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20th Anniversary of Isolation of Human Embryonic Stem Cells: A Personal Perspective

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Following Jamie Thomson's lecture on primate embryonic stem cells (ESCs) at a meeting I had organized in March 1997, in Israel, to celebrate receipt of the Wolf Prize in Agriculture to my colleague and friend Neal First, frozen human embryos donated for research in Israel were shipped to Wisconsin. The five hESC lines (H1, H7, H9, H13, and H14) were established by early 1998 and transferred to my laboratory just before publication of their existence in *Science*, on November 6, 1998. The distribution of the cells from my institute to several laboratories, as early as 1999, enhanced the development of hESC research worldwide. My personal perspective regarding the scientific and political events surrounding this story are presented.

My earliest recollection relevant to this historical perspective goes back 13 years, before the isolation of the first human embryonic stem cell (hESC) lines in 1998. Near Christmas of 1985, at the recommendation of my mentor, Gary Hodgen, I traveled from the Jones Institute for Reproductive Medicine in Norfolk, Virginia, to practice micromanipulation techniques in the Neal First Laboratory of the University of Wisconsin (Madison). This visit not only facilitated the development of micromanipulation methods for assisted reproductive techniques in my IVF laboratory in Haifa in the early 1990s but was also the beginning of a long and lasting friendship with Neal and his family. I kept a close relationship with Neal and his wife Marijo Kent-First, and the three of us published a paper in 1996, a paper on the transition of Y chromosome deletions in *in vitro* fertilization (IVF) babies.

In August of 1995, Jamie Thomson's important publication (communicated to the Proceedings of the National Academy of Sciences by the renowned Neal First), on the isolation of the first primate embryonic stem cell (ESC) line was published (Thomson et al., 1995). Although this publication caught my eye, it attracted relatively little public attention, even though the international race to isolate the first human ESC (hESC) lines was at the start line.

Given the fact that many spare human embryos were kept frozen in my IVF laboratory and could potentially be donated for research on early human development, I decided to join the race. My efforts to establish a collaboration with Jamie were fruitless, until the next opportunity emerged.

Neal First's laboratory became the Mecca for those interested in IVF, nuclear transfer (cloning), and early embryo development of farm animals. In 1996, he was awarded the Wolf Prize in Agriculture (considered as the Nobel Prize in Agriculture). He planned a trip to Israel to collect the prize. To honor Neal's achievement, I organized a mini-symposium on "Nuclear Transfer and Embryonic Stem Cells," scheduled for March, 1997—concomitant with his trip to Israel.

I asked Neal to invite Jamie Thomson to this meeting, which was held at the Faculty of Agriculture of the Hebrew University in Rehovot. Jamie agreed to lecture on primate ESCs.

Surprisingly, the meeting attracted a significant audience, including scientists who later became involved in the early stages of hESC research in Israel. After the meeting, we traveled to the Jordan River and the Sea of Galilee (Figure 1). During his visit, Jamie agreed to work together in the race to isolate the first hESC lines. Thus, it is fair to claim that the history of hESC cells is well rooted in the banks of the Jordan River …

After Jamie left, we communicated by e-mail and discussed the next step. At one point, Jamie considered trying to isolate the cell lines in my laboratory, due to the restrictions on human embryonic research; specifically, as of Federal law in 1996, it was forbidden to destroy a human embryo for scientific research (Winston, 2007). As a result, public funds were not available for that purpose. He also was also concerned about seeking support from a commercial entity. However, the decision was eventually made to perform the work in Wisconsin and to accept the financial support of Geron Corporation.

By May 2, 1997, a proposal for isolating hESC lines from donated frozen embryos was submitted to the Institutional Review Board (IRB) of Rambam Medical Center; it was approved by the committee 2 months later.

The original consent forms were partially based on documents previously submitted and approved by the IRB of the University of Wisconsin, where there were ongoing attempts to isolate the cell lines.

Thirty-six embryos frozen in liquid nitrogen for at least 5 years were donated for this research by couples who signed informed consent.

Due to ignorance, naivety, and time constraints, no Material Transfer Agreement (MTA) was signed before shipping the embryos to Madison. The MTA was important

Figure 1. On the Banks of the Jordan River (March 1997)
Left to right: Marijo Kent-First, Neal First, Jim Bobl, Jamie Thomson, and Joseph Itskovitz-Eldor with his son Idan.

to ensure the proper use of the embryos and to cover issues related to rights of use resulting from the research.

The embryos were transferred to Wisconsin in a liquid nitrogen container ("Dry Shipper") in early January 1998, accompanied by my student Michal Amit. She stayed there for several weeks and kept a detailed record of the methods used to isolate the cell lines.

Soon thereafter, the first hESC lines were established in Wisconsin. The first dissociation and passaging of an ESC colony was accomplished in January 22, 1998, eventually resulting in line H1. This cell line originated from a fresh embryo donated for research at the IVF clinic of University of Wisconsin. In parallel, the frozen embryos transferred to Wisconsin were thawed and cultured to the blastocyst stage. Of the 19 embryos that developed to the blastocyst stage, 13 inner cell masses (ICM) were extracted and cultured on mouse embryonic fibroblasts, resulting in four cell lines (H7, H9, H13, and H14). The relatively large number of high-quality donated embryos, and the newly available Gardner's blastocyst culture media, resulted in a very high yield of ICM. This in turn enabled a "trial and error" approach for employing different techniques to extract the ICM, and the dissociation and passaging of ICM-derived outgrows, facilitating the successful isolation and culture of the hESC lines.

It cannot be stressed enough what the pressure was like at that time to make progress in embryonic research. The race was between labs worldwide, and Jamie's goal was to be first in the world. Hence, one can only imagine the effort that went into a project that culminated in publication within 9 months, including the review cycle.

The development of these five hESC lines in early 1998 was kept strictly confidential. In May 1998, I had the opportunity to attend an NIH-sponsored symposium in Madison on the science and ethics of ESCs. Leading scientists involved in the international effort to derive hESC lines participated in this meeting, but no mention was made of the five hESC lines that were successfully proliferating nearby.

In August 1998, Jamie submitted a paper to *Science* (Thomson et al., 1998), which was accepted for publication. As the expected publication date drew near, tensions were high due to the unpredictable possible responses of the public and media. It was clear that the story would capture media headlines, and this actually happened. To avoid unexpected difficulties related to the publication (November 6, 1998), the vials containing frozen samples from the five cell lines were transferred in a "Dry Shipper" to New York and then via El Al to Israel, before the embargo on the publication was lifted. During the trip, no security issues were raised, and the container was secured under blankets in a closet of the first class cabin.

To the stewardess who wondered about the excitement surrounding the shipment, I had to explain that the valuable but undisclosed research material was being transferred to my laboratory, and that she would learn about it from the media soon. The truth was much more complex. There was a deep fear of vandalism for ethical reasons. In addition, there was fear that pressure from the public would put a stop to ESC research. While at the time this was only speculation, the destruction of embryos did eventually become illegal in some nations.

An MTA was signed prior to shipment to Israel, following intense discussions between the Wisconsin Alumni Research Foundation (WARF) and Rambam Medical Center. Eventually the MTA was signed stipulating that the five cell lines could be used under my supervision, with no clinical use or commercialization allowed.

The cell lines were successfully cultured in my small laboratory on the 13th floor of the Faculty of Medicine, across the street from Rambam Medical Center, the first laboratory to culture hESCs outside the US.

Soon thereafter, the first Israeli hESC lines were established in my laboratory (I3, I4, and I6) by my PhD student Michal Amit (Amit and Itskovitz-Eldor, 2002). The eight cell lines from the H and I series were included in the NIH Registry imposed by the Bush administration, allowing the use of public funds for research using these cells only; the derivation of new cell lines was not allowed. Some of these cell lines were considered to be the "gold standard" for hESC research.

Only in 2009 was the ban on hESC research lifted by President Obama; the NIH required re-registration of the existing cell lines in order to qualify for public funding. The new consent standards were much more stringent; without new consents, cell lines that had become a standard would have been off limits to NIH funded investigators and lost to future clinical use.

The threat to on-going research worldwide based on the cell lines derived from embryos donated in Haifa was real. This, in addition to a visit of the governor of Wisconsin to meet with me at the Technion, placed me under a lot of pressure. Eventually we were able to obtain new consents from the couples who had donated embryos for research some years ago. The consent forms were modified according to the NIH demands, this time including permission for commercialization and the clinical use of the cells and their derivatives.

Soon after the 1998 publication in *Science*, I was approached by Nissim Benvenisty from the Hebrew University and hESCs were transferred to his laboratory; our collaboration produced the first report on the differentiation of hESCs to embryoid bodies (Itskovitz-Eldor et al., 2000). I had also provided the cells to faculty members, Karl Skorecki and Lior Gepstein, who established their own stem cell laboratories.

In parallel, I responded to the requests of other scientists and hESC lines were shipped as early as 2000 to Doug Melton, George Daley, Robert Langer, Oliver Brustle, and Michele Revel, among others.

The early distribution of these hESC lines, together with required know-how and technical support, contributed significantly to the dissemination and development of the research into these magnificent cells.

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