BIOGRAPHICAL MEMOIRS

George E. Seidel, Jr.

July 13, 1943–September 4, 2021
Elected to the NAS, 1992

A Biographical Memoir by
R. Michael Roberts and Jennifer Barfield

George E. Seidel, Jr., an exceedingly modest man, was many things to many people. He was renowned internationally for his scientific contributions to reproductive biology and animal agriculture and as being a kind friend, respected counsellor and a generous, giving colleague who always had an open door and open ear. He was a cattle rancher, a no-nonsense conservationist who cared deeply about the land and its use, and a boss with a relentless work ethic both in relation to his academic activities in teaching and research and to his beef cattle operation. To Sarah, his life and work partner, he was a husband of more than fifty years and father to son Andrew and Andrew's wife Yuan and grandfather to Gregory, and since his passing, Michael. Although at the time of his death, George and Sarah owned more than five hundred breeding beef cattle on their ranch just north of Fort Collins, George was most proud of his birthright as a dairyman, which influenced his approach to life and set the stage for many of his most highly regarded scientific contributions.

Childhood

George was born in 1943 in Virginville, Berks County, Pennsylvania, a small borough approximately sixty miles northwest of Philadelphia. His father, both grandfathers, and four uncles were farmers with diversified crops and animals, but the dominant enterprise was dairy cattle. He was the oldest of four children and, typical of being raised in that environment, was expected to contribute to running the farm at an early age. In George's own words, "I was essentially functioning as an adult by about age 12."[1] Vacations were occasional and brief, and the main social life outside the home revolved around schools, churches, and typical rural institutions, such as 4-H Clubs, county fairs, and farm cooperatives. Seasons dictated farm activities, although animals required daily feed and care, and cows required milking twice daily. His was not an austere lifestyle but it was disciplined and honed responsibility.

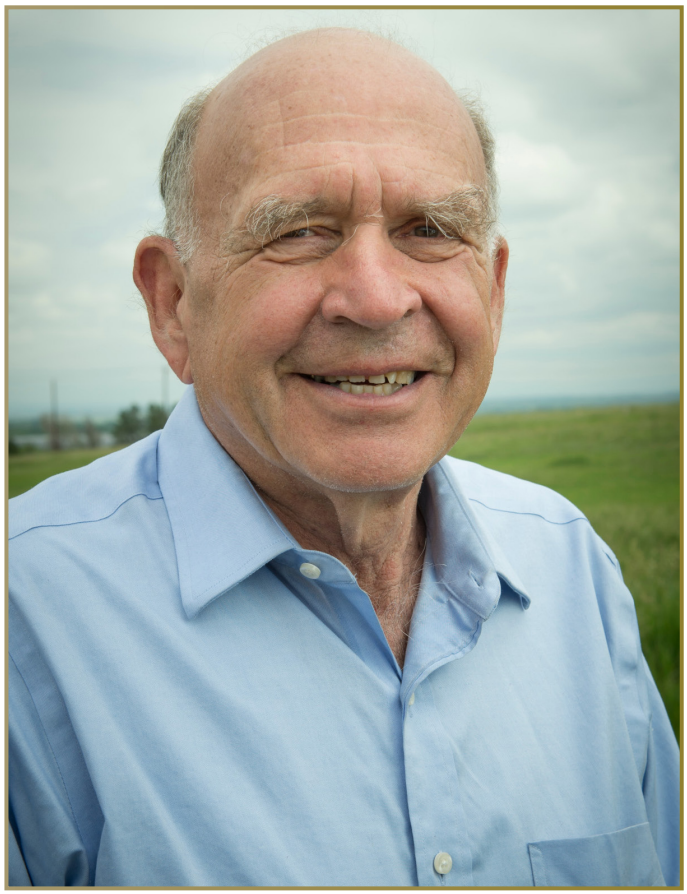

Figure 1 George Seidel at work.

Education and Professional Career

George's early education also reflected much of rural America in the mid-twentieth century. He attended a

one-room school for eight grades located on land leased from his grandmother's farm. The school had no electricity or plumbing, and the teacher brought drinking water in a bucket with a spigot for the students daily. George kept the collapsible drinking cup he used during these years for his entire life. There was one outhouse for boys and one for girls. The building was heated by coal, which, on cold days, was carried from the basement by older students each morning to start a fire in a large round stove.

High school, where George also excelled, was in Fleetwood about five miles from a sharecropped farm on which his family worked. The education he received there provided him the opportunity to be the first in his family to attend college, although his three younger siblings subsequently obtained college degrees as well. George's undergraduate major at Pennsylvania State University was dairy cattle production. Then, in George's words, "I learned that they would pay you to get a masters, I thought that was a heck of a deal. It was a slippery slope from there." As part of this slippery slope, George entered Cornell University and earned a master's degree in 1968 and a Ph.D. in 1970 under the mentorship of Robert "Bob" H. Foote, a talented, hard-working reproductive physiologist with a bubbling enthusiasm for research. After Cornell, George completed a postdoctoral stint at Harvard University in the Department of Anatomy under Donald W. Fawcett, whose laboratory was a world-famous center of excellence in both the art and technology of electron microscopy and whose primary focus was the microanatomy of spermatozoa and the male reproductive system.

In 1971, George was hired as an assistant professor at Colorado State University (CSU) to work on reproduction in bulls and stallions. His hiring, according to George, was based primarily on his master's degree research on bull fertility, but it was his Ph.D. work on superovulation of calves, with the long-term objective of amplifying female reproduction, that launched his career and established his reputation. After several years at CSU, this female-orientated research area evolved to place a greater emphasis on embryo culture, embryo cryopreservation, embryo transfer, and embryo splitting in cattle, topics that we'll expand upon later.

George's master's research on male reproduction also primed the second prong of his career as an academic investigator, namely amplifying male reproduction. His group made major contributions to maximizing sperm production in dairy bulls and improving efficiency of artificial insemination. Beginning in the mid-1990s, his and associated laboratories at CSU launched a highly successful effort to advance sperm sexing and deliver sexed sperm to breeders.

George spent forty years as a regular faculty member at CSU housed in what is now a world-renowned facility, recently renamed the Gordon D. Niswender Animal Reproduction and Biotechnology Laboratory (ARBL).[2] This unit, which began as an artificial breeding service based out of the Department of Physiology and Biophysics in the College of Veterinary Medicine, evolved into a center of research and scholarly excellence on the Foothills Campus of CSU over the course of George's career. George helped form this Program of Research and Scholarly Excellence at CSU, which by nature avoids segregation of graduate student training within departments through housing faculty and students studying animal sciences, equine sciences, clinical sciences, and biomedical sciences in the same facility. ARBL was his research base from the time he was hired as an assistant professor in 1971 to the end of his career. Even though he transitioned to emeritus status in 2011, George continued to collaborate on research projects, write papers, give lectures, and attend meetings until the time of his death. He and Sarah also continued to run their cattle operation.

Research Philosophy

From this short introduction to his career, it must be evident to the reader that George's research ranged from quite basic to very applied. He was the first to admit that many of the techniques for which his laboratory became celebrated were first described by others. His success was in reducing these technologies to practice by simplification and replication and publishing results that people trusted. An example is the commercial development of sexed cattle semen first described for rabbits by Larry Johnson with the USDA but later made practical by the Seidel group for cattle.[3] In George's own words, "we wanted to make it work, take it from a laboratory curiosity to something that worked; that became a goal." And they succeeded.

As George has also stated about his research philosophy: "Although I am driven by broad goals of being a good academician, and sometimes by rather specific goals, I function more like an opportunist. I tend to evaluate the resources around me and try to put them to productive use." This entrepreneurial spirit, which seemed built into George's psyche, was encouraged from the very start of his career by the director of the Animal Reproduction Laboratory at the time, B. W. "Bill" Pickett. In addition to federal grants and some state funds through the university, about half the income needed to support the laboratory was generated by customer services, which included collection and cryopreservation of semen from bulls, and, later, embryo collection and transfer in cattle and horses. Fortunately, this extensive commitment to service activities also provided some distinct advantages, as it allowed many sorts of experiments, with admirable statistical power and low cost, to be superimposed on the service component. Essentially all animals used by the Seidel laboratory, whether from CSU, local breeders, or his own ranch,

were on some kind of experiment, although, for the more basic research, clients' animals or embryos were generally not used. George was adamant about the teaching mission of the university. Thus, although customer services were offered, the approaches used were generally considered experimental until the protocols had been simplified and proven repeatable. Training students was the priority. In George's view, a sign of an effective educator was "teaching yourself out of a job," and indeed, when a critical mass of skilled practitioners had been produced, rather than competing with them, George and ARBL began referring clients to these practitioners while continuing to offer services for more complicated infertility cases.

It is revealing to examine the two, ostensibly surprising, choices of sabbatical leave that George took during his academic career, namely 1978–1979 with Clement L. Markert in the Biology Department at Yale University and 1988–1989 with Rudolph Jaenisch at the Whitehead Institute at the Massachusetts Institute of Technology. Like his postdoctoral pick of Don Fawcett, these sabbatical leaves reflected very much how George accurately surmised the direction in which the science was moving. Neither leave was productive in terms of publications, but each was prescient and intellectually satisfying. Markert was a biochemical geneticist whose laboratory had recently turned its attention to studying embryo development and specifically was attempting to produce homozygous offspring by removing the first pronucleus from fertilized mouse oocytes and suppressing the second meiotic division. In theory, this strategy would have provided a diploid embryo and was, of course, an early attempt at cloning, concepts that must have fascinated George. Although this approach failed, in retrospect because the mouse embryos lacked the differentially imprinted loci that distinguish maternal and paternal genomes and allow complete development to occur, it was a pioneering step towards the concepts of nuclear reprogramming and cloning. George later attempted similar experiments with bovine zygotes at CSU, although these too failed for the same reasons.

George's second leave, with Jaenisch, a developmental biologist who like Markert was pursuing big ideas, was no doubt selected with a view to the long term because of his laboratory's interests in reprogramming, transgenesis, embryonic stems cells, and mechanisms of gene regulation, all topics that clearly could be applied to research on bovine embryos. Recall that this was almost a decade before the birth of Dolly the sheep in 1997 and when cloning mammals was still considered an improbable dream by the dominant band of embryologists who favored the mouse as its model.[4] It's fair to say, however, that the agricultural community was ahead of the game, decidedly less pessimistic, and already achieving some success though embryo splitting and nuclear transfer.

Main Scientific Contributions

George's work in superovulation was among his most significant contributions to the field. In cattle, as in humans, one egg (ovum) is normally released per ovarian cycle for possible fertilization in the oviduct. The process of inducing a female to release more than one egg by pharmaceutical means is commonly called superovulation (or more properly ovarian hyperstimulation) and is necessary to generate the large number of embryos needed for embryo transfer. George's Ph.D. research at Cornell concerned superovulating prepubertal calves, often not much more than two months of age, that can produce dozens of oocytes, although the recovery is highly variable. By combining this approach with in vitro fertilization and embryo transfer to a sexually mature heifer or cow, a young calf can become a genetic mother a year or more earlier than if she had been allowed to mature and be bred at around two years, as is customary.[5] George understood, however, that the standard protocols for inducing superovulation in cattle at the time incorporated the use of equine chorionic gonadotropin (PMSG), which had a number of undesirable side effects, including continual recruitment of new follicles, and which caused a damaging, non-physiological rise in progesterone. As an alternative, the Seidel group decided to use natural follicle-stimulating hormone (FSH), which has a shorter half-life than PMSG and is much less luteotrophic: its ability to stimulate the corpus luteum to produce progesterone is much less than PMSG.[6] The protocol was shown to work best with multiple injections, usually given at half-day intervals for three or four days and usually resulting in release of between five and ten viable eggs, which can be collected from the oviduct by using a non-surgical procedure, also developed by the Seidel laboratory, at the time of estrus and fertilized in vitro.[7] A somewhat similar approach can be used to generate ova from horses. This work is a good example of the Seidel group's ability to codify and reduce to practice a then-emerging technology suitable for on-farm embryo transfer programs.

Under George's guidance, the reproduction laboratory, also became famous for its studies on bisection of embryos to produce identical twins from blastocyst-stage embryos, thereby increasing production of particularly valuable calves.[8] As with superovulation, the group was not the first to exploit this procedure but was able to refine it and make it more efficient. Over the course of only a few years, George and colleagues produced hundreds of twin cattle, sheep, and horses following embryo bisection. These embryos are transferred singly to surrogate females, which can be less prized animals than the genetic mother and instead chosen on the basis of their likelihood of carrying a successful pregnancy to term. Fortunately, offspring from embryo splitting appear to be completely normal both at birth and as adults. The

technology, however, is not entirely simple. It requires considerable expertise, especially in the art of selecting the right embryos for successful transfer that have sufficiently normal morphology and enough cells to trigger maternal responses in the surrogate. Remarkably, with a skilled practitioner, pregnancy rates of around 70 percent can be achieved when high-quality bisected embryos are transferred, a value comparable to that with non-manipulated controls. Even those embryos judged to display an overall "fair" or even "poor" morphology provide pregnancies in the 40 to 50 percent and 15 to 20 percent range, respectively.[9] It should also be noted that it is possible to extend these procedures to produce identical triplets and occasionally quadruplets, but with lowered success rates.

George's work in sexed semen must also be included in any discussion of his contributions to the science. The goal of George's master's research in the Foote laboratory at Cornell was aimed at improving the quality and quantity of bull semen for artificial insemination (or, as he put it, optimizing andrology). Although his laboratory at CSU became renowned for developing reproductive technologies performed on oocytes and early embryos, George returned to andrology in the mid-1990s in a collaborative effort with colleagues in the ARBL and especially with Duane Garner, a pioneer in flow cytometry of sperm, to produce commercial quantities of sexed semen suitable for artificial insemination.[10] Although the typical dose of semen used for inseminating a cow or heifer contains between 10 and 40 million sperm, George suspected that pregnancy rates might not be compromised by using much lower sperm numbers, namely ones that might practically be achieved after separating X- and Y-bearing sperm by flow cytometry and cell sorting. This technology has as its basis the greater (~3.9 percent) content of DNA of X-sperm relative to Y-sperm and depends upon the use of the DNA-specific fluorochrome Hoechst 33342 to distinguish the two kinds of gamete by their relative fluorescent intensities. At the time, this technology was thought only suitable for applications to in vitro fertilization because so few sperm could be separated by sex per unit time. Larry Johnson's landmark discovery that rabbit sperm could be sorted according to DNA content, inseminated into females, and used to provide either male or female-based litters, suggested that the technology might also be successful in cattle. Once more, George and his associates were able to take this basic technology and make it work to provide highly enriched (~85 percent) sperm populations. It also led to the founding of a company, XY Inc., in 1996 (acquired by STgenetics in 2007), with Duane Garner as vice president for research and development. The research establishing the technology required large amounts of private funding, the development of high-speed cell sorters, and engaging farmers and ranchers in numerous field trials using thousands of cattle. It resulted in multiple U.S. patents and many papers in refereed journals. The technology has caused a marked shift in how U.S. dairy cows are bred. Females with the most valuable genetics for producing milk can be bred with X-enriched semen from prize bulls of established fertility to produce heifer replacements for animals cycled out of the herd. The remaining females can, if desired, be inseminated with semen of beef breeds, resulting in calves that will mature into better beef animals than could be provided by purebred dairy calves.

George was a prolific writer. In addition to more than 220 refereed papers, he edited or wrote eight books and published numerous reports, bulletins, and chapters in texts, proceedings, and monographs (we counted some 200). In addition, he penned around eighty "popular" articles for interested non-specialists. George wrote simply and with considerable clarity and, where permitted, with humor.

Ranching

George claimed "he never worked a day in his life" but, in addition to having a full career in academia, he and his wife Sarah were ranchers; Sarah and his son Andrew continue to ranch today. Prior to purchasing his own ranch, George invested in Phantom Canyon Ranches, an enterprise of four contiguous ranches about twenty miles north of Fort Collins as part of an effort to prevent development of the area. George was passionate about preserving large working landscapes. When Phantom Canyon Ranches began to go under, investors had the opportunity to cash out their holdings in land, which is what George did. With those funds and some help from Sarah's father, George and Sarah bought their first ranch, Rabbit Creek, in 1989. Thus began their adventures in ranching, which among other things, were entertainingly detailed in their annual Christmas letter. It was a beef cattle operation, and George was exceedingly proud of it. As many students, colleagues, and scientists, including one of the authors, who visited CSU can attest, a tour of the ranch in one of George's many mechanically suspect vehicles was typically on the itinerary, and almost always a highlight.

Over the years George and Sarah acquired additional ranches, including Waterfall, Koch Pond, Eagle Rock, and in the year before he passed, the Hansen piece. These acquisitions were largely possible because of George investing all his earnings into the ranches. He was able to do this thanks to his parsimonious nature and, perhaps more so, his inability to resist an opportunity. What began with approximately 150 cattle, grew to an annual stocking rate of 350 cow/calf pairs, 8–10 herd bulls, 50 replacement heifers, and 20–30 sale bulls; today the ranch manages just over 800 cattle. The use of his cattle for research began within a few years of purchasing the Rabbit Creek land and is an aspect of his ranch

Figure 2 Seidel cattle drive.

management that continued and expanded until his passing. He also provided access to the ranch to students and colleagues across the university for a variety of educational experiences, from range management classes to training veterinarians and graduate students in a variety of reproductive techniques and protocols. For many, his ranches provided a truly Western experience that included spring calf branding, barn parties, and days-long cattle drives on horseback to public lands at the beginning of the warm months and back again in early fall (Figure 2). These spectacles were complete with cowboy campouts, though today some modern conveniences have been incorporated.

George's love of science, cattle, and the land came together on his ranches in a unique way that represents so much of what was important to him. It should come as no surprise that George was laid to rest in a small fenced cemetery on Rabbit Creek Ranch, with his head and feet in the opposite direction as the other residents, so that he can continue to oversee the haying operations.

Patents and Commercialization of Discoveries

George admitted that he was "never turned on by getting a patent," but he acknowledged that it was important to protect intellectual property and to develop and license it. In all, around thirty patents were filed by XY, but these included many that were foreign and others that were for minor advances that were valuable from the business rather than from a science perspective. The ones pivotal to sex-sorting semen, the so-called foundational patents, include the data showing that you could impregnate cows with many fewer sperm than were used conventionally.[11,12] This technology was the basis for the founding of XY Inc., in which George played a pivotal role and which was originally owned entirely by the CSU Research Foundation—in an entirely new experience for them. It was necessary for someone (George) to negotiate research space from the university, convince investors to buy shares in the company, acquire the necessary equipment (especially high-speed flow cytometers), and ensure that all was done in accordance with the regulations of the university and the State of Colorado. No simple task! George's official role was as principal investigator on the university contract, but he also had the unpaid title of scientific advisor to the company, which allowed him to provide input at board meetings. No CSU personnel, including George, had any financial interest in this enterprise, which now has sales exceeding $100 million and revolutionized the dairy industry in the United States. Royalties received by George from these technologies were substantial and were given back to CSU to provide the ARBL bridge funds for faculty researchers who had previous federal funding and were renewing grant funds. Funds also were used for purchase of large shared equipment to support ARBL research.

International Embryo Technology Society

The International Embryo Technology Society (IETS) began in 1974 as a group of international practicing veterinarians and commercial entities under the name International Embryo Transfer Society. In 1977, George, a decidedly non-veterinarian, was invited to be a (half!) member. At the time, IETS was struggling financially and essentially bankrupt. Bob Gary, who ran an association management company, brought the organization's records to George and Sarah, and in October 1977 they decided to arrange an annual conference managed by the two of them. For the first few years, they handled all aspects of the conference, including catering, which initially was done by ordering plates of food from Safeway and in one instance even keeping food on ice in the bathtub of their hotel room! The first mailings were paid for personally, and neither George nor Sarah took a salary for the first years, with Sarah acting as George's pro bono assistant. Sarah wrote all of the newsletters, including a short digest of research highlights summarized from reprints selected by George. By 1989, IETS was handed over to a professional management company that took over arranging the annual meeting, handling finances, membership lists, and newsletters, and forging a new partnership with the journal *Theriogenology*. Appropriately, Sarah was awarded the Distinguished Service Award from IETS in 2004. George was awarded the Pioneer Award, the highest honor bestowed by the society, in 2008. And after his death, IETS renamed its annual keynote address the George E. Seidel Jr. Keynote Lecture.

Mentoring and Training

During his long career, George was the main advisor or co-advisor to nineteen Ph.D. and forty master's degree trainees, the majority of whom have gone on to fulfilling careers, several in academia but a majority along other tracks.

A surprising number became directors of human IVF laboratories. Many are practicing (or now retired) veterinarians and others have remained involved in the embryo transfer business. All those contacted feel a great debt to the training they received in the Seidel laboratory.

Realizing the growing need for formally trained, skilled personnel as embryologists in both human medicine and animal agriculture, George was also instrumental in developing a one-year, non-thesis specialization for master's students in assisted reproductive technologies (ART) in the College of Veterinary Medicine. The program, which continues, culminates in writing a scholarly paper based on a research project or reproductive topic and the completion of a hands-on internship. It provides students with in-depth laboratory training in in vitro embryo production, largely based on the bovine model, that includes techniques such as in vitro fertilization, embryo and semen cryopreservation and vitrification, embryo biopsy and micromanipulation, and basic maintenance of an assisted reproduction laboratory. At the time, this was a unique offering and remains highly popular and competitive. Its founding again illustrates George's passion for making science useful and accessible to an extended trainee base and satisfying a tangible societal need.

Awards, Fellowships, and Academic Honors

George received considerable recognition for his contributions to animal agriculture. Among his many awards and honors are: the Alexander von Humboldt Award (1983); National Association of Animal Breeders Research Award (1985); Upjohn Physiology Award, American Dairy Science Association (1986); Governor's Award for Science and Technology, State of Colorado (1990); Distinguished Service Award, International Embryo Technology Society (2008); and Animal Physiology and Endocrinology Award, American Society of Animal Science (2008).

George was elected to the National Academy of Sciences and elected a fellow of the National Academy of Inventors. In 2013, he was named an honorary professor at Shandong Agricultural University, Tai-an City, China. In 2020, he received the L. E. Casida Award for outstanding graduate training from the American Society of Animal Sciences.

Final Words

George E. Seidel Jr. died in the early morning hours of September 4, 2021, in Fort Collins, Colorado. George's legacy cannot be easily summarized. His science has left a lasting impact, much of which continues to be foundational to advancements in the reproductive sciences. His legacy is in the land, the hundreds of acres that remain open and under conservation easements, because he valued their preservation and the ranching way of life. He leaves a generation of scientists, practitioners, veterinarians, and medical professionals who were fortunate enough to learn lessons with him that made them better as they forged their own paths in the sciences and beyond. For his family and friends, there is a lifetime of stories to recount and moments to remember that will make us laugh, and sometimes cry, as we miss him deeply. We will be telling those stories throughout our own lifetimes! And while George was recognized during his life with many prestigious awards, there is no doubt that if he could read this memoir, he would certainly cringe at having his accomplishments and person lauded in this way, because above all he was as humble and genuine as they come. But he deserves it and it's the least we can do for him, given all he did for so many of us. As Newton said, we are "standing on the shoulders of giants." George is one of those giants on whom many will stand now and for generations to come.

References

1 Seidel, G. E., Jr. 2022. Translating basic research to animal agriculture. *Annu. Rev. Anim. Biosci.* 10:1–15.

2 Pickett, B. W., et al. 2012. *Sex, Science and Survival in Academe: A History of the Animal Reproduction and Biotechnology Laboratory.* Fort Collins: Colorado State University, Animal Reproduction and Biotechnology.

3 Johnson, L. A. 1995. Sex preselection by flow cytometric separation of X and Y chromosome-bearing sperm based on DNA difference: A review. *Reprod. Fertil. Dev.* 7(4):893–903.

4 Wilmut, I., et al. 1997. Viable offspring derived from fetal and adult mammalian cells. *Nature* 385(6619):810–813.

5 Seidel, G. E., Jr., L. L. Larson, and R. H. Foote. 1971. Effects of age and gonadotropin treatment on superovulation in the calf. *J. Anim. Sci.* 33(3):617–622.

6 Seidel, G. E., Jr. 1981. Superovulation and embryo transfer in cattle. *Science* 211(4480):351–358.

7 Bowen, J. M., R. P. Elsden, and G. E. Seidel Jr. 1978. Non-surgical embryo transfer in the cow. *Theriogenology* 10(1):89–95.

8 Williams, T. J., R. P. Elsden, and G. E. Seidel Jr. 1984. Pregnancy rates with bisected bovine embryos. *Theriogenology* 22(5):521–531.

9 Williams, T. J., R. P. Elsden, and G. E. Seidel Jr. 1984.

10 Garner, D. L., and G. E. Seidel Jr. 2008. History of commercializing sexed semen for cattle. *Theriogenology* 69(7):886–895.

11 U. S. Patent 6,071,689 B; System for improving yield of sexed embryos in animals; https://patentdocs.typepad.com/files/trawnsova_ipr2018-00249_petition.pdf.

12 U. S. Patent6,149,867 B; Sheath fluids and collection systems for sex-specific cytometer sorting of sperm; https://patentimages.storage.googleapis.com/30/52/1f/cd645d027321b0/US6149867.pdf.

Selected Bibiliography

1978 With R. P. Elsden and L. D. Nelson. Superovulating cows with follicle stimulating hormone and pregnant mare's serum gonadotrophin. *Theriogenology* 9:17–26.

1981 Superovulation and embryo transfer in cattle. *Science* 211: 351–358.

1999 With J. L. Schenk, L. A. Herickhoff, and S. P. Doyle. Insemination of heifers with sexed sperm. *Theriogenology* 52:1407–1420.

With J. L. Schenk, T. K. Suh, and D. G. Cran. Cryopreservation of flow-sorted bovine spermatozoa. *Theriogenology* 52:1375–1391.

2000 With S. E. Olson. Culture of in vitro-produced bovine embryos with vitamin E improves development in vitro and after transfer to recipients. *Biol. Reprod.* 62:248–252.

2002 With D. L. Garner. Current status of sexing mammalian spermatozoa. *Reproduction* 124:733-743.

With J. B. Cibelli et al. The health profile of cloned animals. *Nat. Biotechnol.* 20:13–14.

2003 Economics of selecting for sex: The most important genetic trait. *Theriogenology* 59:585–598.

2008 With P. Mazur and S. P. Leibo. Cryopreservation of the germplasm of animals used in biological and medical research: Importance, impact, status, and future directions. *Biol. Reprod.* 78:2–12.

With D. L. Garner. History of commercializing sexed semen for cattle. *Theriogenology* 69:886–895.

2009 With J. L. Schenk, D. G. Cran, and R. W. Everett. Pregnancy rates in heifers and cows with cryopreserved sexed sperm: Effects of sperm numbers per inseminate, sorting pressure and sperm storage before sorting. *Theriogenology* 71:717–728.