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## Theriogenology

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## Review

## A journey through people, places, and projects in equine assisted reproduction

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## A B S T R A C T

*Keywords:*

Equine  
Embryo  
Oocyte  
Fertilization  
Nuclear transfer  
Intracytoplasmic sperm injection

A research study is a product of not only a question and its pursuit but also the people, places, and facilities available at the time. My work in equine assisted reproduction has progressed from embryo transfer to oocyte maturation, oocyte transfer, intracytoplasmic sperm injection, embryo biopsy, embryo vitrification, and cloning, as a result of collaborations with an array of remarkable people. This is a summary of some of the stories behind the studies.

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**1. Introduction**

Budding scientists often think of research as the planned result of a detailed understanding of a problem, a decisive approach by which to study it, and a profound interpretation of the results. However, my experience has been that each project is the product of an interplay of factors including people, cases, places, available resources, and serendipity. What I think of when I review the publications supporting this remarkable honor, receiving the Simmet Prize, are the people and places that led to those studies, and the exciting, or difficult, or surprising times associated with them.

My journey through research started when I began a residency at the University of Pennsylvania's New Bolton Center after 4 years of becoming more and more frustrated with practice. Very soon after starting the residency, in 1982, I knew that I had arrived at exactly the right place. The Section was an amazing collection of people who have turned out to be both collaborators on projects and friends for life. I loved everything about it—Section members would talk about new and old ideas and new and old articles and ask questions and try to figure out answers. There

were clinical questions and physiological questions and endocrinological questions—and everyone knew everything! Bob Kenney, the Section chief (Fig. 1), encouraged thinking analytically, and you knew you had a good idea when you asked him why something happened and he said “Yes.” My first good idea, on reading clinical endometrial biopsies from mares through the winter into the breeding season, was “What stimulates the endometrial epithelium to grow from cuboidal to columnar?” “Yes.”

So that question, in fact, turned into my first research project. The Section had some ovariectomized mares left from another project, and Dickson Varner (who is, over 30 years later, my friend and colleague at A&M) and I designed a study to treat four of the mares with estrogen and then take endometrial biopsies, then treat them with progesterone and then take biopsies. On examining the biopsies, one could see clearly that each hormone separately caused the epithelium to become columnar in a matter of days. Bob was intrigued! I was thrilled! I had found out something that NO ONE EVER KNEW BEFORE! This was great! This was a turning point for me—because not only was it amazing to have figured out the answer but also I found that you got a lot of credit for doing something (research) that you wanted to do! (This still continues to amaze me—there are other things that I also like to do, like ride horses and paint watercolors, but I do not get much credit for them). But in the end, after months of trying

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Fig. 1. Dr. Robert Kenney and the author in 1987.

different methods to analyze the slides, including hours counting cells with eyepiece reticules, I could not figure out a way to accurately quantify the changes in the biopsies for publication and the slides and the initial data from attempts to read them sit in my office to this day. Dickson continues to give me a hard time about not publishing this.

However, the findings from the biopsy study led to my first real research project, the success of which led me to want to go for a PhD, which led to a career in teaching, clinics, and research in equine reproduction. It has been an amazingly fulfilling journey. For this review, instead of discussing the science of that research, because Pete Hansen told me “write whatever you want to!,” what follows are brief summaries of how a few of these studies in equine assisted reproduction came to be.

## 2. University of Pennsylvania

### 2.1. Establishment and maintenance of pregnancy after embryo transfer in ovariectomized mares treated with progesterone

I was intrigued that the endometrial biopsies from the progesterone-treated ovariectomized mares in our study looked exactly like biopsies from cyclic intact mares. This led to the question—was this uterus functional? Could ovariectomized mares treated with progesterone maintain a pregnancy after embryo transfer [1]? Davies et al. [2] had just published an article in which they reported that pregnancy had been established (but lost at Day 55) after embryo transfer in one of four anestrus mules treated with

altrenogest. This gave a hint that it might work! I would never have been able to pursue this question had not Pat Sertich, as a new resident, decided to take on the challenge of starting an equine embryo transfer program in the Section for a client. Thus, we had all the equipment for embryo transfer at Hofmann Center and a budding knowledge of embryo transfer with which to address the question of the ovariectomized mares. We used the four valiant ovariectomized mares from the biopsy study as recipients and used the mares in our teaching herd as embryo donors. Because these were not the most fertile group of mares, it took numerous attempts (each time starting an ovariectomized recipient on progesterone injection 2 days after donor ovulation) before we actually got an embryo from one of the donors. The first embryo transfer I ever did was my first collected embryo into a progesterone-treated, ovariectomized recipient—and she became pregnant! I showed Bob the printout of the 14-day ultrasound examination with a vesicle on it, and he said “Show me tomorrow, when it has grown,” and I did. In all, three of the four ovariectomized mares became pregnant and delivered healthy foals (Fig. 2). I had met Eric Palmer at INRA in Nouzilly, France, the previous year, at Bob’s arrangement, and I wangled to do the associated hormonal assays with Eric because Nouzilly was a lot more interesting than was Philadelphia, my other option!

I went on to expand on this work for my PhD, evaluating use of other progestins [3], use of progesterone in mares with gonadal dysgenesis [4], the importance of synchrony between onset of progesterone treatment and embryo age [5], and the effect of long- and short-term progesterone treatments on uterine protein patterns [6]. Use of nonovulating hormonally-treated mares has become a common clinical procedure.

### 2.2. Clinical significance of aerobic bacterial flora from the uterus, vagina, vestibule, and clitoral fossa of clinically normal mares

This deceptively simple study established information that anyone performing transvaginal manipulations in the mare—that is, doing almost any assisted reproduction



Fig. 2. Mia, one of the three ovariectomized mares in the author’s first research project, and her foal produced via embryo transfer.

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