART) in Japan (Fig. 9). The graph covers the period up to 2020, and the number of ART births has since grown to approximately 70,000. Among them, 2,850 entailed microinjection. Currently, most cases follow pregnancy and delivery via frozen embryo transfer (FET), represented by these gray bars. However, the number of babies born via ICSI, that is, microinjection, actually exceeded 2,850, as fertilized eggs used for FET include those fertilized via ICSI. As this indicates, microinjection has become an indispensable medical technology for ART.

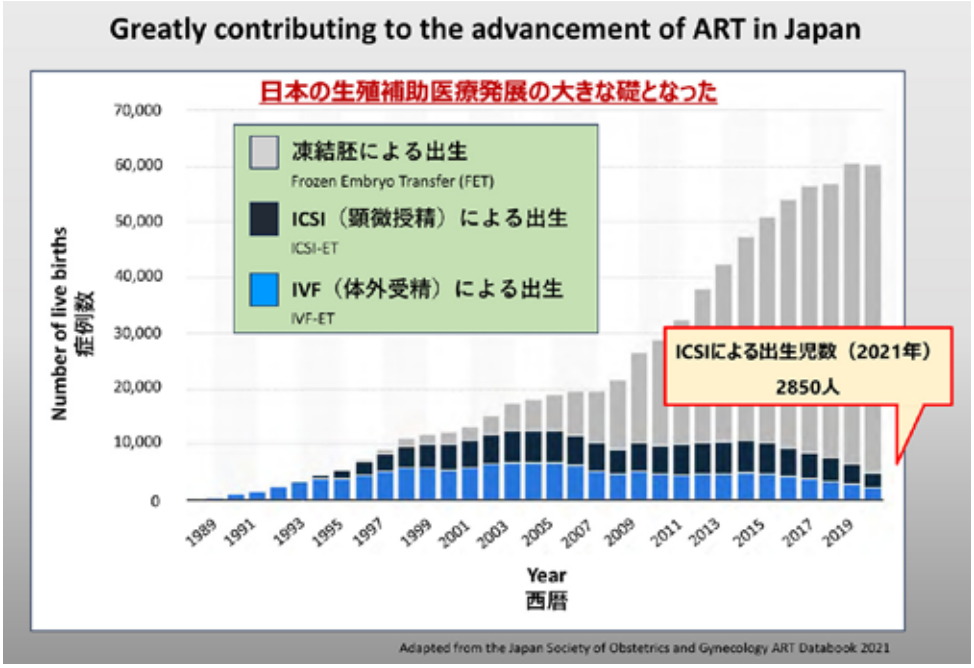

Fig. 9

The first successful use of ICSI in Japan was in 1994. That year, the Fukushima Medical University obstetrics and gynecology group reported an ICSI birth (Fig. 10). Back then, Japan was just the second country to succeed after Belgium, leading the world in ART technology. All members of that Fukushima Medical University ICSI clinical team were obstetricians and gynecologists who had once conducted research at Dr. Yanagimachi's lab under his tutelage.

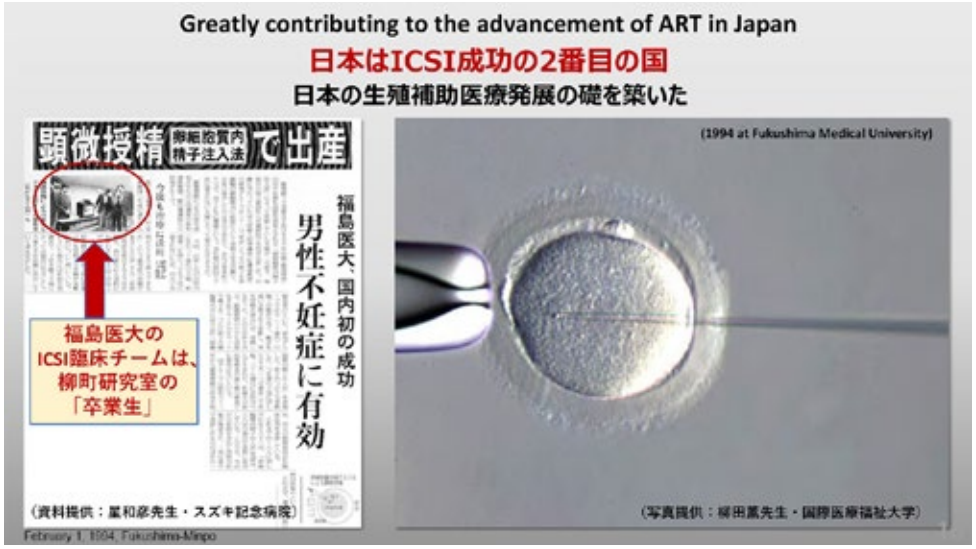

Fig. 10

At that time, researchers had succeeded in microinjection with hamsters and humans, but were having difficulty applying it to mice, the most frequently chosen lab animal. Many "brave" researchers at the Yanagimachi Lab—and I deliberately choose the term "brave"—took on the challenge, but they were nowhere near their goal of widely using mice for experimentation. One such researcher was Dr. Yasuyuki Kimura, who came to study from the Fukushima Medical University Department of Obstetrics and Gynecology. Ultimately, he developed Piezo-ICSI, a new ICSI system using a piezoelectric pulse sensor device, thus dramatically enhancing ICSI using mice. This technology involves sharply yet gently piercing a hole in an egg and injecting sperm. The needle can easily penetrate the firm zona pellucida (Fig. 11).

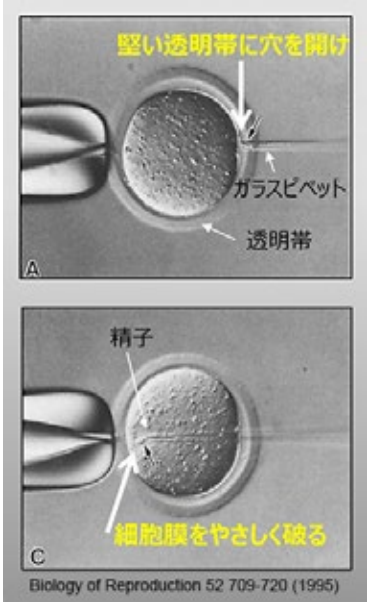

Fig. 11

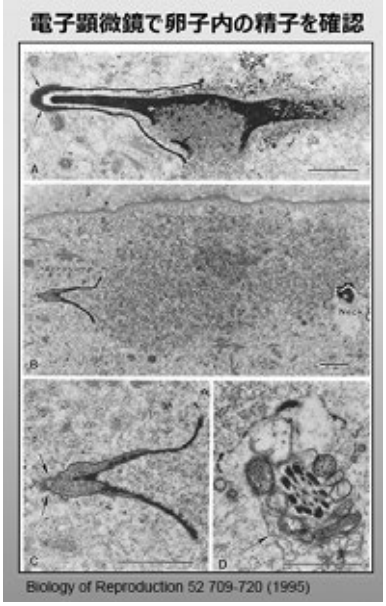

Fig. 12

Fig. 13

The research outlined in his paper used an electron microscope to observe and prove in detail whether or not sperm had entered an egg safely (Fig. 12). This was a method of proof often employed in the Yanagimachi Lab. Live offspring were born from mouse fertilized embryos via ICSI (Fig. 13).

Dr. Kimura found a clue from a very casual conversation with researchers who were visiting the Yanagimachi Lab from abroad and made the most of a dust-covered piezoelectric pulse sensor device left on a shelf in the lab. In this sense, Dr. Kimura's success was not dissimilar to that achieved by Dr. Yanagimachi when he used a laboratory oven in Dr. Chang's Lab to make IVF work.

Piezo-ICSI is now widely used by fertility specialists. They put sperm in an injection pipette with a thin, flat tip, make a hole in the firm zona pellucida with Piezo pulses, move sperm to the tip of the pipette, and squeeze it in through the zona pellucida. When it reaches all the way inside, they gently break the membrane with Piezo pulses to inject sperm into the cytoplasm. This is how Piezo-ICSI works (Fig. 14).

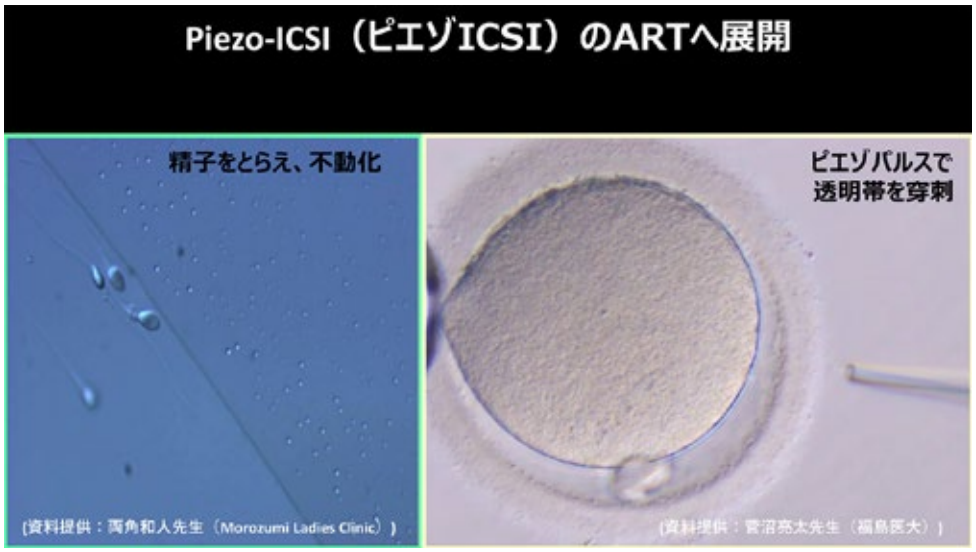

Fig. 14

Dr. Yanagimachi was easy to get along with and didn't put up barriers to others. Everyone was on an equal footing with him in the field of research, be they young researchers or students. In other words, Dr. Yanagimachi took the act of trusting others very seriously. As I remember it, he gave us lots of freedom as far as research was concerned.

But we were often asked if we found what we were doing fun. To say that you "find your work fun," you need to be able to fill in missing pieces, backed by a huge amount of knowledge, which may seem like great fun at first glance.However, to reach the true essence of what he described as "fun," we students needed to accumulate a considerable amount of knowledge.

Like this giant tree, Dr. Yanagimachi was a massive beacon of intellect, around which people from myriad specialties gathered, mingled closely and confidently, and conducted all sorts of research (Fig. 15). Researchers were encouraged to take on new challenges without trepidation. Should they encounter difficulties, they could seek advice from Dr. Yanagimachi. We all shared that kind of sense of security. Under the aegis of Dr. Yanagimachi, researchers felt encouraged to compare notes with those from other fields. Dr. Yanagimachi was also beloved by university staff, as well as other researchers. I always thought that Dr. Yanagimachi had a purity that never wore out, regardless of his age, which attracted many people.

Fig. 15

Let me finish with a personal image. I got married while studying abroad, and we had a wedding ceremony there. The blessing we received from Dr. Yanagimachi, Hiroko-sensei, and my peers at the lab is one of my most cherished memories from that experience.

Thank you very much for listening (Fig. 16).

Fig. 16

Atsuo Ogura

As you heard, I am Atsuo Ogura from the RIKEN BioResource Research Center. Today, I have the pleasure of looking back on Dr. Ryuzo Yanagimachi's achievements. Normally, we would have Dr. Yanagimachi himself here, looking full of life like in this photograph (Fig. 17). Unfortunately, that is not possible now. So, I will talk about what Dr. Yanagimachi would have spoken about if he were here, in memory of him. I will also take the liberty of using slides that he prepared as I speak.

Fig. 17

Dr. Akutsu talked about Dr. Yanagimachi's personality and contributions to medical science. I will follow the timeline of his work, starting with his relationship with Dr. Chang at WFEB, followed by his achievements at UH Mānoa, and finishing with his more recent work.

This is a slide prepared by Dr. Yanagimachi. He worked at Dr. Chang's lab at WFEB between 1960 and 1964. Here you see the "three days a week policy," which I believe refers to what Dr. Akutsu has mentioned: working three days a week for salary and working freely on research of your choice for two days (Fig. 18).

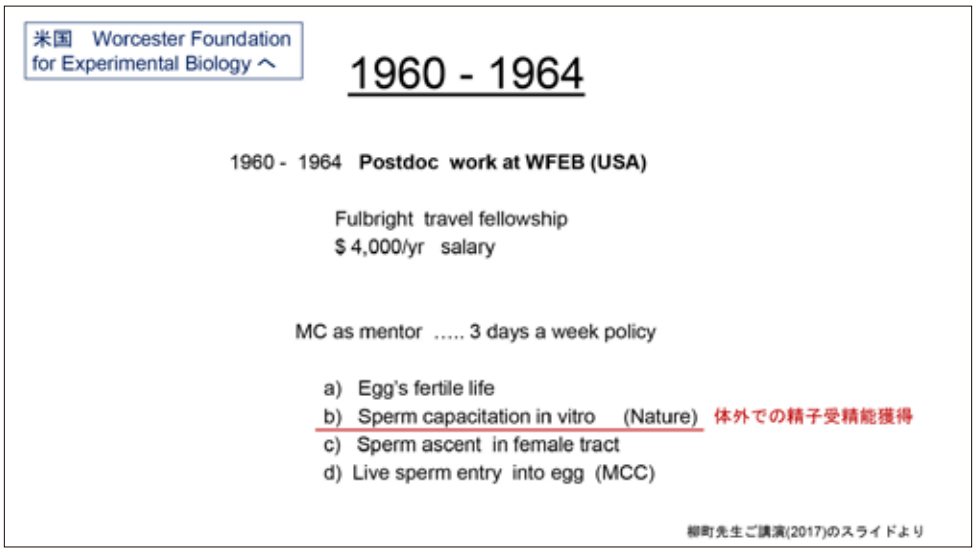

Fig. 18

A key part of his work during this period was "sperm capacitation in vitro," which is indicated

here (Fig. 18). Let me expound on this a bit (Fig. 19). In mammalian fertilization, sperm can't get fertilized immediately, but must undergo a preparatory stage. The terms in red here denote sperm capacitation and hyperactivation, and the acrosome reaction. Fertilization requires these two processes. Dr. Chang and Dr. Yanagimachi were pursuing these two phenomena. In an acrosome reaction, the sperm's bag breaks to release enzymes. After this, it becomes possible for sperm and an egg membrane to be fused, that is, fertilization. Microinsemination skips all this, as shown in this schematic diagram (Fig. 19).

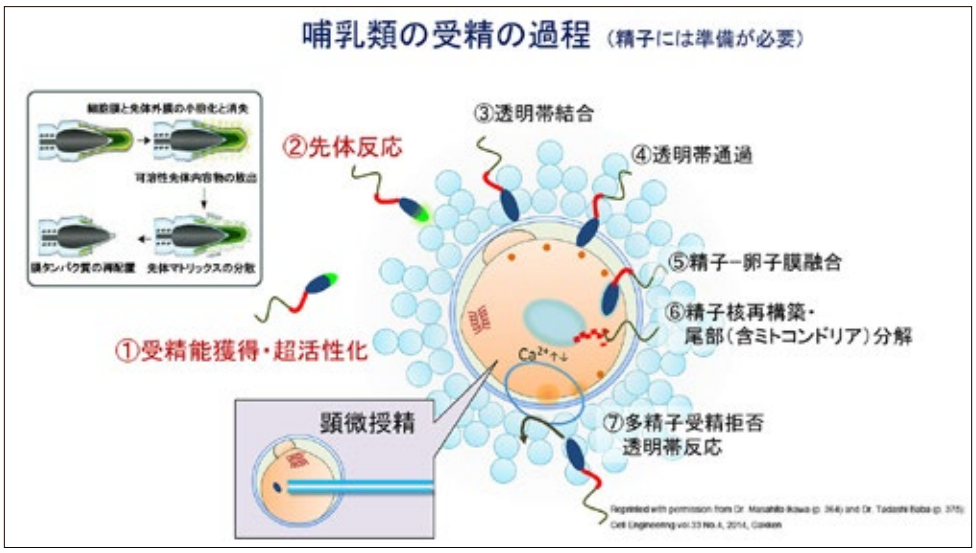

Fig. 19

When Dr. Yanagimachi went abroad to study, there were three great pioneers in the field around the world: Dr. Chang in the US, Dr. Austin in the UK, and Dr. Thibault in France (Fig. 20). Dr. Yanagimachi wrote a letter to Dr. Chang, who agreed to accept him (Fig. 21). Dr. Yanagimachi wrote about this time in a book that he later published, but bear in mind he wasn't doing any work that involved mammals then.

Fig. 20

Fig. 21

He wrote that “M.C. Chang was brave enough to accept me.” Dr. Yanagimachi was that grateful for the opportunity he was given. He placed much trust in his mentor, Dr. Chang, who was the first to call him “Yana.” More than 10 years before their encounter, China and Japan had been in a tumultuous relationship. Dr. Yanagimachi was concerned about that, and dared to ask Dr. Chang, “Why did you accept me?” As he later wrote, Dr. Chang responded by saying, “All scientists are international citizens, and their discoveries should not and could not be monopolized by a particular country.” He was this broad-minded. This spirit of his was instilled in Dr. Yanagimachi and then in us all, and it is a legacy we must cherish.

Another great achievement of Dr. Chang was that he accepted many Japanese researchers after Dr. Yanagimachi. As many of you may know, all the Japanese students listed on this slide went to study under Dr. Chang and made a name for themselves after returning to Japan. You could say that Dr. Chang is a benefactor of reproductive biology in Japan. I hope you will remember this.

Some of Dr. Chang’s prominent work is on in vitro fertilization of rabbits (Fig. 22). It was 1959 before Dr. Yanagimachi went overseas to study. Through in vitro fertilization of rabbits’ eggs, he successfully obtained a rabbit litter. As shown here, the key to this success was that he used sperm that was capacitated in a female rabbit’s body. To put it another way, it was not completely in vitro fertilization.

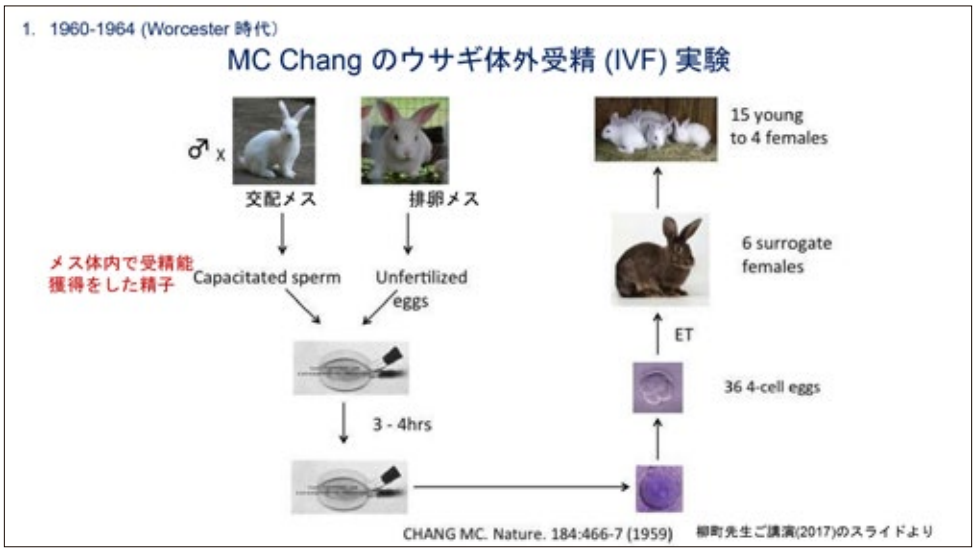

Fig. 22

So, to perform fertilization completely in vitro, Dr. Yanagimachi and Dr. Chang embarked on experiments using hamsters. From this bag called the epididymis that stores sperm, they extracted hamsters' sperm, capacitated it in vitro, and caused an acrosomal reaction. The result was this wonderful fertilized egg (Fig. 23).

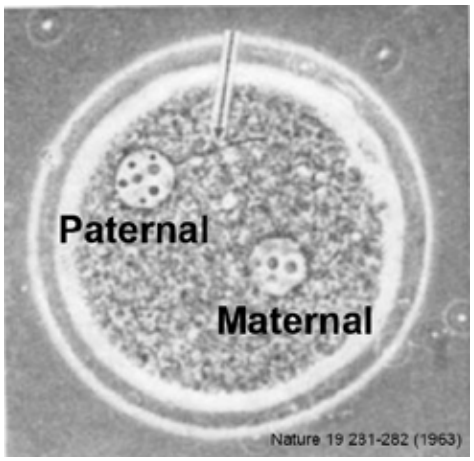

Fig. 23

This nucleus from sperm had a tail. It's a properly fertilized egg that marked the beginning of global fertilization research. It is no exaggeration to say that this opened the way to fertilization studies for researchers worldwide. Now, you might be wondering, "Why hamsters? Why not rabbits or mice?"

I think this is where Dr. Yanagimachi's great prescience had an effect. We have photos of the "bag", acrosome, I mentioned earlier. With a hamster, you could see if an acrosome reaction had occurred with an ordinary phase-contrast microscope (Fig. 24).

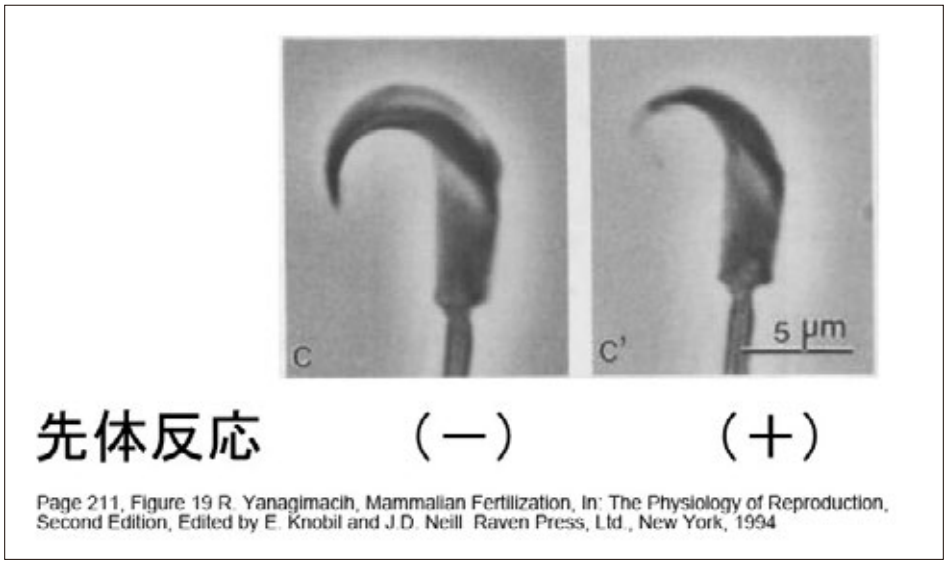

Fig. 24

This means that Dr. Yanagimachi could see under what conditions an acrosome reaction was happening in vitro. Plus, hamster's sperm undergo hyperactivation. Furthermore, for some reason, hamster eggs resist injection, which means they don't die if injected. I'll come back to this later. In any case, thanks to all this, he was able to perform in vitro fertilization and then microinsemination.

I would like to draw your attention to the fact that hamster embryos halt the development process. This is a particular characteristic of hamsters, but embryos stop developing and don't grow into a baby. This is a key point in the process, which I'll also come back to later.

Two years after Dr. Yanagimachi returned home, Dr. Bob Noyes of the University of Hawai'i offered him a position at his university. Dr. Yanagimachi was granted tenure for his first full-time position. Dr. Yanagimachi was also very appreciative of all Dr. Noyes did for him.

This is when Dr. Yanagimachi began to play a big role in research. I hope you can see this, but in really tiny letters, he wrote "38 years old" here on this slide (Fig. 25). I think Dr. Akutsu mentioned that he was a "slow starter." It's true to say he was a "slow starter" in the sense that his career really only took off at the age of 38, but he began doing outstanding work from that point on.

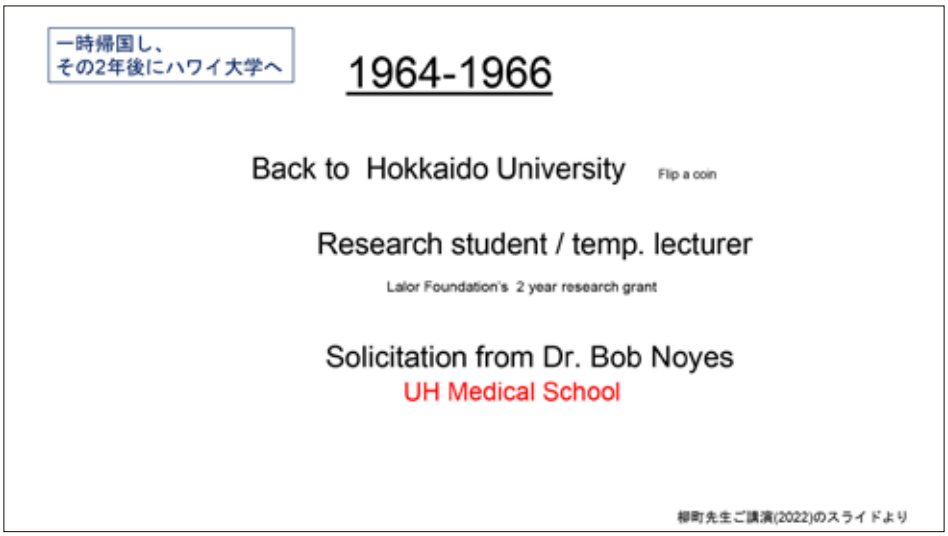

Fig. 25

Some of his major work includes analysis of the process and mechanism of fertilization, and assisted fertilization technologies, such as ICSI and IVF, and assisted fertilization technologies, such as ICSI and IVF, as well as cloning and transgenesis. Here it says "EM," which is short for "Electron Microscopy." As Dr. Akutsu explained, the job of using EM fell upon Hiroko-sensei. I understand that she was originally a child psychology major, but I would like to stress that she learned how to use an electron microscope because it was instrumental to fertilization research at that time and supported her husband over many years.

When I went overseas to study at Dr. Yanagimachi's lab, I was surprised to find just how well the lab was managed. As these photos show, it was set up so anyone could do their experiments. Here you see a 100-page-long protocol called "Hamster Notes," which Dr. Yanagimachi prepared using a typewriter and illustrations he drew himself (Fig. 26). This enabled anyone, including international students, to start their experiments. There is a plethora of things that we could learn from how he managed his lab.

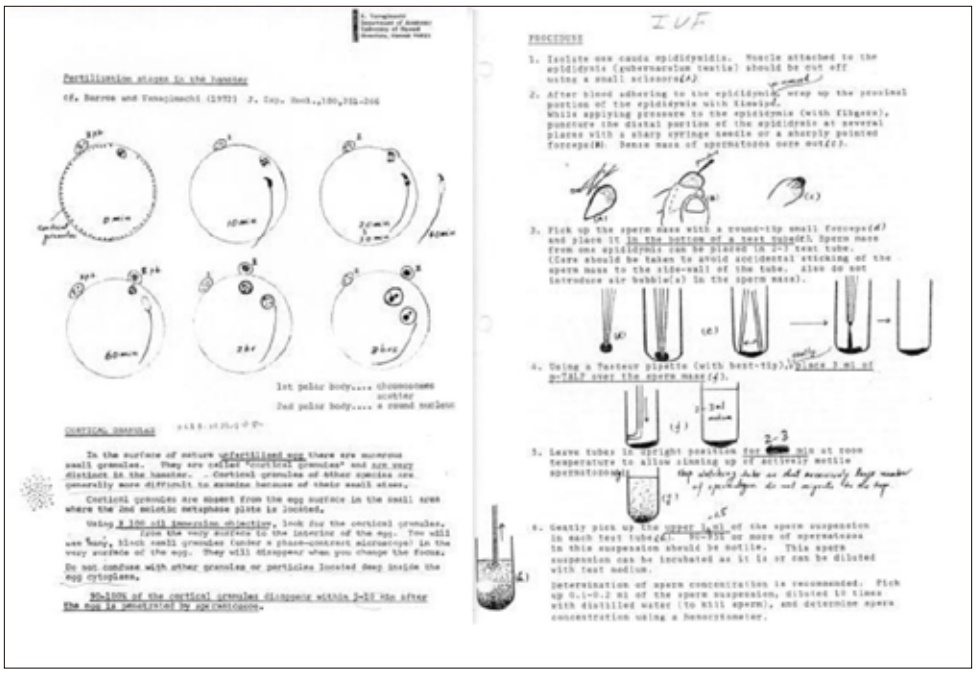

Fig. 26

In 1976, microinsemination was performed successfully for the first time in mammals (Fig. 27). It was done by Dr. Tsuyoshi Uehara from Okinawa, and the key to this success, too, was that he used hamsters. He also used an upright microscope rather than an inverted microscope. So, Dr. Uehara turned things around 180 degrees in his head, and interpreted images upside down and reversed to create the splendid fertilized egg you see here.

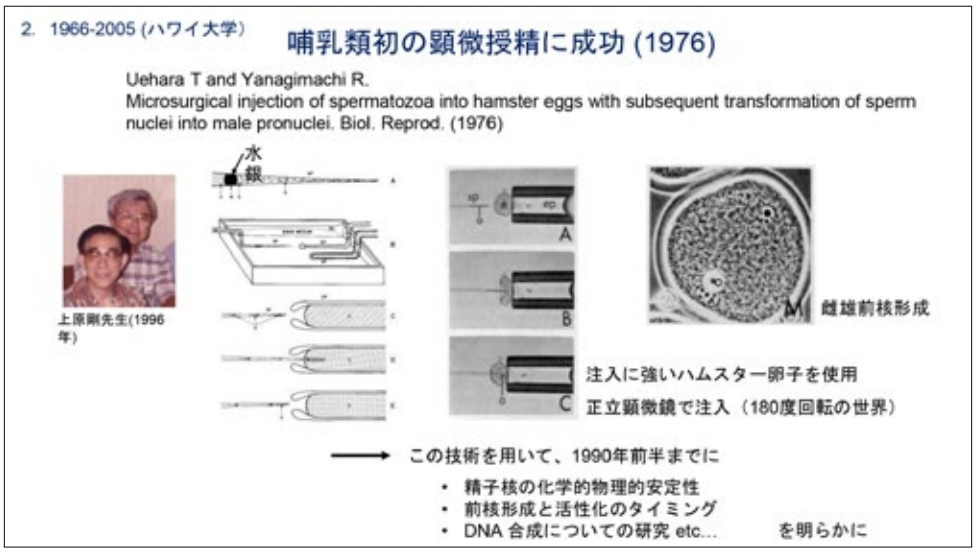

Fig. 27

After this successful attempt, he went on to reveal the basics of fertilization, for example, how sperm enlarge in an egg and under what conditions.

But there was one thing that bothered Dr. Yanagimachi for a long time: he thought for an egg to be called “fertilized,” it would have to develop into a baby. When I joined his lab, his mind seemed occupied with this thought. Hamster eggs may be injected with sperm but do not develop, whereas mice eggs probably would have developed if sperm could be injected. However, there was no way to inject sperm into a mouse egg back then. Once injected, all eggs died.

So, Dr. Yanagimachi came up with a novel idea, which was electrofusion, which could cause microinsemination without injecting sperm (Fig. 28). As the slide shows, sperm cannot physically undergo the electrofusion process. This being the case, they used round spermatid, still round-shaped, unmatured sperm right after meiosis. Using this method, Dr. Yanagimachi and I was able to obtain a litter.

That was how he used microinsemination technology to obtain a litter for the first time. What's more, with a mouse, he was able to obtain a litter from a round spermatid before using sperm.

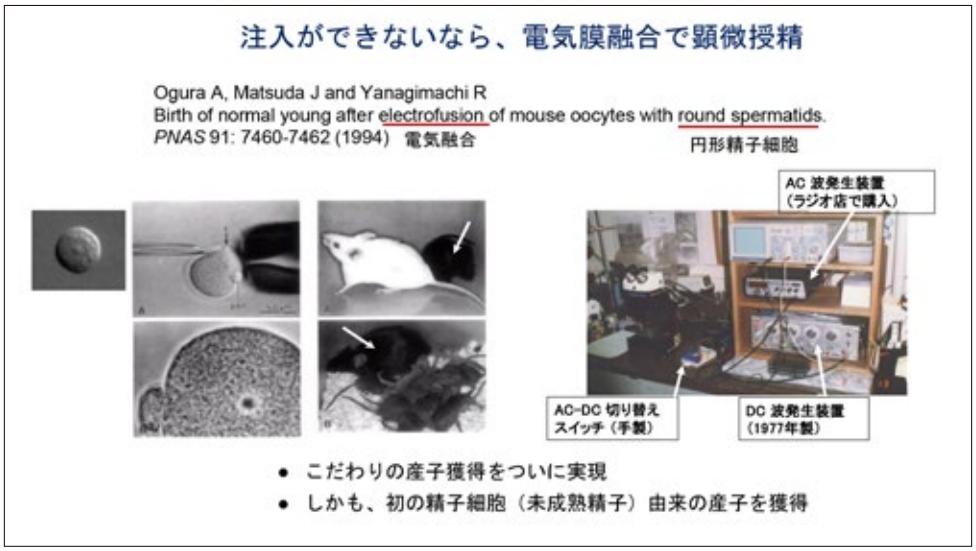

Fig. 28

Because a membrane fusion device was not available at that time, he combined all sorts of instruments. I was astounded to find that in his lab's storehouse, he had kept a DC generator made in 1977. This device generates square waves, the most important form of electricity for electro cell fusion. I recall being surprised by Dr. Yanagimachi's prescience, because he must have bought the DC generator on the assumption that he would eventually conduct such an experiment.

The electrofusion experiment was rather complicated and time-consuming, but in the following year, they succeeded in the microinsemination of mice using a Piezo micromanipulator (Fig. 29). This achievement should be credited to Dr. Yasuyuki Kimura. What was most important about this achievement was that it allowed sperm of any condition to be injected, rather than merely enabling microinsemination. This technology was eventually applied to the nuclear transfer cloning method.

Fig. 29

At this point, the role of hamsters ended, and mice took over the leading role. One such application was to generate baby mice from freeze-dried sperm (Fig. 30). I am sure you are familiar with the concept of freeze drying, which is used for instant coffee or soup. When rehydrated, the freeze-dried matter returns to its original state. By pouring water over freeze-dried sperm and injecting it into an egg, a baby mouse can be conceived almost like magic.

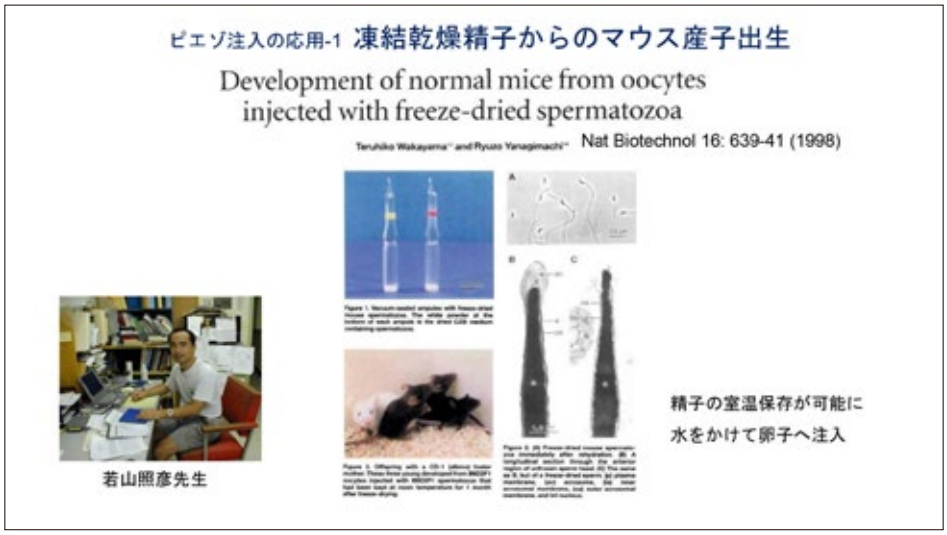

Fig. 30

The experiment was conducted by Dr. Teruhiko Wakayama, who was in Dr. Yanagimachi's lab at the time, and is now a professor at Yamanashi University. The year 1998 was an astonishing year when Dr. Wakayama and Dr. Yanagimachi successfully brought about the birth of a cloned mouse. Named after the "cumulus cell," which was used as a donor cell, this first cloned mouse, Cumulina, was featured on the cover of *Nature* magazine. Cumulina lived for more than two years, and her taxidermy remains are now exhibited at the Smithsonian Museum (Fig. 31).

Fig. 31

It was a monumental event in developmental biology, and the response was nothing short of overwhelming (Fig. 32). The achievement had a tremendous impact, receiving coverage in *The Washington Post* and *The New York Times*, as well as a Governor's award.

Fig. 32

In recognition of the accomplishment, the Institute for Biogenesis Research (IBR) was established at the University of Hawai'i in 2000 (Fig. 33). Needless to say, the founding director was Dr. Yanagimachi. This is a photo taken at that time. Dr. Yanagimachi, Hiroko-sensei, and Dr. Akutsu are all there. Dr. W. Steven Ward, who is here with us today, is also in the back. And this is a photo taken from the IBR website. Dr. Yanagimachi is, of course, here in the photo. IBR continues to evolve as a mecca for developmental biology and reproductive biology.

Fig. 33

Before I conclude, let me talk about Dr. Yanagimachi's more recent activities (Fig. 34). He had long been involved in experiments with mammals. On top of this, he experimented with insects, fish, and rhizocephala. He began working with rhizocephala when he was at graduate school. To be honest, I was ignorant of much of this, and unaware that he did such a large amount of work. I heard about this work from many people, and discovered that he did highly technical and advanced research. In other words, these discoveries affirmed that there was a second, third, or even fourth side to Dr. Yanagimachi that none of us knew about.

Fig. 34

I would like to touch on one of his recent works, which concerns the fact that acrosin, an acrosomal enzyme in hamsters' sperm, is essential for sperm penetration through the zona pellucida, a discovery that Dr. Yanagimachi and I have published recently. As you can see, Dr. Yanagimachi didn't use the "track changes" function in Word files, preferring to use a PDF file to exchange ideas with authors or co-researchers by hand-writing notes on a paper copy (Fig. 35). He also drew illustrations. This document is one of my most treasured mementos.

Fig. 35

This brings him full circle to where he started, with hamsters, which evokes deep emotions in me.

In conclusion, as Dr. Yanagimachi once put it, “You have life only once.” (Fig. 36) He also said, “Find a job you can enjoy, not the one you can tolerate,” advising us to find a job at which we can thrive. Another thing he said was that an early starter may not necessarily be successful, but that you should focus on doing a good job, even if you’re a slow starter. He counseled us to learn from Mother Nature. He also said to seek a job and enjoy it, no matter what anyone says. Try to be the very best in your chosen profession. I think we should convey these messages to younger people.

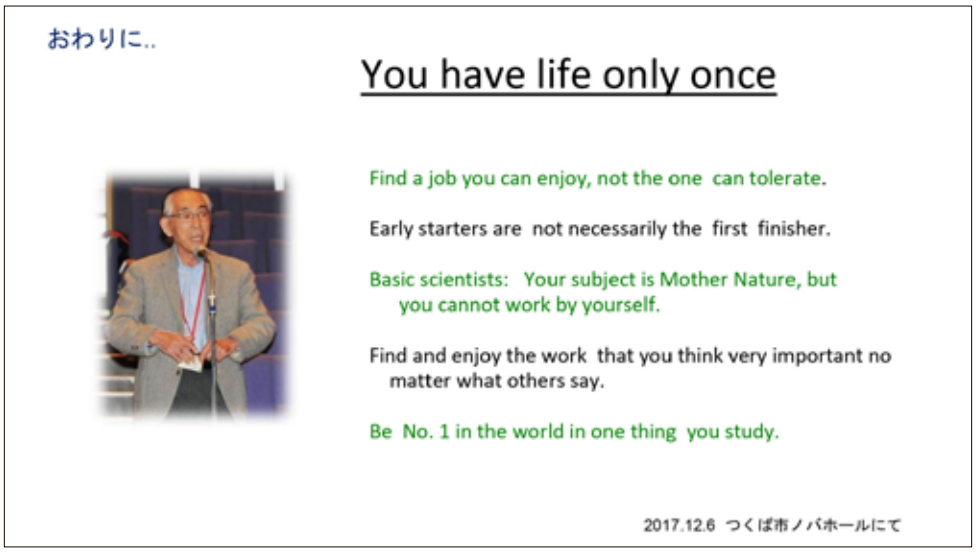

Fig. 36

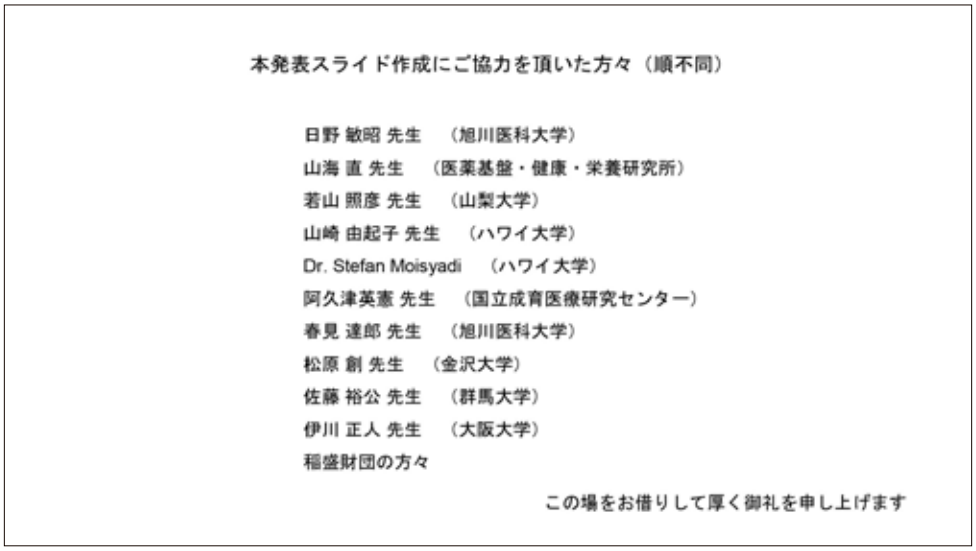

Fig. 37

Finally, this is a list of those who helped me prepare these slides (Fig. 37). I would like to take this opportunity to thank them all.

Thank you very much for listening to my account.