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Reproduction, Fertility and Development

Recipient of the 2022 IETS Pioneer Award: Dr Carol Keefer

Dr Carol L. Keefer, a native of South Carolina, began her scientific career studying Biology at the University of South Carolina where she graduated Magna cum laude in 1974. Her first research experience was working as an undergraduate with Dr Wally Dawson in the Peromyscus mouse colony (later to become the Peromyscus Genetic Stock Center) doing vasectomies and artificial insemination in deer mice while studying sympatric speciation. That initial experience with reproductive biology led Dr Keefer to pursue graduate studies in developmental biology with the distinguished Dr Dick Tasca at the University of Delaware, where she earned a PhD in Biological Sciences in 1981. Dr Keefer revealed that her alternate plan was to study evolutionary genetics, but she was listed as only an alternate for a stipend at Stanford and so chose to study amino acid transport in preimplantation mouse embryos for her PhD dissertation work (Keefer and Tasca 1984). We owe Stanford a debt of gratitude for unknowingly steering Dr Keefer along her path of exemplary contributions to the field of Reproductive Biology! Indeed, Dr Keefer has been a true pioneer in the areas of sperm injection, embryo and somatic cell nuclear transfer, transgenesis, and stem cell research throughout her illustrious career from the 1980s until today. Her contributions have been reflected in over 65 peer reviewed research publications and three book chapters with more than 2400 citations. She has been an invited speaker at more than 40 national and international events and holds two US patents for her novel work in reproductive technologies. Her experience is unique in that she has excelled in clinical, industry, and academic settings, giving her a unique perspective that allows her to think creatively about the challenges facing reproductive biology and assisted reproductive technologies in our current environment.

After her graduate work, Dr Keefer conducted post-doctoral research at Johns Hopkins, and the University of Pennsylvania. The publication derived from her research at John Hopkins on spontaneous oocyte activation in rats (Keefer and Schuetz 1982) provided a crucial key to the subsequent successful cloning of rats by others. She then joined the University of Georgia first as an Assistant Physiologist and shortly thereafter as Assistant Professor in the College of Veterinary Medicine. During this time, she was also involved in establishing one of the first human IVF clinics in the US, Reproductive Biology Associates in Atlanta, Georgia. One of the most impactful findings that Dr Keefer published in the early period of establishing her independent research program was that viable embryos and pregnancies could be obtained following direct microinjection of dead sperm into rabbit oocytes (Keefer *et al.* 1985, 1988; Perreault *et al.* 1988; Keefer 1989). This work paved the way for new methods of sperm storage, and rescue of sperm and genetics from males from whom viable sperm could not be collected. Dr Keefer's program was successfully funded by NIH at the University of Georgia, and was a productive period in her career. However, Carol was attracted to an opportunity in industry with American Breeder's Service (ABS) in 1989 that allowed her to use her embryo manipulation experience to advance research in bovine embryo cloning towards commercial application for genetic improvement. During her time at ABS, Dr Keefer made several crucial advances in embryo cloning technology that led to widespread adoption of the technique in both industry and academia (Keefer *et al.* 1993, 1994; Stice and Keefer 1993; Stice *et al.* 1994, 1996). It became clear through the success of Carol's work that the real value in nuclear transfer may be not in genetic advancement, but in biopharming, or the production of transgenic

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animals for biopharmaceutical production of human medicine. Thus, in 1995 Dr Keefer was recruited to Nexia Biotechnologies in Quebec, Canada. There she led a team in producing transgenic goats via nuclear transfer with transfected donor cells, which secreted recombinant spider silk protein and recombinant human butylcholinesterase in their milk (Gauthier *et al.* 2001; Keefer *et al.* 2001, 2002; Baldassarre *et al.* 2002). At Nexia Dr Keefer served as industry liaison for federal and provincial grants of nearly CAD$1 million to develop technologies supporting the transgenic goat production system. During her time in industry Carol remained an active collaborator with academia, serving as Adjunct Professor in Animal, Dairy, and Veterinary Sciences at Clemson University, and Animal Science at McGill University.

In 2003, Dr Keefer returned to academia with tenure at the University of Maryland, where she remains today as Professor of Animal Sciences within their Biotechnology Initiative. Dr Keefer's research program at Maryland has been continually funded by the USDA and NSF as well as private foundations and competitive internal grants. At Maryland, Carol made a strategic shift in focus to study pluripotent cells, including embryonic stem cells (ESC) in ruminants and mice, feline spermatogonial stem cells, and human teratocarcinoma cells (Keefer *et al.* 2007). Her laboratory was the first to describe induction of trophectoderm lineage differentiation by cytokines in mouse ESC (He *et al.* 2008), demonstrating that ESC could differentiate into both embryonic and placental lineages. Her laboratory also described NANOG expression and unique protein localisation potentially involved in cell differentiation in goat embryos (He *et al.* 2006), as well as control of NANOG expression by the cytokine Noggin in goat embryo derived cell lines (Pant and Keefer 2009). Dr Keefer has established strong collaborative relationships to study other characteristics of stem cells as well, such as measuring the stiffness of stem cells during differentiation (Keefer and Desai 2011; Pillarisetti *et al.* 2011; Ladjal *et al.* 2012). Dr Keefer has also returned to her research roots in some of her recent work, developing exciting new methods for studying metabolism in preimplantation embryos and sperm using metabolomics and fluxomics technologies (Chung *et al.* 2019; Weiner *et al.* 2019).

Dr Keefer also maintains strong collaborative ties with investigators at the Smithsonian Conservation Biology Institute (SCBI). Dr Keefer led efforts to establish conditions for culture of feline spermatogonial stem cells as a means to preserve the genetics of rare and endangered felids (Vansandt *et al.* 2012; Vansandt *et al.* 2016). Dr Keefer has worked with this group on a number of reproductive technologies including embryo culture, estrous cycle synchronisation, reproductive behaviors, sperm quality, embryonic and induced pluripotent stem cells, and *in vitro* follicle culture in a variety of species from cats to cranes over the years (Nestle *et al.* 2012; Collins *et al.* 2014; Fujihara *et al.* 2014; Brown *et al.* 2016, 2017, 2018, 2019; Thuwanut *et al.* 2017; Weiner *et al.* 2019; Zhou *et al.* 2019*a*, 2019*b*).

In addition to maintaining a dynamic and productive research program, Dr Keefer is a vibrant and engaging teacher and mentor. She has developed two new courses for the Animal Science curriculum at Maryland, Experimental Embryology and Animal Biotechnology, in addition to guest lecturing in Physiology of Reproduction, and guiding students in Experiential Learning and Special Problems courses. Over the course of her career, Dr Keefer has mentored many students as a member of their graduate committees and advised/co-advised six MS and seven PhD students, and three post-doctoral trainees, as well as serving as the graduate director of the Animal Sciences program for the past 10 years. Dr Keefer's students have won multiple awards at the university, national, and international levels, reflecting her outstanding guidance. Her influence as a mentor has followed her graduate students into careers in academia, basic research, and conservation.

Finally, the respect and admiration of her colleagues is shown by her election to service in her scientific societies. Of specific interest is her election to President of IETS in 2003, the first woman ever to hold this office. Dr Keefer served on the IETS Board of Governors from 1999 to 2005. She has also chaired the Domestic Animal Biomedical Embryology (DABE) committee of IETS and organised the DABE Symposium in 2015, and served IETS as Program Chair for the Kyoto meeting in 2007. Carol has also been active in IETS on the Education Committee and as the chair of many sessions at our annual conference over the years. She is also an active member of SSR, serving that society on the Nominations and Program Committees. Dr Keefer also serves as a reviewer on both NIH and USDA grant panels. She was invited to serve on the PEW Initiative on Food and Biotechnology Steering Committee in 2005. Her expertise was recognised when she served as one of only three external reviewers for the FDAs Risk Assessment of Animal Cloning in 2006–2007, and for the Canadian Food Inspection Agency's Cloning Risk Assessment in 2008. She also served as a temporary voting member on the FDAs blood products Advisory Committee in 2009 for regulatory consideration of the first biopharmaceutical product produced by transgenic animals. These activities demonstrate Dr Keefer's impact not only on the science of reproductive biotechnologies, but their applications in society.

In summary, Dr Keefer has contributed significantly to the growth of our knowledge, and the use of that knowledge in assisted reproductive technologies for the good of human medicine, the treatment of infertility in humans and endangered species, conservation, and domestic animal genetic improvement. She has left an enduring mark on our field, and on the many of us that call her a mentor, colleague, and friend. The IETS extends our heartfelt congratulations to Dr Carol Keefer as the well-deserved recipient of the 2022 IETS Pioneer Award.

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