A catalyst for change in reproductive science: John D. Biggers as a mentor's mentor

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As we have done in the past, recognizing the true founders of reproductive medicine as we know it today is both an honor and responsibility that we provide to the JARG readership. In this issue of JARG, we take pride in honoring the lifelong achievements of Professor John D. Biggers on the occasion of his 90th birthday. For us personally, and for the many trainees and colleagues who have so generously contributed to this issue, assembling an appropriate tribute for Professor Biggers is a humbling task to say the least. Consider for example the wide-ranging career accomplishments that encompass, from the very beginning, over 60 years of a tireless, critical, and collegial unselfish commitment to so many that have conspired to make reproductive biology and medicine a prominent discipline in biomedical research today. We recognize him for the significance of his contributions to reproductive biology and medicine through the lens of a subset of scientists who have graciously made the following contributions. Hardly can a single journal issue or ASRM symposium achieve a thorough and balanced accounting of the ground-breaking and persistent influences Professor Biggers has had on so many.

With this backdrop, we hope the JARG readership will appreciate our humble efforts at producing a glimpse of Professor Biggers' career from which a sense of the complete scientist will emerge. His accomplishments at the bench are far outweighed by the lasting legacy that lives on in his mentees and by his singular efforts to bring a scholarly interpretation of the evolution of ARTs to his peers, the public, and lawmakers [1]. In fact, it is fair to say that few amongst us can

claim to have made inroads into the debates surrounding IVF, embryo culture, gamete and embryo preservation and cloning to the benefit of the scientific community as a whole and society more broadly. Intent is one thing—implementation quite another!

What follows is fitting testimony to his career accomplishments and impacts built from personal recollections of his colleagues and friends. Next, several papers are contributed from collaborators are complemented by a series of original papers dealing with a range of topics for which he is known and that bear directly on the purview of JARG. Biggers is well-known for his travels around the world as a representative for the World Health Organization and as an invited speaker at meetings that cross many scientific disciplines. A partial list of his role in the Society for the Study of Reproduction (SSR) includes

Founding Member of SSR, 1967 (Figs. 1, 2 and 3)

SSR President, 1968–69

Editor-in-Chief, Biology of Reproduction, 1970–1974

Carl G. Hartman Award Recipient, 1986

Fitting then that we recognize his accomplishments, which cover a span of 60 years, in this special issue of JARG given the influence Biggers' has had on the discourse, basic science discoveries, and clinical impact of his contributions to the field of human ARTs. Congratulations John and thank you for enriching so many lives!

A Tribute to JDB-reflections from your students, colleagues, and friends

In search of the tight junction in mammalian embryos.

David F. Albertini, Ph.D.

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In the early 1970s, with substantial support from the Rockefeller Foundation, the Laboratory for Human Reproduction

Capsule On the occasion of the 90th birthday of Professor John D. Biggers, students, colleagues and friends collaborate in a truly "Biggerian" fashion to offer their appreciation to an inspiring mentor.

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Fig. 1 Copy of certificate noting John D Biggers, DSc, PhD as charter member of the Society for the Study of Reproduction (1967, SSR); Biggers also served as President of SSR 1968–69, Editor-In-Chief of the Societies' journal *Biology of Reproduction* 1970–74, and received the Carl G. Hartman award in 1986

and Reproductive Biology was founded at the Harvard Medical School with a groundbreaking philosophy in mind that seems commonplace today. In true “Crimson” fashion, Harvard went about recruiting several outstanding scientists that would represent leaders in the field of reproductive biology but who would bring distinct (sic complementary) experimental approaches and interests aimed at solving the population crisis that was at hand at the time (Fig. 4). Even with a commitment to advance contraceptive technology, the melding of anatomists, cell biologists, physiologists, biochemists, and clinicians under one roof would have been expected to bring about changes in the way we approach and think about human reproduction. So it was that the likes of John Biggers was joined in this crusade in the early 1970s by a cadre of distinguished scientists including Roy Greep (the first director), Ken Ryan (the second Director) Everett Anderson, Lew, Engel, Claude and Dorothy Vilee, Don Fawcett, Martin Dym, Earl Parr, David Porter, Koji Yoshinaga, David Hamilton, Paul Wassarman and others. As an Anatomy graduate student in the laboratory of Everett Anderson (“Andy”) I had the remarkable opportunity to have trained in this robust environment for the next 4 years and it is during this time that the Anderson and Biggers laboratories initiated the “down-the-hallway” collaboration that would result in the discovery of the tight junction in mammalian embryos [2].

The backdrop for this adventure was the following. Tom Ducibella had joined Andy’s lab to study the nature of cell interactions during development of the mouse embryo and as it happened, we shared office/lab space and received formal training in embryo culture and electron microscopy over a 3 year span of time that would carry on for many in the future. It was during this time that a revolution in cell biology was taking place thanks to the introduction of a new technique

known as freeze fracture electron microscopy. The Anatomy Department at HMS established one of the first facilities in the United States where this technique could be performed. In true *carpe diem* spirit, Andy sent me off to building B where the Balzer’s apparatus was located to learn this technique from the resident expert, Dan Goodenough. Within a year, I had acquired so much experience making high quality replicas mainly of ovarian tissue, the subject of my Ph.D. thesis, that many faculty sought me out to train their students, technicians, and postdocs. Andy, in the spirit of LHRRB collaboration, brought this to the discussion table with John Biggers whose lab was in the midst of characterizing the transepithelial resistance of trophoblast in the rabbit embryo. The late Dale Benos was undertaking the membrane physiology of trophoblast and it soon became clear that bringing a morphological dimension to this project would be advisable. Enter Ducibella and Albertini (Fig. 4).

John took great enjoyment in sharing his nascent wisdom on the mammalian embryo in very different ways with his students. I remember well the day he showed Tom, Andy, and I Professor Mulnard’s time-lapse movie of development in the mouse embryo and being in awe of the sequences in which compaction, and blastocoel expansion, collapse and re-expansion were vividly in evidence [3]. This experience launched the search for the mammalian tight junction.

Tom thus began collecting and sectioning mouse embryos at various stages of preimplantation development and, being the freeze-fracture expert of the group, I set about collecting enough embryos to make a go at finding intercellular junctions with our focus being on compacted morulae and expanded blastocysts. While Andy fully appreciated the technical challenges ahead, which would be best described as finding a needle in a haystack, John really had no idea how difficult it would be to locate an embryo in a vast sea of platinum replicas where an occasional 100 μm object may have left the footprint we were looking for. What John was aware of was the publication of a seminal paper from the Goodenough laboratory that had demonstrated that the structure of tight junctions, specifically the number of strands located at the apical border of many epithelial cells, was nicely correlated with the transepithelial resistance that could be recorded across an epithelium. In fact, since the number of strands was greatest when transepithelial resistance was maximum, he predicted that the rabbit or mouse trophoblast should be well-endowed with some sort of record-breaking number of strands. If only science were so simple!

After months of preparing replicas of glycerol droplets in which Tom and I were sure we had placed hundreds of mouse embryos, and taking literally hours and hours to scan these in the electron microscope (with the priority set on this project, I was given permission to use the top-of-the-line Phillips 300 TEM that Andy and Don Fawcett shared, but only after hours), I came up empty handed. Meanwhile, Tom was making great progress with the TEM of sections and even had

Fig. 2 Letter received from Dr Howard W. Johnson, Jr, MD recognizing Professor Biggers on the occasion of his 90th birthday

evidence using extracellular tracers that indeed John was right—these were looking like the tightest of tight junctions imaginable. This scenario did little to earn me praise or attention from John and Andy. In fact to say that I was feeling like my days as a graduate student were threatened would be putting it mildly. Without the freeze fracture data, our study would be woefully incomplete. It was time to rethink our approach.

The next chapter was what I like to call a Krogh moment. After feeling like I was wandering aimlessly in the Sahara desert of platinum replicas, the eureka moment arrived when John brilliantly suggested that we use a larger embryo, like those of the rabbit, which were being regularly used by Dale Benos in the Biggers laboratory. At last, the details of intercellular junctions within the mammalian embryo came into sight, and indeed the properties of the tight junction were what we had predicted but with one disconcerting caveat. The number of strands was far less than the expectations we had set. Despite John's disappointment, we had enough data in hand to proceed with our publication that appeared about 1 year before Terry Magnuson reported similar results in the mouse. The search was over and from this experience I did gain a modicum of respect from John that has translated into a career long collaboration for which I am most grateful.

There are many more anecdotal observations I could share originating from the founding years of the LHRRB and the beginnings of my relationship with John. I will close with a final memory that again sets the tenor not only for the LHRRB in the 1970s, but for a budding graduate student, provided perspective for what was to lie ahead as my career evolved.

Fridays at noon, the LHRRB seminars were held in the Roy Greep room on the first floor (Fig. 5). The seating plan was conspicuously hierarchical—Professors dutifully sat in the first row, and from front to back were arranged Associate then Assistant Professors, followed by postdocs, technicians and yes, graduate students at the back of the room (and closest to the tea and coffee that would be made following questions at the end of the seminar) (Fig. 5). Many notable speakers were paraded through the seminars series of the 1970s including the likes of M.C. Chang, Etienne Beaulieu, Cornelia Channing, A. Rees Midgely, Kent Christenson and so many others who were or would become leaders in the field. In the emerging spirit of tradition, two behaviors became apparent. First, with the front row fully occupied by the Professors, I noticed that with rare exception a collective slumber party was consistently in evidence. That is, nearly all of our leaders were catching up on apparently much-needed sleep nodding asynchronously like a wave left to right and back. Most remarkable to me was the not uncommon occurrence of waking up as the speaker was concluding their presentation. And, those who had enjoyed the deepest slumber were in fact the very first to ask probing and insightful questions that would launch a vigorous discussion for the rest of the audience to enjoy! How could this be? As an aging scientist I can admit to have gradually added this technique to my repertoire but remain bemused as to the mechanisms underlying this fascinating phenomenon (Fig. 6).

The second behavior on display at these gatherings was more predictable. As mentioned, the graduate students assumed residence at the back of the seminar room and given the dominance of questioning from the front, rarely did anyone ask questions from the bleacher section we occupied—with one notable exception. Week in and week out, I often sat next to an older woman who wore many layers of clothing consistently topped off with a shawl that was partly wrapped around her head. While I found her appearance rather odd, she was the only brave soul who asked questions from back there and her questions gave clear indications of her familiarity with the topic of the seminar—especially when it had to do with embryos, eggs, or ovaries! Who was she? It was John who first pointed out to me that she was Ms. Miriam Menkin, a legend in her own right. Miriam, as many of you may know, was the driving force behind the research of John Rock and Arthur Hertig during the years of the famous egg hunts that yielded the earliest descriptions of human development, which remain today as classics in human embryology textbooks. She also

Fig. 3 Newspaper article originally published November 1, 1979 in The Ledger-Star titled “Both sides swap barbs over test-tube babies.” This article represents one of the many times Biggers expert opinion was solicited from the media during the early years of human IVF

was co-author with John Rock on the first reported case of in vitro fertilization in the human but that is another story.

In conclusion, I hope John that these memories, in a small way, demonstrate how deeply indebted I am to you for your mentorship and friendship. Besides being a role model as a scientist, I have gained so much insight from you into the world of reproduction and how to convey to our peers and the public a morally-based conviction of what our profession should be. Happy 90th birthday!

**Ryuzo “Yana” Yanagimachi, D.Sc.
Prof. Emeritus.
University of Hawaii**

I first met John Biggers in the spring of 1964 when I visited him at the School of Veterinary Science of the University of

Pennsylvania (Fig. 7). It was several months before I returned to Japan after 4 years of working at the Worcester Foundation for Experimental Biology (Shrewsbury, Massachusetts) as M. C. Chang’s postdoc fellow. Ralph Gwatkin and Ralph Brinster were among fellows who were studying under his mentorship at that time. I also met him during the summer of the same year at his summer house in Woods Hole, Massachusetts. He must have been a faculty member of an NIH-funded summer training course of embryology in the Marine Biological Laboratory. Colin Austin, Charles Metz and Albert Monroy were among his guests of the evening, I recall.

John and I took different courses, but I was fortunate to be able to work with him in a National Cooperative Program of Mouse Sperm Cryopreservation sponsored by the National Center of Research Resources during 2001–2003 and published one paper with him [4]. His team developed an “Evaporative Drying” method which I thought would be very difficult. I congratulate his group on this startling success.

While I was working on sperm-egg fusion, I found that zona pellucida-free eggs of the golden hamster can fuse with spermatozoa of the same and many other species including humans, provided they are acrosome-reacted. I found that a medium that John and others developed for mouse embryos was suited for this study and called it BWW medium (Biggers, Whitten and Whittingham) (Fig. 8) [5]. John Biggers’ many contributions including analyses of the metabolism of preimplantation embryos, improvement of mammalian embryo culturing media, and his mentoring many superb young fellows remain unsurpassed. I cannot imagine how today’s field of experimental embryology in mammals would be if he were not in this field of study (Fig. 9).

Remembering and appreciating John D. Biggers

Dianne Moore Smith, Ph.D.

If I look back about 50 years, my first memories of Dr. Biggers are from the Physiology Dept. at the University of Pennsylvania. In the early 60’s J.D.B. was giving a series of lectures for the graduate students on mammalian oocyte maturation, fertilization and early embryonic development. I was working in a neuroendocrine lab at the time and knew very little about developmental biology, mammalian or otherwise. Dr. Biggers’ lectures captured my interest and ultimately led to a change in the direction of my studies. Dr. Biggers was very approachable and agreed that I should join his group.

After prelims, I began the daily jitney ride, along with several others in Biggers’ Lab and the Veterinary School, from West Philly across the Schuylkill River, to the King Ranch Lab in the Lippincott Building. The process of choosing a thesis topic was difficult for me. I was still a scared kid from Saskatchewan, overawed by the romance of the research

Fig. 4 Koji Yoshinaga at the Laboratory of Human Reproduction and Human Reproductive Biology (LHRRB), Harvard Medical School, Boston MA. Circa 1974

environment and unsure of my own creative and organizational skills. Finally Dr. Biggers called me into his office and said that if I didn't choose a topic and get under way, he would choose it for me! This was the rude awakening I needed and I stopped procrastinating and chose "Implantation in the Mouse".

In 1964 I had a break from mice and spent the summer at the Marine Biology Lab (MBL) at Woods Hole, Mass. Dr. Biggers was a staff member, as was his good friend C.R. Austin, in the Fertilization and Gamete Physiology Program directed by Charles Metz. All the students had projects utilizing the gametes of invertebrates so abundant at Woods Hole. For me it was an introduction to molecular embryology, although my project was neuroendocrine in nature. I also became familiar, with the practical application of precise experimental design and rigorous statistical testing that JDB

demand. I remember sitting for hours and clanking away at an old Monroe calculator in a room full of such devices!

It was not all work at Woods Hole. I remember many pleasant hours exploring Cape Cod with Dr. Biggers and his family, watching *dynoflagellates* light up the sea water and fireflies flit and flash at night and learning the biochemistry of these incredible sights. Also memorable was a New England lobster boil on the beach for all the staff and students in the Gamete Physiology Program. It was quite a summer. Thank you, John.

Eventually enough implantation data were collected and analyzed and it was time to write a thesis. I wrote several drafts and finally stayed with John and his family over one agonizing weekend when he ignored his wife and kids (much to Mrs. Biggers' annoyance!) and tore the whole thing apart. For two long days we reorganized and rewrote. He told me my

Fig. 5 Plaque situated in the Greep Seminar Room, LHRRB, Harvard Medical School

Fig. 6 Celebrating the Ph.D. defense of David Albertini in May, 1975. From left to right Richard Schultz, Albertini, and Professor Everett Anderson. Photograph taken by Dr. Rich Wilkinson

syntax was “positively Germanic” and where did I learn to string out so many modifying clauses in one sentence! I was crushed as I thought I’d done quite a good job! Another life lesson, also long remembered and the experience recalled each and every time I wrote a grant application, paper for publication or, in later years, procedure manuals for IVF labs.

Over the years in Dr. Biggers’ lab, I got to know fellow students Ralph Brinster and David Whittingham quite well. I remember some good times at New Bolton Center where we all went to help with the collection and identification of oocytes flushed from the oviducts of superovulated cows. I was in awe of the skills of the large animal veterinarians and how much more difficult it was to flush bovine compared to mouse oviducts.

I graduated from Penn in 1966 and went off to California to post doc with Lajos Piko and then Luciano Zamboni. Meanwhile Dr. Biggers moved to Johns Hopkins. I spent a couple of months in his lab there on a short sabbatical leave. I don’t remember much about the work but do remember meeting Alan Schuetz and Roger Donahue. I also remember a fantastic outing with John and his family to Chincotique on the Assateague National Seashore to view a rare total eclipse of the sun. I can’t remember if we saw any ponies that day but one could not leave the area without learning all about these famous and much publicized creatures.

At some point in the early 70’s Dr. Biggers visited California. We went sailing and walked on the beach. He told me about his plans to move to Harvard Medical School and outlined the program project he was developing at the Laboratory for Human Reproduction and Reproductive Biology (LHRRB), a new facility directed by Roy Greep (Fig. 10). John invited me to join his group. I accepted and moved to Boston in 1972. Others in the group included Paul Wasserman, Richard Schultz, Tony Bellve, Susan Heyner, Doug Powers, Mary Nekola and Ed Lee. Koji Yoshinaga was in Dr. Greep’s group

and I remember him as a fine scientist and a real gentleman. The LHRRB put on an excellent seminar program which featured guest lecturers, all experts in their respective fields of Reproductive Biology. There were also short courses given by Harvard Medical School scientists and invited guests from different disciplines, also well known for their contributions to basic reproductive biology and clinical practice. It was an exciting and stimulating environment and one that was designed to foster collaboration between investigators within our program project and between other LHRRB groups.

At Harvard I learned several more life lessons. Dr. Biggers was a master at the fine art of writing grants and he taught us well. I realized that I was a good instructor for individuals and small study groups but a poor communicator in front of large classrooms packed with medical students. I also discovered that I hated academic politics and was happiest working in my lab, publishing the data and continuing to study Reproductive Biology.

I left the LHRRB in 1979. A short time later I joined Dr. Mel Taymor and his fellows in Clinical Endocrinology at the Beth Israel Hospital where we established the first IVF program in Boston. IVF involved a combination of reproductive endocrinology, embryonic development and implantation physiology. It was tailor-made for my theoretical and laboratory skills. Dr. Biggers was once again a valued consultant for me and also for Dr. Taymor in this undertaking.

In 1983 I left Boston and saw John infrequently in the ensuing years. Once, in the 90’s, he visited my IVF lab in Anaheim. He stayed with us and I remember that we went out for an excellent Vietnamese dinner. I think of him often and remember and appreciate the valuable lessons learned during my years of close association with him. I am delighted that he was able to locate me after such a long time. I very much look forward to the reunion in Boston.

Happy 90th Birthday John and many more to come.

Fig. 7 (Left-right) John D Biggers Ryuzo Yanagimachi

Fig. 8 Founding members BWW media formulation designed by John Biggers, Wes Whitten and David Whittingham (left to right). Photograph and labeling compliments of Ryuzo Yanagimachi

Fig. 9 John Biggers, Anne McLaren and Koji Yoshinaga. Taken at a meeting 1992 in Bordeaux, France

Professor John D. Biggers: mentor, scientist, naturalist and friend

Catherine Rackowsky, Ph.D.

Brigham and Women's Hospital, Harvard Medical School

It was more than 35 years ago, in the mid 1970's. Although Professor John Biggers and I worked on the same floor of the Laboratory of Human Reproduction and Reproductive Biology ("the LHRRB") at Harvard Medical School and I knew who he was, both of us being British and not yet "formally introduced", it would take more than a year for us finally to talk. I was looking for another lab to complete my post-doctoral work. John's lab at the other end of the hallway seemed a-buzz with energy, filled with scientists and trainees from all over the world and I knew that I wanted to be a part of that. Yet, as a post-doc, it was with great trepidation that I asked John's administrative assistant, Eleanor Sokol, for an appointment. How would I be received by a scientist of such international acclaim? I had no funding, yet my interest in the embryo and implantation seemed to fit perfectly with his laboratory's focus on early mammalian development. While I carried my fears right to John's door and into that first meeting, all worries were completely allayed after that first conversation. I quickly learned that John selflessly puts trainees ahead of him: it was his "policy". Within the hour, we had designed experiments to investigate the role of prostaglandins in blastocyst formation and had also outlined a Lalor Foundation application which provided the basis for a successful application.

My life as a post-doctoral fellow under John's tutelage and guidance was enriched in enumerable ways. From a scientific perspective, I reinforced the most important appreciation of the rigors of experimental design, the importance of using appropriate statistical tests and the vagaries of the "p-value" which had previously been only a "fleeting devil among numbers"!! I also quickly learned that John not only

encouraged, but expected, independent thinking and the pursuit of original ideas – this was, in essence, the spirit of John's laboratory. I was fortunate to have "been raised" in a lab with a similar philosophy during my PhD years with Brian Heap in Cambridge. However, there were others in the lab that needed more guidance; for them I saw firsthand how John and his more experienced laboratory members provided guidance, but never spoon-fed. This highly supportive environment led to enormous productivity; failure was simply not an option in the Biggers' lab no matter what it took (Fig. 11).

Fast-forward several months after joining John's lab to a very specific occasion that would mark one of the more important moments in my career. The impact of this was so great that I still distinctly recall what I was doing and where I was sitting when John approached me. I was sitting at my desk with the fume hood blasting to dry a recently run chromatogram just far enough away to keep my hair from singing. As I sat there, I heard John asking if I'd like to contribute to a special report he had been asked to write for the then Department of Health, Education and Welfare (DHEW) on the potential support of using federal dollars for research involving in vitro fertilization and embryo transfer. If so, he continued, he would take me to Washington to participate in DHEW hearings for consideration of federal funding for clinical IVF. I was filled with excitement, thrilled with this opportunity and in awe that "the" Professor John Biggers, the one of world renown in the field of mammalian embryology, was inviting me to participate in such an important undertaking. Of course I accepted and several months later found myself sitting around a large oval table with John, a number of politicians, a couple of religious leaders whose names I have lost with time, an ethicist (Dr. Leon Kass), and an infertile couple, discussing the science and ethics of IVF in animal models, and as a treatment option for human infertility. Not only did I get to participate in a policy-making process so early in my career but also, and more importantly, I was reminded again of John's generosity to provide a young trainee with every possible opportunity for career advancement and the gathering of a larger context for our work and the battles that needed to be fought.

The big lesson learned from these early observations was that it is critically important to provide an atmosphere of scientific enquiry and autonomy, yet with structure and sufficient support to bring mentees up and beyond. I have tried to carry this with me as a mentor and supervisor.

Aside from the wonderful role model as an investigator and mentor, I would be remiss not to mention two other vignettes that shed light on the depth and breadth of John's personality and interests. His sense of humour is extraordinary, although I will say that much of what "we Brits" find amusing gets lost on the average American! And then there is John the nature-lover (Fig. 12).

My first glimpse of his deep appreciation of the natural world came from a weekend trip that he, a few others and I

Fig. 10 John Biggers and colleagues in front of the LHRRB (courtesy of Professor Henry Leese)

took one summer's day to Mount Washington, New Hampshire. As we made the lengthy climb up, John started to describe the Alpine flowers we would find at the top. His enthusiasm and excitement were contagious; when we finally arrived I was certainly not disappointed – stretched out before us were multi-coloured carpets of low-growing, exquisitely formed flowers, some of which were almost too small to see with the naked eye. For you see, Alpine flowers are adapted to hugging the ground in their harsh and high habitats. John was beaming from ear to ear as only he can! His connection with nature's beauty and creativity was contagious and we must have spent the best part of an hour passing around the binoculars to become absorbed in this miniature and wonderful

world. The following Monday, I did not see Professor Biggers in quite the same light. It was as though he had become much more human and we shared something that had been a gift to both of us.

To sum up the enormous impact John has had on my life: I would not have remained a scientist if it had not been for John: he steered me away from being an antique furniture restorer when I was discouraged by a difficult unfunded period in my late twenties! He has encouraged my passion for mammalian eggs and embryos; he has taught me the rigors of scientific investigation and the importance of expressing one's opinion even if it may lead to controversy; he has shown me, by example, how mentoring should provide young trainees a

stimulating, supportive yet freeing environment to explore and pursue novel ideas. Perhaps most of all, he and his wife Betsey Williams, are very dear friends to my husband, Henry Devine and me. Thank you, John (and Betsey), for all of it! (Fig. 13).

On John Biggers

Professor Henry Leese
University of York

I first met John Biggers outside Foyle's Bookshop in London, in 1979. The background to this meeting was that I was a new Lecturer (Assistant Professor) at York University in the UK and my senior professor, and former Post Doctoral supervisor, Ramsey Bronk, a US citizen and son of a famous US scientist, Detlev Bronk, had said he was visiting family friends in Boston and wondered if there was anyone he might contact on my behalf, since he knew I was interested in taking a sabbatical in the USA. I knew immediately that the lab in which I would most like to work was John's since, with Claude Lechene and R.M. Borland, he had made measurements of the microenvironment in the mouse oviduct which tied in closely with my interest in Fallopian tubal physiology [6].

Thus, Ramsey met with John who said he would be interested in my visiting his lab but felt that we should meet up first before deciding to go ahead. It so happened that John was about to visit his long-time research colleague and good friend, Anne McLaren [7] in London and so, through the good offices of Anne, who I already knew, I ended up waiting to meet John outside Foyle's Bookshop, on a rainy day, if I remember correctly. John and I met up and took a short bus ride to Anne's lab in the Medical Research Council Mammalian Developmental Biology Unit, of which she was the head. The meeting went well and it was agreed that I should apply for funds to the UK Royal Society to visit John's lab in Harvard Medical School for 6 months in 1980. The project was, if I remember correctly to measure the nutrient content of nanoliter-sized samples of mouse oviduct fluid.

The Royal Society bid was successful and I turned up in John's lab in June 1980. On either the first or second evening I was in Boston, he invited me to his house where I met Catherine Racowsky and Roger Pedersen who happened to be staying with him prior to going to a Gordon Conference at Colby Sawyer College. He had also arranged for me to attend the conference and I travelled there and back with them all in his car – where amongst other things, I discovered he was an authority on the life of the American blues singer Bessie Smith (1894–1937)! John, Catherine and Roger were most generous in their welcome as were the delegates I met at the Gordon Conference. For me, this warmth and openness is a characteristic of people in the USA and contrasts with the Anglo-Saxon

Fig. 11 Photograph taken at the Culture Club meeting in 1992 at the Marine Biological Laboratory, Woods Hole, MA. Culture Club 1992 included John Eppig, John Biggers, Don Wolf, Richard Schultz, Bob Foote, Barry Bavister and Richard Tasca. Photograph courtesy of Richard Tasca

reserve that tends to typify the English. What's more, these friendships have continued to the present day. As for the Gordon Conference; it was an eye-opener and turned out to be the first of a number I attended. Gordon Conferences are unrivalled for their capacity to promote scientific discussion at the highest level in convivial surroundings.

At some stage during this period, John uttered the words that would change my life! He was concerned at the prospects of my generating much data on the mouse oviduct fluid project in only 6 months and said instead:

I suggest you work on mouse embryos.

While taken aback, I needed no persuading; John was a world authority on mouse embryos and as for The Royal Society – well, those were the days when good science was more important than following a grant proposal to the letter and after all, mouse embryos are what reside in mouse oviduct fluid!

And so I began research on mouse embryos in the LHRRB (Laboratory of Human Reproduction and Reproductive Biology) and never looked back. The work with John led to a paper in *Analytical Biochemistry* [8] in which we measured the ATP, ADP and AMP content of single mouse embryos using the ultramicrofluorometric methods in use in the LHRRB. In addition to John and Claude, the work could not have been done without invaluable help from Ed Mroz.

I would like to mention two more characteristics of the Biggers' lab over the years: the first is the phenomenal number of graduate students, post docs, and visitors who have passed through it – a roll call in our field. The second can be illustrated by an anecdote which illustrates his philosophy on collaborative work. When I was about to leave his lab in January 1981 I said that I would obviously seek his permission to pursue any research which built on the work initiated in his lab. John immediately put me straight on that and said that I could do whatever I

Fig. 12 Sperm Club meeting held at Harvard University on June 1–2, 2000. Principal investigators in the cooperative program are in italics. The others are co-investigators. Standing from left to right: Andrzej Bartke, Mehmet Toner, *Larry Mobraaten*, *Alan Trounson*, *Ismail Kola*, *John Biggers*, Richard Tasca. Sitting: *Ryuzo Yanagimachi*, Kent Lloyd, David Whittingham and Jim Overstreet. “We elected John to be the permanent Chairman of our Sperm Club meetings at this meeting.” Photograph courtesy of Richard Tasca

liked! – that he never imposed any restriction on the research that people who had visited the lab wished to do subsequently. This was the good advice I have adopted throughout my own career, recommended to others and would never change.

About the history of The Culture Club

Richard Tasca, Ph.D.
NIH, NICHD

I was specifically hired by NIH in 1984 to stimulate and encourage research in the area of human in vitro fertilization in the aftermath of the birth of Baby Louise in England. However, NIH was banned from supporting human embryo research so my task was to figure out ways to support human embryo research through research on nonhuman organisms. So, I put together a cooperative agreement program (not grants or contracts) that would establish a group of outstanding investigators to work in collaboration with each other and with NIH (I served as the Research Coordinator) towards the specific goal of improving the culture conditions for nonhuman preimplantation research and of course, including the critical element of egg quality. We held three meetings a year over the whole 14 years and not a single principal investigator missed a meeting, such was their dedication to the cause. So, The Culture Club was started in 1986 and then recompleted in 1991 and again in 1996. Altogether, a period of 14 years resulting, collectively, in over 240 publications. The results were outstanding and obviously long-lasting. The Culture Club developed KSOM/AA, CZB, NCSU-23, and the HECM series and these media are all still in use in many labs around the world. To our surprise, it only took 3 years for a human IVF lab in Virginia to test the CZB medium. KSOM/AA is the base medium for Global, one of the successful human embryo culture media that is also used in many human IVF clinics around the world. John Biggers played a key role in the

development of KSOM as he introduced the simplex optimization approach.

From primate follicle culture to dried sperm: thirteen years in the Biggers Lab (1992–2005)

Lynda K. McGinnis, Ph.D.
University of Kansas Medical Center

I first met John Biggers in 1992. I had recently completed a Master's degree studying ovine and porcine embryo culture and John invited me to move to Massachusetts to work with him in

Fig. 13 2011 SSR meeting in Portland Oregon. Standing from left to right: Romana Nowak, Lynda McGinnis, Paul Burgoyne, Gloria Lee. Seated: Betsey Williams, John Biggers

his laboratory at the New England Regional Primate Research Center. I knew of John's many publications on embryo culture media and was thrilled with the chance to work with him. John had recently received a renewal of the "culture club" grant (National Cooperative Program on Non-Human In Vitro Fertilization and Preimplantation Development; John's grant "Culture Media For Preimplantation Development" was continuously funded 1989–1997). When I join the group, Joel Lawitts and John were nearing completion of their studies on the original design of the Potassium Simplex-Optimized Medium (KSOM) for embryo culture [9]. The goal of our work with the primates was to develop an in vitro system for the culture of primate follicles. In 1965, Ira Fritz, Wan Kyoo Cho and John Biggers had presented their results of whole ovary culture [10]. More recent studies from Roger Gosden's laboratory [11–15] had developed media for the culture of explanted follicles. John's initial primate studies were conducted with Lorna Johnson, Bob Becker, Abdelmoneim I. Younis and David Albertini and resulted in publications describing the effects of superovulation regimen on rhesus monkey estrogen levels, follicle growth, oocyte maturation and embryonic development [16–18]. For the culture of whole follicles, I began with the Gosden lab's culture media formulations and tested various substrates in an attempt to develop a 3-dimensional culture system that would allow follicles and oocytes to grow and mature in a more natural state. Matrigel, a complex solution of extracellular matrix, was a relatively new product on the market at that time and John agreed this would be worth a try. The initial studies were conducted with mouse follicles and resulted in the publication of a 3-dimensional follicle culture system in which follicles grew from secondary to large antral (>400 µm diameter) stage during 3 days of culture; fully grown oocytes that responded to in vitro LH by maturing to metaphase-II and "ovulating" from the follicles [19]. We repeated these studies with the rhesus monkey and found that primate follicles also grew well in our Matrigel culture system (McGinnis, Johnson, Albertini and Biggers unpublished, 1995). We also used this culture model for the study of the cyclin D2 knock-out mouse in conjunction with Piotr Sicinski [20], thus proving that granulosa cells require cyclin D2 to support proliferation but not estrogen production in response to FSH.

During this time, we were also studying cryopreservation of non-human primate oocytes with Abdelmoneim Younis and Mehmet Toner. This research was conducted on *Cynomolgus* monkey oocytes to determine the effects of osmotic and cryo-stress on primate oocytes and the characteristics of water permeability during freezing [21]. The cryopreservation studies were later continued by Fouad Trad on human oocytes [22].

In 1995, John decided to close his lab at the primate center and refocus the studies to embryo culture media. So, I closed the primate lab and moved everything into John's main laboratories at the Harvard Medical School in Boston. At this time, the lab included three post-docs, Jay Baltz, Greg Erbach and

Piyush Bhatnagar. The same week that I moved into Boston, Jay left the lab and started his new faculty position at the Ottawa Hospital Research Institute. Shortly thereafter, John's last graduate student, Susan Palmieri returned to the lab following her time as post-doc in Germany at the EMBL [23]. Over the next 10 years, the primary focus of John's work continued towards the optimization of KSOM embryo culture media [24]. However, we had great adventures delving into other fields as well.

It was not uncommon for other Harvard associated researchers to contact John asking for assistance in studying their non-fertile knock-out mice. I was lucky to be in the lab at this time. John gave me the opportunity to work with Mary Donohue and Jordan Kreidberg conducting studies to understand the causes of embryonic failure in mice lacking the transcription factors Yin-Yang 1 and Wt1, respectively [25, 26]. One of the funniest experiences was when someone walked into the lab carrying a frozen six-legged male mouse wrapped in a plastic bag and asked if we could resurrect the sperm to generate live offspring! I was conducting studies on in vitro fertilization (IVF) along with Michael Summers so John suggested we try IVF. We kept the male on-ice for a few days while females could be set-up for egg collections. I then extracted the frozen cauda epididymis from the six-legged male and thawed it in modified Tyrodes media. To our amazement, there were actually motile sperm present, although very few. I then collected mature metaphase-II oocytes from CF1 females and attempted an IVF. Unfortunately, it was not successful. If this happened today, we would try this with ICSI using the methods recently perfected by Gloria Lee and Jie Liu, the current members of John Biggers and Mehmet Toner's research laboratory [27].

One amazing feature of these years that I must mention: All of the studies that we conducted in John's lab from 1997 to 2002 were accomplished in a laboratory that was constantly moving. It was decided by the Department of Cell Biology that John's lab should move to new space ... space that would be made available at some time in the distant future. In the meantime, we were forced to move out of his original labs in the old LHRRB building and sent into a semi-nomadic lifestyle. We moved the lab five times in 5 years but the science never slowed down. Over twenty publications resulted from John's lab while it was moving around Boston. The lab finally came to rest when we merged with the laboratories of Mehmet Toner within the Center for Engineering in Medicine, Massachusetts General Hospital that was physically located inside the Shriner's Burn Hospital for Children. From this location, Mehmet and John are continuing their shared research projects.

In 1997, we ventured into the field of embryonic stem cells when John was awarded an R41-R42 grant titled "Kit for Constructing ES Cell/Blastocyst Chimeras". The "awardee organization" was Cell and Molecular Technologies, Inc. The goal was to design an optimized culture media for the isolation and culture of embryonic stem (ES) cells. About this time, Liben Zhu joined the lab and worked day and night, 7 days a week

collecting blastocysts and isolating ES cell lines. I planned and prepared the media that we would test. Our idea was that ES cells originate from an embryo and since mammalian embryos grow best in simple defined media, we would use KSOM as the base medium for a new ES cell culture system. The media worked as we'd theorized it would. Liben had great success in isolating ES cell lines from C57/Bl6 so we decided to try a strain that no one had yet been able to isolate....FVB. With our media and Liben's daily care, we were able to isolate what was probably the first FVB ES cell line. In conjunction with Joel Lawitts at the Beth Israel Transgenic Laboratory, ES cells were injected into C57/Bl6 blastocysts or aggregated at the 8-cell stage and yielded live chimeric pups that were then crossbred to produce all white mice. Tail samples from these mice and the ES cells were sent to Jackson Laboratories in Bar Harbor for genetic testing thus proving the ES cells were pure FVB (Zhu, McGinnis, Lawitts and Biggers unpublished data). These ES cells were then sent to Cell and Molecular Technologies, Inc along with the final formulation of the culture media. With the sale of this company, I'm not sure what happened to these cells or the ES cell media formulations designed under John's guidance.

In 1999, John's research took a new turn when he was awarded a U01 titled "Young from stored and impaired mouse sperm" from NICHD. This grant was part of the NIH-NICHD Consortium on Mouse Sperm Cryopreservation, with other grants awarded to Ryuzo Yanagimachi at the University of Hawaii, Alan Trounson at Monash University and Larry E. Mobraaten at the Jackson Laboratories. Sub-awards within John's grant went to Mehmet Toner at Massachusetts General Hospital/Harvard Medical School and to Nejat Olgac at the University of Connecticut. In John's lab, we were studying the effect of mouse strain on in vitro fertilization with a purpose to determine if it was the egg or the sperm that made some strains notoriously difficult to fertilize in vitro. This was far more complicated than it seemed and the factors of environment and day-to-day variations in the mouse colony had huge effects on the outcome week-to-week. Perhaps the most exciting portion of this grant was the sperm desiccation studies with Sankha Bhowmick and Mehmet Toner. Methods for freeze-drying of human and bovine sperm were published in 1954–57 [28, 29] and many studies on freeze-drying have been conducted since then. Mehmet and John's idea was to preserve sperm by desiccation at room temperature preferably, with long-term storage at room temperature or simple refrigeration. Mehmet's research group had already proven success with desiccation of mammalian cells so the idea was to translate these methods for preservation of sperm cells [30]. Sankha developed a chamber in which we placed a glass slide and a small drop of sperm. The chamber was then connected to a nitrogen gas tank permitting the desiccation of sperm samples under controlled conditions of airflow and temperature. Mehmet's studies with

other cells including oocytes had shown the benefit of adding trehalose as a protectant. The base salt solution was provided by Yana ... the same base medium that he was using for freeze-drying of sperm. The result was amazing. Sperm dried to 0.3 % water can be stored at 4C for months, rehydrated with water, injected into eggs and produce live offspring [31–36].

I left the lab in 2005 to pursue a PhD degree in the laboratory of David Albertini. But the sperm and embryo studies are still ongoing in Mehmet and John's lab with a goal to improve the methods until survival of dessicated sperm stored at room temperature equals the viability from current methods of ultra-low temperature freezing and liquid nitrogen storage.

A quick search of PUBMED for John D. Biggers, finds 177 publications (as of August 2013). I'm sure there are many more articles, book chapters and symposia contributions that are missing from this list. At 90 years young, he has co-authored eight new publications in the past year.

To my colleague, mentor and friend John D. Biggers, I thank you for all the wonderful years and look forward to many more to come.

Romana Nowak, Ph.D.

University of Illinois – Urbana

Dear John,

I would like to send my heartiest congratulations to you on your 90th birthday! I look forward to celebrating it with you and other colleagues later this year. It has been a pleasure and honor to know you and work with you during my time in the Laboratory of Human Reproduction and Reproductive Biology. It is through you that I became excited about embryo development and implantation. It is through you that I learned to appreciate the beauty of confocal microscopy and imaging methods for embryos. I also learned so much from you about strategies in grant writing and the importance of standing up for oneself. You also demonstrate every day that being a scientist should not preclude one from participating in a broad range of intellectual and creative activities. I wish you many more years of enjoyment.

With very best wishes,

Romana Nowak

Greg Erbach, Ph.D

Dear John,

Congratulations on your 90th birthday. I might speak for many of your colleagues who find it remarkable you can look back on such a long and distinguished career, and even more remarkable you are yet looking *forward* to making even more contributions to your fields of interest. Your intellect, diligence and wide scholarship have been an inspiration to me. I especially relish our discussions

about the impact of our work on the world. And you're a fun travelling companion, too. I thank you again for your guidance in my postdoctoral work, for your example, and for your friendship.

Best wishes always,
Greg Erbach

A tribute to John Biggers: On The Politics of Memory

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The Bridge Center, London, UK

The title is taken from Raul Hilberg's beautiful autobiography, "The Politics of Memory" [37]. Raul Hilberg, a dedicated scholar, searched avidly for the truth. In the opening to his autobiography he quotes the poet and scholar H. G. Adler, "History without tragedy does not exist, and knowledge is better and more wholesome than ignorance".

Marlow in Joseph Conrad's *Heart of Darkness* comments, "It is impossible to convey the life sensation on any given epoch of one's existence, that which makes the truth, its meaning- its subtle and penetrating essence. It is impossible. We live, as we dream, alone" [38]. The tone is melancholic. The words were spoken in complete darkness; a metaphor. If the truth be known it is hard to know oneself, let alone another person. We have personal reflections and memories of events. It is to be hoped some are shared. I have chosen my words with care whilst reflecting on a very dear colleague. What are historians, if not journalists looking backwards?

John Dennis Biggers was born of a different time. He learned his craft in a different era. His life has spanned much of the twentieth century and to date all of the twenty first. He is one of those rare scientists who wish to understand nature. He is searching for truth; most look for explanations. It is of the fox and the hare. His journey began before the cacophony of din trumpeted by giant publicity machines present with enormous fanfare the smallest scientific discovery and medical advance. *Veritas vitae magistra* became *partum verum*. We are now bombarded by a massive opinion industry and to quote Schelling, "The entire age has become a committee."

In 1993 I was a Clinical Fellow in Reproductive Endocrinology and Infertility at the Brigham and Women's Hospital, Harvard Medical School in Boston. My arrival in Boston was not the result of deep deliberations, but a more typical English way of drift only to be justified much later by post-factum rationalisation. The clinical fellowship required completion of a research project. What to do about this matter? Intracytoplasmic sperm injection (ICSI) for the treatment of male factor infertility had recently been added to the assisted reproductive technology (ART) armamentarium. ICSI however was never studied in detail in animal model systems before its widespread use in human IVF. A few lone voices had expressed concern about

possible potential long term effects on children conceived through the use of ICSI, particularly as more and more extreme forms of male infertility were being treated with the technique. Alas, the train had already left the station. We now know that the ICSI procedure carries additional risks. I decided to set up an animal model system to study the ICSI procedure. It seemed to be good science at the time, but upon reflection a bad idea, even foolhardy. I was a fulltime clinician with very little protected time to undertake basic research. There were however interesting questions to pose about the ICSI procedure. What were the effects of bypassing all the natural barriers to normal fertilisation? Why did ICSI work? I had previously investigated micromanipulation and microinjection at the syncytial blastoderm stage of development of the fruit fly, *Drosophila melanogaster*. How difficult could ICSI be in an animal model system, such as the mouse oocyte? I had no idea of what I was getting into and discovered very quickly the challenges of mouse ICSI. It was only later following a report from Kimura and Yanagimachi's laboratory using a piezo-driven pipette did ICSI of the mouse oocyte become routine [39]. I was able to achieve low levels of normal fertilisation of mouse oocytes using ICSI, but the resulting 2PN zygotes failed to cleave. More discouraging was the fact that my control zygotes flushed from the oviducts of mated females also failed to cleave. A laboratory technician suggested I discuss the matter with "an English guy in the LHRB (The Laboratory of Human Reproduction and Biology) who knew something about embryo culture". I arranged to meet John in his office on the fourth floor of the LHRB.

The meeting with John went well. The office not surprisingly was piled high from floor to ceiling with books and papers. John's attire was typical of an English academic, not bespoke, more sprezzatura. John suggested IVF-generated mouse 2PN zygotes would be a better control for ICSI-generated embryos. He further suggested using KSOM for embryo culture. I promptly proceeded to modify KSOM after reading the somewhat limited literature on mouse IVF. I was able to achieve consistently high fertilisation rates >90–95 % with both inbred and F1 hybrid strains using KSOM supplemented with increased concentrations of glucose and bovine serum albumin (BSA). The presence of high glucose did not block the second cleavage division of IVF-generated mouse embryos. This was an interesting finding as the prevailing view overwhelmingly considered glucose inhibitory to early embryo development. It was certainly the view in clinical embryology. Sadly, our times favour dogma. The great satirist Jonathan Swift wrote in his "Thoughts on Various Subjects". When a true genius appears in the World, you may know him by this infallible Sign: that the Dunces are all in Confederacy against him." We soon showed it was possible to achieve complete pre implantation mouse embryo development following IVF of an outbred mouse strain with a single medium, modified KSOM, and live offspring after embryo transfer (Summers et al., 1995). This was most gratifying. John had previously asked the question, "Can a single medium be developed which

supports development throughout the pre implantation period?” [40]. The answer is an unequivocal, yes. I gave up the ICSI project and turned my attention to embryo culture media.

A few years later John was preparing to move his office. Much of the work fell to Lynda McGinnis who discovered during the sorting process prior to the move a number of 8×10 inch black and white photographs of John. The photographs date from the time of John’s successful collaboration with the late Anne McLaren during their time together in the Canine Block at the Royal Veterinary College, London. John previously wrote a short article outlining his recollections of this period of study with Anne McLaren [41]; it speaks of a grander time. I asked John if I might have one of the photographs which he graciously agreed to sign at my request. I, in turn, informed him of my intention to mount and frame the photograph in a manner similar to the signed and framed photograph of Walter Heape hanging in his office. John had a quizzical expression, paused in thought and then responded, “Yes, but Walter Heape is dead.” The signed photograph hangs in my office in London, alongside a framed frontispiece of an issue of *Human Reproduction Update*, which contains our review on embryo culture.

Parenthetically, John’s interest in the life of Walter Heape is perhaps of no surprise to those who have followed his work over the years. He previously wrote a highly readable article on the centenary of Heape’s embryo transfer experiments [42] and is currently completing a biography of his life and work. The Heape’s family home was in Prestwich, near Manchester. However, Walter Heape’s place of birth has been documented as both Prestwich and London. John asked me to research the matter as I had recently returned to London for family reasons. I was able to confirm Walter Heape was born on April 29th 1855 in Brompton in the County of Middlesex. John now has a facsimile of the original birth announcement from the Register of Births.

John, it seems to me, has in many ways always conducted his scientific studies from the perspective of an enthusiastic amateur. In this regard he is more like the naturalists of the Victorian era. It is hard to imagine many occasions when he left his office at Harvard carrying an attaché case overloaded with journals and papers to read. I did not know John as a young man. We met later. Perhaps he was even more impetuous and driven earlier in his career. His approach to research is thoughtful, deliberate and methodical. René Dubos’s book on the life and work of Thomas Avery comes to mind. Dubos quotes Avery as deprecating hurry and would recall “the words of an old black patient who watched, with amused surprise, the young doctors rushing about the wards of the John Hopkins Hospital: ‘What’s your hurry, Doc? By rushing that way, you passes by much more than you catches up with!’”. Avery was 67 years old when first reporting on deoxyribonucleic acid as the transforming factor of pneumococcus. From the book it is clear Avery realised the deep implications of his findings, but with typical modesty did not put it into print. It is however clearly noted in his letters. John was of a similar age when first applying sequential simplex optimisation

Fig. 14 Sperm Club members attending a meeting at the Jackson Laboratories, Bar Harbor, Maine. From left to right, Michael Summers, Lynda McGinnis, Monika Ward, David Whittingham, John Biggers

to the development of culture media [43]. Both men share many admirable qualities, such as patience, caution, humility, and relentless perseverance, along with a mellowed savagery and a quiet dignity that age and wisdom bring.

The importance of mentors cannot be over stated. I came from chemistry to biology and then medicine. I was attracted to chemistry because of precision and order, and was influenced by the writings and publications of Sir Robert Robinson, Lord Alexander Todd of Trumpington and Robert Burns Woodward. Interestingly, they were all Scotsmen. In the field of biology, I read John Biggers, Anne McLaren and Beatrice Mintz. Each author is possessed of a certain clarity and purpose.

John has published well-over 250 research papers, commentaries and reviews. The output is quite remarkable. He has never shown a tendency to be an empire builder. His interests cover reproductive physiology, embryo culture, cryobiology, sperm preservation, statistics and theory of experimental design, and the history and ethics of reproductive biology. When considering the body of work contributed by John, along with his work on behalf of the World Health Organisation (WHO) and the United Nations Educational, Scientific and Cultural Organisation (UNESCO) it is surprising that he has not received wider recognition in his county of birth. Perhaps this is a discussion for another time. For now, it is a pleasure to have shared with John a common interest. I have benefited enormously for having met and collaborated with John Biggers during the past 20 years. I am sure many of his colleagues feel the same way.

For John Biggers

Monika Ward, Ph.D.
University of Hawaii

When I was a PhD student working at the Institute for Human Genetics in Poland, I was performing so called ‘hamster test’

Fig. 15 Gathering of John's colleagues and friends at the 2011 SSR meeting in Portland, Oregon

constantly to investigate function of sperm from men with various spermiogenic defects. The culture media I was using in this assay was the BWW medium, or Biggers-Whitten-Whittingham medium. Never did I anticipate in these days that I would meet any of the famous scientists whose last names provided the acronym for this medium. And yet, it turns out that I have met them all, talked to them all, worked closely with one and collaborated with another!

I was introduced to John during the first meeting of scientists involved in the NICHD funded 'Sperm Club' consortium grant, held in summer of 2000 at the Harvard Medical School, Boston. I was a member of the Hawaii team co-lead by Ryuzo Yanagimachi and David Whittingham, and John was a leader of the Harvard team and hosted this meeting. I was a fresh post-doc then, and quite overwhelmed by those surrounding me. I remember vivid discussions on a variety of issues pertaining to the project, with John, not surprisingly, directing those related to embryo culture and his famous KSOM. I also recall my observation that when we all moved to the cafeteria for lunch, still actively (read: loudly) engaged in discussions on the subject, which by now was mouse electro-ejaculation, that other cafeteria attendees were conspicuously moving away from our vibrant group with strange looks on their faces (Fig. 14).

After this first meeting at Boston I had the privilege of seeing John at least every year during our formal grant-related gatherings. At each of these meetings I remember John from two angles. One, not surprisingly, was his extensive knowledge in the arena of embryo culture and beyond. The other, was his adventurous spirit. As far as I can tell, and I am sure I was not privy to many of his whereabouts, he always took advantage of being in a different place and was off to explore. After our 2000 meeting in Australia John went to Tasmania, and I heard some stories later on about wild rafting in caves. When we met at the Jackson Lab in 2001 we were all

hiking in the Acadia mountains (Fig. 15). I also heard stories, and have seen photos, from other adventurous trips of John, including a relatively recent one to Antarctica. Scientifically, I have always enjoyed interacting with John and discussing projects. He was very focused on experimental design and statistical analyses, but also willing to offer advice on essentially anything else. We have one joint publication for which John performed the statistical analyses so complex that it still sends shivers down my spine.

I will conclude by saying that John has always been a scientific hero to me. And upon meeting him in person, this feeling was only reinforced by discovering that he is not only a great scientist but also a wonderful human being. Happy Birthday John!

Monika Ward

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