

The 2023 Bartlett Address

The Journey of a Theriogenologist

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It is truly an honor to be recognized by my professional association for my efforts in Theriogenology. I thank my nominators and the boards of ACT, SFT, and the Foundation for this amazing honor and I humbly accept this year's Bartlett award, knowing that many other deserving members continue to humble me and amaze us all. I follow in the footsteps of many of my Theriogenology heroes whom I have long admired and respected.

[Caution to the Reader: From here forward, the reader should only continue if interested in excruciating detail about one person's life story! I have detailed my journey in Theriogenology for posterity but not for the typical reader.]

My journey in Theriogenology has been a convoluted trip with roots in my undergraduate studies at Michigan State University in the state where I grew up. The trip is marked by many meaningful relationships and opportunities in which I received advice and opinions about career routes and strategies for success. The journey is also one of great humility. Veterinary practice is a great humbler. Some failures also marked the journey but I feel strongly that the roads NOT taken gave me as much meaning and experience as the roads I took. For some milestones, I had opportunities denied and for other milestones, my choices led me to great opportunity, random and deliberate choices, but at every step the people I have met turned into wonderful experiences, lifelong friendships, and deep rewards. It's all about the people for me in Theriogenology. Friendships. Great advice that bends the road traveled and not traveled. Did I mention lots of opportunities for laughing?

I have never been disappointed by the genuine warmth and support from our profession. My journey is partly marked by the faculty positions and residency opportunities that didn't happen! This led me to a pivotal Texas A&M University (TAMU) opportunity and lifelong acquaintances, friendships and collaborations. Faculty positions at CSU, Georgia, Auburn, Kentucky, and Wisconsin, also didn't pan out but in each case I met wonderful people in our profession. I didn't realize it at the time but these roads not taken served to broaden and deepen my network in Theriogenology for the long haul.



Getting into the Therio Zone ... Medicine

My journey begins as an undergraduate student at Michigan State University, where as a relatively unfocused undergraduate I enrolled in a Dairy Reproduction course in Animal Science and I was hooked on reproduction. I spent a year as an undergraduate senior in a research laboratory in Anthony Hall where I learned about working with cattle and hormones. Memorably, my mentors made me stand up and give an intimidating seminar presentation that buckled my knees but had to have built character.

That opportunity allowed me to land at Oregon State University where my mentors, Fred Stormshak, Don Holtan, and Lloyd Swanson taught me, and my graduate student colleagues, about management and physiology of cattle, sheep, and horses. Although these Animal Scientists were (probably) disappointed I wanted to be a vet, I was accepted into the College of Veterinary Medicine at Michigan State. When I finally got to 3rd year, I was excited for Theriogenology. I had bought the textbook (Current Therapy in Theriogenology edited by David Morrow) a year earlier! On the first day of Theriogenology class, Dave Morrow knew us all by name and he called on me minutes into the first class, gulp. I grew to know and respect Dr. Morrow, and I also learned (and got awesome advice) from him and Ed Mather, Carla Carleton, Resident Cindy Smith and Rick Bridges, and Dean Neely, the latter stoking my equine interests. Dave Morrow was among the nerdiest people I've known, in the best way, but he was

genuine and loved students! He always wore a tie, even when palpating. One day, when I was a senior student in the LA Clinic, a young veterinarian named Dickson Varner, gave an interview seminar in semen freezing in stallions. I was impressed, of course, but didn't know the impact of this for a few years yet to come. Also, during my time as a vet student, I had an opportunity as a research intern at the San Diego Zoo where I was involved in an IVF project with Barbara Durrant in goats as a model for African and Sub-Saharan antelope. This fed my passion for wildlife and conservation reproductive biology. I also met and became friends with Vic Shille who was spending a sabbatical year at the Zoo. Vic was a wonderful and brilliant mentor and Theriogenologist and I benefitted from much advice and Vic's warm personality. Vic also had ties to UC Davis and that impacted later.

Possibly the most valued advice I received about my future career was from Dave Morrow who told me I must get out into vet practice if I wanted to be a resident and ultimately a professor of Theriogenology. I joined a 5-person Dairy practice on the Eastern Shore of Maryland in which I had my own herds and relationships with farmers. Except for this one guy who shall remain nameless. That is, until the day he had a cow with an RDA and uterine torsion, and then he needed me and gave me a chance. Sadly, I lost that cow, but the dairyman saw how hard I worked to resolve the problem, and we went on to several years of humbling vet work.

During my time in Maryland, while being a dairy herd vet, I also filled in evenings in the small animal clinic. A nice young woman came in one evening with two baby rabbits. Would I tell her if they were sisters or brothers? No Idea. I called my boss for advice and he suggested I look at their bottoms and if they looked the same, just say they were sisters. It seemed reasonable. Of course, I ran into that woman in a grocery store a few weeks later and she told me the bunnies' babies were doing fine, and did I want one or two? Humbled again. Now I knew I needed advanced training!

It was in that practice that I met my colleague and wonderful friend, Peyton Jones, who in turn, introduced me to Tom Bowman, Maryland Thoroughbred breeding, Winfield Farm and Northern Dancer, and other notable Thoroughbred farms. Tom was a presence at Winfield's (later Northview Stallion Station) and, at that time, was just adapting ultrasound and twin management into equine practice. Tom was incidentally the first Repro resident at New Bolton Center so there was lineage in common. I fell in love with horse breeding and Peyton introduced me to Dale Paccamonte, then a resident at University of Florida. Dale, also an MSU grad, had previously been in the same practice. We still talk about the stories from that dairy practice! When I interviewed at Florida for a residency, Dale hosted and we became friends and colleagues. This was the first opportunity I didn't get (I wasn't offered the residency) but gained some wonderful friends in Theriogenology with Michelle LeBlanc and Dale. A few months later, Michelle called me to tell there was an out-of-cycle residency available at Texas A&M University and I immediately called Ron Elmore, and four weeks later, I arrived in College Station.

College Station, as a Yankee, opened my eyes to the joys of the South – food, cattle, horses, open space, two-stepping and waltzing, chicken-fried steak, BBQ to name a few. Literally millions of stories about Texas



Texas A&M Residency, College Station, TX

livestock, people and ranches! I also met my wonderful wife, Becky, who was a 3rd year TAMU vet student at the time. She later provided another journey for me in reproduction, ultimately making me a father of two boys, but that is a story for another time...

It was here that I was mentored by an amazing team consisting of Dickson Varner, Terry Blanchard, Ron Elmore, and Kathy Bretzloff. Surgeons David Morris, Tex Taylor and Jeff Watkins offered huge learning opportunities and an amazingly colorful Field Service team. Humbled along the way, of course. Dave Morris was a surgeon, but he was also a Theriogenologist and a super nice person and great mentor. Here we learned about bull penile surgeries, rodeo bulls, and vaginal prolapse management. Dave was also a role model for the hopelessly disorganized. I also bonded with my fellow residents, Lloyd Kloppe, Steve Brinsko and Pat Meyers. I was replacing Lloyd but in the few months we overlapped, I learned so much from this master of equine Therio and we were friends for years. Sadly, Lloyd recently passed and this devastated many colleagues and friends. Hats off to Lloyd! Jim Rugila was also a latter resident and officemate. Chickens in the office! Pat and I were the 'Meyers Bros' – unrelated but put together by personality and as Northerners out of water. Both Lloyd and Pat finished their residencies in my first year but Steve and I held the fort for better than a year and a half and he has been a close friend to this day. Steve was truly one of the funniest people I'd ever known. Steve had file folders of jokes-printed and organized by topic. Either every Friday afternoon, or whenever we had downtime, out came the files. The laughing and biting sarcasm about situations and ultimate ironies almost never stopped to this day. Texas pasture skiing, whereby one was towed after roping an unruly cow that broke loose, took shape as a potential Olympic event.

When I began to study for ACT boards, I realized that I needed to have "the red book", Dr. Steve Roberts' massive state-of-the-art of Theriogenology sprung from his decades at Cornell,

I couldn't afford to buy the book on a resident's salary. In those days before email, I sent off a letter (using stamps and an envelope, of course) to Steve Roberts, then 'retired' in Woodstock Vermont, explaining that I really wanted and needed the book. In pretty short time, I received the book still in its wrapper compliments of Dr. Roberts along with a warm personal note that he hoped it would help. A few years later, I would face Dr. Roberts across a table as an examiner for the ACT board practical exam.

At Texas A&M, I developed a special and long-lasting relationship and collaboration with Dickson who mentored me as an



Texas A&M: "I've Been Everywhere, Man."

older than typical resident. Dickson was a master of modern Therio and a great doctor but also had a keen eye for historical context.

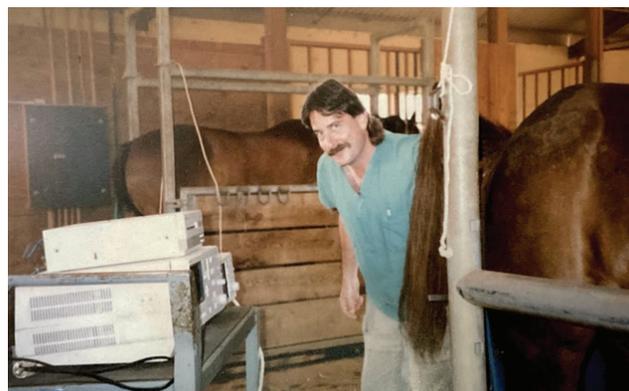
Terry Blanchard was a superb Theriogenologist but also an amazing vet. He had a wealth of practice and life experience, which was incredibly valuable as a mentor to me. We spent most time on stallions and mares but also beef cattle and tropical breeds: *Bos indicus* and *taurus*. Some of our learning (about life and vet practice) was done after hours in the Field Service trucks on our own with the humbling presence of students. The TAMU experience was hard and gritty and the case-load was sometimes crippling. But we learned and taught from all the richness. I had opportunities to work on rhino and giraffe reproduction with Dr. Dewey Kramer and his group in the Reproductive Sciences Laboratory. Add to the mix, the Theriogenology work at the Texas prison system for bull BSEs and horse breeding. This was an exceedingly rich mixture of clinical experience and teaching opportunities that set the stage for many years of wide-eyed memories and stories.

Steve, Dickson, and I also played music together and (deserv- edly) won a talent contest at TAMU!

After the TAMU residency, I became a resident veterinarian at a large Standardbred breeding farm in Northern Illinois where I put to use my TAMU training. A Texas beach girl, Becky was really looking forward to living in real winter and snow in Northern Illinois but was definitely ready to move on to the next adventure, in California, a few years

later. What I suspected but had not appreciated, or prepared for, was that foals were everything and that repro was just a means to get foals on the ground! I learned an amazing amount of foal medicine along with practical obstetrics and neonatology – we delivered approximately 250 foals per year, so there was always something. During my third breeding season, the state had a large strangles outbreak and many horses were sick. A few died. I learned to perform field tracheotomies for the severe cases and had numerous sick adult and juvenile horses. This was above and beyond the endemic levels of *Rhodococcus equi* infections existing on the farm. It was time to refocus and pursue research and teaching...

Dr. John Hughes at UC Davis offered me an opportunity to run the California side of a now-famous synthetic GnRH implant study for FDA approval as a funded PhD student opportunity. I was accepted as a doctoral student at the University of California. The project was a multi-center collaboration between Colorado State and UC Davis with a large number of mares at each institution to gain approval for clinical use of deslorelin or Ovuplant. This experience allowed me to learn about the FDA approval process and do graduate studies with John Hughes, George Stabenfeldt, Irwin Liu, Bob Bondurant, Jan Roser, along with many others at UC Davis. My graduate student friends were Pat McCue, Mats Troedsson, and Peter Daels; under the training of George and John we became lifelong colleagues and friends. I can never forget my first meeting with George as he played jazz piano beautifully for me in his office and provided tremendous mentoring. I channeled his coolness and have always kept a guitar in my office. Dr. Hughes was also a wonderful mentor and we became good friends with LaVonne and John.

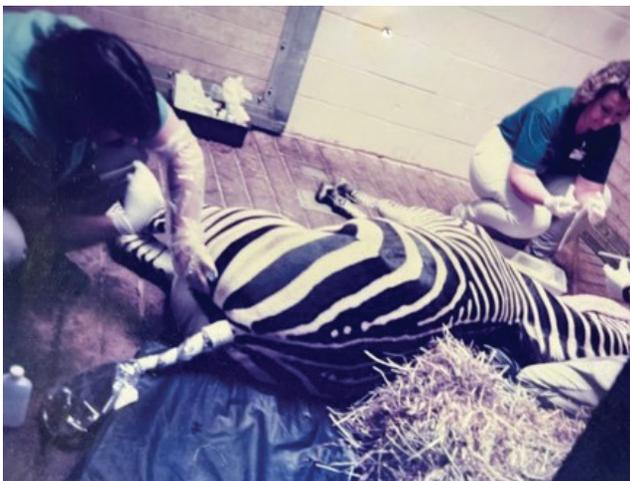


PhD graduate student, UC Davis

As a graduate student, my first course was an amazing fertilization biology class taught by a team of amazing and well-known reproductive biologists. I developed a deep interest in Andrology and male subfertility in horses and I was focused on the cell and molecular biology of sperm. Studies focused on acrosomal function of horse sperm and interaction with oocytes and we developed the beginnings of a program to understand the role of sperm membrane function in fertility. I developed experience working with fluorescence microscopy, along with acrosomal biology and the sperm surface membrane enzyme system. In the context of fertilization, we began to think of sperm function in livestock related to sperm-egg interaction, of which the acrosomal reaction was the first step.

Being in the Therio Zone ... Science and Life

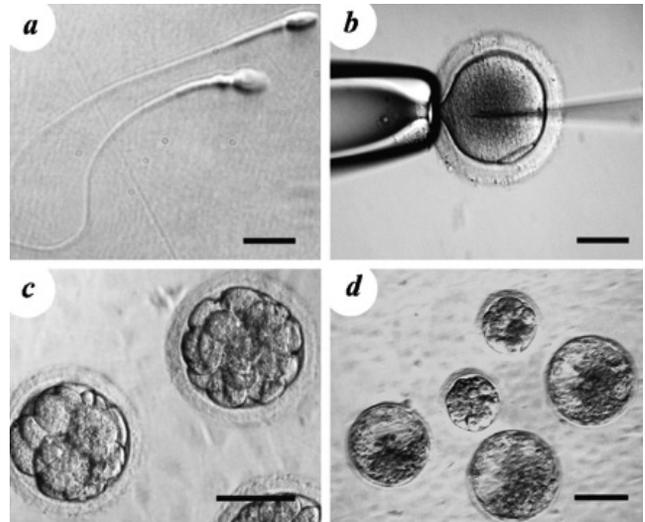
After a postdoctoral fellowship on contraceptive studies with non-human primates with the human andrology and fertility group at UC Davis School of Medicine, I joined the faculty as Assistant Professor at New Bolton Center, in the School of Veterinary Medicine, University of Pennsylvania. In addition to a large equine caseload that included a state-of-the-art neonatal ICU, I joined a well-established team that performed more stallion BSEs and semen shipping than most places around the world. Bob Kenney had recently retired, and it turned out, Bob was still around for another couple years and always could be counted on for wisdom, burning sarcastic wit, and knowledge. Pat Sertich, Sue McDonnell, Dick McFeely, and Regina Turner (graduate student at the time) rounded out a great team, along with amazing and several hard-working selfless residents including Tamara Dobbie and Sylvia Bedford. John Hurtgen, another master of the game, was nearby in New Freedom and I never failed to learn from many things about stallions from John along the way. Ina Dobrinski joined the faculty 'in town' (Philadelphia) in Ralph Brinster's lab and we developed a long-term friendship as she began to master mammalian germ cell biology, which began in this unequalled lab at Penn. I was able to obtain external funding (USDA, Grayson) for stallions and the lab published a few articles on acrosomal function and hyaluronidase of the sperm surface membrane in stallion sperm function and capacitation. Here, Pep Rutllant from Spain joined the lab as postdoc initially (and then as lecturer at UC Davis) and we collaborated for many years with a deep friendship.



Philadelphia Zoo Zebra Mare BSE.

After four years as Assistant Professor at New Bolton Center, I accepted an offer to join a basic science department in the Veterinary School at the University of California, Davis. This opportunity would lead me out of clinical medicine but allowed a scientific career to expand. It was at this time my academic and personal life began to take shape. Our toddlers grew into teens, and then young men. Numerous generations of dogs and cats... We made a life in Davis California and now have many close friends and colleagues! A lifetime of wonderful Theriogenology colleagues and students met me: Al Conley, Barry Ball, Bob Bondurant, Linda Munson, Jim Maclachlan, Irwin Liu, Joannie Rowe, countless residents and graduate students who are now accomplished Theriogenologists and Reproductive Biologists around the world. In addition, many international colleagues and collaborators in Germany,

Australia, Italy, France, UK, The Netherlands, Belgium, Japan, and Spain.



[Honaramooz, Li MW, Penedo MC, et al: Accelerated maturation of primate testis by xenografting into mice. *Biol Reprod.* 2004 May;70(5):1500–1503.]

At UC Davis, my research and teaching took hold. In addition to Theriogenology teaching, I took on gross anatomy teaching and I surprisingly developed a love and expertise for this fundamental discipline's ability to reach 1st and 2nd year vet students. Most Theriogenology programs don't historically engage these early years of the vet curriculum and I really enjoyed the refreshing enthusiasm of first year DVM students. I was course leader for many years of the basic Gross Anatomy course and I was recently awarded a School of Veterinary Medicine Faculty Teaching Award (2022) and a UC Davis (2023) Distinguished Teaching Award for Professional and Graduate Education. I also began serving the ACT first as board member in the mid-2000s and then as Treasurer for six years during this time. Many more ACT and SFT friendships!

Being in the Research Zone...

My research journey is a bit more convoluted. At UPenn and at UC Davis, my lab's work on acrosomal function and sperm membrane biology focused on stallion sperm function. We developed a record of publications and presentations in this area that eventually encompassed sperm mitochondrial function and mitochondrial genetics as related to oxidative metabolism in sperm from stallions, dogs, and, more recently, fish. Our research in sperm physiology and function has been focused on oxidative stress due to cryopreservation conditions and aging in stallions, dogs, and bulls for the past 20+ years. In our canine work, the lab has collaborated with Guide Dogs for the Blind and we've received generous funding from the Great Dane Club of America for the canine aspects of this work in which we began to explore the effects from male age and sperm function in large populations of breeding Labradors and Danes.

Along the way, we realized that sperm function must really put into context of fertilization to more fully understand the role of this remarkable and unique cell. For this reason, the lab

began moving into the direction of the sperm's role in fertilization physiology and this drew us into the sperm's role in early embryo development. Several studies focused on oxidative stress in stallion and rhesus monkey sperm with collaborations with Barry Ball's lab at UC Davis and Peter Sutovsky's lab at the University of Missouri. However, I will highlight some of the unique opportunities we've had in recent years.

As in life, the failures, or at least the roads not taken (or unable to be taken) were also important. Many side roads emerged and I will tell a few stories about that. In the early years as faculty member at UC Davis, my lab collaborated with the UC Davis lab of the esteemed Dr. John Crowe, a world-renowned lyobiologist (freeze drying). Together, we put a strong effort forward on attempts to understand cryobiology of sperm (which was new to John) through membrane phase behavior that led to attempts to freeze-dry sperm using the intracellular sugar trehalose, which is used naturally by many desert creatures to withstand seasonal and decades-long drought. Since sperm cells are known to already have the one of the lowest water contents of any mammalian cell, it made sense to try to load these cells with trehalose and freeze-dry them for long-lasting preservation. In the end, we never were able to recover live or motile sperm, but we did determine that stallion and monkey sperm could be dried to zero water content and maintain good morphology and even could be used to fertilize eggs using intracytoplasmic sperm injection (ICSI). Additionally, we reported that immature rhesus monkey spermatogenesis could be re-established when testicular explants were transferred to immunocompromised mice in an acceleration of spermatogenesis- earlier than the donor was entering puberty. The mice hosted testicular explants that grew and maintained monkey spermatogenesis! Furthermore, we were able to then freeze-dry the matured sperm harvested from the mice and used these sperm for ICSI to make viable monkey embryos. Since then, numerous species have successfully reported ICSI embryos fertilized with dried sperm. Someday, the 'code' will be cracked to preserve sperm without liquid nitrogen!

A side project from this collaboration, set our lab on the path to understand phase transitions in stallion sperm during cooling. This work had not been performed previously in horses and allowed us to begin to think about stallion sperm membranes existing in multiple phase states, which include crystalline and gel states with varying lipid melting points in a single cell's membrane that opened an understanding of how these cells withstand, or do not withstand, low temperature. Perhaps some males have differing success in cryopreservation due to their membrane phase traits?

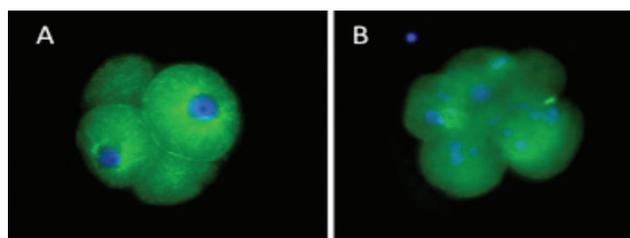
Another offshoot of our work was the use of oxidative stress in stallion and dog sperm, which we then applied to zebrafish. We demonstrated that zebrafish sperm were equally susceptible to oxidative stress as mammalian sperm. This was particularly interesting because fish sperm are exposed routinely to highly varying osmotic and oxidative conditions (and temperatures) in which these cells can persist with high success rates. Lessons such as these can be applied to our growing understanding of livestock sperm stress.

Several years ago, we participated in a series of studies of the marine invertebrate, Elkhorn coral in the Caribbean. The latter are struggling around the world to survive as the great coral reefs are under increasing global pressure

primarily due to warming and pollution. The Elkhorn coral (*Acropora palmata*) of the Caribbean are highly endangered and form the basis of complex habitat for countless ocean animals and plants. Beginning in the 1980s, the elkhorn coral population declined 97 percent from warming-related white band disease. My lab had the opportunity to participate in a cryopreservation project conducted by the Smithsonian Institution along with several major international aquariums for a field study in Puerto Rico. In this international collaboration, we performed the cryopreservation studies of sperm and accompanying studies on embryos for this endangered hermaphroditic species. Again, collaborations and friendships became long-lasting with several National Zoo and Smithsonian investigators. We were able to send several UC Davis veterinary students to the National Zoo in Washington, DC over the years for research internships.

Elkhorn coral are known to broadcast spawn naturally only during a few days per year at night after a full moon so the team needed to be ready to jump into action when the spawn began. When they spawn, the hermaphroditic coral simultaneously release sperm and eggs (they do not self-fertilize), which float to the surface and mix. The fertilized motile zygotes then sink to the bottom and begin to attach to rocks and substrate. These organisms are deeply susceptible at this specific time to predation and hostile environmental conditions. So there is a tiny window in which to collect the gametes. This required a team of professional night divers to monitor for the beginning spawn each night around the second week of August. When the spawning coral were spotted, the lab teams sprung into action and retrieved samples of the rising sperm and eggs released by each hermaphroditic individual, which are released and mix at the surface to provide genetic diversity. Our goal was to cryopreserve as much genetic material as possible, to optimize the freezing process so that the aquariums and conservation biologists could propagate the baby coral under controlled conditions. The project is ongoing and successful for in vitro management, but still remains unknown if repopulation is possible in the Caribbean reefs.

As sperm function needed to be understood during sperm-oocyte interaction, our lab developed a non-human primate model for early events following ICSI fertilization and combined the oxidative stress model for early embryo development.



A. normal 4-cell rhesus monkey embryo showing intact nuclei and cytoskeleton. B. Abnormal 4-cell embryo resulting from ICSI using oxidatively stressed sperm. Note multiple abnormal micronuclei and chromatin fragmentation. Confocal fluorescence image (600x); [Burrue V, Klooster K, Barker CM, et al: Abnormal early cleavage events predict early embryo demise: sperm oxidative stress and early abnormal cleavage. *Sci Rep* 2014 Oct 13;4:6598.]

We induced oxidative stress on monkey sperm and then used ICSI to inject sperm into mature Meiosis II oocytes. We found that oxidative damaged sperm showed somewhat normal cleavage division but improper nuclear structure and function evidenced by fragmented nuclei and micronuclei: a condition that mimicked embryo development resulting from the use of sperm from aging men. We began to incorporate time-lapse imaging in near real-time for monkey embryos, as had been reported for human *in vitro* embryos in which we defined the timing and durations of each mitotic division in early embryos leading to blastocysts. This led to equine embryo time-lapse studies.

As the lab became more experienced with ICSI in non-human primates, we began to experiment with ICSI in horses and our program has now developed into a robust equine research and commercial ICSI program. We reported the first all-UC Davis ICSI foal in 2018 as the program was begun.



The birth of UC Davis's first ICSI foal, Petri.

Although monkey embryos are more or less transparent and easy to monitor *in vitro*, horse oocytes and embryos are very optically dense and cell divisions (in addition to being barely able to see sperm during ICSI) are quite challenging to observe and record due to this dark appearance under most light microscopes. A new optical imaging system allowed us to perform kinetic monitoring of horse embryos in near real-time. Dr. Pouya Dini joined our faculty in 2020 and together we have developed a research and clinical ICSI program in the past several years along with a great collaboration and friendship!

In our lab at UC Davis, we developed a state-of-the-art commercial ICSI laboratory to complement our research efforts that has performed hundreds of ICSI sessions annually for shipped and in-house aspirated immature oocytes for horse breeders from around the US. Although not every ICSI session results in a frozen embryo, we can report encouraging results both commercially and for research that have allowed us to collect real-time imaging data for each embryo growing in the

lab. Knowing the timeline for certain specific cellular events to occur has allowed us to gain confidence in predicting which embryos are most likely to survive vitrification and result in a viable and healthy foal. Using imaging and molecular techniques our lab is studying the events that occur in the oocyte prior to fertilization, the zygote immediately after fertilization, and the resulting embryo. Further studies have focused recently on using the non-invasive time-lapse imaging system to measure and monitor oocyte maturation and cumulus expansion and we are able to predict which oocytes will most likely succeed following maturation and ICSI. We are extending these studies into single cell transcriptomics to understand the difference between *in vitro*-produced and *in vivo*-produced embryos, the embryonic capsule, embryo pulsation: all the while teaching an old dog new tricks along the way!

Conclusions

To conclude, I have had a lifetime of amazing experiences in Theriogenology, friendships and collaborations and life lessons. I hope my impact on each has been as positive for others as it has been for me. I am indebted to my Theriogenology colleagues for many years of sharing expertise in reproduction and for long-lasting, and new, friendships.

A few assorted lessons to pass on

Don't speak too loudly or enthusiastically about reproductive biology and pathology in restaurants-not everyone appreciates what we do!

Don't hesitate to talk to the leaders and founders of our specialty! There is a wealth of information and you will not find nicer people in the world.

Do a better job listening than talking. It's been said your ears will never get you in trouble!

