

Reproduction and Perinatology Update

A publication of the D.H. Barron Reproductive and Perinatal Biology Research Program and the Center for Perinatal Outcomes Research



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UF paper selected by *Endocrine News* as one of the five most important endocrine discoveries of 2016

Each year, *Endocrine News* talks to editors of journals published by the Endocrine Society to identify the top discoveries in endocrinology for the year. For 2016, one of the discoveries highlighted was work from the University of Florida on the mechanism by which estrogen signals to its target cells that was published in the March 2016 issue of *Endocrinology* (Nanjappa et al., *Endocrinology* 157: 2909-2919).

Steroid hormone receptors are present in the nucleus and, when bound to ligand, interact with DNA to alter transcription of target genes. However, for decades it has been known that about 5% of steroid hormone receptors such as estrogen receptor 1 (ESR1, also known as estrogen receptor alpha), are located in cell membranes. The function of membrane ESR1 (mESR1) has been extensively debated, partly due to the lack of good model systems to determine their role in normal estrogen signaling.

In a paper published in *Endocrinology*, the laboratory of **Paul Cooke**, Dept. of Physiological Sciences, used a recently developed transgenic mouse that expresses normal nuclear ESR1 but lacks membrane ESR to demonstrate that the lack of mESR1 leads to extensive abnormalities in sperm, testis and other organs that are major ESR1 targets, such as the efferent ductules, and causes progressive infertility. These results indicate that mESR1 plays a previously unrecognized major role in mediating the estrogen effects that support male reproductive development and function.

As pointed out by the editors of *Endocrine News*, the discovery proves that estrogen signaling through membrane receptors is vital and this knowledge could lead to new therapeutic tools, including a male contraceptive.

Read the article in
Endocrine News at

<http://endocrinenews.endocrine.org/eureka-top-endocrine-discoveries-2016/>



Lead author Manjunatha
Nanjappa



Senior investigator Paul
Cooke

Contribute IDC to Support Our Program

Our reproductive biology effort is supported by the Center for Perinatal Outcomes Research. This is a source of funds that are generated by indirect costs from us - up to 7.5% of IDC that otherwise goes to the Deans Office can be designated to the Center. These funds are used to invite speakers to campus, organize retreats and are used as a source of seed grants. Please consider designating some of your IDC to the Center

Identify Our Program on Your Next Paper

Please consider including the D.H. Barron Reproductive and Perinatal Biology Research Program as one of your affiliations

Faculty, Student, Postdoc and Alumni News

Gregory Christman, J. Wayne Reitz Professor and Chair of Reproductive Biology in the Dept. of Obstetrics & Gynecology, was Chairman of the Organizing Committee for the 2nd World Congress on Polycystic Ovarian Syndrome held October 5-7, 2016 in Orlando Florida.

Manju Nanjappa, a postdoctoral scientist in the laboratory of **Paul Cooke**, received the Thomas S.K. Chang Trainee Travel Award to attend the 2016 American Society of Andrology Meeting in New Orleans. His talk there received the Trainee Merit

Award, the ASA's highest student award. For more about Nanjappa's accomplishments see the story on page 1.

Sofia Ortega successfully defended her doctoral dissertation in December and was awarded the PhD degree. She is currently a postdoctoral scientist in the lab of Tom Spencer at the University of Missouri.

Alice Rhoton-Vlasik, an associate professor in the Dept. of Obstetrics & Gynecology, gave an invited lecture entitled "Options for Fertility Preservation in Adolescents: GnRH

Agonists or Not? at the 2016 Annual Meeting of the North American Society for Pediatric and Adolescent Gynecology in Toronto in April 2016.

Paula Tribulo, a doctoral student in the AMCB in the Hansen lab, has been elected as Vice President of the UF Chapter of Sigma Xi, the Scientific Research Honor Society.



Research Highlight - The Evolutionary Origin of Cartilage Development

Oscar Tarazona and Martin J. Cohn, Dept. of Molecular Genetics and Microbiology

Our laboratory is interested in the molecular mechanisms of embryonic development as they relate to two areas of biology; the causes of congenital malformations (birth defects), and the relationship between embryonic development and the evolution of anatomical form. In this research highlight, We report on a study aimed at understanding the evolutionary origin of the genetic program for cartilage development.

The evolution of novel cell types led to the emergence of new tissues and organs during the diversification of animals. In vertebrate embryos, the primary cell type found in the developing skeleton is the cartilage cell, or chondrocyte, which synthesizes cartilage matrix. The origin of the chondrocyte, therefore, was central to the evolution of the vertebrate endoskeleton. Cartilage is generally considered to be a vertebrate-specific tissue; however, reports of "cartilage-like" tissues in invertebrates can be found in the classical zoology literature. This raises the possibility that the genetic program for cartilage development and the chondrocyte as a cell type could have an unexpectedly ancient evolutionary origin.

To investigate this question, we first compared the structure and composition of

Research Highlight (continued) – Evolution of Cartilage

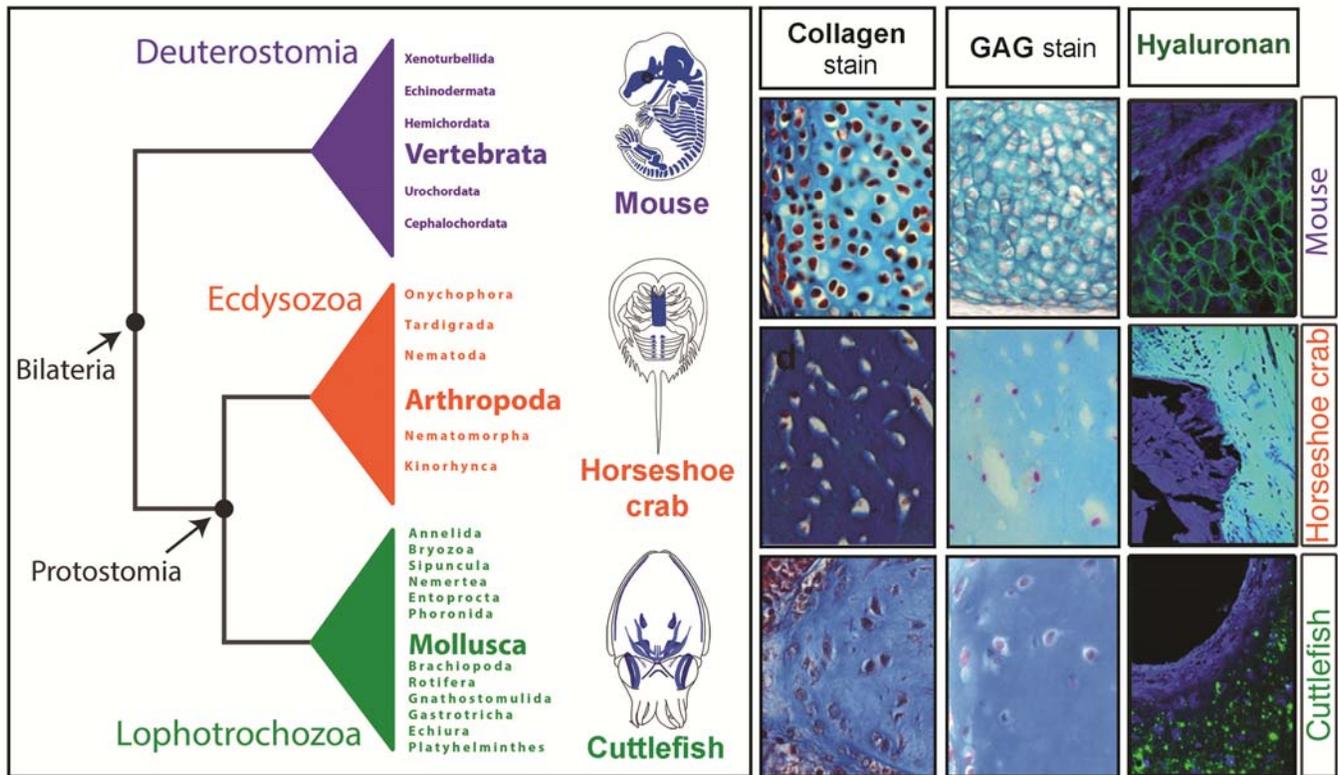


Figure 1. Phylogenetic distribution of cartilage and “cartilage-like” tissues in bilaterally symmetrical animals (left). Structural and histochemical similarities among cartilages of mouse, horseshoe crab, and cuttlefish (right).

the cuttlefish (a cephalopod mollusk) and the horseshoe crab (an arthropod). We found that cuttlefish and horseshoe crab cartilage is remarkably vertebrate-like, consisting of cells embedded in abundant extracellular matrix that is rich in collagen, acidic glycosaminoglycans, and hyaluronan (Fig. 1).

The major extracellular matrix protein in vertebrate cartilage is type II collagen, encoded by the *Collagen2 α 1* (*Col2 α 1*) gene. We identified pro-orthologs of the *Col2 α 1* gene, which we designated *ColA*, in cuttlefish and horseshoe crab genomes. Analysis of *ColA* expression in invertebrate embryos revealed that transcripts localize to cartilage forming cells (Fig. 2a and 2b). We then asked whether the same cell signaling proteins and transcription factors that regulate *Col2 α 1* in vertebrate chondrogenesis (cartilage development) are expressed during invertebrate chondrogenesis. The hedgehog signaling pathway plays essential roles in early vertebrate chondrogenesis, activating transcription of the cartilage master genes *Sox5/6/9*, which directly regulate *Col2 α 1*, and controlling chondrocyte proliferation and differentiation. *Sox9* and β -catenin have opposing functions in vertebrate chondrogenesis; they inhibit one another’s transcriptional activity, and, as such, cartilage formation requires degradation of the anti-chondrogenic protein β -catenin in *Sox9*-expressing cells.

In the developing cartilaginous skeletons of cuttlefish and horseshoe crab embryos, we identified hedgehog-expressing cells immediately adjacent to a population of pre-cartilaginous cells that express *Sox5/6/9* orthologs (designated *SoxE* and *SoxD*), just as in vertebrate embryos. Functional manipulations of these pathways in cuttlefish embryos showed that antagonism of the hedgehog pathway or stabilization of β -catenin inhibited expression of *ColA* and blocked cartilage development, mimicking their roles in vertebrate chondrogenesis.

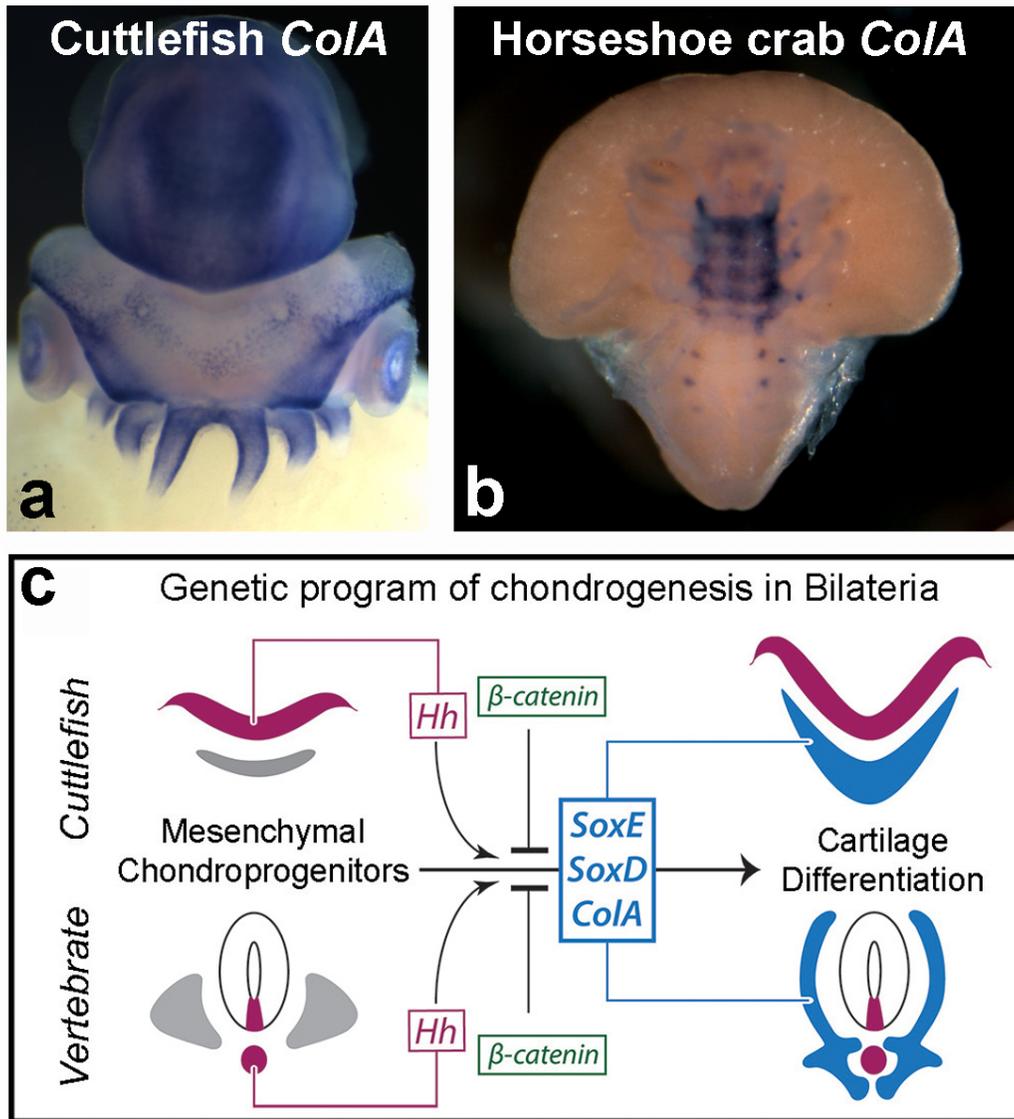


Figure 2. Whole mount in situ hybridization showing expression of the ancestral *CollagenA* gene in embryonic cuttlefish (a) and horseshoe crab. (c) Comparison of the gene network that regulates cartilage development in the cuttlefish funnel cartilage (top) and mouse vertebral cartilage (bottom).

Taken together, these data demonstrate that true chondrocytes exist in vertebrates and invertebrates, highlighting an unexpectedly deep conservation of this cell type and of the genetic program for cartilage development. Our findings suggest that the core kernel of the gene network that (a) specifies cartilage cell type identity and (b) orchestrates cartilage development was likely present in the common ancestor of all bilaterally symmetrical animals (bilateria). Finally, these results raise the potential for the use of invertebrates as model organisms to study chondrogenesis, cartilage physiology, and regeneration.

 This work was published in Tarazona et al. 2016, The genetic program for cartilage development has deep homology within Bilateria. Nature 533: 86-89.

Schedule Spring Seminar Series

Reproductive & Perinatal Biology Seminar, Wednesday 4:00-5:00 PM
D.H. Barron Conference Room Medical Sciences Building M-304

- February 1: **Joseph Kramer, PhD, HCLD**
Current and Future Practice of Donor Egg IVF
 Director, IVF & Andrology Laboratory, UF Health Reproductive Medicine
- February 8: **Pete Hansen, PhD**
Developmental programming in the preimplantation period – the view from the cow
 Distinguished Professor and L.E. “Red” Larson Professor, Department of Animal Sciences, University of Florida
- February 15: **Robert S. Egerman, MD, FACOG**
Diabetes, Pregnancy and Drinking the Kool-Aid: A clinician's perspective on assessment and treatment
 Professor, Department of Obstetrics & Gynecology, Maternal Fetal Medicine and Department of Medicine, General Internal Medicine, University of Florida
- February 22: **Alice Rhoton-Vlasak, MD**
Updates in Reproductive Biology
 Associate Professor, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Florida
- March 1: **Ki Park, MD**
Adverse Pregnancy Outcomes and Long Term Cardiovascular Risk in Women
 Assistant Professor, Division of Cardiovascular Medicine, Department of Medicine, University of Florida
- March 22: **John Bromfield, PhD**
A role for seminal fluid in promoting optimal pregnancy outcomes
 Assistant Professor, Department of Animal Sciences, University of Florida
- March 29: **Kirk Conrad, MD**
Relaxin Redux! An Update
 Professor, Departments of Physiology & Functional Genomics and Obstetrics & Gynecology, University of Florida
- April 5: **Gregory Christman, MD**
Changing Clinical Reproductive Medicine Practice with Level 1 Trial Evidence
 J. Wayne Reitz Professor and Chief, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Florida
- April 12: **Rachel Piersanti, DVM**
Oocyte expression of epigenetic machinery is altered in response to inflammatory mediators
 PhD Candidate, Department of Animal Sciences, University of Florida
- April 19: **Andrew Antolic, BS, MPH**
Chronic maternal hypercortisolemia in late gestation, alters the cardiac transcriptome and fetal ECG at birth
 PhD Student, Department of Pharmacodynamics, University of Florida
- April 26: **Rex Hess, PhD**
Small tubules, surprising discoveries: Efferent ductules of the male
 Professor Emeritus, Comparative Biosciences, University of Illinois College of Veterinary Medicine
- Luiz Renato de Franca, PhD**
Spermatogonial stem cell biology and transplantation in vertebrates
 Professor, Department of Morphology, Federal University of Minas Gerais, Brazil and Director, National Institute for Amazonian Research

Recent Contracts and Grants

NIH. Membrane estrogen receptor 1 mediation of epigenetic effects of estrogen. P. Cooke (PI). R03 HD087528). 2016-2018, \$100,000.

USDA/NIFA. Breeding climate-smart beef cattle. R Mateescu (PI), PJ Hansen (coPI) and M Elzo (coPI). 2017-2020, \$733,0000.

Zoetis. Molecular fingerprint of embryos with high competence to establish pregnancy. PJ Hansen, J Block and JJ Bromfield (P). 2017, \$93,300.

Selected Publications

Bromfield, JJ. A role for seminal plasma in modulating pregnancy outcomes in domestic species. *Reproduction* 2016; 152:R223-R232.

Conrad KP. G-Protein-coupled receptors as potential drug candidates in preeclampsia: targeting the relaxin/insulin-like family peptide receptor 1 for treatment and prevention. *Hum Reprod Update* 2016; 22:647-64.

Daetz R, Cunha F, Bittar JH, Risco CA, Magalhaes F, Maeda Y, Santos JE, Jeong KC, Cooke RF, Galvão KN. Clinical response after chitosan microparticle administration and preliminary assessment of efficacy in preventing metritis in lactating dairy cows. *J Dairy Sci* 2016; 99:8946-8955.

Deatsman SL, Rhoton-Vlasak AS, Vasilopoulos T. Age and fertility: A study on patient awareness. *JBRA Assist Reprod.* 2016; 20:99-106.

Hao C, Gely-Pernot A, Kervarrec C, Boudjema M, Becker E, Khil P, Tevosian S, Jégou B, Smagulova F. Exposure to the widely used herbicide atrazine results in deregulation of global tissue-specific RNA transcription in the third generation and is associated with a global decrease of histone trimethylation in mice. *Nucleic Acids Res* 2016; 44:9784-9802.

Hoppe BS, Harris S, Rhoton-Vlasak A, Bryant C, Morris CG, Dagan R, Nichols RC, Mendenhall WM, Henderson RH, Li Z, Mendenhall NP. Sperm preservation and neutron contamination following proton therapy for prostate cancer study. *Acta Oncol.* 2017; 56:17-20.

Jeon SJ, Cunha F, Ma X, Martinez N, Vieira-Neto A, Daetz R, Bicalho RC, Lima S, Santos JE, Jeong KC, Galvão KN. Uterine microbiota and immune parameters associated with fever in dairy cows with metritis. *PLoS One* 2016; 11:e0165740.

Larkins CE, Enriquez AB, Cohn MJ. Spatiotemporal dynamics of androgen signaling underlie sexual differentiation and congenital malformations of the urethra and vagina. *Proc Natl Acad Sci U S A* 2016; 113:E7510-E7517.

Martinez N, Sinedino LD, Bisinotto RS, Daetz R, Risco CA, Galvão KN, Thatcher WW, Santos JE. Effects of oral calcium supplementation on productive and reproductive performance in Holstein cows. *J Dairy Sci* 2016; 99:8417-8430.

Mumford SL, Legro RS, Diamond MP, Coutifaris C, Steiner AZ, Schlaff WD, Alvero R, Christman GM, Casson PC, Huang H, Santoro N, Eisenberg E, Zhang H, Cedars MI for the NIH/NICHD Reproductive Medicine Network. Baseline AMH level associated with ovulation following ovulation induction in women with Polycystic Ovary Syndrome. *J Clin Endocrinol Metab* 2016; 101:3288-3296.

Nanjappa MK, Hess RA, Medrano TI, Locker SH, Levin ER, Cooke PS. Membrane-localized estrogen receptor 1 is required for normal male reproductive development and function in mice. *Endocrinology* 2016; 157:2909-2019.

Ortega MS, Rocha-Frigoni NA, Mingoti GZ, Roth Z, Hansen PJ. Modification of embryonic resistance to heat shock in cattle by melatonin and genetic variation in *HSPA1L*. *J Dairy Sci* 2016; 99:9152-9164.

Selected Publications (continued)

Santoro N, Eisenberg E, Trussell JC, Craig L, Gracia C, Lozano L, Huang H, Alvero R, Casson P, Christman G, Coutifaris C, Diamond M, Legro R, Robinson RD, Schlaff WD, and Zhang H for the Reproductive Medicine Network: Fertility related quality of life from two RCT cohorts with infertility: unexplained infertility and polycystic ovary syndrome. *Hum Reprod* 2016; 31:2268-2279.

Zarate MA, Chang EI, Antolic A, Wood CE. Ketamine modulates fetal hemodynamic and endocrine responses to Pozor M, Freeman D, Troedsson M, Brown M, Morton A, Smith A, McNaughten J. Anatomical variations in epididymal-testicular fusion in stallions and their possible clinical significance. *Equine Vet J* 2016;48:490-495.

Work in Progress Meetings

The WIP meetings are on hiatus this spring to give WIPers time to recharge with new ideas and data. The WIP will reconvene in the fall.

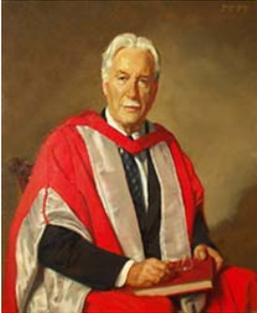
NOTES FROM VIRGIL ON HORSE BREEDING



Horse and Foal, 5th/6th Century, Eastern Roman Empire

Now waneth our care for the sires, our care for the dams hath begun.
When at last they wander in foal, when the tale of the months hath run,
 These let none suffer to pull at the yoke of the ponderous wain,
 Nor to clear at a bound the highway, in fiery race to strain
 Far over the meadow-land, nor in rushing floods to be swimming.
Upon treeless lawns let them graze, and beside slow brooks full-brimming,
Where the moss billows softly, the bank is in deepest greenness arrayed.
By caves be they sheltered, and overscreened by the rocks' cool shade.

Virgil (1st Century BC), *The Georgics*, Book III, *The Georgics of Virgil* by Arthur S. Way (London: Macmillan and Co., 1912)



**DH Barron Reproductive
& Perinatal Biology
Research Program**

University of Florida

**Repro & Perinatal
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Send items of interest to
P.J. Hansen at
Hansen@animal.ufl.edu

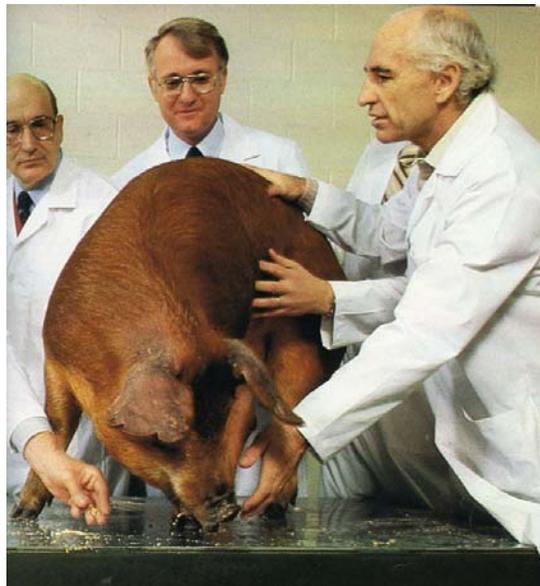
We're on the Web!
www.perinatal.ufl.edu

About the D.H. Barron Reproductive and Perinatal Biology Research Program

History: The Repro program was founded in 1969 by Donald Henry Barron, Fuller Bazer and others. Seminars have been held continuously since that time. Donald Henry Barron (1905-1993) came to UF as the J. Wayne Reitz Professor of Reproductive Biology after a career at Cambridge and Yale. His research in fetal physiology lead to his being referred to as the Father of Scientific Obstetrics and the Father of Fetal-Placental Physiology. Known to his colleagues and students as "Dr. B.", his portrait is on the masthead. In 1969, Fuller Bazer, currently the O.D. Butler Chair in Animal Science at Texas A&M University, was an assistant professor in the Dept. of Animal Sciences. Since then, he has become one of the pioneers in understanding the nature of communication between the embryo and mother. Among the many recognitions he has received was the Wolf Prize in Agriculture in 2003.

Mission: To foster collaborative, multidisciplinary, and integrative approaches to basic and translational research that (i) improves the health of pregnant women and their babies, (ii) enhances the reproductive success of agriculturally important animals and wildlife, and (iii) prepares the next generation of scientists in these research disciplines.

Scope: Basic, translational and clinical research aimed at understanding (i) the biology of reproduction in humans and animals from fertilization to delivery and early postnatal development, and (ii) genetic, epigenetic or environmental influences that cause abnormal pregnancy outcomes, including those influences that predispose the mother and offspring to adult diseases.



SSR Turns 50!

Shown here in a February 1983 story in *Life* entitled "Producing the Perfect Porker" is Phil Dziuk of the University of Illinois (right). Dziuk is considered as the founding father of the Society for the Study of Reproduction, Founded in 1967, the SSR celebrates its 50th Birthday this year and will hold its Golden Anniversary Meeting in Washington DC 1 on July 13-16, 2017. Like the interdisciplinary reproductive biology group at UF, the SSR has continually stressed the synergies that can be realized by fostering communication broadly among scientists interested in reproductive processes.