MARCH/APRIL 2002
Supplement

# Journal of ANDROLOGY

## American Society of Andrology

***27th Annual Meeting***

***April 24–27, 2002***
***Seattle, Washington***

***Program and Abstracts***

Published by THE AMERICAN SOCIETY OF ANDROLOGY

# schedule at a glance

**Wednesday, April 24, 2002**

8:00 am **Andrology Laboratory Workshop**
The Andrology Laboratory of the Future: Impact of the Genomic and Proteomic Revolutions (concludes at 5:00 pm)

5:30 pm **Welcome and Opening Remarks**
5:45 pm **Distinguished Andrologist Award**
6:00 pm **Serono Lecture**
- Of Genes and Genomes
*David Botstein, Stanford University*

7:15 pm **Welcome Reception**

**Thursday, April 25, 2002**

8:00 am **Solvay/Unimed Lecture**
- Androgen Therapy in the Older Man-Where Are We Now and Where Are We Going?
*Lisa Tenover, Emory University*

8:55 am **Distinguished Service Award**
9:05 am **Biopore Lecture**
- Global Analysis of Germline Gene Expression
*Sam Ward, University of Arizona*

10:00 am **Coffee Break and Exhibits**
10:30 am **Concurrent Oral Sessions I, II, III**
- Basic and Clinical Aspects of Male Sexual Dysfunction
- Hormonal Control and Cellular Aspects of Spermatogenesis
- Basic and Clinical Aspects of Sperm Maturation in the Epididymis

12:15 pm **Women in Andrology Luncheon**
- Perspectives on Success and Satisfaction as a Woman in Andrology

1:30 pm **Symposium I: HIV**
- Male Circumcision and HIV Acquisition and Transmission *Ron Gray, Johns Hopkins University*
- HIV: How is it Transmitted and Who is at Risk?
*Dave Phillips, Population Council*
*Christopher Miller, Univ. of CA - Davis*

3:00 pm **Refreshment Break and Exhibits**
3:30 pm **Women in Andrology Lecture**
- Paternal Exposure to Cyclophosphamide: Mechanisms and Consequences
*Barbara Hales, McGill University*

4:30 pm **Poster Session I**
7:00 pm **Trainee Colloquium & Soiree**

**Friday, April 26, 2002**

7:00 am **Past Presidents' Breakfast**
8:00 am **AUA Lecture**
- Nerve Grafting During Radical Prostatectomy
*Ed Kim, Univ. of Tennessee*

8:55 am **Young Investigator Award**
9:05 am **Buckeye Lecture**
- Androgenic Regulation of Apdptosis in the Prostate Gland and in Prostate Cancer
*R. Buttyan, Columbia University*

10:00 am **Coffee Break and Exhibits**

10:30 am **Symposium II: Proteomics/Genomics: Will This Revolutionize Andrological Research?**
- Genetics and Biology of Adult Male Germ Cell Tumors
*Raju S.K. Chaganti, Memorial Sloan-Kettering*
- Modeling Human Disease Through Transgenesis
*Sarah Comerford, UTSWMS & HHI*
- How to Find Cellular Proteins that Sense the Environment *David Garbers, HHMI*

12:00 pm **Laboratory Science Lunch**
1:30 pm **Symposium III: Do Gene Defects Impact Male Reproduction: If So, How?**
- The Battle of the Sexes: *Sry* and the Control of Testis Organogenesis
*Blanche Capel, Duke Univ. Med. Ctr.*
- Natural Potent Androgens: Lessons from Human Genetic Models
*J. Imperato-McGinley, Cornell University*
- Genetic Defects in Male Reproduction
*Stephanie Seminara, Harvard University*

3:00 pm **Refreshment Break and Exhibits**
3:15 pm **Plenary Lecture**
- The Ins & Outs of PSMA, the Evolving and Intriguing Tale of its Prostate Biology & Tumor Targeting *W.D. Heston, Cleveland Clinic*

4:05 pm **Schering Lecture**
- Bioethics and Stem Cell Research
*Laurie Zoloff, San Francisco State Univ.*

5:00 pm **Poster Session II**
7:00 pm **Banquet**

**Saturday, April 27, 2002**

8:00 am **Latin American Lecture**
- Phenotypes of Sperm Pathology: A Journey from Descriptive Morphology to Molecular Genetics
*Hector Chemes, Buenos Aires Children's Hospital*

9:00 am **Symposium IV: Are there Estrogen Effects on the Testis?**
- Uncovering a Role for Estrogen Receptors in Male Reproduction *Ken Korach; NIEHS*
- Estrogen Effects on the Developing Testis: Direct or Indirect Effects? *Richard Sharpe, Edinburgh*
- Developmental Effects of Estrogenic Chemicals
*Fred vom Saal, Univ. Missouri-Columbia*

10:30 am **Break**
10:45 am **Symposium V: The Molecular Basis of Erectile Physiology:** From Bench to Bedside (Sponsored by an unrestricted educational grant from the Bayer Pharmaceutical Division) *Wayne Hellstrom, Tulane University; George Christ, Albert Einstein College of Medicine; Thomas Mills, Medical College of Georgia; Arthur Burnett, Johns Hopkins Hospital)*

12:15 pm **Business Meeting & Awards Presentation**

12:45 pm **Conclusion of Annual Meeting**

2:15 pm **Postgraduate Course: Andrology: Genes, Hormones & Environment**
- Session 1- Prostate Cancer: Update and Diagnostics (including gene-based)

**Sunday, April 28, 2002**

7:20 am **Postgraduate Course**
- Session 2-Infertility and Testicular Cancer: Genetics, Environment, Counseling and Testing (concludes at 10:45 am)

# president's welcome

Welcome to the 27th Annual Meeting of the American Society of Andrology. The Annual Meeting is an important means, perhaps the most important means, by which ASA seeks to fulfill its missions, which are to advance and promote basic knowledge of the male reproductive tract and of clinical andrology, to foster interdisciplinary collaboration and communication, and to attract young investigators to the field. I am certain that you will find these missions fulfilled at this meeting, thanks to Dr. Erv Goldberg, Program Chair, Dr. Jacquetta Trasler, Postgraduate Course Chair and their committees. The symposia, state-of-the-art lectures and Postgraduate Course reflect the unique partnership of basic scientists and clinicians that ASA represents. The sessions are designed to be multi-disciplinary, often are bench-to-bedside, and span new areas of clinical andrology, endocrinology, urology, infertility, contraception, cell biology and molecular biology. Among the topics to be discussed are: scientific and medical implications of the human genome, germline gene expression, HIV transmission, reproductive toxicology, radical prostatectomy, prostate cancer, proteomics/genomics, gene defects, gene therapy, bioethics and stem cell research, sperm motility, estrogen effects on the testis, and erectile dysfunction. I believe it fair to say that, through the efforts of Drs. Goldberg, Trasler and their committees, the 27th Annual Meeting reflects the diverse interests of ASA members exceptionally well, and, at the same time, takes us beyond our comfort zones --- exactly what a vibrant meeting should do.

Perhaps the least visible component of a successful meeting is local arrangements. Fortunately, we have a very strong Local Arrangements Committee in Seattle, chaired by Dr. Alvin Matsumoto. If all goes smoothly, part of the reason is the hard, behind-the-scenes work of Dr. Matsumoto and his committee. (If all is not smooth, blame the President.)

We also all owe a debt of gratitude to Sabrina Ritchie and Kent Lindeman of the ASA business office, Holland-Parlette Associates. Their roles in organizing a meeting such as this, and, as importantly, in the day-to-day running of the Society, are essential. I am most appreciative.

Finally, you should all be aware that the annual meeting, and therefore our Society, is benefiting greatly from the generosity of its sponsors. Indeed, the sponsorship of the meeting is growing --- and needs to. We cannot, and must not, take the support we receive lightly. Please express your thanks to our sponsors when you visit the exhibits and when you attend the lectures and symposia sponsored by them.

With any luck, the year 2002 will be better than 2001! Hopefully, our 27th Annual Meeting will be among its highlights. I hope you enjoy it, and gain greatly from it.

**Barry R. Zirkin, PhD.**
*President, American Society of Andrology*

past presidents of the american society of andrology

| | | | | | |
|---|---|---|---|---|---|
| 1975-1977 | Emil Steinberger | 1984-1985 | Rudi Ansbacher | 1992-1993 | Ronald S. Swerdloff |
| 1977-1978 | Don W Fawcett | 1985-1986 | Anna Steinberger | 1993-1994 | Bernard Robaire |
| 1978-1979 | C. Alvin Paulsen | 1986-1987 | William D. Odell | 1994-1995 | Glenn R. Cunningham |
| 1979-1980 | Nancy J. Alexander | 1987-1988 | Larry L. Ewing* | 1995-1996 | Marie-Claire Orgebin-Crist |
| 1980-1981 | Philip Troen | 1988-1989 | C. Wayne Bardin | 1996-1997 | Arnold M. Belker |
| 1981-1982 | Richard M. Harrison | 1989-1990 | Rupert Amann | 1997-1998 | Terry T. Turner |
| 1982-1983 | Richard J. Sherins | 1990-1991 | Howard Nankin | 1998-1999 | Richard V. Clark |
| 1983-1984 | Andrzej Bartke | 1991-1992 | David W. Hamilton | 1999-2000 | Barry T. Hinton |
| | | | | 2000-2001 | J. Lisa Tenover |

* Deceased

March 27-29 Testis Workshop
March 30-1 ASA

# table of contents

# American Society of Andrology

## OFFICERS

| | |
|---|---|
| President | Barry R. Zirkin, PhD |
| Vice President | Jon L. Pryor, MD |
| Treasurer | Christopher J. DeJonge, PhD, HCLD |
| Secretary | Terry R. Brown, PhD |
| Past President | J. Lisa Tenover, MD, PhD |

## MEMBERS OF THE EXECUTIVE COUNCIL

Janice L. Bailey, PhD; Adrian S. Dobs, MD, MHS ; Joanna Ellington, DVM, PhD; Janice Evans, PhD; Marc Goldstein,MD, FACS; Michael D. Griswold, PhD; Jonathan P. Jarow, MD; Gary Klinefelter, PhD; Patricia L. Morris, PhD; Pasquale Patrizio, MD; Kenneth P. Roberts, PhD; Carlos A. Suarez-Quian, PhD

## COMMITTEE CHAIRS

| | |
|---|---|
| 2002 Program Chair | Erwin Goldberg, PhD |
| 2002 Local Arrangements | Alvin Matsumoto, MD |
| Andrology Laboratories | Gregory Kopf, PhD |
| Archives & History | Jean Fourcroy, MD, PhD, MPH |
| Awards | Ashok Agarwal, PhD, HCLD |
| | Joanna Ellington, DVM, PhD |
| Constitution & Bylaws | D.N. Rao Veeramachaneni, DVM, PhD |
| Development | J. Lisa Tenover, MD, PhD |
| Finance | Gail Prins, PhD |
| Future Meetings | Bernard Robaire, PhD |
| Industrial Relations & Exhibits | Charles Muller, PhD |
| International Liaison | Carlos Morales, DVM, PhD |
| Laboratory Science Forum | Aniela Bollendorf, MT |
| Membership | Marc Goldstein, MD, FACS |
| | Matthew Hardy, PhD |
| Minority Affairs | Samir Subas Raychoudhury, MVSC, PhD |
| Nominating | Wael Salameh, PhD |
| Publications | Paul Cooke, PhD |
| Society Liaison | Wayne Hellstrom, MD, FACS |
| Trainee Affairs | Stuart Ravnik, PhD |

## JOURNAL OF ANDROLOGY EDITORIAL OFFICE

6-160 Jackson Hall, 321 Church Street SE, Minneapolis, MN 55455
Phone: (612) 625-1488 • Fax: (612) 625-1163 • Email:andrology@lenti.med.umn.edu
Editors-in-Chief: David W. Hamilton, PhD and Jon L. Pryor, MD
Editorial Assistant: Michele Lewis

## EXECUTIVE OFFICE

ASA • 2950 Buskirk Avenue, Suite 170 Walnut Creek, CA 94596
Phone: (925) 472-5910 • Fax: (925) 472-5901 • Email: asa@hp-assoc.com • www.andrologysociety.com
Executive Director: Sabrina Ritchie • Administrative Manager: Katka Loomisova

## NOTICE TO READERS

All events will be held at the Seattle Sheraton Hotel and Towers. Room names are listed directly beneath the lecture/event name in the detailed program of events. Every effort has been made to ensure that the information printed here is correct; however, details (especially room assignments) are subject to change. Be sure to check the hotel reader board for the most up-to-date information while you are at the meeting.

# general information

## additional information

For additional information on ASA's 27th Annual meeting, contact ASA staff via phone at (925) 472-5910, fax at (925) 472-5901 or email asa@hp-assoc.com or visit ASA's website at http://www.andrologysociety.com.

## annual banquet

The 2002 banquet will be held at the Sheraton Seattle Hotel & Towers on Friday, April 26 at 7:00pm. Tickets can be purchased for $60.00 per person. Extra tickets can be purchased on-site.

## exhibits

ASA's Exhibit Hall features equipment and information pertinent to andrologists and will be open April 25-26 (Thursday-Friday). Make a point to visit all the exhibitors to be eligible to win the raffle drawing (held in the Exhibit Hall on Friday afternoon).

## hotel accommodations

The official hotel for the 27th Annual Meeting of the American Society of Andrology is the Sheraton Seattle Hotel & Towers. For reservations, please call (800) 204-6100 or (206) 447- 5555. Don't forget to mention you are attending the ASA Annual Meeting to receive the reduced room rate. The following room rates are being offered to ASA attendees until March 20, 2002. After that regular room rates may apply, so we urge you to make your reservations as soon as possible. Rates are $176 per person (single/double occupancy). A limited number of rooms have been set aside for students at the $131 per person rate. These will be filled on a first-come, first served basis.

Overflow hotel accommodations can be made at the West Coast Grand Hotel which is located 2 blocks from the Sheraton. Rates are $162 per night, single/double occupancy and can be made by calling (800) 325-4000 or (206) 971- 8000. Please indicate you are with the American Society of Andrology to receive group rate.

## laboratory science forum

The 2002 Laboratory Science Forum will be held at 12:00 noon on Friday, April 26. Lunch tickets are available for $30 per person.

## opening reception

Join us on Wednesday, April 24 at 7:15 pm for a Welcome Reception to connect with friends and colleagues. Admission to the reception is included in your registration fee.

## poster sessions

Poster sessions will be held Thursday (4:30 pm-7:00 pm) and Friday (5:00 pm-7:00 pm) in Grand Ballroom A-B and the Grand Ballroom Lobby. Poster boards will be 4' X 8'. Presenters should put up their posters the morning of their session and stand near their posters during the formal poster session to answer questions about their research and findings. Posters must be removed at the close of the session. Any posters remaining the following morning will be removed.

## registration

The meeting registration and information desk will be open at the following times for on-site registration:

| | |
|---|---|
| Wednesday, April 24 | 7:00 am-6:00 pm |
| Thursday, April 25 | 7:00 am-6:00 pm |
| Friday, April 26 | 7:00 am-6:00 pm |
| Saturday, April 27 | 7:00 am-5:00 pm |
| Sunday, April 28 | 7:00 am-10:00 am |

## slide preview room

The Juniper Room, located on the second floor of the hotel, will be available from 7:00 am until 11:00 pm, Wednesday, April 24 through Saturday, April 27 for previewing slides and preparing presentations.

## trainee colloquium & soirée

The Trainee Colloquium will begin at 7:00 pm on Thursday, April 25 with the Soirée following immediately thereafter. Both events are free of charge and open to everyone.

## transportation

The Sheraton Seattle Hotel & Towers is located in downtown Seattle 20 minutes from the Seattle Tacoma Airport Transportation options from the airport to the hotel include Airporter shuttles which leave every 1/2 hour ($8 one way) and taxi ($25-30 one way).

## women in andrology

The Women in Andrology group, which exists to promote the visibility and contributions of women within the Society and the field of Andrology, meets for lunch each year at ASA's Annual Meeting. This year's luncheon will be held Thursday, April 25 at 12:15pm and will highlight "Perspectives on Success and Satisfaction as a Woman in Andrology." Tickets are $30 per person.

# message from the program chair

It is a pleasure to welcome you to the 27th Annual Meeting of the American Society of Andrology. The Program Committee and I hope that we have provided a rich and diverse menu of topics presented by our colleagues engaging in outstanding research and for our trainees exploring the ambience of a National Meeting early in their careers. It has been a real challenge to follow our last meeting held in conjunction with the International Society of Andrology. While our Program is considerably smaller, it should be satisfying and there should be increased opportunity for the personal interactions that make such gatherings memorable. Both clinical and basic research is well-represented in this years program. We begin with the Serono lecturer, Dr. David Botstein, introduced by Dr. David Garbers on Wednesday evening. Botstein will present his views on the scientific and medical implications of the human genome. David Botstein is Professor and Chairman, Department of Genetics, Stanford University and a member of the National Academy of Sciences. He began his theoretical contributions on linkage mapping of the human genome in 1980 by suggesting, with collaborators, that restriction fragment length polymorphisms (RFLPs) could be used to produce a linkage map of the human genome and to map the genes that cause disease in humans.His laboratory is taking a broad, interdisciplinary approach to study the biology shared by all eukaryotes. Ultimately they seek to apply this knowledge toward an understanding of human disease mechanisms. The annual American Urological Association Lecture will be presented by Dr. Ed Kim, a urologist at the University of Tennessee, who will discuss his pioneering work on Nerve Grafting During Radical Prostatectomy. The Ernst Schering Research Foundation Lecturer this year is Dr. Laurie Zoloff, San Francisco State University, who will address the timely issue of Bioethics and Stem Cell Research. The Latin American Lecture entitled "Phenotypes of Sperm Pathology: A Journey from Descriptive Morphology to Molecular Genetics" will be presented by Hector Chemes, Buenos Aires Children's Hospital, Argentina. The American Society of Andrology/Women in Andrology Lecture will feature Dr. Barbara Hales speaking about "Paternally Mediated-Developmental Toxicity". Five outstanding panels of scientists have been assembled to present cutting edge advances of basic and clinical relevance in Symposia on HIV Risks and Transmission (Gray, Phillips and Miller); Proteomics and Genomics in Andrological Research (Chaganti, Comerford, Garbers); Gene Defects and Male Reproduction (Capel, Imperato-McGinley, Seminara); Estrogen Effects on the Testis (Korach, Sharpe and vom Saal); and, with the support of an unrestricted educational grant from Bayer Pharmaceutical Company, a Symposium on Erectile Dysfunction organized by Dr.Jonathon Jarow, moderated by Dr. Wayne Hellstrom. Featured speakers are Drs. Arthur Burnett, George Christ and Tom Mills. The program is augmented by the Biopore sponsored lecturer, Sam Ward, University of Arizona, on "Global Analysis of Germline Gene Expression"; a Plenary Lecture by W.D. Heston, Cleveland Clinic, entitled " The "Ins & Outs of PSMA, the Evolving and Intriguing Tale of its Prostate Biology & Tumor Targeting "; the Buckeye Lecture on "Androgenic Regulation of Apoptosis in the Prostate Gland and in Prostate Cancer" presented by R. Buttyan, Mem. Sloan Kettering during the Solvay/Unimed Plenary session. Lisa Tenover will discuss Hormone Replacement Therapy in the male during the Solvay/Unimed Plenary session. Members, trainees and guests of the Society will participate in three concurrent oral sessions and two poster sessions where the latest ongoing research in the field of andrology will be presented and discussed by the meeting participants at large. ASA invites all to enjoy this full menu of scientific advances in the incomparable setting of the Pacific Northwest.

**Erwin Goldberg, PhD**
*Chair, 2002 Program Committee*

**Program and Abstract Review Committee:**
*Terry Brown, Chung Lee, Ken Tung, Lisa Tenover, Jon Pryor, Mary Ann Handel, Mike Griswold, Gail Prins, Bernard Robaire, Stu Ravnik, Chris DeJonge, Barry Hinton, Patricia Olds Clarke, Dorrie Lamb, Richard Bronson, Matt Hardy, Greg Kopf, Art Matsumoto, Debbie O'Brien, Jon Jarow and Barry Zirkin.*

# distinguished andrologists

| | | | | | |
|---|---|---|---|---|---|
| 1976 | Roy O. Greep & M.C. Chang | 1977 | Robert E. Mancini | 1978 | Robert S. Hotchkiss |
| 1979 | Thaddeus Mann | 1980 | John MacLeod | 1981 | Alexander Albert |
| 1982 | Eugenia Rosemberg | 1983 | Kristen B.D. Eik-Nes | 1984 | Mortimer B. Lipsett |
| 1985 | Robert H. Foote | 1986 | Alfred D. Jost | 1987 | Emil Steinberger |
| 1988 | Yves W. Clermont | 1989 | C. Alvin Paulsen | 1990 | Marie-Claire Orgebin-Crist |
| 1991 | Philip Troen | 1992 | C. Wayne Bardin | 1993 | Anna Steinberger |
| 1994 | Richard J. Sherins | 1995 | Rupert P. Amann | 1996 | J. Michael Bedford |
| 1997 | Brian P. Setchell | 1998 | Ryuzo Yanagimachi | 1999 | Richard D. Amelar |
| 2000 | Bayard T. Storey | 2001 | Frank French | | |

*The Distinguished Andrologist Award is sponsored by the American Society of Andrology.*

# serono award lectureship

David Botstein, Ph.D. is currently the Professor and Chairman of the Department of Genetics at the Stanford University School of Medicine. Botstein was educated at Harvard (A.B. 1963) and the University of Michigan (Ph.D. 1967). He joined the faculty of the Massachusetts Institute of Technology, where he rose through the ranks from Instructor to Professor of Genetics. In 1987 he moved to Genentech, Inc. as Vice President - Science and in 1990 he moved to his present position: Stanford W. Ascherman, MD, Professor and Chairman of the Department of Genetics, Stanford University School of Medicine.

Dr. Botstein's research has centered on genetics, especially the use of genetic methods to understand biological functions. The bacteriophage P22 was the focus of his earliest research, which included studies of DNA replication, recombination, head assembly and DNA maturation. Dr. Botstein also contributed to understanding of the regulation and evolution of temperate bacteriophages. In the early 1970's Dr. Botstein turned to budding yeast (Saccharomyces cerevisiae) and devised novel genetic methods to study the functions of the actin and tubulin cytoskeletons. Other scientific interests of the Botstein laboratory include protein secretion (both in bacteria and yeast) and the use of localized random mutagenesis technologies to understand protein structure/function relationships. Finally, Dr. Botstein began his theoretical contributions on linkage mapping of the human genome beginning in 1980 by suggesting, with collaborators, that restriction fragment length polymorphisms (RFLPs) could be used to produce a linkage map of the human genome and to map the genes that cause disease in humans. Recent research activities include studies of yeast genetics, genomics and cell biology including the development (with J. Michael Cherry) of the Saccharomyces Genome Database; and (with P. O. Brown) the development of DNA microarray technology and analysis methods and their application to classifying and understanding human cancers.

Dr. Botstein was elected to the U.S. National Academy of Sciences in 1981 and to the Institute of Medicine in 1993. He has won several awards, notably the Eli Lilly Award in Microbiology (1978), the Genetics Society of America Medal (1988) and the Allen Award of the American Society of Human Genetics (1989). He served on many policy-making and peer-review committees, most recently the NAS/NRC study on the Human Genome Project (1987-88), the NIH Program Advisory Panel on the Human Genome (1989-90) and the Advisory Council of the National Center for Human Genome Research (1990-1995).

Serono Lectureship Recipients

| | | | | | |
|---|---|---|---|---|---|
| 1980 | C. Alvin Paulsen | 1981 | Pierre Soupart | 1982 | Kevin J. Catt & Maria L. Dufau |
| 1983 | J. Michael Bedford | 1984 | C. Wayne Bardin | 1985 | David M. De Kretser |
| 1986 | Ronald S. Swerdloff | 1987 | Roger V. Short | 1988 | Roger Guillemin |
| 1989 | Frank S. French | 1990 | David C. Page | 1991 | Tony M. Plant |
| 1992 | Yves Clermont | 1993 | Leroy Hood | 1994 | Michael D. Griswold |
| 1995 | Marie-Claire Orgebin-Crist | 1996 | Norman B. Hecht | 1997 | Patrick C. Walsh |
| 1998 | Jurrien Dean | 1999 | Neal First | 2000 | Bert O'Malley |
| 2001 | John D. Gearhart | | | | |

*The Serono Lectureship is sponsored by Serono Laboratories, Inc.*

# distinguished andrologist award

Dr. Geoffrey Malcolm Hasting Waites is the recipient of the 2002 Distinguished Andrologist Award. Emeritus Professor Waites has had a highly distinguished career in Andrology over the past 40 years. Dr. Waites graduated from the Universities of Birmingham and Cambridge, UK with BSc, MA, PhD, and ScD (Doctor of Science) degrees. His academic career has included positions as: Associate Professor of Physiology, University of Sydney, Australia; and Professor & Chairman, Physiology & Biochemistry and Dean, University of Reading, UK.

Dr. Waites' research also included studies at the British, French and Australian Government Science Institutes. In 1983, he was invited to Geneva, Switzerland as Consultant and Staff Member, for the World Health Organization (WHO). He then became the appointed manager of the WHO Male Task Force of the Special Program of Research, Development and Research Training in Human Reproduction (HRP).

Dr. Waites has over 200 publications comprising invited reviews, chapters in books, and research papers on: cardiovascular and reproductive physiology; blood flow; endocrine and metabolic control of the testis and the blood-testis barrier; the epididymis; the scrotum and thermoregulation; and antifertility applications (chlorinated sugars). Also, his contributions include clinical and scientific publications reporting WHO-supported studies and clinical trials and WHO technical manuals and policy reports.

Dr. Waites' principal accomplishments include: research contributions into the physiology of the blood-testis barrier; the scientific coordination of the multi-centre WHO trials establishing the contraceptive efficacy of male hormonal methods; development of the now universal standard for semen analysis the WHO Laboratory Manual for Semen Analysis and Cervical Mucus Interaction (2nd -4th editions); and establishment of WHO andrology workshops in developing countries (China, Chile, Indonesia, Kenya, Morocco, Vietnam,) and in Russia. Dr. Waite was also a founding member of the British Andrology Society and the founder member and President of the International Society of Andrology (1993-1997).

Dr. Waites' colleagues were most impressed with his dedication to improving the standards of andrology on a global basis. "Dr. Waites has uniquely been able to translate his accomplishments as a researcher into programmatic developments that have markedly enhanced the development of andrology over the past few decades". "Very few andrologists have had such a tremendous scientific impact worldwide". "Geoff has acted as a mentor for andrology in emerging countries, and for this is revered around the world". For his global impact in the field of Andrology, the American Society of Andrology is honored to award Dr. Waites the 2002 Distinguished Andrologist Award.

# distinguished serviceaward

Terry T. Turner, PhD is recipient of the 2002 Distinguished Service Award. Dr. Turner is currently a Professor of Urology and Cell Biology at the University of Virginia School of Medicine. He received his PhD from the University of Georgia in 1974 with Dr. Doyle Johnson. His postdoctoral studies were at the Department of OB/GYN at University of Texas Health Science Center at San Antonio with Dr. Carl Pauerstein. Since that time, Dr. Turner has been at UVA, including some early work with Dr. Stuart Howards who adapted in vivo micro puncture techniques for study of epididymal and seminiferous tubule function. His internationally recognized work has been funded by the NICHD and the NIDDK. This work has been primarily in the areas of: 1) epididymal epithelium regulation and the role of this unique microenvironment on sperm maturation; and 2) testicular mechanisms of vascular pathology and oxidative stress leading to disruption of spermatogenesis. These studies have resulted in over 100 publications to date.

In addition to his scholarly activities, Dr. Turner has played a pivotal role in the American Society of Andrology since its inception. This includes being a charter member of the society and attending every meeting since inception! Dr. Turner's list of activities in the society include: member of the Executive Council 1979-1982; Chair, Student Affairs Committee, 1981-1984; Chair, Awards Committee, 1986-1987; Chair, Nominating Committee, 1992; Chair, 1995 Program Committee, 1993-1995; Chair, Long Range Planning Committee, 1996; Secretary, 1988-1992; Editorial Board, Journal of Andrology, 1993-1997; Vice-President, 1996; President, 1997; Immediate Past President, 1998; Reviewer of manuscript for Journal of Andrology, 1980-present; Co-Chair of ASA-sponsored conference, the Third International Conference on the Epididymis, Charlottesville, VA, May 29-June 1, 2002.

Dr. Turner's valuable role in our society is reflected in the letters of nomination sent on his behalf. "Terry represents the 'conscience' of our society and has always been one of its strongest supporters". "Terry has served the society in many ways and selflessly. I can not think of anyone that would merit this distinction more". "Terry's enthusiasm for the field of Andrology, as well as his great stories and sense of humor were a major reason that I joined the ASA as a trainee. He has helped shaped a culture of scientific openness and fun that is so unique to our organization".

Because Dr. Terry Turner has served the American Society of Andrology with distinction and has worked for many years to create an organization we are all proud of, we are honored to award him with the 2002 Distinguished Service Award.

Distinguished Service Award Recipients

| | | | | | |
|---|---|---|---|---|---|
| 1994 | C. Alvin Paulsen | 1995 | Andrzej Bartke | 1996 | Philip Troen |
| 1997 | Marie-Claire Orgebin-Crist | 1998 | Rupert P. Amann | 1999 | David W. Hamilton |
| 2000 | Bernard Robaire | 2001 | Gail S. Prins | | |

*The Distinguished Service Award is sponsored by the Genetics & IVF Institute*

# young andrologist award

Christopher L.R. Barratt

Christopher was born in a small village in Wales. His interest in biology led him to undertake a BSc in Zoology at Swansea University where he thoroughly enjoyed the student lifestyle and played rugby at every opportunity. Following a short period as a secondary school teacher, which he found less interesting than hoped, he decided to pursue his interest in human reproduction. His PhD supervisor was Jack Cohen and during this challenging educational 3 year period, he realized the importance of experimental design and tolerance of supervisors. At 27 and feeling like an eternal student, he realized it was time to earn some money, so he secured a position with Ian Cooke in Sheffield. The initial post was as an embryologist in a natural cycle IVF program which was thought by many in the human reproductive arena to be a lot of hard work for minimal reward i.e. low live birth rate per treatment cycle started. Fertilization failure was a significant issue (only one egg!) which stimulated his interest in sperm function and led to the development of the male reproduction research program. During this period, he became interested in teaching semen analysis and couldn't understand why most people were unable to accurately count sperm. This provided the impetus to develop structured training courses, first in the UK as part of the British Andrology Society, and then throughout Europe, as part of ESHRE. Scientifically, he was fascinated by the transport of sperm through, and interaction with, the human female reproductive. Logically these investigations included interaction of selected sperm populations with the egg vestments, an area that remains his main focus. Christopher moved back to Birmingham in 1997 to direct the assisted conception centre and become Professor of Reproductive Medicine at Birmingham University. Interestingly, in the current climate of single embryo transfer it is possible that the hours spent investigating natural cycle IVF may turn out to be a vital learning experience.

Christopher is a firm believer in interdisciplinary research and always trying to employ people who have complementary clinical and scientific skills to himself. Additionally, he is very keen to nurture young scientists, encouraging them, when appropriate, to develop into independent investigators.

His professional activities include: Associate Editor of *Human Reproduction*; Editorial board member for *Journal of Andrology* and Editorial board member for *Human Fertility*.

Young Andrologist Award Recipients

| | | | |
|---|---|---|---|
| 1982 | L.J.D. Zaneveld | 1983 | William B. Neaves |
| 1984 | Lonnie D. Russell | 1985 | Bruce D. Schanbacher |
| 1986 | Stephen J. Winters | 1987 | Ilpo T. Huhtaniemi |
| 1988 | Larry Johnson | 1989 | Barry T. Hinton |
| 1990 | Luis Rodriguez-Rigau | 1991 | Patricia M. Saling |
| 1992 | Gary R. Klinefelter | 1993 | Robert Chapin |
| 1994 | Wayne J.G. Hellstrom | 1995 | Christopher DeJonge |
| 1996 | Paul S. Cooke | 1997 | Gail A. Cornwall |
| 1998 | William R. Kelce | 1999 | Stuart E. Ravnik |
| 2000 | Matthew P. Hardy | 2001 | Jacquetta Trasler |

*The Young Andrologist Award is sponsored by the Texas Institute for Reproductive Medicine and Endocrinology, P.A.*

New Investigator Award Recipients

| | | | |
|---|---|---|---|
| 1983 | Thomas T. Tarter | 1984 | Peter S. Albertson |
| 1985 | Randall S. Zane | 1986 | Mark A. Hadley |
| 1987 | Peter Grosser | 1988 | Stuart E. Ravnik |
| 1989 | Tracy L. Rankin | 1990 | Donna O. Bunch |
| 1991 | Robert Viger | 1992 | John Kirby |
| 1993 | Michael A. Palladino | 1994 | Linda R. Johnson |
| 1995 | Mehdi A. Akhondi | 1996 | Wei Gu |
| | | | Daniel B. Rudolph |
| 1997 | Loren D. Walensky | 1998 | Dolores D. Mruk |
| 1999 | Jacques J. Tremblay | 2000 | Jeffrey J. Lysiak |
| 2001 | Alexander T.H. Wu | | |

*The New Investigator Award is sponsored by the West Michigan Reproductive Institute, P.C.*

# new investigator award

The New Investigator Award is given to the ASA trainee member with the best abstract and research presentation at the Annual Meeting. The award encourages trainee members to submit and present their best work and contribute to the scientific excellence of the Society.

The recipient of the 2002 New Investigator Award will be announced during ASA's Annual Business Meeting, to be held Saturday, April 27, 2002 at 12:15 pm.

# thank you sponsors

## The American Society of Andrology

*wishes to thank the following organizations for their generous support of the 2002 Annual Meeting, Postgraduate Course and Laboratory Workshop*

*Gold Club*-a minimum of $10,000 contribution to an ASA endowment fund

**Buckeye Urology and Andrology, Inc.**

*Silver Club*-a minimum of $5,000 contribution to an ASA endowment fund

**West Michigan Reproductive Institute, P.C.**

*Sustaining Sponsor*-a minimum of $500 contribution to ASA for 5 years or more

**American Urological Association**
**California Cryobank**
**Genetics & IVF Institute**
**Hamilton Thorne Research**
**Serono Laboratories**
**Texas Institute for Reproductive Medicine and Endocrinology**

*Sponsors*

**Bayer Pharmaceutical Division**
**BIOPORE, Inc.**
**Ernst Schering Research Foundation**
**Fertility Solutions, Inc.**
**Glaxo Smith Kline Research & Development**
**Lilly ICOS**
**Merck & Co., Inc.**
**Sigma-Tau Consumer Products, a divison of Sigma-Tau Pharmaceuticals**
**UniMed Pharmaceuticals Inc, a Solvay Pharmaceuticals, Inc. Company**

*The American Society of Andrology gratefully acknowledges these contributors to the various ASA Endowment or Asset Funds for the past year:*

**$1,000 - $4,999**
Gail Prins, PhD
University of Minnesota

**$250-$999**
Frank French
Bayard Storey, PhD
J. Lisa Tenover, MD, PhD

**$100-$249**
Christopher De Jonge, PhD
Robert Foote, PhD,MS
Matthew Hardy, PhD
Alan Menge, PhD
Charles Muller, PhD
Jon Pryor,MD
Carlos Suarez-Quian, PhD

**$1-$99**
Andrzej Bartke, PhD
Sally Perreault-Darney, PhD
Edward Eddy, PhD
David Hamilton, PhD
Gabor Huszar, MD
Masaichi Kimura, MD,PhD
Jose Ballesca Lagarda
Marvin Meistrich, PhD
Dirk Propping, MD,PhD
Omar Fernandez-Sandoval, MD
Rosario Tapia-Serrano
Richard Sherins, MD
Hiroki Shima, MD,PhD
Hitoshi Takeshima, MD
Christina Wang, MD
Nancy Warner, MD
Jan Wolski, MD

# course objectives & CME credit information

## annualmeeting

Following this program the participant should be able to:

- Relate the technological advances of the human genome project to clinical importance
- Understand the effects and consequences of hormone replacement therapy in the male
- Understand the role of genes and genetic defects in male reproduction
- Be aware of risks and transmission of HIV
- Recognize the toxic effects of cyclophosphamide exposure
- Assess the procedure of nerve grafting during radical prostatectomy
- Describe the role of genetics and gene therapy in prostate cancer
- Understand the latest developments in the area of erectile dysfunction
- Recognize estrogen effects on the testis

The University of Minnesota designates this continuing medical education activity for 16.5 hours in category 1 credit toward the AMA Physician's Recognition Award. Each physician should claim only those hours of credit actually spent in the educational activity.

## postgraduatecourse

Following the program the participant should be able to:

- Describe the clinical epidemiology of prostate cancer in different populations
- Identify the role of estrogens in the development and promotion of prostate disease as well as new research and treatments for androgen-refractory prostate cancer
- Evaluate how basic science advances in genetics and endocrinology will influence the clinical management of prostate cancer
- Describe the reproductive consequences (e.g. infertility birth defects) of the father's exposure to drugs and chemicals
- Understand the role of cancer susceptibility genes and environmental exposures in the pathophysiology of testicular cancer
- Describe the role of genetic testing and counselling in clinical andrology

The University of Minnesota designates this continuing medical education activity for 7.0 hours in category 1 credit toward the AMA Physician's Recognition Award. Each physician should claim only those hours of credit actually spent in the educational activity.

Both the Annual Meeting and Postgraduate Course have been planned and implemented in accordance with the Essentials and Standards of the Accreditation Council for Continuing Medical Education through the joint sponsorship of the University of Minnesota and the American Society of Andrology. The University of Minnesota is accredited by the ACCME to provide continuing medical education for physicians.

# agenda

**Wednesday, April 24, 2002**

**Andrology Laboratory Workshop**
The Andrology Laboratory of the Future: Impact of the Genomic and Proteomic Revolutions WEST ROOM

8:00 am **Introduction**
*Gregory S. Kopf, Ph.D.,Wyeth-Ayerst Research*
*Pasquale Patrizio, M.D., Univ. of Pennsylvania*

8:15 am **Session I**: Application of Gene Technology to the Andrology Lab of the Future
*Michael Griswold, Ph.D., Washington State Univ.*

9:00 am Diagnostic Markers for the Future Andrology Laboratory coming from Basic Science Research
*Norman B. Hecht, Ph.D., Univ. of Pennsylvania*

9:45 am **Refreshment Break**

10:15 am **Session II**: Genetics of Male Infertility
*Renee Reijo, Ph.D., Univ. of California, San Francisco*

11:00 am Automated Instrumentation and the Detection of Sperm DNA Integrity for Diagnosis and Prognosis of Male Infertility
*Donald P. Evenson, Ph.D., So. Dakota State Univ.*

12:00 pm **General Discussion**

12:15 pm **Lunch**

1:30 pm **Session III**: Calcium Signaling Pathways During Fertilization: How They Work and How They Go Astray
*Harvey M. Florman, Ph.D., Univ. of Massachusetts*

2:15 pm Defining a Molecular View of Fertilization: Mapping the Sperm Cell Surface
*Timothy Quill, Ph.D., Cecil & Ida Green Ctr. For Reprod. Bio. Sci.*

3:00 pm **Refreshment Break**

3:30 pm **ProteinChip® Technology**: Pioneering Clinical Proteomics for Predictive Medicine
*Deborah Diamond, Ph.D., Ciphergen Biosystems, Inc*

4:15 pm **General Discussion**

4:45 pm **Summary**
*Gregory S. Kopf, Ph.D. Pasquale Patrizio, M.D., and Charles Muller, Ph.D.*

5:30 pm **Welcome and Opening Remarks**
GRAND BALLROOM C

5:45 pm **Distinguished Andrologist Award**
GRAND BALLROOM C

6:00 pm **Serono Lecture**
GRAND BALLROOM C
• Of Genes and Genomes
*David Botstein, Stanford University*
introduced by *David Garbers*

7:15 pm **Welcome Reception**
CIRRUS BALLROOM

**Thursday, April 25, 2002**

8:00 am **Solvay/Unimed Pharmaceuticals Lecture**
GRAND BALLROOM C
*Chair: Rupert Amann*
• Androgen Therapy in the Older Man-Where Are We Now and Where Are We Going?
*Lisa Tenover, Emory University*

8:55 am **Distinguished Service Award**

9:05 am **Biopore Lecture**
GRAND BALLROOM C
*Chair: Mary Ann Handel*
• Global Analysis of Germline Gene Expression
*Sam Ward, University of Arizona*

10:00 am **Coffee Break and Exhibits**
GRAND BALLROOM B & C

10:30 am **Concurrent Oral Sessions I, II, III**
**SESSION I:** Basic and Clinical Aspects of Male Sexual Dysfunction
GRAND BALLROOM C
*Chairs: Arthur Burnett and Ron Lewis*

1. **The effect of as-needed tadalafil treatment of erectile dysfunction in men with diabetes**
*H. Padma-Nathan, G. Anglin, A. Yu, S.J. Whitaker*

2. **A single oral dose of vardenafil had no acute effect on sperm motility in healthy males**
*T. Taylor, R.J. Bauer, G. Rohde, F. Kiesewetter*

3. **Endothelial nitric oxide synthase gene transfer restores nitric oxide production and erectile function in diabetes**
*T.J. Bivalacqua, D. Adams, H.C. Champion, D.Y. Yang, A.B. Abdel-Mageed, W.J.G. Hellstrom*

4. **Long term efficacy of sildenafil citrate following radical prostatectomy (RP): 3-year follow-up**
*R. Raina, D.R. Nelson, A. Agarwal, M.M. Lakin, E. Klein, C.D. Zippe*

5. **Long term efficacy and compliance of intracorporeal (IC) penile injections in patients with erectile dysfunction (ED) following radical prostatectomy**
*R. Raina, M.M. Lakin, R.A. Saleh, A. Agarwal, C.D. Zippe*

6. **A comparison of long term penile damage after definitive prostate radiation therapy (RT) vs. radical retropubic prostatectomy (RRP) in men with post treatment ED: Is there a difference?**
*A. McCullough, N. Mani, S. Telegrafi*

# agenda cont.

**SESSION II:** Hormonal Control and Cellular Aspects of Spermatogenesis
WEST ROOM A
*Chairs: Debbie O'Brien and Stu Ravnik*

7. **Contraceptive efficacy of a depot androgen and progestin combination in men**
*D. J. Handelsman, L. Turner, S. Wishart, A. J. Conway, P.Y. Liu, E. Forbes, R. I. McLachan*

8. **Protein kinase a (PKA) enhancement of gata-4-mediated gene expression: A link with hormone-activated cell signaling**
*J.J. Tremblay, F. Hamel, R.S. Viger*

9. **Use of the yeast three-hybrid system to elucidate ternary protein interactions during meiosis**
*C.A. Kersten, S.E. Ravnik*

10. **Activation of the mitochondria-dependent apoptotic pathway in heat-induced testicular germ cell apoptosis in fas ligand-defective mice**
*A.S. Hikim, H.H. Lue, S. Rodriguez, P.H. Yen, C. Wang, R.S. Swerdloff*

11. **Expression and inhibition of recombinant GAPDS, the sperm-specific isoform of glyceralde hyde 3-phosphate dehydrogenase**
*WeiDong Qu, P.L. Magyar, K. Miki, M. Goto, D.A. O'Brien*

12. **TPX-1 Binding studies of the mouse outer dense fiber protein TPX-1**
*D.M. Hickox, D.M. de Kretser, M.K. O-Bryan*

**SESSION III:** Basic and Clinical Aspects of Sperm Maturation in the Epididymis
WEST ROOM B
*Chairs: Matt Hardy and Patricio Morales*

13. **PC2-The cystatin-related epididymal spermatogenic (CRES) protein is an inhibitor of the prohormone processing serine protease PC2**
*G.A. Cornwall, A. Cameron, I. Lindberg, D. Hardy, N. Hsia*

14. **Potential role of a Na+/HCO3- cotransporter in mouse sperm capacitation**
*I. Demarco, F. Espinoza, J. Sosnik, J. Hockensmith, G. Kopf, A. Darszon, P.E. Visconti*

15. **PDC-109 enables epididymal bull sperm to bind to oviductal epithelium TM**
*T. Gwathmey, G.G. Ignotz, S. Suarez*

16. **The nicotinic acetlycholine receptor is involved in the ZP3-initiated acrosome reaction of human sperm**
*C.M. J. Bray, J.-H. Son, S. Meizel*

17. **Sperm proteasomes are involved in human fertilization**
*P Morales, M. Kong, C. Pasten, E. Pizarro*

18. **Injection of sperm nuclear halos into oocytes results in normal paternal pronuclei chomosomes**
*Mohaar, M.A. Szczygiel, W.S. Ward*

12:15 pm **Women in Andrology Luncheon**
FULLER'S RESTAURANT (LOBBY LEVEL)
• Perspectives on Success and Satisfaction as a Woman in Andrology

1:30 pm **Symposium I:** HIV - How is it Transmitted and Who is at Risk
GRAND BALLROOM C
*Chair: Nancy Alexander*
• Male Circumcision and HIV Acquisition and Transmission *Ron Gray, Johns Hopkins University*
• HIV: How is it Transmitted and Who is at Risk?
*Dave Phillips, Population Council*
• Biology of HIV Transmission
*Christopher Miller, Univ. of CA - Davis*

3:00 pm **Refreshment Break**
GRAND BALLROOM A & B

3:30 pm **Women in Andrology Lecture**
GRAND BALLROOM C
*Chair: Sally Perrault*
• Paternal Exposure to Cyclophosphamide: Mechanisms and Consequences
*Barbara Hales, McGill University*

4:30 pm **Poster Session I**
GRAND BALLROOM A & B
*(Sponsored by Fertility Solutions)*

7:00 pm Trainee Colloquium & Soirée
CEDAR ROOM
•Andrology in the Public Health Arena-From Seamen to Semen *Steven Schrader, NIOHS*

**Friday, April 26, 2002**

7:00 am **Past Presidents' Breakfast**
PILCHUCK ROOM

8:00 am **AUA Lecture**
GRAND BALLROOM C
*Chair: Jon Pryor*
• Nerve Grafting During Radical Prostatectomy
*Ed Kim, Univ. of Tennessee*

8:55 am **Young Investigator Award**
GRAND BALLROOM C

9:05 am **Buckeye Lecture**
GRAND BALLROOM C
*Chair: Terry Brown*
• Androgenic Regulation of Apoptosis in the Prostate Gland and in Prostate Cancer
*R. Buttyan, Columbia University*

10:00 am **Coffee Break and Exhibits**
GRAND BALLROOM A & B

10:30 am **Symposium II:** Proteomics/Genomics: Will This Revolutionize Andrological Research?
GRAND BALLROOM C
*Chair: John Herr*

• Genetics and Biology of Adult Male Germ Cell Tumors
*Raju S.K. Chaganti, Memorial Sloan-Kettering*

• Modeling Human Disease Through Transgenesis
*Sarah Comerford, UTSWMS & HHMI*

• How to Find Cellular Proteins that Sense the Environment *David Garbers, UTSWMS & HHMI*

12:00 pm **Laboratory Science Lunch**
CIRRUS BALLROOM

1:30 pm **Symposium III:** Do Gene Defects Impact Male Reproduction: If So, How?
GRAND BALLROOM C
*Chair: Norman Hecht*

• The Battle of the Sexes: *Sry* and the Control of Testis Organogenesis
*Blanche Capel, Duke Univ. Med. Ctr.*

• Natural Potent Androgens: Lessons from Human Genetic Models
*J. Imperato-McGinley, Cornell University*

• Genetic Defects in Male Reproduction
*Stephanie Seminara, Harvard University*

3:00 pm **Refreshment Break**
GRAND BALLROOM A & B

3:15 pm **Plenary Lecture**
GRAND BALLROOM C
*Chair: Gail Prins*

• The Ins & Outs of PSMA, the Evolving and Intriguing Tale of its Prostate Biology & Tumor Targeting
*W.D. Heston, Cleveland Clinic*

4:05 pm **Schering Lecture**
GRAND BALLROOM C
*Chair: Bernard Robaire*

• Bioethics and Stem Cell Research
*Laurie Zoloff, San Francisco State Univ.*

5:00 pm **Poster Session II**
GRAND BALLROOM A & B

7:00 pm **Banquet**
GRAND BALLROOM C

**Saturday, April 27, 2002**

8:00 am **Latin American Lecture**
GRAND BALLROOM C
*Chair: Carlos Suarez-Quian*

• Phenotypes of Sperm Pathology: A Journey from Descriptive Morphology to Molecular Genetics
*Hector Chemes, Buenos Aires Children's Hospital*

9:00 am **Symposium IV:** Are there Estrogen Effects on the Testis?
GRAND BALLROOM C
*Chair: Rex Hess*

• Uncovering a Role for Estrogen Receptors in Male Reproduction *Ken Korach; NIEHS*

• Estrogen Effects on the Developing Testis: Direct or Indirect Effects? *Richard Sharpe, Edinburgh*

• Developmental Effects of Estrogenic Chemicals
*Fred vom Saal, Univ. Missouri-Columbia*

10:30 am **Break**

10:45 am **Symposium V:** The Molecular Basis of Erectile Physiology: From Bench to Bedside
GRAND BALLROOM C
*Moderator: Wayne Hellstrom, Tulane University*
(Sponsored by an unrestricted educational grant from the Bayer Pharmaceutical Division)

• Nitric-Oxide-Mediated Penile Erection: Neuroregulatory and Blood Flow-Dependent Mechanisms *Arthur Burnett, Johns Hopkins Hospital*

• Channels as Molecular Targets for Improved Therapy of Erectile Dysfunction
*George Christ, Albert Einstein College of Medicine*

• Inhibition of Tonic Contraction: A New Way to Approach ED?
*Thomas Mills, Medical College of Georgia*

12:15 pm **Business Meeting**
GRAND BALLROOM C

12:45 pm **Conclusion of Annual Meeting**

**Postgraduate Course**
**Saturday, April 27, 2002**

**Andrology: Genes, Hormones & Environment**

2:15 pm **Introduction**
GRAND BALLROOM C
*Jacquetta Trasler, Course Chair*

**Session 1-** Prostate Cancer: Update and Diagnostics (including gene-based)

2:30 pm Epidemiology of Prostate Cancer
*Janet L. Stanford, Fred Hutchinson Cancer Research Center*

3:05 pm Estrogens and Prostate Cancer
*Gail Prins, University of Illinois*

3:40 pm **Refreshment Break**

4:10 pm Androgen Refractory Prostate Cancer-New Insights, New Treatments
*Martin Gleave, Univ. of British Columbia*

4:45 pm Prostate Cancer: How Basic Science Advances Will Impact Clinical Management
*Paul Lange, University of Washington*

5:20 pm **Discussion -**Two 5-10 minute viewpoints (2 speakers), Discussion (all speakers)

6:00 pm **Dinner**

# agenda cont.

**Sunday, April 28, 2002**

**Session 2-**Infertility and Testicular Cancer: Genetics, Environment, Counseling and Testing

| | |
|---|---|
| 7:20 am | **Introduction**<br>GRAND BALLROOM C |
| 7:30 am | Male Contribution to Birth Defects: Basic Aspects/Mechanisms<br>*Bernard Robaire, McGill University* |
| 8:05 am | Male Contribution to Birth Defects: Clinical Aspects<br>*Wendi Robbins, UCLA* |
| 8:40 am | **Refreshment Break** |
| 9:00 am | Testicular Cancer: Environmental Origins vs. Cancer Susceptibility Genes<br>*Richard Sharpe, University of Edinburgh* |
| 9:35 am | Genetic Testing and Counselling in Andrology (Genes, Environmental Exposures)<br>*Pasquale Patrizio, Univ. of Pennsylvania* |
| 10:10 am | **Discussion-**Two 5-10 minute viewpoints (2 speakers) Discussion (all speakers) |
| 10:45 am | **Conclusion** |

# future meetings

## 28th Annual Meeting of the American Society of Andrology

Saturday, March 29-Tuesday, April 1, 2003
Sheraton Crescent Hotel
Phoenix, Arizona USA

*For additional information on all meetings, contact:*
American Society of Andrology
2950 Buskirk Avenue, Suite 170
Walnut Creek, CA 94596
Phone (925) 472-5910
Fax (925) 472-5901
Website www.andrologysociety.com

# poster session I

***Sponsored by Fertility Solutions***

19 CHARACTERIZATION OF UNIQUE TRANSCRIPTION OF GERM CELLS FROM THE MOUSE TESTIS WITH THE USE OF AFFYMETRIX GENECHIP® ARRAYS / *D.J. McLean, D.J. McLean , P.J. Friel, J.R. McCarre, M. D. Griswold*

20 AN INITIAL PROFILING OF MESSENGER RNA EXPRESSION FROM MICRODISSECTED STAGE-SPECIFIC TUBULES BY OLIGONUCLEOTIDE ARRAY / *T.B. Guo, J. Toppari, W.A. Saleme*

21 HYPOSPERMATOGENESIS: CAN WE BLAME THE CALCIUM CHANNEL?
*C. Millan, I.R. Hurley, J.L. Marmar, S. Benoff*

22 THE TFIIH BASAL TRANSCRIPTION COMPLEX IS ABUNDANTLY EXPRESSED DURING MEIOSIS IN THE MOUSE TESTIS / *S.E. Ravnik, E.A. Whitmire, W.L. Boston, P. L. Frisbie*

23 CKS2 IS UPREGULATED DURING MEIOSIS AND BINDS THE MEIOTIC ISOFORM, CDK2fl / *E.N. Attaya, S.E. Ravnik*

24 CHARACTERIZATION OF THE ROLE THAT RARa PLAYS IN SPERMATOGENESIS BY EXAMINATION OF THE RARa KNOCKOUT MICE / *T.J. Doyle, K.W. Braun, D. McLean, P. Shane, M.D. Griswold, K.H. Kim*

25 INHIBITION OF SPERM PRODUCTION IN MICE BY ANNEXIN V MICROINJECTED INTO SEMINIFEROUS TUBULES: A POSSIBLE ETIOLOGY OF PHAGOCYTIC CLEARANCE OF APOPTOTIC SPERMATOGENIC CELLS AND MALE INFERTILITY / *Y. Maeda, A. Shiratsuchi, M. Namiki, Y. Nakanishi*

26 SPERMATOGENESIS AFTER ORCHIECTOMY ALONE TREATMENT FOR NON-SEMINOMA STAGE I TESTICULAR CANCER / *V. Mamaladze*

27 RESTORATION OF SPERMATOGENESIS AND FERTILITY IN AZOOSPERMIC MUTANT MICE BY SUPPRESSION AND REELEVATION OF TESTOSTERONE FOLLOWED BY ICSI / *A. Tohda, K. Matsumiya, T. Okuno, M. Okabe, H. Kishikawa, A. Tsujimura, K. Dohmae, A. Okuyama, Y. Nishimune*

28 SEMINIFEROUS EPITHELIUM CYCLE LENGTH IN Bolomys lasiurus (RODENTIA, MURIDAE)
*C.D. de Lima Nessralla, L.R. FranÁa*

29 IDENTIFICATION OF RETINOIC ACID INDUCED GENES IN MOUSE TESTIS BY cDNA MICROARRAY ANALYSIS
*A.J. Oudes, J.C. Rockett, D.J. Dix, K.H. Kim*

30 CYSTATIN TE-1, A CYSTATIN-RELATED GENE EXPRESSED IN THE TESTIS AND EPIDIDYMIS
*Y. Li, M.O. Robinson, P.J. Friel, D.J. McLean, M.D. Griswold*

31 INVOLMENT OF GGA IN THE LYSOSOMAL TRANSPORT OF PROSAPOSIN; A PROTEIN EXPRESSED IN SERTOLI CELL / *S. Lefrancois, A.J. Hassan, C. Morales*

32 RAB GTPases ARE KEY PLAYERS IN TIGHT (TJ) AND ANCHORING JUNCTION (AJ) DYNAMICS IN THE TESTIS
*A.S. Nga Lau, D.D. Mruk*

33 SERTOLI CELL NUMBER INCREASES MARKEDLY DURING INFANCY IN THE RHESUS MONKEY, BUT TO A LESSER EXTENT THAN THAT DURING PUBERTY IN THIS SPECIES / *D.R. Simorangkir, T.M. Plant , G.R. Marshall*

34 A NEW PROCEDURE TO PURIFY ADULT RAT AND MOUSE LEYDIG CELLS / *A. Salva, G.R. Klinefelter, M.P. Hardy*

35 ISOLATION AND FUNCTION OF ADULT RAT SERTOLI CELLS / *M. Anway, J. Folmer, B.R. Zirkin*

36 CYCLINS EXPRESSION IN SOMATIC CELLS OF THE TESTIS IN NEONATAL WISTAR RATS
*F.M. Pereira, G.C. Bregunci, G.D. Cassali, R.A. Hess, L.R. FranÁa*

37 SITE-DIRECTED MUTAGENESIS OF THE StAR PROMOTER REVEALS THE REQUIREMENT OF MULTIPLE C/EBP AND GATA ELEMENTS FOR PKA-DEPENDENT SYNERGY BETWEEN GATA-4 AND C/EBPfl
*F. Hamel, J.J. Tremblay, R.S. Viger*

38 TUMOR NECROSIS FACTOR-a INDUCES NEUTROPHIL RECRUITMENT TO TESTICULAR VENULES: CHARACTERIZATION OF SIGNALING PATHWAYS / *J..L. Lysiak, T.T. Turner*

39 TESTOSTERONE INHIBITS PRODUCTION OF 25-HYDROXYCHOLESTEROL IN TESTICULAR MACROPHAGES
*Y.O. Lukyanenko, J.C. Hutson*

# poster session I

40 PERCUTANEOUS NEEDLE TESTICULAR BIOPSY IN INFERTILE MEN
*J.K. Wolski, P. Kluge, B. Biarda, K. Koziol, P. Lewandowski*

41 SEASONALITY OF CRYPTORCHIDISM AND HYPOSPADIAS IN GREECE: EPIDEMIOLOGICAL RELATIONSHIPS / M.
*Charalampos, D. Demetriades, S. Antypas, N. Sofikitis*

42 PROGESTERONE RECEPTOR EXPRESSION, LOCALIZATION, AND ACTIVITY IN RAT TESTES
*Y.H. Lue, C. Wang, A.P. Sinha Hikim, C.M. Yamamoto, A. Leung, V. Atienza, M. Diaz-Romero, R.S. Swerdloff*

43 THE EFFECTS OF REPRODUCTIVE HORMONES ON COLOCALIZATION OF CORTACTIN WITH F-ACTIN IN ISO LATED RAT SERTOLI CELLS / *K.M. Wolski, J. Hushen, D.F. Cameron*

44 POTENTIAL ESTROGEN REGULATION OF HENSIN, AN EPITHELIAL TERMINAL DIFFERENTIATION GENE IN THE MALE REPRODUCTIVE TRACT / *Q. Zhou, Q. Al-Awqati, R. Nie, K. Carnes, D. Lubahn, R.A. Hess*

45 THE INITIATROR CASPASE 9 AND THE EXECUTIONER CASPASES 3 AND 6 ARE INVOLVED IN GERM CELL APOPTOSIS FOLLOWING GONADOTROPIN DEPRIVATION IN RATS
*G. Palomeno, A.P. Sinha Hikim, Y.H. Lue, V. Atienza, C. Wang, R.S. Swerdloff*

46 ANDROGEN RECEPTORS WITH A PATHOLOGICALLY EXPANDED POLYGLUTAMINE TRACT FORM AGGREGATES WHICH ALTER NEURITE TRANSPORT IN MOTONEURONAL CELLS
*L. Martini, S. Simeoni, F. Piccioni, P. Pozzi, I. Andriola, V. Poletti*

47 A PHARMACOKINETIC STUDY OF ORAL TESTOSTERONE UNDECANOATE (ORG 538)
*B.D. Anawalt, J.K. Amory, C. Wang, R.S. Swerdloff, A.S. Dobs, A.W. Meikle, J. Elbers, N.S. Houwing*

48 CAN AGE-RELATED DECREASES IN LEYDIG CELL TESTOSTERONE PRODUCTION BE RESTORED WITH LH?
*H. Chen, M.P. Hardy, B.R. Zirkin*

49 A MORPHOMETRIC ANALYSIS OF TESTICULAR FUNCTION IN SENESCENCE ACCELERATED MICE
*N. Manabe, Y. Akiyama, M. Sugimoto, M. Kiso, K. Komatsu, M. Shimabe, H. Miyamoto*

50 DETECTION OF PARTIAL ANDROGEN DEFICIENCY IN AGING MALES AND ITS IMPACT. PRELIMINARY REPORT
*R.T. Serrano, R.S. Echeverìa, V.J. Lezana, M.J. Torres, A.J. Moreno*

51 CALORIC RESTRICTION AFFECTS GENE EXPRESSION IN THE EPIDIDYMIS OF THE BROWN NORWAY RAT
*K. Jervis, B. Robaire*

52 ESTIMATION OF ENDOGENOUS LUTEINIZING HORMONE-TESTOSTERONE SECRETORY COUPLING IN THREE MAMMALIAN SPECIES/ *J.D. Veldhuis, D.M. Keenan, I. Clarke, S. Alexander, C.H. G. Irvine*

53 IN UTERO EXPOSURE TO PERSISTENT ORGANIC POLLUTANTS (POPS) AFFECTS GENE EXPRESSION IN THE FETAL TESTIS / *A. Adeeko, D. Li, J.M. Trasler, B.F. Hales, B. Robaire*

54 GESTATIONAL EXPOSURE TO ETHANE DIMETHANESULFONATE (EDS) ALTERS DEVELOPMENT OF THE MOUSE TESTIS / *D. Tarka, J.D. Suarez, N.L. Roberts, J.M. Rogers, M.P. Hardy, G.R. Klinefelter*

55 IN UTERO EXPOSURE TO TRIBUTYLTIN ALTERS CELLULAR JUNCTIONS IN ADULT RAT VENTRAL PROSTATE
*J. Barthelemy, D.G. Cyr*

56 TESTOSTERONE LEVELS IN MALE PESTICIDE APPLICATORS AND THE SEX OF THEIR CHILDREN
*S.E. Holland, V.F. Garry, S.M. Schrader, J.S. Kesner, EA. Knecht*

57 SPERM MOTILITY IN HSF1 KNOCKOUT MICE AFTER HEAT SHOCK IS ASSOCIATED WITH FERTILITY DEFICITS
*L.F. Strader, S.D. Perreault, J.C. Luft, D.J. Dix*

58 ABNORMAL SPERM MORPHOLOGY AND OFFSPRING SEX RATIO IN MALE FUNGICIDE APPLICATORS
*S.M. Schrader, T.W. Turner*

59 OFF-SITE SEMEN ANALYSIS IN MEN INVESTIGATED FOR REPRODUCTIVE TOXICITY: PRESERVATION OF SPERM SHAPE AND THE CHROMATIN ANILINE BLUE STAINING PATTERN / *C. Celik-Ozenci, L. Vigue, G. Huszar*

60 SPERM COUNT VS BLOOD LEAD GENERAL LINEAR MODEL: MULTIPLE STUDIES FROM WORKSITE
*W.J. Moorman, P.B. Shaw, T.W. Turner, S.M. Schrader*

61 POLYCHLORINATED BIPHENYLS INDUCED ALTERATIONS OF MORPHOLOGY AND CYTOSKELETON IN MCF-7 HUMAN BREAST CANCER CELLS / *S. Raychoudhury , A.F. Flowers*

62 THE INFLUENCE OF ANTISPERM ANTIBODIES ON THE ACROSOME REACTION
*C. Bohring, J. Skrzypek , W. Krause*

63 HUMAN SPERM WITH SEVERELY DEFECTIVE MORPHOLOGY VARY WIDELY IN THEIR ABILITY TO PENETRATE ZONA-FREE HAMSTER EGGS / *R.A. Bronson, S. Bronson, L. Oula*

64 DIFFERENTIAL EFFECTS OF GLUCOSE AND FRUCTOSE ON THE LOCATION OF GLYCOGEN SYNTHESIS IN MATURE DOG SPERMATOZOA
*T. Rigau i Mas, J.M. Fernandez-Novell, M.J. Palomo, J. Ballester, J.J. Guinovart, J.E. Rodriguez-Gil*

65 THE PRODUCTION OF NITRIC OXIDE IN BULL AND FISH SPERM
*R.W. Atherton, D.S. Bohle, C. Montville, C.S. Cruickshank, A.D. McBean*

66 A COMPARATIVE ANALYSIS OF ENDOGENOUS PLATELET-ACTIVATING FACTOR CONTENT IN MAMMALIAN SPERM / *W.E. Roudebush, J.A. Cano, J.B. Massey, C.W. Elsner, A.A. Toledo, J.R. Diehl*

67 POSSIBLE ROLE OF INTRACELLULAR CALCIUM IN THE HEAD-TO-HEAD AGGLUTINATION OF BOAR SPERMATOZOA/ *H. Harayama*

68 PROBABILITY OF SPERM WITH ACTIVATED KILLER ENZYMES TO HAVE DAMAGED MEMBRANES
*U. Paasch, S. Grunewald, H.J. Glander*

69 REACTIVE OXYGEN SPECIES INDUCE DEOXYRIBONUCLEIC ACID (DNA) DAMAGE IN EQUINE SPERM
*J. Bamber, B.A. Ball*

70 ANDROLOGICAL PARAMETER DIFFERENCES BETWEEN IDENTICAL GENOMIC BULLS
*C. Lessard, J.F. Bilodeau, J.L. Bailey, P. Leclerc, R. Sullivan*

71 BULL FERTILITY - COMMERCIAL OUTCOME DATA INAPPROPRIATE TO ESTABLISH UTILITY OF A DIAGNOSTIC TEST OF SPERM QUALITY / *R. Amann, R.H. Hammerstedt*

72 THE FATE OF BOVINE OOCVTES INTERACTING IN VIVO WITH 1, 2, OR 3 SPERM; IMPLICATIONS OF DATA INTERPRETATION/ *R.P. Amann, R. G. Saacke*

73 EFFECT OF WEIGHT AND AEROBIC TRAINING ON STEROID HORMONES AND SEMEN PROFILES
*W. H. Park, K. H. Rha, K. J. Cho, M.S. Lee*

74 SEMEN QUALITY SCORE - A NOVEL METHOD TO PREDICT SUCCESSFUL OUTCOME IN DONOR INSEMINATION FOR COUPLES WITH MALE FACTOR INFERTILITY
*P. Ranganathan, I. Bandaranayake, J. Chauhan, M. Oder, D. Nelson, A. Agarwal*

75 LONGITUDINAL STUDY OF SEMEN QUALITY AFTER INTERMITTENT EXPOSURE TO AIR POLLUTION
*J. Rubes, D. Zudova*

76 SEMEN QUALITY OF SPERM DONORS IN LOS ANGELES AND PALO ALTO: EFFECTS OF TIME, TEMPERATURE, AND LOCATION / *I.M. Fowler, P. Kraft, K.T. Berhane, E. Kim*

77 SEASONAL VARIATION OF SEMEN PROFILES IN HEALTHY YOUNG MEN IN KOREA
*K.H. Rha, W.H. Park, J.Y. Noh, S.W. Han, M.S. Lee*

78 INTER-SAMPLE VARIABILITY IN POST-THAW MOTILITY AND MOTION KINETICS OF CRYOPRESERVED HUMAN SPERM
*R.A. Saleh, R.K. Sharma, J. Chauhan, M. Oder, N. Esfandiari, E. Nada, R. Raina, B. Paige, D.R. Nelson, A. Agarwal*

79 ASSISTED REPRODUCTION USING HAMSTER SPERMATOZOA GENERATED INTO XENOGENEIC TESTES
*S. Tsabalas, Ch. Mamoulakis, A. Kaponis, D. Baltogiannis, X. Giannakopoulos, I. Miyagawa, A. Tasos, N. Sofikitis*

80 "MAPPING" PERCUTANEOUS NEEDLE TESTICULAR BIOPSY IN NON-OBSTRUCTIVE AZOOSPERMIA
*J.K. Wolski, P. Kluge, B. Biarda, K. Koziol, P. Lewandowski*

# poster session 1

81 COMPARISON OF TWO DENSITY GRADIENT PREPARATIONS FOR SPERM SEPARATION FOR MEDICALLY ASSISTED CONCEPTION: EFFECT ON RECOVERY, CLEAN-UP, MOTILITY, AND MOTION PARAMETERS
*W.A. Roudebush, J.A. Cano, M.H. Durrance, M.C. Holmes, M.A. Witt, S.M. Slayden, H. I. Kort*

82 CRYOPRESERVED SPERM FROM TESTICULAR SPERM OBTAINED POST-HUMOUSLY RESULTS IN SUCCESS FUL DELIVERY AFTER INTRACYTOPLASMIC SPERM INJECTION (ICSI)
*M.L. Check, J.H. Check, D. Summers-Chase, J.K. Choe, R.W. Johnson*

83 PREGNANCY RATES WITH FROZEN TESE SPERM ARE HIGHLY DEPENDENT ON AGE OF FEMALE PARTNER
*P. Studney, M. Puccini, L. Ray, L. Nelson, C. Niederberger, L. Ross, G.S. Prins*

84 CASE STUDY: PREGNANCY RESULTING FROM IVF FOLLOWING ICSI USING SPERM HEAD WITH DETACHED TAIL / *B.R. Emery, C. Thorp, P. Kuneck, L. Erickson, D.T. Carrell*

85 ICSI- AND ROSI-MEDIATED TRANSGENESIS IN THE MOUSE / *H. Akutsu, H. Kusakabe, T. Osada, R. Yanagimachi*

# poster session 2

87 GENE EXPRESSION PROFILES IN THE VENTRAL, DORSAL AND LATERAL LOBES OF THE BROWN NORWAY RAT PROSTATE / *T.R. Brown, Y. Tian*

88 DIFFERENTIAL EXPRESSION OF NITRIC OXIDE SYNTHASE ISOFORMS IN HUMAN PROSTATE (BPH, PROSTATIC CARCINOMA AND NORMAL) TISSUE
*Y.S . Mehta, J. Bivalacqua, D. Sarma, K. Moparty, R. Davis, A. B. Abdel-Mageed, S. Sikka*

89 PROTEIN PROFILING OF EXPRESSED PROSTATIC SECRETIONS / *C.H. Muller, D. Diamond, R.E. Berge*

90 INCREASED TISSUE FACTOR (TF) EXPRESSION IN PROSTATE CANCER: ROLE AS NEOPLASTIC MARKER IN EXFOLIATED PROSTATE CELLS IN POST-PROSTATE MASSAGE URINE
*M.J. Wilson, T.J. Kappel, R. Bach, R. Haller, J.W. Slaton, N.F. Wasserman, B. Quast, A.A. Sinha*

91 THE ASSOCIATION BETWEEN ANDROGEN RECEPTOR AND EGF RECEPTOR INHIBITS EGF-INDUCED INVASION IN PROSTATE CANCER CELL LINES / *L. Bonaccorsi, V. Carloni, M. Muratori, G. Forti, E. Baldi*

92 LONG-TERM EFFICACY OF LHRH AGONIST THERAPY AND ANTI-ANDROGEN MONOTHERAPY IN THE TREAT MENT OF LOCALIZED (T1-3) PROSTATE CANCER
*R. Raina, E.E. Cherullo, W. Conrad, D.R. Nelson, A. Agarwal, C.D. Zippe*

93 EARLY USE OF VACUUM CONSTRICTION DEVICE (VCD) FOLLOWING RADICAL PROSTATECTOMY (RP) FACILITATES EARLY SEXUAL ACTIVITY AND POTENTIAL RETURN OF ERECTION
*R. Raina, R.A. Saleh, J. Chauhan, A. Agarwal, C. D. Zippe*

94 DIETARY ISOFLAVONES INDUCE APOPTOSIS IN PROSTATE CARCINOMA
*G.P. Risbridger, R.A. Jarred, C. Dowling, S. McPherson, A. Husband, J. Pedesen, M.F. Frydenberg*

95 IS TESTOSTERONE AND PSA RELATED IN ELDERLY POPULATION?
*K.H. Rha, J.W. Jung, Y. D. Choi, S. W. Han, S. J. Hong, M. S. Lee*

96 ACCUMULATION OF MACROPHAGES IN THE TESTIS, EFFERENT DUCTS AND EPIDIDYMIS OF CATHEPSIN A DEFICIENT MICE / *N. Korah, A. díAzzo, L. Hermo*

97 SHORT AND LONG-TERM EFFECTS OF THE ANTI-ESTROGEN ICI 182,780 ON THE TESTIS AND EFFERENT DUCTULES, WITHOUT CHANGES IN TESTOSTERONE 1
*C.A. Oliveira, K. Carnes, Q. Zhou, R. Nie, D.E. Kuehl, G.L. Jackson, L.R. FranÁa, M. Nakai, R.A. Hess*

98 GERM CELL-SPECIFIC EXPRESSION OF A CYSTATIN-RELATED EPIDIDYMAL SPERMATOGENIC (CRES) PRO MOTER-CAT TRANSGENE / *N. Hsia, J.P. Brousal, S.R. Hann, G.A. Cornwall*

99 EFFECT OF 5 ALPHA-REDUCTASE (5a-R) INHIBITION ON GENE EXPRESSION IN THE EPIDIDYMIS /
*N. Henderson, B. Robaire*

100 ANALYSIS OF THE 5' UPSTREAM REGION OF RAT 5 ALPHA REDUCTASE (5a-R) TYPE / *S. Seenundun, B. Robaire*

101 FURTHER CHARACTERIZATION OF THE MURINE EPIDIDYMAL SPAM1 (PH-20) / *P.A. Martin-DeLeon, H. Zhang*

102 AN ANTIMICROBIAL ROLE FOR THE EP2/HE2 GENE IN THE EPIDIDYMIS / *O. Froehlich, C. Po, L.G. Young*

103 CHARACTERIZATION AND IDENTIFICATION OF CAPACITATION-ASSOCIATED PIG SPERM TYROSINE PHOSPHO PROTEINS / *C. Reyes-Moreno, C. Dube, S. Tardif, J.L. Bailey*

104 PHOSPHOPROTEOME ANALYSIS OF CAPACITATED HUMAN SPERM CELLS
*P.E. Visconti, S.S. Ficarro , O. Chertihin, F. White, A. Westbrook, F. Jayes, P. Kalab, J. Shabanowitz, J.C. Herr, D. Hunt*

105 DIFFERENT SIGNAL TRANSDUCTION PATHWAYS ARE INVOLVED DURING HUMAN SPERM CAPACITATION INDUCED BY BIOLOGICAL AND PHARMACOLOGICAL AGENTS / *J. Thundathil, E. de Lamirande, C. Gagnon*

106 ZONA PELLUCIDA-INDUCED ACROSOME REACTION IN THE HUMAN: DEPENDENCY ON ACTIVATION OF PERTUSSIS TOXIN-SENSITIVE GI PROTEIN AND CALCIUM INFLUX, AND PRIMING EFFECT OF PROGESTERONE AND FOLLICULAR FLUID / *M. Morshedi, A. Schuffner, H.S. Bastiaan, H. Duran, Z.Y. Lin, D.R. Franken, S. Oehninger*

107 ACROSOME REACTION IN HUMAN SPERM: ROLES OF CAPACITATION, TYROSINE PHOSPHORYLATION AND MEMBRANE EXTERNALIZATION OF PHOSPHATIDYLSERINE
*M. Morshedi, A. Schuffner, Z.Y. Lin, H. Duran, M. Hsiu, D.R. Franken, S. Oehninger*

108 COMBINATION OF DETERGENT AND DTT CAUSES PATERNAL CHROMOSOME DAMAGE
*M.A. Szczygiel, R. Yanagimachi, W.S. Ward*

109 MALE INFERTILITY IN NECTIN-2 DEFICIENT MICE / *S Mueller, T. A. Rosenquist , R.A. Bronson, E. Wimmer*

110 FATTY ACID PROFILE OF SPERMATOZOA FROM TERATOSPERMIC DOMESTIC CATS
*B. Pukazhenthi, M. Ollero, B. Teter, J.G. Howard, J.G. Alvarez, D.E. Wildt*

111 SPERMATOGENIC TRAIT DIFFERENCES IN THE NORMOSPERMIC VERSUS TERATOSPERMIC DOMESTIC CAT
*K. Stoebel, K. Jewgenow, S. Blottner, D.E. Wildt, B.S. Pukazhenthi*

112 TERATOZOOSPERMIA - MOLECULAR BACKGROUND OF SPERM DYSFUNCTION / *M. Szczygiel, M. Kurpisz*

113 EXPRESSION ANALYSIS OF THE Y CHROMOSOME GENES IN IDIOPATHIC AZOOSPERMIA AND SEVERE OLIGOZOOSPERMIA / *E. Koh, H. Suzuki, M. Namiki*

114 CARNITINE TREATMENT OF OLIGOSPERMIC MEN IN IUI/IVF PROCEDURE / *S. Micic, N. Lalic, N. Bojanic*

115 RELATIONSHIP BETWEEN AGE OF MAN, SPERM DNA FRAGMENTATION AND INFERTILITY
*D. Evenson, M. Virro, J. Brannian, C. deJonge*

116 THE DEGREE OF SPERM DNA FRAGMENTATION AND/OR STABILITY TO PREDICT THE OUTCOME OF INTRAUTERINE INSEMINATION / *M. Morshedi, H. Duran, S. Taylor, S. Oehninger*

117 DEVELOPMENT OF AN INEXPENSIVE ARTIFICIAL VAGINA FOR SEMEN COLLECTION FROM RABBITS
*C.K. Naughton, D. Nelson, A.J. Thomas, Jr.*

118 A NOVEL HOME-USE TEST DEVICE FOR MOTILE SPERM
*L. Bjorndahl, I. Brewis, E. Barratt, B. Ola, D. Bradley, K. Sharif, M. Afnan, C. Barratt*

119 A DOUBLE CHAMBER DEVICE FOR ADVANCED SPERM TESTING: SEMEN ANALYSIS AND AN ASSAY FOR SPERM MATURITY BY HYALURONIC ACID (HA) BINDING / *G. Huszar, C. Celik-Ozenci, A. Jakab, L. Vigue*

120 HUMAN OR ANIMAL FERTILITY - EVALUATION OF OUTCOME TO ESTABLISH UTILITY OF A DIAGNOSTIC TEST OF SPERM QUALITY OR DETECT A CHANGE IN FERTILIZING POTENTIAL OF SPERM
*R.P. Amann, R.H. Hammerstedt*

121 PRESENCE OF TIMP-2 ON SPERM CORRESPONDS TO RERTILITY OF RANGE BEEF BULL
G.R. Dawson, M.E. Bellin, J.N. Oyarzo, H.E. Hawkins, M.J. Arns, R.L. Ax

# poster session 2

122 NONGENOMIC PROGESTERONE RECEPTORS IN HUMAN SPERMATOZOA
*M. Luconi, L. Bonaccorsi, G. Forti, E. Baldi*

123 REDUCTION OF SEMINAL ANTIOXIDANT CAPACITY IN INFERTILE MEN WITH LEUKOCYTOSPERMIA: A PROSPECTIVE STUDY / *R.A. Saleh, R.K. Sharma, J. Chauhan, M. Oder, N. Esfandiari, D.R. Nelson, E.A. Nada, R. Raina, A.J. Thomas, Jr., A. Agarwal*

124 MEIOTIC ABNORMALITIES IN AZOOSPERMIA AND BILATERAL VARICOCELE / *G. Tritto, M.O. North*

125 INFLUENCE ON THE PREGNANCY OF MOTILITY OF FRESH AND FROZEN-THAWED TESTICULAR SPERMATOZOA IN OBSTRUCTIVE AZOOSPERMIA
*J.T. Seo, J.M. Yun, Y.S. Lee, Y.S. Park, H.S. Lee, S.H. Lee, H.K. Byun, H.W. Youm, S.J. Song, I.S. Kang*

126 EFFECTS OF TWO TECHNIQUES OF LEFT VARICOCELECTOMY (VRCL) ON FERTILIZATION AND EMBRYONIC CAPACITY FOR IMPLANTATION / *A. Chatzikyriakidou, Ch. Mamoulakis, A. Kaponis, X. Giannakopoulos, I. Miyagawa, D. Tsalikis, D. Yiannakis, N. Sofikitis*

127 PREVALENCE OF VARICOCELE IN CHILDREN AND ADOLESCENTS IN NAMHAE, KOREA; 3-YEAR FOLLOW-UP STUDY / *K.H. Rha, J.H. Kim, B.H. Kim, D.H. Kwon, D.Y. Lee, S. W. Han, M. S. Lee*

128 THE USE OF CYANOACRYLATE IN NEW TECHNIQUE OF VASOVASOSTOMY
*J. Chavarria-Guevara, G.M. Sanchez, J. Garcia, N.E. Foyo, A.J. Moreno, R. Tapia-Serrano*

129 FERTILITY AFTER CANCER: A PROSPECTIVE REVIEW OF ASSISTED REPRODUCTIVE OUTCOME WITH BANKED SEMEN SPECIMENS / *P. Ranganathan, J. Chauhan, M. Oder, F. Pasqualotto, J. Hallak, H. Kobayashi, A.J. Thomas*

130 TADALAFIL PROVIDES PROMPT RESPONSE AND EXTENDED PERIOD OF RESPONSIVENESS FOR THE TREAT MENT OF MEN WITH ERECTILE DYSFUNCTION / *H. Padma-Nathan, K. Saikali, V. Watkins, A. Yu, S.J. Whitaker*

131 TADALAFIL SIGNIFICANTLY IMPROVES ERECTILE FUNCTION IN MEN WITH ED, INTEGRATED ANALYSIS OF REGISTRATION TRIALS / *H. Padma-Nathan, S. Wei, A. Yu, S.J. Whitaker*

132 TRANSPENILE DELIVERY OF MICROVASCULOKINETIC DRUGS (TRAPS) IN ERECTILE DYSFUNCTIONS. LASER DOPPLER FLOWMETRY STUDY / *G. Tritto*

133 ARE COMPLICATIONS NECESSARILY MORE COMMON WITH COMPLEX PENILE PROSTHESIS IMPLANTATION?
*R. Wang, V.A. Cancellaro, K.W. Lennox, R.W. Lewis*

134 PROSPECTIVE STUDY OF PREOPERATIVE AND POSTOPERATIVE NOCTURNAL PENILE TUMESCENCE (NPT) IN MEN UNDERGOING BILATERAL NERVE SPARING RADICAL PROSTATECTOMY (BNSRRP)
*A. McCullough, L. Levine, H. Padma-Nathan*

135 LONG-TERM INTRACAVERNOUS (IC) THERAPY RESPONDERS CAN POTENTIALLY SWITCH TO SILDENAFIL CIT RATE AFTER RADICAL PROSTATECTOMY (RP) / *R. Raina, R.A. Saleh, J. Chauhan, S. Ausmundson, M. M. Lakin*

136 COMBINATION THERAPY: SILDENAFIL CITRATE ENHANCES SEXUAL SATISFACTION IN VACUUM CONSTRIC TION DEVICE (VCD) FAILURES FOLLOWING RADICAL PROSTATECTOMY (RRP)
*R. Raina, R.A. Saleh, J. Chauhan, A. Agarwal, M.M. Lakin, C.D. Zippe*

137 SILDENAFIL PLUS SERTRALINE IN THE TREATMENT OF PREMATURE EJACULATION / *A.F. Lozano*

138 PEDIATRIC HERNIORRHAPHY AS AN UNUSUAL CAUSE OF TESTICULAR PAIN: MECHANISM AND PATHOPHYSIOLOGY / *J.I. Sandlow, C.G. Christiansen*

139 IS THE YACHIA CORPORAL PLICATION FOR PEYRONIE'S DISEASE STILL EFFICACIOUS? *B.G. Boyd, B.G. Buffi G. Boyd, Peter A. Ruff, Matthew Zelinski*, Run Wang, Ronald W. Lewis, P.A. Ruff, M. Zelinski, R. Wang, R.W. Lewis*

140 THE EFFECT OF TREATMENT OF SPERM WITH SUBNORMAL HYPOOSMOTIC SWELLING TEST (HOST) SCORES WITH THE PROTEIN DIGESTIVE ENZYME CHYMOTRYPSIN ON SUBSEQUENT VIABLE PREGNANCY RATES PRs) AFTER INTRAUTERINE INSEMINATION (IUI) / *M.L. Check, D. Kiefer, J.H. Check, W. Hourani, A. Bollendorf*

141 EFFECT ON PSA LEVELS OF THE NOVEL, DUAL 5a REDUCTASE INHIBITOR,DUTASTERIDE WITH MARKED SUP PRESSION OF DIHYDROTESTOSTERONE / *R.V. Clark, T.H. Wilson, C.G. Roehrborn, G. Andriole*

142 DIFFERENTIAL CELLULAR PROLIFERATION AND APOPTOSIS OF HUMAN CAVERNOSAL CELLS IN RESPONSE TO OXIDATIVE STRESS AND ANTIOXIDANTS / *S.C. Sikka, H. Wang, W.J. G. Hellstrom*

143 INDEXING ATYPICAL CYTOLOGY TO NMP22 DECREASES FALSE-POSITIVE RESULTS / *R. Raina, H. Klepacz, J. Chauhan, L. Ponsky, S. Sharma, D.R. Nelson, A. Agarwal, C.D. Zippe*

144 TARGETED MULTICOLOR FLUORESCENE IN SITU HYBRIDIZATION (FISH) DETECTS TRANSITIONAL CELL CAR CINOMA (TCC) IN PATIENTS WITH ATYPICAL TRANSITIONAL CELLS BY URINARY CYTOLOGY / R. Raina, M. *Skacel, L. Liou, A. Agarwal, R.R. Tubbs, C.D. Zippe*

# index of abstract authors

# 1

**THE EFFECT OF AS-NEEDED TADALAFIL TREATMENT OF ERECTILE DYSFUNCTION IN MEN WITH DIABETES**

H. Padma-Nathan[*1], G. Anglin[*2], A. Yu[*3], and S.J. Whitaker[*3] for the International Tadalafil Study Group. [1]University of Southern California, Los Angeles, USA; [2]Eli Lilly Canada, Scarborough, Canada; [3]ICOS Corporation, Bothell, USA.

**Objectives:** Tadalafil is a potent and selective inhibitor of phosphodiesterase type 5 under review for marketing approval for the treatment of erectile dysfunction (ED). The purpose of this multicenter, double-blind, placebo-controlled study was to assess the efficacy and safety of tadalafil taken as needed by diabetic men with mild-to-severe ED. **Methods:** Two hundred sixteen men (mean age: 56 years) with mild-to-severe ED and diabetes (type 1 or 2) were enrolled. Baseline (BL) International Index of Erectile Function (IIEF) scores and Sexual Encounter Profile (SEP) diary information were collected during a 4-week treatment-free run-in period. Patients were randomized to receive placebo (PBO) or tadalafil 10 or 20 mg for 12 weeks. Endpoints included the change from BL in IIEF Erectile Function domain scores and SEP diary data, and the percentage of "yes" responses to a global assessment question (GAQ). **Results:** Tadalafil 10 and 20 mg significantly improved IIEF Erectile Function domain scores when compared with PBO (change from BL: tadalafil 10 mg [6.4] and 20 mg [7.3], versus PBO [0.1]; $P$ <0.001). In addition, tadalafil 10 and 20 mg improved erectile function (IIEF Erectile Function domain) and successful intercourse attempts (SEP Questions 2 and 3) in a greater percentage of diabetic patients than PBO. Both doses of tadalafil were superior to placebo in the percentage of "yes" responses to GAQ (tadalafil 10 mg [56%] and 20 mg [64%], versus PBO [25%]; $P$ <0.001). Tadalafil 10 and 20 mg were generally well tolerated. There were no treatment-related changes in lab values, ECGs, or vital signs. Most adverse events were mild to moderate in intensity. The most common treatment-related adverse events occurring in more than 5% of tadalafil-treated patients were dyspepsia and headache, which were mild to moderate in intensity. **Conclusions:** In this study, tadalafil 10 and 20 mg demonstrated superiority to PBO when administered as needed for the treatment of mild-to-severe ED in diabetic men and was well tolerated.

# 2

**A SINGLE ORAL DOSE OF VARDENAFIL HAD NO ACUTE EFFECT ON SPERM MOTILITY IN HEALTHY MALES**

R.J. Bauer, MD, G. Rohde, PhD, Bayer AG, Pharmaceutical Division, Wupperthal, Germany; T. Taylor, Bayer Corporation, West Haven, CT, and F. Kiesewetter, MD, Friedrich-Alexander-University of Erlangen, Erlangen, Germany.

**Background**: Vardenafil, a novel, potent, selective PDE5 inhibitor. is currently under development for the treatment of erectile dysfunction. PDE5 inhibitors may influence testicular function by elevating cGMP. cGMP may be involved in numerous processes relating to testicular function, including sperm motility **Objective**: To determine the effects of a single dose of vardenafil 20 mg on indices of testicular function, pharmacokinetics, and safety in healthy subjects. **Methods**: In this randomized, double-blind, crossover study, 16 healthy male subjects (24-43 years) received a single oral dose of placebo or vardenafil 20 mg. At screening (-1 to -2 weeks) and 1.5 hours after dosing, ejaculate and blood plasma were obtained. **Results**: No patient experienced a change in motility WHO (Grade A+B) >20% following vardenafil 20 mg. Vardenafil did not alter other sperm parameters.

| Sperm Parameter, mean (SD) | Placebo | Vardenafil 20.mg |
|---|---|---|
| Motility WHO grade (A+B), % | 61.7 (8.7) | 63.1 (9.2) |
| Concentration, $10^6$/ml | 90.4 (49.8) | 89.4 (52.2) |
| Density, $10^6$ | 346.8 (220.1) | 333.9 (219.7) |
| Vitality, % | 91.8 (3.4) | 91.0 (3.9) |
| Morphology, % normal | 50.4 (7.9) | 53.4 (7.4) |

Vardenafil did not alter ejaculate concentrations of α-glucosidase or fructose. The amount of vardenafil present in the ejaculate was 0.0002 (±0.0001) % of the administered 20 mg oral dose. The most common adverse events included headache and vasodilatation at a mild level of severity. **Conclusion:** Based on WHO criteria, a single dose of vardenafil 20 mg did not have an acute effect on sperm motility and a variety of other sperm parameters.

# 3

**ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE TRANSFER RESTORES NITRIC OXIDE PRODUCTION AND ERECTILE FUNCTION IN DIABETES** Trinity J. Bivalacqua, Dave Adams, Hunter C. Champion, Dae Yul Yang, Asim B. Abdel-Mageed, and Wayne J.G. Hellstrom.

Diabetes causes a decrease in cavernosal nitric oxide (NO) bioavailability that is associated with a diminished erectile response. The purpose of this study was to determine whether adenoviral-mediated overexpression of endothelial nitric oxide synthase
(ENOS) could restore NO production and erectile function. Three groups of animals were utilized: 1) age matched control rats, 2) streptozotocin (STZ)-induced diabetic rats (65 mg/kg ip) transfected with AdCMV·gal, and 3) STZ-rats with AdCMVeNOS. One day after transfection, these study animals underwent cavernosal nerve stimulation (CNS) to assess erectile function. eNOS and nNOS protein levels (Western blot), NOS activity (L-arginine to L-citrulline conversion), cGMP and nitrate + nitrite levels (ELISA) were assessed in cavernosal tissue from all three groups of animals. Diabetic animals had significant decreases in cavernosal nNOS, NOS activity, cGMP and nitrate + nitrite levels. One day after administration of AdCMVeNOS, eNOS protein, NOS activity, cGMP and nitrate + nitrite levels in the corpora cavernosa were significantly increased ($P<0.05$) to levels similar to control animals. STZ-rats had a significantly decreased erectile function as determined by CNS when compared to control rats. The increase in cavernosal pressure in response to CNS was enhanced in STZ-rats transfected with eNOS that was similar to control rats. Enhanced expression of eNOS employing an adenoviral vector restored erectile function in the diabetic rat. These data suggest that gene-transfer of eNOS may constitute a new therapeutic intervention for the treatment of diabetic ED.

# 4

**LONG TERM EFFICACY OF SILDENAFIL CITRATE FOLLOWING RADICAL PROSTATECTOMY (RP): 3-YEAR FOLLOW-UP** Rupesh Raina*, David R Nelson, Ashok Agarwal, Milton M Lakin, Eric A Klein, Craig D Zippe, Cleveland, OH

To evaluate long term efficacy and safety of sildenafil for the treatment of erectile dysfunction (ED) after RP. Data from 41 patients who responded to sildenafil therapy at 1 year following RP were stratified according to the type of nerve sparing (NS) procedure: bilateral NS, unilateral NS, and non-NS. A telephone survey was conducted during the first year of sildenafil usage and repeated 3 years later. Sildenafil was prescribed at a dose of 50 mg, and increased to 100, if needed. The responses to the abridged 5-item International Index of Erectile Function (IIEF) questionnaire, the number of patients' attempts/successful intercourse, partner satisfaction, and side effects were assessed. At 3 years, 71% (29/41) patients were still responding to sildenafil. Thirty-one percent (9/29) of these respondents had augmented their dose from 50 to 100mg. The drop out rate was 29% with 50% (6/12) discontinuing because of the return of natural erection; only 5 patients dropped out because of gradual loss of efficacy. The abridged IIEF item shows no difference in 1 yr and 3 yr scores in either of the nerve-sparing groups. Eighty-five percent of patients were sexually satisfied and 95% were able to achieve and maintain erection in more than 65% of attempts. The most common side effect at 3 years was: headache (12%), flushing (10%), and abnormal color vision (2%). No patient discontinued the drug at 3 years because of side effects. Following radical prostatectomy, patients with erectile dysfunction that respond to sildenafil continue to show excellent long-term efficacy and compliance.

# 5

**LONG TERM EFFICACY AND COMPLIANCE OF INTRA-CORPOREAL (IC) PENILE INJECTIONS IN PATIENTS WITH ERECTILE DYSFUNCTION (ED)FOLLOWING RADICAL PROSTATECTOMY (RP).**

R. Raina*, M.M. Lakin*, R.A. Saleh*, A. Agarwal, C.D. Zippe

The objective of this study was to determine the efficacy, frequency and long term compliance of IC penile injection in patients with ED following RP. The International Index of Erectile Function (IIEF) questionnaire and a follow-up profile sheet were sent to 75 patients using IC injection therapy for treatment of ED following RP. The Responses to IIEF questions 3 (frequency of penetration), 4 for (maintenance of erection) and 7 (sexual satisfaction) were scored from 0 (no intercourse) to 5 (always). The efficacy score (ES) was calculated by totaling responses to all three questions (0 = no intercourse, 1-5 = poor, 6-10 = moderate, 11-15 = good). The mean age of patients was 61 years. Prostaglandin E1 (PGE1)was used in 61% and Timex solution (PGE I, papavarine, phentolamine) in remaining 39%. The mean preoperative values for questions 3, 4 and 7 were 4.8, 4.8, and 4.8 respectively and the mean ES was 14.4. Following surgery (with a minimum follow up of 12 months), the mean scores for questions 3, 4 and 7 decreased to 1.4, 1.3, and 1.4, with an ES of 4.2. These scores increased significantly to 3.9, 3.8, and 3.61 and ES to 11.1 after IC injection therapy. There was no significant difference in responses between nerve sparing (NS) (n = 45) and non-NS groups (n = 30) or the type of medications used for IC therapy. Of the 75 patients, 39(52%) discontinued treatment due to problem with the IC. Overall, the efficacy score was good in 66.7%, moderate in 10.7%and poor in 22.7%of the patients. While oral therapy is the first option for erectile dysfunction following NS radical prostatectomy, the long term efficacy and compliance of IC makes it an excellent salvage option in patients who fail sildenafil and a first option in patients following non NS surgery.

# 6

A COMPARISON OF LONG TERM PENILE DAMAGE AFTER DEFINITIVE PROSTATE RADIATION THERAPY (RT) VS RADICALRETROPUBIC PROSTATECTOMY (RRP) IN MEN WITH POST TREATMENT ED: IS THERE A DIFFERNCE?

Neritan Mani, MD, Shpetim Telegrafi MD Andrew McCullough MD

New York University School of Medicine, NY

Objectives: Patients are frequently quoted comparable long term potency statistics with definitive radiation vs. radical prostatectomy. Though initially favoring radiation, after 3 years potency statistics are roughly equal. We asked ourselves whether the penile duplex doppler results in men presenting with ED after RT were likewise comparable. We retrospectively compared penile doppler parameters in men presenting with ED to an aged matched cohort after RRP.

Methods:
Color duplex doppler scans in10 men presenting for treatment of ED after definitive RT for prostate cancer were reviewed. Peak systolic velocities (PSV), systolic rise times (SRT) and resistive indices (RI) were examined. All men were potent before RT, and chose RT because of the alleged better potency preservation. The scans were compared to an aged match cohort of 11 men with a comparable time from radical prostatectomy. All men had failed sildenafil.

Results:
7/10 men had external beam radiation. Duplex doppler studies in men presenting with ED after RT show significantly more end organ damage in the form of both arterial disease and veno-occlusive dysfunction. (see chart)

| | Mean Age Yrs | Mean Time from treatment (months) | PSV (cm/sec) | SRT (sec) | RI |
|---|---|---|---|---|---|
| RT | 66 | 30 | 24.6 | 0.155 | 0.742 |
| RRP | 64 | 36 | 63 | 0.088 | 0.97 |
| P Value | 0.45 | 0.4 | 0.001 | 0.001 | 0.001 |

Conclusions:
Although the long term results of potency preservation with RT are comparable to RRP, men with ED after RT have considerably more severe end organ damage and may require more invasive treatments for their ED. This should be taken into account when counseling patients prior to treatment.

# 7

**CONTRACEPTIVE EFFICACY OF A DEPOT ANDROGEN AND PROGESTIN COMBINATION IN MEN.** L Turner*, S Wishart*, AJ Conway*, PY Liu*, E Forbes*, RI McLachan, DJ Handelsman. Department of Andrology, Concord Hospital, ANZAC Research Institute, University of Sydney, Sydney NSW 2139 & Prince Henry's Institute of Medical Research, Monash Medical Centre, Clayton, Victoria 3168, Australia

While hormonal male contraception has been theoretically feasible for decades with formal proof of principle demonstrated by the landmark WHO male contraceptive efficacy studies by the early 1990ís, development of practical male contraceptive regimens has lagged. Following the landmark WHO studies, no male contraceptive efficacy studies using depot or combination hormonal regimens have been reported. We have completed the first study to estimate the contraceptive efficacy of a depot hormonal regimen, an androgen/progestin combination, in men. Fifty-five men in stable relationships seeking a change in contraceptive method were administered testosterone (four 200 mg implants, every 4 or 6 months) and a synthetic progestin (300 mg depot medroxyprogesterone acetate, DMPA, every 3 months). Once sperm output was suppressed (<1M/mL, two consecutive months), men entered a 12 month efficacy phase during which other contraception was ceased. Sperm output was monitored monthly. There were no pregnancies in 426 person-months (35.5 person-years) of efficacy exposure (upper 95% confidence limit 8% per annum). Mean sperm density fell rapidly (by 88% @1 month and 98% @2 months) allowing nearly all men to enter efficacy within 3 months (50% @ 1 month, 83% @ 2 months, 94% @ 3 months). Only 2/55 (3.6%) men were unable to enter efficacy due to non-suppression. A few men treated with T implants at 6 month intervals demonstrated androgen deficiency symptoms &/or escape of spermatogenic suppression (predicted by suboptimal gonadotropin suppression) between months 5-6; men receiving T implants at 4 months intervals had no androgen deficiency nor loss of gonadotropin and sperm output suppression. Recovery was slower than anticipated (median time 6-9 months to sperm density >20M/mL) presumably related to DMPA kinetics. Discontinuations were for protocol (12), personal (10) and medical (5) reasons but there were no serious adverse effects related to drug exposure. We conclude that the first prototype depot androgen/progestin combination regimen provides high contraceptive efficacy with satisfactory short-term safety but slow recovery of spermatogenesis. Larger and longer studies with purpose-developed depot combinations are required to clarify the overall safety and efficacy of the promising depot approach to hormonal male contraception.
This study was supported by CONRAD.

# 8

**PROTEIN KINASE A (PKA) ENHANCEMENT OF GATA-4-MEDIATED GENE EXPRESSION: A LINK WITH HORMONE-ACTIVATED CELL SIGNALING.** J.J. Tremblay, F. Hamel*, R.S. Viger, CHUL Research Centre, Laval University, Québec, PQ, Canada.

Gonadal gene expression is tightly regulated by pituitary trophic hormones. Hormone binding triggers activation of an intracellular signaling pathway that typically involves cAMP production, activation of PKA, and phosphorylation of target transcription factors (TFs). The GATA TFs have recently emerged as a novel family of regulators of gonadal gene expression. Several target genes have been identified for GATA TFs including StAR, MIS, aromatase, inhibina, and SF-1. Interestingly, the expression of most of these genes is regulated by trophic hormones. The downstream molecular effectors of these hormonal signals, however, have yet to be fully understood. Therefore, we tested the intriguing possibility that members of the GATA family, particularly GATA-4 (G4), may represent such effectors. To mimic cAMP-induced promoter activity, the PKA catalytic subunit was cotransfected in CV-1 fibroblasts along with G4 and different GATA-dependent gonadal promoters. The transcriptional potential of G4 on the StAR, aromatase, and inhibina promoters was markedly enhanced by PKA but not PKC. PKA also greatly enhanced G4/SF-1 synergism on the aromatase and inhibina promoters, whereas G4/C/EBP fl synerism on the StAR promoter was strictly PKA-dependent. Taken together, these data suggest that G4 is a target for PKA. Using an in vitro kinase assay, we show that G4 is indeed phosphorylated by PKA. Moreover, cAMP treatment of MA-10 Leydig cells increases G4 DNA binding activity without affecting its expression level, suggesting that G4 phosphorylation alters its DNA binding properties. Thus, G4 constitutes a novel effector of hormone-induced gonadal gene expression. Supported by CIHR.

# 9

USE OF THE YEAST THREE-HYBRID SYSTEM TO ELUCIDATE TERNARY PROTEIN INTERACTIONS DURING MEIOSIS.
C.A. Kersten and S. E. Ravnik, Dept. of Cell Biology and Biochemistry, Texas Tech Medical Center, Lubbock, TX 79430

The cell cycle is tightly controlled by cyclins and Cdks which bind together and phosphorylate downstream target proteins. Little is known about the specific molecular role of cyclins and Cdks during the meiotic cell cycle as opposed to the events of mitosis. The meiotic cell cycle is controlled, in part, by cyclin A1 and its Cdk binding partner Cdk2β. Since cyclin A1 (and hence the complex) is required for meiosis, we tested the hypothesis that the complex interacts with other proteins to carry out its biochemical function. Therefore, we used the yeast three-hybrid system as a means to further elucidate the role of the cyclin A1/Cdk2β complex by determining which proteins interact in a ternary protein complex. Approximately 7 x $10^6$ clones from a mouse testis cDNA library in the pGAD10 vector (Clontech) were screened using both mouse cyclin A1 + Cdk2β expressed from the pBridge vector (Clontech) in the yeast strain AH109. Potential interactors will grow on nutrient selective media and turn blue in the presence of x-alpha galactosidase due to expression of conditional nutritional and reporter genes, respectively. To date, approximately 1600 clones have been isolated and further selected based on growth and x-alpha gal reactivity. Of these putative interactors, several have significant similarity to known proteins in the database. Of particular interest are 1) the 5' nuclear cap binding protein subunit 1 which may suggest a role for Cylin A1/Cdk2β in processing of specific meiotic mRNAs such as suggested for Cyclin E, and 2)the mouse SCF complex protein Skp1 which is involved in the protein degradation pathway. Continuing experiments include further characterization of these clones by biochemical binding studies such as immunoprecipitation, and expression analysis.

# 10

**ACTIVATION OF THE MITOCHONDRIA-DEPENDENT APOPTOTIC PATHWAY IN HEAT-INDUCED TESTICULAR GERM CELL APOPTOSIS IN FAS LIGAND-DEFECTIVE MICE.** AP Sinha Hikim, YH Lue, S Rodriguez*, PH Yen*, C Wang, and RS Swerdloff, Departments of Medicine and Pediatrics, Harbor-UCLA Medical Center, and Research and Education Institute, Torrance, CA.
In studies using the Fas ligand (FasL)-defective gld mice that express a nonfunctional form of FasL, we have shown that heat-induced germ cell apoptosis is not blocked, thus, providing evidence that the Fas signaling system is not required for heat-induced germ cell death. We now report on the role of the mitochondria-dependent death pathway in mild heat-induced germ cell apoptosis in FasL defective mice. Scrota of adult (8 weeks old) wild type and gld mice were exposed to 22 C (control) or 43 C for 15 minutes, and the animals were killed 2 or 6 h after heat treatment. In situ TUNEL assay showed massive apoptosis of germ cells, as previously reported by us, between 2 and 6 h after heat exposure specifically at stages I-IV and XI-XII in both wild type and gld mice. Western bot analysis showed no obvious alterations in FasL levels while Fas levels appeared to be slightly lower in the testes of gld mice 6h after heat treatment. Consistent with our previous observations in rats, a redistribution of Bax from its normal cytoplasmic to perinuclear localization was clearly evident in heat susceptible germ cells of both wild type and gld mice. The redistribution of Bax is further accompanied by activation of the initiator caspase 9 and the executioner caspase 3 but not 7. Taken together, these results indicate selective involvement of the mitochondria-dependent apoptotic pathway in heat-induced germ cell apoptosis.
Supported by a grant from NIH (HD39293).

# 11

**EXPRESSION AND INHIBITION OF RECOMBINANT GAPDS, THE SPERM-SPECIFIC ISOFORM OF GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE** WeiDong Qu1,3*, PL Magyar1*, K Miki2*, M Goto2*, DA O'Brien1 1Laboratories for Reproductive Biology, Dept of Cell & Developmental Biology, University of North Carolina, Chapel Hill, NC, 2NIEHS, NIH, Research Triangle Park, NC, 3 Dept of Environmental Health, Fudan University, Shanghai, China
GAPDS, a novel glycolytic enzyme in mammalian sperm, is tightly bound to the fibrous sheath and appears to be required for hyperactivated motility. Several studies indicate that sperm GAPDS is more sensitive to substrate inhibition than the isozyme expressed in somatic cells (GAPD). We have expressed recombinant mouse GAPD and a truncated GAPDS (tGAPDS) as GST fusion proteins to compare activities and inhibition in vitro. The truncated form is 70% identical to GAPD and lacks only the N-terminus that anchors GAPDS to the fibrous sheath. Soluble fusion proteins were purified and GST was removed by thrombin cleavage. Western blots confirmed the size (36,000) and specific immunoreactivity of each isozyme. Recombinant GAPD and tGAPDS had comparable activities in assays measuring reduction of the NAD cofactor. a-chlorohydrin, which is metabolized by sperm to a potent substrate inhibitor of GAPDS, had no effect on the recombinant is ozymes. Synthesis of an active metabolite, such as (S)-3-chlorolactaldehyde, will be required to compare substrate inhibition of GAPD and GAPDS. Adenine nucleotides that act as competitive inhibitors of NAD cofactor binding caused dose-dependent inhibition of both GAPD and tGAPDS. Inhibition of both isozymes was >65% at 10 mM ATP. However, ADP and cyclic AMP showed more marked inhibition of somatic GAPD compared to tGAPDS. Further analyzes of recombinant enzyme inhibition may identify new contraceptive strategies.

# 12

**BINDING STUDIES OF THE MOUSE OUTER DENSE FIBER PROTEIN TPX-1**
**D.M. Hickox*, D.M. de Kretser, M.K. O'Bryan*** Monash Institute of Reproduction & Development, Monash University, Melbourne, Victoria, 3168, Australia

To fully understand the processes underlying fertilisation, the molecular mechanisms behind sperm tail function must be characterised. Towards this aim, the mouse gene, *tpx-1,* is currently under investigation. *Tpx-1* encodes a 1.6 kb transcript which is translated into a protein of 25kDa and 27kDa and is localised to the acrosome and the outer dense fibers of the sperm tail. Tpx-1 has been implicated in Sertoli cell – germ cell adhesion and is a member of the cysteine-rich secretory protein (CRISP) family. CRISPs are found in various cell types, including several secretory glands and T cells. Characterisation of *tpx-1* will offer an increased understanding of both sperm function and CRISP biology as a whole, having relevance to both infertility and contraceptive research.

Towards determining the function of tpx-1, a yeast two-hybrid approach has been taken. The region coding for the mature tpx-1 protein was used as bait to screen a testis expression library. One hundred and fifty two yeast colonies expressing putative tpx-1 binding partners have been detected. Of these positive clones, several represent duplicates and previously unidentified cDNAs. Four putative interacting proteins have been selected for further characterisation: MSJ-1, RANBP-9, basigin and a novel CRISP we have named CRISPY. Preliminary data provides evidence for the co-expression of these proteins with tpx-1, both temporally and locally. Protein binding will be confirmed using co-immunoprecipitation studies, which are in progress. The combination of molecular biological and biochemical approaches will provide further support for interaction between tpx-1 and these proteins and will also provide insight into the role of tpx-1 in sperm function and/or development.

# 13

**THE CYSTATIN-RELATED EPIDIDYMAL SPERMATOGENIC (CRES) PROTEIN IS AN INHIBITOR OF THE PROHORMONE PROCESSING SERINE PROTEASE PC2.** G.A. Cornwall,1, A. Cameron*2, I. Lindberg*3, D. Hardy*1, and N. Hsia1 1 Texas Tech University Health Sciences Center, Lubbock, TX., 2 University of Bristol, U.K., 3 Louisiana State University Health Sciences Center, New Orleans, LA. We previously established that the CRES protein defines a new subgroup in the Family 2 cystatins of cysteine protease inhibitors. However, CRES lacks two of the three sequence motifs necessary for cysteine protease inhibition and thus may not function as a classic cystatin. Furthermore, unlike the ubiquitous expression of the cystatins, CRES expression is limited to the proximal caput epididymidis, round spermatids, and the anterior pituitary gonadotrophs. We tested whether recombinant CRES protein functions as a protease inhibitor and inhibits the hydrolysis of Z-Phe-Arg-MCA by the cysteine proteases papain and cathepsin B. As expected, cystatin c was a potent inhibitor of both papain and cathepsin B, but CRES showed little to no inhibition of the two proteases. Because CRES protein is present in the pituitary gland, a site of high levels expression of prohormones and PC2, we next tested whether CRES inhibits the serine protease PC2. In contrast to cystatin c which showed little inhibition of PC2, CRES exhibited a dose-dependent inhibition of Pyr-Arg-Thr-Lys-Arg-MCA hydrolysis by PC2. Kinetics of inhibition by CRES were mixed-type with nanomolar Kis and predominantly competitive inhibition at lower CRES concentrations. CRES did not inhibit other serine proteases including trypsin nor the prohormone processing enzymes PC1 and furin. We conclude that CRES preferentially inhibits PC2 in vitro and suggest that CRES is an endogenous inhibitor of PC2 or PC2-like proteases.

# 14

**POTENTIAL ROLE OF A NA+/HCO3- COTRANSPORTER IN MOUSE SPERM CAPACITATION** I. Demarco(1), F. Espinosa(2), J. Sosnik(1), J. Hockensmith(3), G. S. Kopf(4), A. Darszon(2) and P. E. Visconti(1). Department of Cell Biology (1) and Biochemistry and Molecular Genetics (3), Univ. of Virginia. Charlottesville, Va; Department of Genetics and Molecular Physiology, Institute of Biotechnology, UNAM, Mexico (2); Center for Research on Reproduction & Women's Health, Univ. of Pennsylvania, Philadelphia, Pa (4). Mammalian sperm acquire fertilization capacity after residing in the female tract for a finite period of time. The physiological changes occurring in the female reproductive tract that renders the sperm able to fertilize constitute the phenomenon of "sperm capacitation Capacitation is associated with an increase in the tyrosine phosphorylation of a subset of proteins. This increase, as well as the process of capacitation itself, is regulated by a HCO3-/cAMP-dependent pathway involving protein kinase A (PKA). Zeng et al. (1995) reported that capacitation is accompanied by hyperpolarization of the sperm plasma membrane. Here we present evidence that, in addition to its role in the regulation of adenylyl cyclase, HCO3- might also have a role in the regulation of plasma membrane potential in mouse sperm. Addition of HCO3- but not Cl- induces a hyperpolarizing current in mouse sperm plasma membranes. This HCO3--dependent hyperpolarization was not observed when Na+ was replaced by the non-permeable cation choline+. Replacement of Na+ by choline+ also inhibited the capacitation-associated increase in protein tyrosine phosphorylation. The lack of an increase in protein tyrosine phosphorylation was overcome by the presence of cAMP agonists in the incubation media. Both the lack of a hyperpolarizing HCO3- current as well as the inhibition of the capacitation-dependent increase in protein tyrosine phosphorylation in the absence of Na+ suggest that a Na+/HCO3- cotransporter is present in mouse sperm and is coupled to events regulating capacitation.

# 15

**PDC-109 ENABLES EPIDIDYMAL BULL SPERM TO BIND TO OVIDUCTAL EPITHELIUM TM** Gwathmey, GG Ignotz*, SS Suarez; Dept Biomedical Sci, Cornell Univ, Ithaca, NY 14853

After insemination, mammalian sperm are sequestered in the oviduct by binding to its mucosal epithelium. Sequestration of sperm in this reservoir evidently serves to maintain their fertility until ovulation occurs. Sperm bind to the epithelium via a lectin on the plasma membrane overlying the acrosome. In cattle, the sperm lectin binds to a fucosylated molecule on the epithelial surface. We identified the bull sperm lectin as PDC-109, a secretory protein of the seminal vesicles.ÜBecause sperm do not encounter PDC-109 until exposure to seminal plasma, we investigated whether epididymal sperm would bind to oviductal epithelium, and, if not, would addition of PDC-109 confer binding ability. Explants of epithelium, pooled from oviducts of 3 cows, were prepared just before each experiment. They consisted of irregular balls of epithelium, with cilia beating on the outer surface. Sperm were flushed from the caudal epididymis, washed in TALP medium, incubated in 250 ug/ml PDC-109 or TALP alone for 20 min, washed again, and added to explants. Washed ejaculated sperm were used as a positive control for binding. Binding was measured as the number of sperm per 0.1 mm sq of explant surface. Fewer epididymal sperm bound to explants ($p < 0.01$; mean +/- SEM: 6 +/- 1.7) than did ejaculated sperm (34 +/- 6.5). However, addition of PDC-109 to epididymal sperm increased binding density (33 +/- 4.5) to that of ejaculated sperm.ÜEjaculated and epididymal sperm membrane extracts were subjected to polyacrylamide gel electrophoresis and western blot analysis with antibody to PDC-109. A 16.5 kDa protein labeled by anti-PDC-109 was detected in ejaculated, but not epididymal, sperm extracts. These results indicate that PDC-109 confers on epididymal sperm the ability to bind oviductal epithelium. USDA grant 2001-35203 11132

# 16

**THE NICOTINIC ACETLYCHOLINE RECEPTOR IS INVOLVED IN THE ZP3-INITIATED ACROSOME REACTION OF HUMAN SPERM** Chris Bray, Jung-Ho Son and Stanley Meizel. Department of Cell Biology and Human Anatomy, School of Medicine, University of California at Davis, One Shields Avenue, Davis, California 95616-8643

Previously, work from this laboratory has shown that two neuronal receptor/channels, the glycine receptor and the GABAA receptor were involved in the mammalian sperm acrosome reaction (AR) initiated by the egg zona pellucida (ZP) and progesterone respectively. In the present report, we present the first data suggesting that another neuronal receptor/channel, the nicotinic acetylcholine receptor (nAChR) is involved in the human AR initiated in vitro by a purified recombinant human ZP protein, ZP3 (rhZP3) expressed in CHO cells. In a recent report, we were able to demonstrate that rhZP3 possesses biological activity as it is able to rapidly stimulate the AR in capacitated human sperm. Preincubation of capacitated human sperm with the highly specific nAChR anatagonist alpha bungarotoxin (100 nM) significantly inhibited the AR initiated by rhZP3 ($n = 6$, $p < 0.05$) but not AR initiated by the calcium ionophore ionomycin ($n = 3$) or progesterone ($n = 4$). Sperm viability and motility were not effected by alpha bungarotoxin. Acetylcholine (10 μM) significantly initiated the human AR, ($n = 4$), and alpha bungarotoxin (100 nM) significantly inhibited that initiation ($n = 3$). These results suggest ion flux via the nAChR is important to the human AR initiated by the ZP. Research supported by NIH grant 33369 to S.M. The rhZP3 was a generous gift from Dr. J. D. Harris.

# 17

**SPERM PROTEASOMES ARE INVOLVED IN HUMAN FERTILIZATION.**

P. Morales, M Kong*, C. Pasten*, E. Pizarro*. Faculty of Health Sciences. University of Antofagasta. Antofagasta, Chile.

Fertilization in mammals comprises the sequential interactions of the sperm with the cumulus oophorus, zona pellucida (ZP), and oocyte plasma membrane. Here we report the presence of proteasome activity in human sperm and its participation during the fertilization process. Sperm proteasome activity was measured using the fluorogenic substrate Suc-Leu-Leu-Val-Tyr-AMC in the presence or absence of different concentrations of the inhibitor clasto-lactacystin [beta]-lactone. The participation of proteasomes during sperm-ZP binding, ZP- and progesterone- (P) induced acrosome reactions (AR), and P-induced Ca2+ influx was also evaluated. Motile sperm, obtained through a Percoll gradient, were incubated in modified Tyrodeìs medium (2.6% BSA) at 37 -"C and 5% CO2. Sperm-ZP binding was evaluated using the hemizona assay; ZP-induced AR was evaluated incubating several ZP with sperm for 1 min. Some zonae were then fixed (pulse); the rest were transferred to a sperm-free drop and incubated for 30 min (chase). Sperm-ZP bound after chase and pulse were evaluated for AR. P-induced AR was evaluated incubating overnight capacitated sperm with 6.9 µM P for 15 min. AR was scored using the supravital dye Hoechst 33258 and the PSA-FITC lectin. Ca2+ influx was evaluated using fura-2AM and a spectrofluorometer. Results indicated that intact as well as sperm extracts possess proteasome activity, susceptible to inhibition by lactacystine; sperm-ZP binding was not inhibited by lactacystine; and both ZP- and P-induced AR was inhibited by lactacystine. Preliminary dat suggest that P-induced Ca2+ influx was also inhibited by lactacystine. In conclusion, human sperm proteasomes are involved in the exocytosis of the acrosome, perhaps in events upstream of Ca2+ influx. FONDECYT 1011051.

# 18

**INJECTION OF SPERM NUCLEAR HALOS INTO OOCYTES RESULTS IN NORMAL PATERNAL PRONUCLEI CHROMOSOMES.** Isaac Mohar*, Monika A. Szczygiel*, and W. Steven Ward*. Institute for Biogenesis Research, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, HI.

Mouse sperm nuclei extracted with 2 M NaCl and 10 mM dithiothreitol to remove the protamines and histones retain their overall morphology, but lose all elements of chromatin structure except the organization of DNA into loop domains. These loops are attached at their bases to the structural element of the sperm nucleus, the nuclear matrix. The DNA appears as a halo surrounding the sperm nucleus as the DNA loops extrude from the nucleus and have been called nuclear halos. We have suggested that this sperm DNA loop domain organization is required for embryogenesis, and have developed a technique to inject mouse sperm nuclear halos into oocytes to test this hypothesis. Here, we report that sperm nuclear halos can decondense properly, form pronuclei, and that these pronuclei have normal chromosomes in 32% of the injections. These data suggest that sperm nuclear halos retain all the information necessary for normal chromosomal organization, and that micromanipulation of sperm nuclear halos can be accomplished without damaging the DNA. When eggs were allowed to develop in vitro, none of the nuclear halo injected oocytes developed to blastocysts. It is not clear whether this is because of a technical problem yet to be solved, or an indication that other chromatin structures that are destroyed by the 2 M NaCl extraction are also required for development. However, the data do indicate that it is possible to micromanipulate sperm nuclear halos without damaging the DNA. This is remarkable given that in sperm nuclear halos most of the DNA is completely devoid of protein and is essential naked as loops extruding from the nuclear matrix.

# 19

**CHARACTERIZATION OF UNIQUE TRANSCRIPTION OF GERM CELLS FROM THE MOUSE TESTIS WITH THE USE OF AFFYMETRIX GENECHIP™ARRAYS.**

D.J. McLean*, D.J. Pouchnik*, P.J. Friel*, J.R. McCarre2*, M.D. Griswold. Center for Reproductive Biology, Washington State University, Pullman, WA and Department of Biology, Univesity of Texas at San Antonio, San Antonio, TX. We have used Affymetrix GeneChip™ oligonucleotide arrays to characterize the gene expression in several specific cell types of the testis. Testicular cell types from outbred Swiss (CD-1) mice include primitive type A spermatogonia and primitive Sertoli cells from 6-day old mice, type A and type B spermatogonia from 8-day old mice, and pachytene and round spermatids from adult mice. Total RNA from each sample was used to generate target to probe MGU74Av2 gene arrays containing probe sets for approximately 12,000 genes. The percentage of GeneChip™ genes expressed were as follows: type A spermatogonia-37.2%, type B spermatogonia-42.9%, pachytene spermatocytes-28.5%, round spermatids-34.2% and Sertoli cells-37.0%. Genes such as lactate dehydrogenase C and transition protein 1 had high expression patterns in pachytene spermatocytes but not type A spermatogonia. Similarly, type A spermatogonia had high expression of genes associated with extracellular matrix binding such as laminin while round spermatids had high expression of sperm tail genes. Comparison analysis demonstrated that type A spermatogonia expressed 1221 unique genes when compared to pachytene spermatocytes and round spermatids. Likewise, pachytene spermatocytes expressed 320 unique genes and round spermatids expressed 572 unique genes. Further analysis will focus on identifying and verifying the expression patterns of novel genes associated with specific cell types of the testis to utilize this technology as an approach to identify contraceptive targets. Supported by HD10808 from NICHD.

# 20

**AN INITIAL PROFILING OF MESSENGER RNA EXPRESSION FROM MICRODISSECTED STAGE-SPECIFIC TUBULES BY OLIGONUCLEOTIDE ARRAY** T.B. Guo*, J. Toppari, W.A. Salameh, Dept. of Medicine, Harbor-UCLA Medical Center, Torrance, CA, USA and Dept. of Physiology, Univ. of Turku, Turku, Finland

Transcriptional changes in a given biological process can be studied by use of DNA array technology. Though gaining acceptance, this technology has yet to be widely applied to the testis, partly due to its intrinsic extraordinary heterogeneity of cell types and their stages of development. The meiotic transcriptional program in budding yeast has been characterized by array experiments. Genes were grouped based on their temporal expression patterns during meiosis. In trying to understand meiosis initiation in mammals, i.e. signals that govern the progression from type B spermatogonia to preleptotene spermatocytes, we have prepared RNA samples from stage-specific adult mouse seminiferous tubules by transillumination-assisted microdissection. Here we describe our initial oligonucleotide microarray experiment to compare the mRNA expression changes between stages V and VII. Affymetrix arrays were processed according to manufacturerìs specifications and were analyzed by the GeneChip software. The staining intensities were comparable and the background was low. Housekeeping genes like bactin, GAPDH, cyclophilin, all were abundantly expressed and constant. Out of 6500 probe sets, a vast majority did not change significantly, but 221 (3.4%) were judged to have increased expression levels from stages V to VII and 624 (9.6%) were decreased. Overall, the gene expression patterns and levels agree well with the literature. For example, some postmeiotic genes such as transition proteins and Hsc70 were increased. SCP2, also described in the yeast array, was moderately expressed. Components of Wnt pathway were upregulated. Using a similar approach, these results will be substantiated in a developmental study.

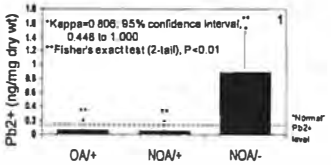
# 21

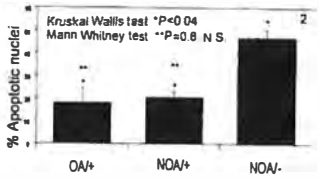
HYPOSPERMATOGENESIS: CAN WE BLAME THE CALCIUM CHANNEL?
[1]C. Millan, [1]I.R. Hurley, [2]J.L. Marmar, [1]S. Benoff. [1]North Shore-Long Island Jewish Research Institute, Manhasset, NY, [2]Robert Wood Johnson Medical School, Camden, NJ

Our Affymetrix Genechip studies indicated that pro-epoptotic genes in testis of Sprague Dawley rats are up-regulated compared to controls when given drinking water containing 0.3% lead acetate for 1 week. TUNEL assay confirmed that apoptosis in seminiferous epithelium correlated with lead intake ($P < 0.04$). Lead often competes for calcium binding sites. Modulation of intracellular calcium through L-type voltage-dependent calcium channels (L-VDCC) effects apoptosis in neural, pancreatic, cardiac and prostatic tissues. In brain, this mechanism involves, at least in part, altering splicing of calcium transporters (Guilarte *et al.*, Mol Brain Res 76:299-305, 2000) under the control of calcium/calmodulin-dependent protein kinase type IV (CaMK IV)-responsive RNA elements (Xie and Black, Nature:410:936-9, 2001). We have observed L-VDCC mRNAs are deleted in exons 7 and/or 8 (responsible for metal ion sensitivity) in ~70% (25/36) of testis biopsies of men with non-obstructive azoospermia (NOA). The goal of this study was to determine the plausibility of a mechanistic connection between ion channel structure and lead-induced apoptosis leading to hypospermatogenesis.

We probed lead levels, apoptosis and L-VDCC mRNA sequence (by RT-PCR) in human NOA (n=14) testis biopsies, using obstructive azoospermia (OA; n=3) controls. Elevated testicular lead levels correlated with presence of deleted L-VDCC transcripts (Figure 1; "+" =full length L-VDCC; "-" = deleted); also elevated testicular apoptosis with deleted L-VDCC transcripts (Figure 2). Testis L-VDCC mRNAs contained CaMK IV splicing signals. Immunocytochemistry indicated that both full-length and deleted transcripts encoded L-VDCC antigenic epitopes that localized to plasma membranes of seminiferous epithelium. L-VDCC proteins regulated as per our mechanism are appropriately expressed on the cell surface. These data suggest that alteration of calcium homeostasis is an important intermediary step in various forms of hypospermatogenesis.

(Supported by NIEHS/NIH Grant Nos. ES 06100 and ES 10496 to SB)




# 22

**THE TFIIH BASAL TRANSCRIPTION COMPLEX IS ABUNDANTLY EXPRESSED DURING MEIOSIS IN THE MOUSE TESTIS.** **S.E.** Ravnik, E.A. Whitmire*, W.L. Boston*, and P.L. Frisbie*, Dept. of Cell Biol. & Biochem., Texas Tech Medical Center, Lubbock, TX 79430.

Cell cycle passage requires the activity of cyclin/Cdk complexes. For full kinase activity, the Cdk subunit must be phosphorylated by another cyclin/Cdk complex made up of Cyclin H/Cdk7. Cyclin H and Cdk7 also functions in transcriptional regulation by interaction with the TFIIH basal transcription factor complex and phosphorylation of RNA polymerase II. During our studies of cyclin H and Cdk7 during meiosis, we observed that these proteins are abundant in male mouse germ cells undergoing meiosis, similar to other proteins involved in basic transcriptional mechanisms, such as RNA polymerase II and the TATA Binding Protein. This is in contrast to mitotic cells, where Cdk7 levels and activity decline at M-phase. Since cyclin H and Cdk7 function as integral members of the TFIIH complex in mitotic cells, we have examined the meiotic expression of MAT-1, p62, and p89, other components of the TFIIH complex. Our analysis of mRNA and protein expression reveal that, like Cyclin H and Cdk7, TFIIH components are abundantly expressed in male meiotic germ cells and show an increase in the expression levels as germ cell development proceeds. Our data, along with other published reports suggests that overexpression of components of the basal transcription machinery play a key role in maintenance of gene transcription during spermatogenesis. [Funded in part by the South Plains Foundation and NICHD].

# 23

**CKS2 IS UPREGULATED DURING MEIOSIS AND BINDS THE MEIOTIC ISOFORM, CDK2β.**

*E.N. Attaya and S.E. Ravnik*, Dept. of Cell Biology and Biochemistry, Texas Tech Medical Center, Lubbock, TX 79430

Cell division is controlled, in part, by cyclin dependent kinases (Cdks) which, when bound to a cyclin, are activated and phosphorylate various downstream targets. Using the alternatively spliced Cdk2 isoform, Cdk2β, we used the yeast two-hybrid system to identify binding partners of Cdk2β. Since Cdk2β may be a key player in meiosis due to its expression in prophase of meiosis I in spermatogenesis, we screened an adult mouse testis cDNA library identifying the mouse homolog of Cks2 (CDC28 kinase subunit). Cks proteins have been reported to be required for entry into mitosis, and may target the cyclin-Cdk complex to different substrates. Despite the work performed to elucidate the role of Cks proteins in mitosis, their exact function remains unclear. To date, there are no data on the function of Cks proteins during meiosis. Here we have examined the expression of Cks2 with quantitative PCR using the Roche Light Cycler System and *in situ* hybridization. Cks2 is more abundant in day 17 and adult mice than Cks1 and is only weakly detected in mutants which lack germ cells. Cks2 mRNA is localized to pachytene and diplotene spermatocytes, whereas Cks1 shows no such localization. The localization of Cks2 in meiotic germ cells was confirmed using western and immunohistochemistry analyses. Furthermore, binding of Cks2 to Cdk2β was shown biochemically using immunoprecipitation and the specificity of Cks2 by peptide blocking experiments. Our experiments are consistent with the hypothesis that Cks2 is the predominant meiotic Cks and not Cks1. [Supported by grant #1FY99-601 from the March of Dimes (SER) and #5 T32 HD07271-16 from the NICHD (ENA).]

# 24

**CHARACTERIZATION OF THE ROLE THAT RAR-alpha PLAYS IN SPERMATOGENESIS BY EXAMINATION OF THE RAR-alpha KNOCKOUT MICE**

T.J. Doyle*, K.W. Braun*, D McLean*, Patricia Shane*, M.D. Griswold, K.H. Kim, School of Molecular Biosciences, Center for Reproductive Biology, Washington State University, Pullman, WA

Retinoic acid (R-alpha) has been shown to be necessary for spermatogenesis. An essential mediator of RA action in the testis is the retinoic acid receptor a (RAR-alpha). RAR-alpha knockout (KO) male animals are sterile. RARalphaKO testicular morphology shows varying degrees of degeneration, from seminiferous tubules completely degenerated and void of germ cells, similar to the testicular morphology of vitamin A-deficient animals, to seminiferous tubules that have a full complement of germ cells. However, RAR-alpha protein expression was not detected in the testis of KO animals by Western blot and immunohistochemical analyses. Epididymal sperm count of KO animals was 10% of the number found in the epididymis of wild type animals. To investigate the possible mechanism of germ cell loss, analysis was performed to detect apoptotic cells. There was an increase in the number of germ cells undergoing apoptosis in the testis of KO animals when compared to that in wild type animals. These results suggest that RAR-alpha expression in the testis is necessary for increased survival of germ cells. To investigate whether RAR-alpha in the germ cells was important for their survival, we used spermatogonial transplantation technique. Wild type germ from C57/BL Rosa mice were injected into the testis of RAR-alpha KO recipient animals, followed by a 130-day recovery period. Immunostaining for RAR-alpha on the injected testicular sections revealed that seminiferous tubules were colonized and repopulated by Rosa germ cells. These results suggest that RAR-alpha in germ cells is important for increased survival of germ cells.

# 25

**INHIBITION OF SPERM PRODUCTION IN MICE BY ANNEXIN V MICROINJECTED INTO SEMINIFEROUS TUBULES: A POSSIBLE ETIOLOGY OF PHAGOCYTIC CLEARANCE OF APOPTOTIC SPERMATOGENIC CELLS AND MALE INFERTILITY** Yuji Maeda*, Akiko Shiratsuchi*, Mikio Namiki and Yoshinobu Nakanishi*, Department of Urology, Kanazawa University School of Medicine and the Graduate School of Medical Science, Kanazawa University

Many differentiating spermatogenic cells die by apoptosis during the process of mammalian spermatogenesis. However, apoptotic spermatogenic cells are hardly detectable by histologically examining the testis, probably due to the rapid elimination of dying cells by phagocytosis. Previous in vitro studies showed that Sertoli cells selectively phagocytose dying spermatogenic cells by recognizing the membrane phospholipid phosphatidylserine (PS) exposed to the surface of spermatogenic cells during apoptosis. We examined here if PS-mediated phagocytosis of apoptotic spermatogenic cells is occurring in vivo. For this purpose, the PS-binding protein annexin V (AV) was microinjected into the seminiferous tubules of live mice, and their testes were histologically examined. The injection of AV caused no histological changes in the testis, but significantly increased the number of apoptotic spermatogenic cells as assessed by the TUNEL staining. The number of Sertoli cells did not change in the AV-injected testes, and AV itself did not induce apoptosis in primary cultured spermatogenic cells. These results indicated that AV inhibited the phagocytic clearance of apoptotic spermatogenic cells and suggested the occurrence of PS-mediated phagocytosis of those cells in vivo. Finally, the injection of AV brought about a significant reduction in the number of spermatogenic cells in the seminiferous tubules and of sperm in the epididymis under certain experimental conditions. This suggests that the elimination of apoptotic spermatogenic cells is required for the production of sperm.

# 26

**SPERMATOGENESIS AFTER ORCHIECTOMY ALONE TREATMENT FOR NON-SEMINOMA STAGE I TESTICULAR CANCER**

V. Mamaladze, National Cancer Center, Tbilisi, Georgia

OBJECTIVE: To review patterns of spermatogenesis in a long-term follow-up of pts. with stage I non-seminoma testicular cancer treated by orchiectomy alone and to assess correlation between spermatogenesis recovery and FSH level. MATERIAL AND METHODS: From 1983 to 1996, 54 pts. with stage I non-seminoma testicular cancer treated by orchiectomy alone who were disease-free were analyzed by the requirement of pre- and posttreatment sperm count (SC), Sperm morphology, motility, penetration of the spermatozoa and FSH level. Sperm characteristics were assessed at the baseline, 1 year and >=2 years after orchiectomy, using conventional methods. SC were classified as normo- (NS - >10x106/ml), olygo- (OS Ò 1to 9x106/ml) and asoospermic (AS - <1x106/ml). Serum FSH level was analyzed by radioimmunoassay. The mean value of two samples from each pts. was used. Median follow-up time was 42 months. The chi-square and log-rank tests were used for statistical analysis.

RESULTS: At baseline 29 pts. (54%) were NS, 14 (26%) were OS and the remaining 11 pts. Ò AS. During follow-up of 1 year the number of NS pts. increased to 42 (78%) ($p<0.05$). The mean sperm count was increased from 22 to 34x106/ml during the same period of time ($p<0.05$), without any statistically significant increase after 2 years. There were no significant difference concerning other parameters of the sperm analyzes. The FSH level was in correlation with SC recovery. Mean SC increased significantly in 46 pts. with the normal FSH values, whereas there was no increase in the same parameter on the elevated baseline FSH pts. ($p<0.05$). CONCLUSION: Our data suggest that spermatogenesis recovery is noted within 1 year after orchiectomy alone for stage I non-seminoma testicular cancer and recovery is highly associated with serum FSH level.

# 27

**RESTORATION OF SPERMATOGENESIS AND FERTILITY IN AZOOSPERMIC MUTANT MICE BY SUPPRESSION AND REELEVATION OF TESTOSTERONE FOLLOWED BY ICSI**

A.Tohdal*, K.Matsumiya1, T.Okuno2*, M.Okabe*, H.Kishikawal*, A.Tsujimural*, K.Dohmae3*, A.Okuyamal, Y.Nishimune4*. 1Dept. of Urology, Osaka University Medical School, 2Genome Information Research Center, 4Research Institute for Microbial Diseases, Osaka University, Suita, Japan. 3Dept. of Food Science and Nutrition, Mukogawa Women's University, Nishinomiya, Ja an.

Male mice homozygous for the mutant juvenile spermatogonial depletion (jsd) gene show spermatogonial arrest and an elevated intratesticular testosterone level, like many other experimental infertility models such as those with irradiation- or chemotherapy-induced testicular damage. In this category of infertile males, suppression of the testosterone level induces spermatogonial differentiation to spermatocytes but no further. In the present study with jsd mice, we carried out induction of spermatogenesis first to spermatocytes by suppression of testosterone levels using a GnRH antagonist, Nal-Glu, at a dose of 2.5mg/kg/day for 4 weeks, and then to elongated spermatids by withdrawal of Nal-Glu. Spermatids were seen in the cross-sections of seminiferous tubules in all mice treated by administration and subsequent withdrawal of Nal-Glu. Four weeks after withdrawal of Nal-Glu, some of the germ cells differentiated into elongated spermatids. Thus, in this model, testosterone suppresses spermatogonial differentiation, although it stimulates meiosis. Furthermore, we successfully rescued the male sterility in jsd mice by subsequent ICSI using the elongated spermatids induced by the programmed hormone therapy.

# 28

**SEMINIFEROUS EPITHELIUM CYCLE LENGTH IN BOLOMYS LASIURUS (RODENTIA, MURIDAE)** C.D.L Nessralla*, L.R. França. Lab. of Cellular Biology. Dept. of Morphol., Federal University of Minas Gerais - Belo Horizonte - MG - Brazil - 31270-901.

Bolomys lasiurus is a rodent species of grassland and savanna, largely distributed in eastern South America. For this reason, this species is being frequently utilized in studies involving ecological approaches. Nevertheless, the literature related with the reproductive biology in this species is still scarce. In this regard, the main objective of the present work was to investigate the seminiferous epithelium cycle (SEC) in Bolomys, using the acrosomic method. Thirteen adult animals (60g of BW) were sacrificed during the breeding season. To estimate the duration of spermatogenesis the animals received 60µCi of tritiated thymidine i.p. 1h, 8 and 20d before sacrifice. The testes were perfused-fixed through the left ventricle with 4% glutaraldehyde. Tissue samples were embedded in plastic and routinely prepared for histological and autoradiographic evaluation. The mean frequencies (%) of the XII characterized stages of SEC, based on 350 seminiferous tubule cross-sections for each animal, were as follow: I, 4.5; II, 4.7; III, 16.4; IV, 6.2; V, 3.8; VI, 6.6; VII, 19.0; VIII, 6.5; IX, 12.8, X, 5.5; XI, 6.9; XII, 7.2. The most advanced germ cells labeled at 1h, 8 and 20d after thymidine injections were, respectively, preleptotene/leptotene at stage IX, pachytene at stage IX, and elongate spermatids at stage III. In association with the mean frequencies found for each stage, the autoradiographic analysis showed that the spermatogenic cycle in Bolomys lasted 7.8 days. This value is one of the lowest found for the mammalian species investigated up to date. Considering that the entire spermatogenic process takes approximately 4.5 cycles to be completed, its total duration in Bolomys is very short, lasting 35.1 days. Financial support: CNPq.

# 29

**IDENTIFICATION OF RETINOIC ACID INDUCED GENES IN MOUSE TESTIS BY CDNA MICROARRAY ANALYSIS**

Asa J. Oudes*1, John C. Rockett*2, David J. Dix2*, and Kwan Hee Kim1* 1School of Molecular Biosciences, Center for Reproductive Biology, Washington State University, Pullman, WA 99164. 2United States Environmental Protection Agency, Research Triangle Park, NC 27711.

Vitamin A is essential for spermatogenesis. A deficiency of dietary vitamin A has been shown to cause a loss of germ cells and reproductive dysfunction in mammals. The action of vitamin A is mediated by retinoid receptors, which are ligand-dependent transcription factors. One of the retinoid receptors, retinoic acid receptor alpha (RAR-alpha), is critical for spermatogenesis. RAR-alpha knockout mice are sterile and suffer from reduced viability. We hypothesize that many genes vital to testis function are regulated by RAR-alpha. Thus, it will be important to identify and characterize the genes that RAR-alpha controls in the testis. A mouse Sertoli cell line (MSC-1) and testis from vitamin A-deficient (VAD) mice were used to produce pools of mRNA that have enhanced or repressed levels of RAR-alpha controlled gene expression. mRNA products from MSC-1 cells were converted to cDNA by reverse transcription and hybridized with two cDNA microarrays, one mouse testis specific and the other general mouse cDNA. Analysis of cDNA arrays revealed the expression of Seven in Absentia-1a (SIAH-1a) and T-cell Death Associated Antigen-51 (TDAG51) were increased in MSC-1 cells with retinoic acid treatment. Realtime PCR assays of cDNA derived from the testis of VAD mice treated with retinol confirmed that retinoids enhance the expression of SIAH-1a and TDAG51 in vivo. Finally, the protein expression pattern of SIAH-1a and TDAG51 in VAD and normal mouse testis was determined by immunohistochemistry.

# 30

**CYSTATIN TE-1, A CYSTATIN-RELATED GENE EXPRESSED IN THE TESTIS AND EPIDIDYMIS** Ying Li*, Monty O. Robinson*, Patrick J. Friel*, Derek J. McLean* and Michael D. Griswold. Center for Reproductive Biology, Washington State University, Pullman, WA 99164

We have used differential display reverse transcriptase Polymerase Chain Reaction (DD-RT PCR) to examine Sertoli cell gene expression. As a result, a new member of the mouse cystatin family has been cloned. The cDNA sequence contains an open reading frame encoding a putative 21-residue signal peptide and a mature protein of 117 amino acids, including four highly conserved cysteine residues and motifs of importance for the inhibitory activity of Family 2 cystatins. Unlike other cystatins, the Cystatin TE-1 gene does not contain specific highly conserved motifs thought to be necessary for cysteine proteinase inhibitory activity. Northern blot analysis revealed that the Cystatin TE-1 gene is expressed in testis and epididymis, with no expression detected in other tissues examined. In situ hybridization showed that Cystatin TE-1 mRNA was mainly localized to Sertoli cells and was dramatically restricted in its expression to the proximal caput region of the epididymis. In addition, the CystatinTE-1 transcript decreased after castration, implying that this gene is regulated by androgens. However, testosterone administration failed to maintain cystatin TE-1 expression in the epididymis. Unilateral castration resulted in the dramatic decrease of the Cystatin TE-1 mRNA from the castrate epididymis, but not from the intact epididymis where the connection between the testis and epididymis was maintained. These observations suggest that testicular factors or hormones other than androgens may be involved in the regulation of Cystatin TE-1 gene expression. The highly restricted expression and unusual regulation suggests that CystatinTE-1 may have a very specialized role in the testis and epididymis. Supported by HD 10808 from NICHD.

# 31

INVOLMENT OF GGA IN THE LYSOSOMAL TRANSPORT OF PROSAPOSIN; A PROTEIN EXPRESSED IN SERTOLI CELL

Stephane Lefrancois, Ahmed J. Hassan and Carlos R. Morales
Department of Anatomy and Cell Biology, McGill University, Montreal, Quebec, Canada

Proteins destined for the lysosomes use two well-known transport mechanisms. Soluble proteins such as cathepsin B use the mannose 6-phosphate (M6P) receptor, which in turn interact with GGA while integral membrane proteins such as LAMP-2 interact with AP-3. GGA and AP-3 recruit clathrin that causes the formation of vesicles that are targeted to the lysosomal compartment. Prosaposin is a soluble lysosomal protein that does not use the M6P receptor and cannot directly interact with AP-3. However, we hypothesize that through a novel receptor, prosaposin must use either the GGA or AP-3 mechanism to be packaged into clathrin coated vesicles to be targeted to the lysosomes. In this study, we examine COS-7 cells transfected with a dominant negative construct for GGA along with a human fibroblast cell line that is deficient in AP-3. Cells were grown either on cover slips for confocal microscopy or embedded in Lowicryl for electron microscopy and immunostained with prosaposin, cathepsin B, or LAMP-2 antibody. Our results show that prosaposin is absent from lysosomes of cells that where transfected with a dominant negative construct of GGA, but present in the lysosomes of AP-3 mutant cells. This suggests that prosaposin must interact with an unknown receptor that in turn binds GGA, which recruits the necessary components to form a clathrin coated vesicle to target prosaposin to the lysosomal compartment. (Supported by CIHR)

# 32

**RAB GTPases ARE KEY PLAYERS IN TIGHT (TJ) AND ANCHORING JUNCTION (AJ) DYNAMICS IN THE TESTIS.** Ann S.N. Lau[1,2*] and Dolores D. Mruk[1]. [1]Population Council, New York, NY; [2]The University of Hong Kong, Department of Zoology, Hong Kong.

Previous *in vitro* studies from this laboratory in which rat Sertoli cell (SC) cultures or Sertoli-germ cell (SC-GC) cocultures were utilized, have unequivocally demonstrated changes in the expression of several target molecules during TJ and AJ assembly. However, the identities, not to mention the characterizations, of signaling molecules, which would be responsible for regulating these events remain largely unknown. This study sought to determine the roles of Rab GTPases, in particular Rab13 and Rab8B, small GTP-binding proteins, in TJ and AJ dynamics in the testis. When primary SCs were cultured at high cell density ($0.5x10^6$ cells/cm$^2$) on Matrigel™-coated dishes for 7 days, it was demonstrated that the steady-state Rab13 mRNA level increased significantly, which coincided with the assembly of TJs, as assessed by transepithelial electrical resistance (TER) measurement and passive diffusion of FITC-dextran (M$_r$ 4.4 and 35.6 kDa) across the SC epithelium, and transient induction of TJ-component genes. We, however, failed to detect changes in the expression of Rab13 when SCs were cultured at low cell density ($0.05x10^6$ cells/cm$^2$) in which inter-SC TJs did not form. More importantly, the inclusion of testosterone ($10^{-7}$ M), which was previously shown to enhance TER and stimulate occludin and ZO-1 expression, into SC cultures stimulated Rab13 expression approximately 6-fold, demonstrating TJ component molecules are modulated by androgens. Whereas changes in the Rab13 mRNA level appeared to be restricted to times of TJ assembly, this was not true for Rab8B, which was implicated to participate in AJ dynamics. For instance, Rab8B expression decreased following AJ assembly when freshly isolated GCs, consisting largely of spermatogonia, spermatocytes and elongate spermatids, were added onto SCs, which were previously cultured for 5 days on Matrigel™-coated dishes, at a SC:GC ratio of 1:5. To confirm our hypothesis that Rab8B participates in AJ assembly, we examined the expression of Rab8B by using a reversible *in vivo* model of late-stage GC depletion, in which animals were administered with different regimens of 1-(2,4-dichlorobenzyl)-indazole-3-carbohydrazide (Cheng *et al.*, 2001; *Biol. Reprod.* 65:449-461). However, Rab8B expression tumbled during detachment of GC from the epithelium. Although conventional wisdom would suggest a stimulation of Rab8B expression during AJ disassembly, the loss of GC (adult GC express approximately 5-fold more Rab8B than adult SC) from the epithelium would explain this decrease in testicular Rab8B expression. Collectively, these results have broadened the scope of study of TJ and AJ dynamics in the testis.

# 33

**SERTOLI CELL NUMBER INCREASES MARKEDLY DURING INFANCY IN THE RHESUS MONKEY, BUT TO A LESSER EXTENT THAN THAT DURING PUBERTY IN THIS SPECIES.**

D.R. Simorangkir, T.M. Plant and G.R. Marshall, Department of Cell Biology and Physiology, and Medicine, University of Pittsburgh School of Medicine, Pittsburgh, P A

We have previously established that puberty in the rhesus monkey is associated with a 6-fold increase in Sertoli cell (SC) number that is driven by the elevation in gonadotropin secretion occurring at this stage of primate development (Marshall and Plant, Biol Reprod, 54:1192, 1996). SC proliferation also occurs prior to puberty in this species, but it remains to be determined whether the increase in SC number during this phase of development is restricted to the first few months of life, when, as at the time of puberty, robust LH and FSH secretion is occurring. In order to clarify this issue, 4 newborn (<2 days of age) and 4 infantile (4-5 months of age) males were castrated 2hr after receiving an iv injection of BrdU. Tissue was fixed in Bouinìs and SC numbers were estimated using standard histomorphometry, and expressed as SC number/testis (mean ± SD). The number of SC was 4-fold greater ($p<0.05$, Studentìs t test) in infants (156 ± 49 x 106) than in newborns (42 ± 12 x 106). These values may be compared to those previously reported by us for juvenile (approximately 15 months old) and fully adult males of 286 ± 121 x 106 and 1780 ± 452 x 106, respectively. From the foregoing results, we conclude that SC proliferation during prepubertal development in the rhesus monkey occurs predominantly during infancy, when gonadotropin levels are elevated. Nevertheless, in this higher primate, the pubertal phase of development, which occurs several years after birth when gonadotropin secretion is reestablished, contributes the greatest increase to the final complement of SC in the adult testis. Supported by HD08610.

# 34

**A NEW PROCEDURE TO PURIFY ADULT RAT AND MOUSE LEYDIG CELLS.**

A. Salva*, G.R. Klinefelter l, M.P. Hardy. Population Council, New York, NY and 1 Reprod Tox Branch, US EPA, Research Triangle Park, NC.

From the mid-1980's onwards, Leydig cells have been purified by a multi-step procedure that includes centrifugal elutriation and Percoll density gradient sedimentation in order to study their regulatory biology. The purpose of the elutriation step is to eliminate contaminating testicular cells that have lower sedimentation velocities than Leydig cells, including sperm, condensed and round spermatids, endothelial cells, small germ cells and red blood cells. However, elutriation requires complex training and involves expensive equipment. The objective of this study was to test a new procedure for Leydig cell purification that follows Percoll density gradient sedimentation with centrifugation in a discontinuous gradient of bovine serum albumin (BSA, 10%, 5%, and 2.5%) at 60 x g for 10 minutes. Leydig cells were then collected from the 10% BSA layer. We assessed whether the yield, purity and/or capacity for testosterone (T) production of Leydig cells isolated by the new BSA method were comparable to fractions isolated by an established elutriator-purified method (Klinefelter et al, Biol Reprod 1987, 36: 769-783). The average yield of rat Leydig cells was two million cells/testis, the purity was 97%, and luteinizing hormone (LH) stimulated T production was 722 ng/million cells/3 h. The average yield for mouse Leydig cells was 72000/testis, the purity was 98%, and LH stimulated T production was 3888 ng/million cells/3 h. These numbers were comparable to results obtained by the elutriator method. We conclude that Leydig cells may be isolated efficiently without centrifugal elutriation, by a new procedure that includes a step of discontinuous BSA gradient centrifugation.

# 35

ISOLATION AND FUNCTION OF ADULT RAT SERTOLI CELLS. M.D. Anway*, J. Folmer*, B. R. Zirkin. Div. Reprod. Biol., Johns Hopkins Univ., Baltimore, MD.

Much of what we know of adult Sertoli cell regulation and function has come from in vitro studies of immature cells and from manipulation in vitro. However, many Sertoli cell functions are regulated by conditions that are difficult to replicate in vitro. Our objective was to adapt methods to isolate Sertoli cells rapidly and purely enough to make it possible to treat rats in vivo and then assess Sertoli cell function immediately after their isolation. Testes were first dispersed in collagenase followed by trypsin digestion and then in collagenase/hyaluronidase/DNase/trypsin inhibitor. The dispersed cells were then subjected to hypotonic shock and then filtered through nylon mesh filter. This procedure takes approximately 4 hours. From a single testis, we obtain 4 - 8 million Sertoli cells, with an average yield of about 6 million/testis. The purity, determined by morphological analysis of plastic-embedded cells or after staining for tyrosine-tubulin, ranged from 70-90%. The contaminants typically included intact germ cells (5%), myoid cells (5%) and ruptured cells (5%). Protamine 2 mRNA was not detected in the Sertoli cell preparations, but SGP-2, transferrin, espin, tsx, and cox-1, all Sertoli cell-specific genes, are highly expressed. As expected, age and LH-suppression did not result in changes in Sertoli cell SGP-2 mRNA. In contrast, transferrin mRNA was expressed in young adult rats, increased at age 20 months, and decreased after LH suppression (8 wk, testosterone-estradiol implants). These results suggest that it will be possible to experimentally manipulate Sertoli cells in vivo and then isolate the cells quickly and purely enough to study the effects of experimental manipulation without culturing the cells after their isolation. Supported by NIH grants AG08321 and U54-HD36209.

# 36

**CYCLINS EXPRESSION IN SOMATIC CELLS OF THE TESTIS IN NEONATAL WISTAR RATS** F.M. Pereira*, G.C. Bregunci*, G.D. Cassali*, R.A. Hess1, L.R. França; Dept. of Morphol., Federal University of Minas Gerais, Belo Horizonte - MG - Brazil - 31270-901. 1University of Illinois, Urbana-Champaign, USA.

Eukariotic cell proliferation involves an orderly sequence of events that results in the duplication and division of all cell components. In this process, the cyclin-dependent kinases (CDKs) play an essential role in the regulation of cell proliferation, being activated during specific phases of cell cycle. Although it is well known that Sertoli cell proliferation that occurs during testis development is regulated by FSH and thyroid hormones, there are few data in the literature concerning the type of cyclins involved in Sertoli cell division. In this regard, the main objective of the present study was to investigate the expression of different cyclins in Sertoli cells of Wistar rats, during the period these cells are proliferating actively. Testes from four day old rats were fixed in 2% paraformaldehyde for 2h, and routinely embedded in paraplast. Sections with 4-5µm in thickness were obtained to perform immunocytochemistry detection of cyclins A, B1, D1, D2, and D3, utilizing specific monoclal ab (LabVision, Neomarkers, CA). Except for D3 cyclin, Sertoli cells in young rats were labeled for all other cyclins investigated. However, the percentage of cells labeled for each cyclin investigated showed high variation, being approximately 30% for D1, 15% for D2, 10% for A, and 5% for B1. Additionally, it was observed that peritubular myoid cells and mesenchymal cells (presumably Leydig cell precursors) showed labeling for all cyclins investigated. The present study is the first to perform a more detailed investigation of cyclins expression in somatic cells of the testis in rats, during the early period of postnatal testicular development. Supported by Capes and NIH grant HD35126.

# 37

**SITE-DIRECTED MUTAGENESIS OF THE STAR PROMOTER REVEALS THE REQUIREMENT OF MULTIPLE C/EBP AND GATA ELEMENTS FOR PKA-DEPENDENT SYNERGY BETWEEN GATA-4 AND C/EBPß.** F. Hamel*, J.J. Tremblay, R.S. Viger, CHUL Research Centre, Laval University, Québec, PQ, Canada.
Steroidogenic acute regulatory protein (StAR) regulates cholesterol transport in steroidogenic tissues. StAR gene expression is induced by trophic hormones predominantly through cAMP production and activation of protein kinase A (PKA). Several transcription factors (TFs) are known to regulate the StAR promoter such as SF-1, C/EBPß, and GATA-4 (G4). Although these TFs are key regulators of basal StAR promoter activity, how these factors are integrated as downstream effectors of the hormone stimulatory signal remains poorly understood. We have recently shown that PKA enhances G4-mediated activation of the StAR promoter and that synergy between G4 and C/EBPß is strictly PKA-dependent. The StAR promoter contains one GATA and two C/EBP elements. To determine which elements are required for PKA-dependent synergy between G4 and C/EBPß, we performed 5' deletion analyzes and site-directed mutagenesis. Mutation of the GATA element abolished G4-mediated activation and PKA-dependent synergy with C/EBPß, highlighting the strict requirement for this element. Deletion or mutation of the distal high affinity C/EBP element abrogated PKA-dependent synergy, indicating that the distal C/EBP element is essential and the proximal low affinity C/EBP element is not. Interestingly, mutation of the distal C/EBP element into a GATA element restored PKA-dependent synergy. This suggests that the low affinity C/EBP element, although not sufficient, nonetheless contributes to hormone-induced StAR promoter activity. This contribution, however, appears to require stabilization by neighboring DNA-bound proteins. Thus, the level of C/EBPß and phosphorylation of G4 likely act as signals for acute StAR expression.

# 38

**TUMOR NECROSIS FACTOR-alpha FACTOR INDUCES NEUTROPHIL RECRUITMENT TO TESTICULAR VENULE CHARACTERIZATION OF SIGNALING PATHWAYS.** Jeffrey J. Lysiak and Terry T. Turner. Departments of Cell Biology and Urology, University of Virginia, Charlottesville VA 22908
Ischemia-reperfusion (IR) of the murine testis results in germ cell-specific apoptosis (GCA). Previous studies from this laboratory have demonstrated that testicular IR-induced GCA is dependent upon the recruitment of neutrophils to subtunical venules of the testis. Recent studies have also shown that there is an increase in the proinflammatory cytokine tumor necrosis factor (TNF)-alpha lesion molecule, E-selectin, and activation of the stress-related kinase, c-jun N-terminal kinase (JNK) after IR of the murine testis. The present study was performed to determine if the increase in TNF-alpha expression observed after IR of the testis is involved in the cell signaling events leading to E-selectin expression and subsequent neutrophil recruitment to the testis. Mice were either given an intrascrotal injection of 500 ng TNF-alpha or vehicle alone and sacrificed at 1, 2, and 4 hours after injection for the removal of testes for histological examination and for protein isolation. Histological results revealed that TNF-alpha caused an increase in neutrophil adhesion to subtunical venules. Preliminary results of western blot analyses indicate that total testicular JNK remains unchanged and phosphorylated JNK, the active form of the enzyme, is increased at 1 hr after TNF-alpha injection. These findings indicate that the increase in TNF-alpha observed after IR of the testis may stimulate neutrophil recruitment through a JNK activation pathway. Interestingly, NFKB activation is also known to be downstream of TNF-alpha signaling in other tissues. We will determine if NFKB is also involved in the stimulation of E-selectin after IR of the testis. Supported by RO1-DK53072 and an AFUD Fellowship.

# 39

**TESTOSTERONE INHIBITS PRODUCTION OF 25-HYDROXYCHOLESTEROL IN TESTICULAR MACROPHAGES** Yevgeniya O. Lukyanenko* and James C. Hutson. Texas Tech University Health Sciences Center, Lubbock, TX.
Testicular macrophages reside in close association with Leydig cells and produce IL-1, IL-6, TNF a and 25-hydroxycholesterol, all of which influence Leydig cells. While the cytokines are generally thought to inhibit steroidogenesis, 25-hydroxycholesterol is readily converted to testosterone and thereby increases steroidogenesis. Macrophages produce a 25-hydroxylase that converts cholesterol to 25-hydroxycholesterol. Because most endocrine and paracrine mechanisms receive negative feedback, it was hypothesized that production of 25-hydroxycholesterol by testicular macrophages may be regulated by testosterone. This hypothesis was tested by measuring steady-state levels of 25-hydroxylase mRNA and production of 25-hydroxycholesterol by adult rat testicular macrophages treated with testosterone. We found that high concentrations of testosterone significantly inhibited 25-hydroxycholesterol production and steady-state levels of 25-hydroxylase mRNA after 24 hr. It was also found that LPS and 25-hydroxycholesterol itself also inhibited 25-hydroxycholesterol production. Preliminary studies indicate that the rate of degradation of 25-hydroxylase mRNA was not influenced by testosterone. These studies demonstrate that the production of 25-hydroxycholesterol by testicular macrophages is negatively regulated by testosterone suggesting the presence of a negative feedback regulatory loop for the paracrine interaction between macrophages and Leydig cells. This not only provides additional data concerning the regulation of this oxysterol at the molecular level but also supports the hypothesis that 25-hydroxycholesterol is involved in physiologically relevant processes. This finding further supports the concept that the interaction between macrophages and Leydig cells is physiologically relevant.

# 40

**PERCUTANEOUS NEEDLE TESTICULAR BIOPSY IN INFERTILEMEN**
J.K.Wolski(1,2),P.Kluge(3)*,B.Biarda(1)*,K.Koziol(1)*,
P.Lewandowski(1)*,(1)Infertility Center"Novum",Warsaw,POLAND
(2)Clinic of Urology & (3)Department of Pathology, The Children's Memorial Health Institute, Warsaw, POLAND
Aims: Histological evaluation of testicular tissue is the important predictive factor in diagnosis of infertile men. The obtaining testicular specimens using open biopsy was reported by patients as a painful and embarrassing and sometimes hospitalization was required.This work presents our experience with percutaneous needle testicular biopsy(PNTB). Material & methods: During 10.96-06.01,in "Novum", 527 PNTB of 519 testis were done in 262 men (mean age 31.5 yo) with: Azoospermia-241,cryptozoospermia-13,anejaculation-8.In 8 men PNTB were performed twice,5 men had one testis.Biopsies were done from both testes,under general anesthesia,with 1.6 needle from HepafixΔ set(B.Braun,Germany),1-2 specimens were obtained.Since 1999,together with histological examinations, parts of testicular tissue were frozen for ICSI-TESA. Mean time of PNTB was 10-20 minutes and all procedures were ambulatory.Each patient was leaving "Novum",after clinical observation,2 hours later.No antibiotic was used and only paracetamol was prescribed as an analgesic drug. After 7 days the surgical control and explanation of histopathological result was done and conception planning was established. Results: Specimens from 514(97.5%) testes were adequate for histopathological examination and good enough for clinical resolutions.There were no influences for properly clinical qualifications in 13(2.5%) inadequate specimens. In 7/262 (2.7%) men complications were noticed:6-hematoma of testis treated by operation( l) and by puncture(5) and 1-prologated scrotal pain treated by antibiotic and non-steroid anti-inflammatory drugs. No longish breaks in work were observed. Conclusions:Percutaneous needle testicular biopsy (PNTB) is simple, safe minimal invasive,histological sufficiently and relatively cheap diagnostic procedure in infertile men.

# 41

**SEASONALITY OF CRYPTORCHIDISM AND HYPOSPADIAS IN GREECE: EPIDEMIOLOGICAL RELATIONSHIPS**

Ch. Mamoulakis,[1,2] D. Demetriades,[*1] S. Antypas,[*1] N. Sofikitis,[2] [(1)]Dept of Pediatric Surgery, Aghia Sophia Children's Hospital, Athens, Greece, [(2)]Dept of Urology, Ioannina University, Ioannina, Greece

**OBJECTIVE:** To examine seasonal trends of cryptorchidism and hypospadias in Greek population.

**MATERIALS AND METHODS:** Data on one thousand one hundred seventy eight males with true isolated cryptorchidism and 542 with simple hypospadias born between 1991-1998 were received and analysed from three major paediatric centre databases. All 630,087 boys born during the same period of time were used as a comparison group. Seasonality by month of birth was evaluated using Edwards' model with adjusted frequencies and exact angles.

**RESULTS:** Cryptorchid and hypospadiac births followed a monthly cyclic distribution during the year, well described by the equation of the simple harmonic oscillation ($x^2$ of good-fitness=5.99 with 11 df, P>0.75 for cryptorchidism, $x^2$ of good-fitness=14.38 with 11 df, P>0.1 for hypospadias). The maximum and minimum incidence rate was observed in March and September respectively for cryptorchidism ($x^2_{EDWARDS}$=14.27 with 2 df, P<0.005), and between October/November and April/May respectively for hypospadias ($x^2_{EDWARDS}$= 6.98 with 2 df, P<0.05). Both the 3rd and the 1st trimester (crucial embryonic periods of the final testicular descent and the differentiation-development of the male urethra respectively) of cryptorchid and hypospadiac gestations respectively showed a statistically significant incidence in winter.

**CONCLUSIONS:** Both congenital anomalies show a documented seasonal variation in Greece. Since both crucial embryonic periods of the final testicular descent and the differentiation-development of the male urethra are considered to be androgen-dependent, the existence of an androgen stimulator that follows a cyclic pattern of variation with a trough (diminished androgen production by the fetal testis) in winter may be speculated. Under these settings it could be hypothesized that the coincidence of the 1st or 3rd trimester of a potentially genetically influenced gestation with the winter period, could lead to the phenotypical expression of hypospadias or cryptorchidism respectively. hCG is a possible example of such an androgen stimulator since it shows seasonal variation with lower levels in winter. Since no significant differences in daylight length are found among seasons in Greece, the detection of a statistically significant seasonal variation may suggest that factors other than light are involved in the pathogenesis of cryptorchidism and hypospadias. Seasonal alterations of weather temperature may be given as a possible example of an alternative cause contributing via the above described mechanism to the seasonal variations observed.

# 42

**PROGESTERONE RECEPTOR EXPRESSION, LOCALIZATION, AND ACTIVITY IN RAT TESTES.** Lue YH, Wang C, SinhaHikim AP, Yamamoto CM*, Leung A*, Atienza V*, Diaz-Romero M*, Swerdloff RS. Div. Of Endocrinology, Dept. of Medicine, Harbor-UCLA Medical Center Research and Education Institute, Torrance, California.

Human male contraceptive studies have demonstrated that the administration of combined testosterone (T) and progestin induces a more rapid suppression of spermatogenesis than with T alone. We hypothesize that progestins alone through progesterone receptors (PRs) have direct actions on testes. Using the Sprague-Dawley rats (60 days old) as a model, we determined PR expression, localization, and binding characteristics in the adult testis. One testis from each animal was removed for progestin binding activity and PR protein expression by Western bloting. The contralateral testes were fixed by vascular perfusion with Bouinìs solution, embedded in paraffin for determining PR localization by immunohistochemistry. The results show that there is specific binding of labeled progesterone to testicular cytosolic preparations. Progesterone, 17a-hydroxyprogesterone and pregnenolone displaced labeled progesterone from the testicular cytosol, whereas testosterone, estradiol and other synthetic progestins had no effect. Western blot analyzes show PR-A and PR-C expression is more abundant than PR-B in testes. Immunohistochemical analyzes indicate that PR-A is mainly localized intracellularly in pachytene spermatocytes, Sertoli and Leydig cells, and PR-C, a truncated form of PR-A and PR-B, is localized to cell membranes of round spermatids and intracellularly in pachytene spermatocytes. Moderate PR-C staining was observed in the somatic cells. We conclude that progesterone acting through PRs may have direct actions on spermatogenesis.

# 43

**THE EFFECTS OF REPRODUCTIVE HORMONES ON COLOCALIZATION OF CORTACTIN WITH F-ACTIN IN ISOLATED RAT SERTOLI CELLS** K.M.Wolski, J.Hushen*, D.F.Cameron; Department of Anatomy,University of South Florida College of Medicine, Tampa, FL

FSH results in peripheralization of F-actin in isolated rat Sertoli cells inducing "binding competency" of these cells. In coculture, testosterone stimulates round spermatid (step 1-8) attachment to Sertoli cells in a dose response fashion only when the Sertoli cells are binding competent. This priming effect of FSH appears associated with actin distribution by yet unknown mechanisms. Cortactin, an actin bundling protein, localizes to the cortical cytoskeleton and has been shown to cross-link F-actin in vitro. It has been identified in the seminiferous epithelium in a pattern similar to actin distribution in Sertoli cells in vivo. The purpose of this study was to identify the presence of cortactin in isolated rat Sertoli cells, to determine its distribution pattern relative to F-actin, and to define the effects of reproductive hormones on its localization. The effects of FSH, DHT, testosterone, and estradiol were observed in Sertoli cell monocultures and Sertoli-spermatid cocultures plated on a Matrigel substrate. Cortactin was present in all Sertoli cells. FSH appeared to have an effect on cortactin localization, as it did on F-actin localization within the cell. Steroid reproductive hormones did not appear to result in a noticeable redistribution of either F-actin or cortactin. The localization of cortactin following induction of the actin-rich Sertoli-spermatid junctional complexes by round spermatids in coculture is currently under investigation. The inductive effect of FSH on Sertoli cell binding competency is associated with the peripheralization of F-actin, which may be regulated, in part, by cortactin distribution within the cell.

# 44

**POTENTIAL ESTROGEN REGULATION OF HENSIN, AN EPITHELIAL TERMINAL DIFFERENTIATION GENE IN THE MALE REPRODUCTIVE TRACT**

Qing Zhou1, Qais Al-Awqati*2, Rong Nie1, Kay Carnes*1, Dennis Lubahn*3, Rex A. Hess1 1 Dept Vet Biosci, Univ of Illinois, Urbana,IL 61802; 2Dept. Med/Physiol, Columbia University, NY; 3Biochemistry & Child Health, Univ of Missouri, Columbia, MO

The disruption of estrogen receptor alpha (ERa) gene in rodents resulted in fluid reabsorption defect in efferent ducts, dilution of sperm and male infertility. Our previous studies showed the epithelium lack of ERa has dramatic decrease in the microvillus border, the loss of apical endocytotic vesicles and the uptake of luminal fluid. It is obvious that the morphological and functional characteristics of terminally differentiated epithelial cells have been lost in these cells. Hensin, an extracellular matrix glycoprotein secreted by epithelium, was reported in many tissues to mediate the terminal differentiation of the epithelium. Here, we hypothesized that hensin is the gene mediating the estrogen actions in male efferent ducts for the final establishment of the highly polarized and differentiated epithelial cells. cDNA probes from CUB2 domain and 3'-UTR of hensin mouse homologue, CRP-ductin, were used to test the expression of this gene in adult male rat by Northern blot analysis. A 7 Kb product was detected in efferent ducts, epididymis, vas deferens and prostate in a high abundance, which share the same size of hensin intestine splicing variant CRP-ductin. A 5Kb product that has same size as hensin taste bud splicing variant ebnerin was also detected in efferent ducts. In 55-day ICI 172,780 treated adult rat, the expression of 7Kb product was decreased significantly. Our data suggested that there is an estrogen regulation on hensin expression in male reproductive tract. Further study need to be done to correlate the estrogen regulation of hensin gene and terminal differentiation of efferent ducts epithelium. Supported by NIH grant HD35126.

# 45

**THE INITIATROR CASPASE 9 AND THE EXECUTIONER CASPASES 3 AND 6 ARE INVOLVED IN GERM CELL APOPTOSIS FOLLOWING GONADOTROPIN DEPRIVATION IN RATS.** G Palomeno*. AP Sinha Hikim, YH Lue, V Atienza*, C Wang, RS Swerdloff, Department of Medicine, Harbor-UCLA Medical Center and REI, Torrance, CA.

Withdrawal of gonadotropins and/or intratesticular T induces germ cell apoptosis. The molecular mechanisms by which it does so are not well understood. The execution of most if not all apoptosis requires caspase activation. The purpose of the present study was to examine the possible involvement of the key initiator and executioner caspases in germ cell apoptosis triggered by withdrawal of gonadotropins. Groups of adult male rats were given a daily injection of vehicle for 14 days or GnRH antagonist (GnRH-A) for 2, 5, and 14 days. Germ cell apoptosis involving exclusively stages VII-VIII was achieved by day 5. Within the study paradigm, the highest number of dying cells occurred by day 14, at which time a modest but significant increase in the incidence of apoptosis was also noted at stages other than VII-VIII. Immunocytochemical analysis revealed increased expression of Bax as well as active initiator caspase 9 and the executioner caspases 3 and 6 in dying germ cells. Unlike our heat model, we found no redistribution of Bax or the active executioner caspase 7 to the perinuclear region of the apoptotic germ cells. These results suggest that: 1) withdrawal of gonadotropins and consequently intratesticular T induces germ cell apoptosis possibly via a mechanism involving the initiator caspase 9 and the executioner caspases 3 and 6 and 2) these executioners may play a non-redundant role in programmed germ cell death in a stimulus-specific manner. Our understanding of the cascade responsible for reversible loss of germ cells after gonadotropin deprivation may allow us to focus on specific targets for male contraceptive development. Supported by a grant from NIH (HD39293).

# 46

**ANDROGEN RECEPTORS WITH A PATHOLOGICALLY EXPANDED POLYGLUTAMINE TRACT FORM AGGREGATES WHICH ALTER NEURITE TRANSPORT IN MOTONEURONAL CELLS.** L. Martini, S. Simeoni*, F. Piccioni*, P. Pozzi*, I. Andriola*, A. Poletti*. Istituto di Endocrinologia, and Centre of Excellence for Neurodegenerative Diseases, Via Balzaretti 9, 20133, Italy.

Expansion of triplet (CAG)n repeat sequences in the androgen receptor (AR) gene have been associated to a particular type of neurodegeneration in humans. The patholohy, Spinobulbar muscular atrophy (SBMA) or Kennedy's disease, is an X-linked recessive disorder characterized by motoneuron loss. The repeat gives an elongated polyglutamine (polyGln) tract in the AR and confers a toxic gain-of-function to the mutant protein. Using immortalized motoneurons (NSC34) transfected with the wt or mutant ARs, we have shown that SBMA AR has a cytoplasmic diffuse distribution in transfected cells, which exhibits reduced growth/survival rate. After testosterone activation SBMA AR aggregates (cytoplasmic and neuropil inclusions) but cell viability increased; thus, cytoplasmic aggregates do not play a role in cell death. To analyse the possible toxic role of neuropil aggregates on motoneuronal functions, we have co-transfected NSC34 with chimeric constructs of green fluorescent protein and either the SBMA AR (GFP-AR.Q48) or wtAR (GFP-AR.Q0) plus a blueFP containing a mitochondrial localization signal (mtBFP). We have found that, in motoneuronal cells with neuropil aggregates the distribution of mitochondria is altered in neuronal processes. Confocal microscopy showed that the same cells have a marked reduction in f-actin polymerized fraction; they also show significant changes in cell morphology and in motor proteins involved in axonal transport. Thus, aggregates physically alter neurite transport and may deprive neuronal processes of factors/components important for axonal functions. Telethon (Italy) Grant 1283 and Italian Ministry of Health are acknowledged.

# 47

**A PHARMACOKINETIC STUDY OF ORAL TESTOSTERONE UNDECANOATE (ORG 538)** BD Anawalt, JK Amory, University of Washington, Seattle; C Wang, RS Swerdloff, Harbor UCLA Research and Education Institute, Torrance; AS Dobs, Johns Hopkins School of Medicine, Baltimore; AW Meikle*, University of Utah, Salt Lake City; J Elbers, NS Houwing, Organon, Oss, The Netherlands

Objective: A pharmacokinetic trial of an orally administered testosterone undecanoate (TU) preparation (Org 538) was conducted in 49 men (means: age, 45.2 y; weight, 90.9 kg; BMI, 27.6) with primary or secondary testosterone (T) deficiency (morning total T, <=3.0 ng/mL). Methods: This multicenter, randomized, parallel-group, open-label trial compared oral dose regimens of TU for the 24-hour pharmacokinetic profiles of serum total T after 28 days of administration. Men were randomized to four regimens of TU, all with meals: 120 mg/d (40 mg tid), 160 mg/d (40-40-80 mg), 160 mg/d (80 mg bid), and 240 mg/d (80 mg tid). Blood was sampled before the a.m. TU dose on days 1, 3, 7, 14, and 21. Serial blood samples were collected for up to 48 hours after the morning dose on day 28. Results: For all groups, steady state was reached by day 3. On Day 28, the mean Cavg for T ranged from 3.4 ng/mL (40 mg tid) to 7.4 ng/mL (80 mg tid). On Day 28, the mean Cmax of T was 6.76, 8.41, 10.8, and 14.5 ng/mL for the 120, 160 (40-40-80), 160 (80 bid), and 240 mg groups. On Day 28, the mean tmax for T was 10.5, 14.0, 14.0, and 8.50 h for the 120, 160 (40-40-80), 160 (80 bid), and 240 mg groups. Serum T was within the normal range for 77% (40 mg tid) to 95% (80 mg bid) of the day. About 80% of the men who took 80 mg bid or tid but <50% of those who took 40 mg tid or 40-40-80 mg had minimum and average T levels within the normal range. No serious adverse events were observed. Conclusion: Oral administration of 120-240 mg/d of TU for up to 28 days safely and effectively restored serum T to low- or high-normal values (normal range 2.5-9.

# 48

CAN AGE-RELATED DECREASES IN LEYDIG CELL TESTOSTERONE PRODUCTION BE RESTORED WITH LH? H. Chen*, M. P. Hardy, B. R. Zirkin. Div. Reprod. Biol., Johns Hopkins Univ., Baltimore, MD; and Population Council, New York, NY.

Aging in Brown Norway rats is associated with reduced Leydig cell testosterone production. To address the mechanism by which aging Leydig cells become steroidogenically hypofunctional, Leydig cells from young and old rat testes were isolated and cultured with luteinizing hormone (LH) for 3 days in vitro. Leydig cells isolated from young rats that had received LH-suppressive testosterone implants for 10 days served as positive controls. The ability of young control Leydig cells to produce testosterone at high levels was sustained over a 3-day culture period. Testosterone production by cells from young LH-suppressed rats increased over this period, almost to control levels. In contrast, culture of the steroidogenically hypofunctional old Leydig cells with LH failed to increase their testosterone production, suggesting that LH stimulation cannot reverse the steroidogenic deficits of old Leydig cells. Reduced numbers of LH binding sites characterized Leydig cells from old rats as well as LH-suppressed young rats. However, whereas Leydig cells from the young LH-suppressed rats produced cAMP at the high levels of young control cells, the old cells produced far less cAMP, suggesting that old Leydig cells have defects in the LH-cAMP signaling cascade. When stimulated with forskolin, old cells produced the same amount of cAMP as young control and young LH-suppressed cells, suggesting that adenylate cyclase is maintained in the old cells. Taken together, these results suggest that inefficient LH signal transduction may explain the reduced steroidogenesis that characterizes old Leydig cells. Supported by NIH grant AG08321.

# 49

**A MORPHOMETRIC ANALYSIS OF TESTICULAR FUNCTION IN SENESCENCE ACCELERATED MICE.** N. Manabe*, Y. Akiyama*, M. Sugimoto*, M. Kiso*, K. Komatsu*, M. Shimabe*, H. Miyamoto*, Unit of Anatomy and Cell Biology, Department of Animal Sciences, Kyoto University, Kyoto 606-8502, Japan

The Senescence Accelerated Mice (SAM) are inbred strains derived from AKR mice and contain two related strains, SAM prone (SAMP) and SAM resistant (SAMR). The SAMP has a shorter lifespan and demonstrates accelerated aging. In this study, we examined age-related changes in testes in SAM mice by morphometry. During immature periods, the absolute numbers of spermatogonia, primary spermatocytes, spermatids, and Sertoli cells in SAMP were greater than those in SAMR. Significant differences were noted in number of spermatogonia at 6 weeks old, primary spermatocytes at 3, 5, and 6 weeks, round spermatid at 5 and 6 weeks old, Sertoli cells at 3 and 6 weeks old. Round spermatid in SAMP appeared earlier than in SAMR. In contrast, to immature period, these cell numbers in SAMP at 40 weeks old were less than those in SAMR at the same age, although it was not significant in Sertoli cell number. Downward trends in these parameters appeared in SAMP from 20 to 40 weeks old, while there were no significant changes in SAMR. The volume of seminiferous tubules in SAMP was greater than that in SAMR at 5 weeks old but similar at advanced age. The absolute number and volume of Leydig cells in SAMP were larger than that in SAMR at 3 to 6 weeks old. While the number of Leydig cell in SAMP was not changed from 20 to 40 weeks in age, significant decrease was observed in volume of cells. This decrease was not noted in age-matched SAMR. Consistent with the changes in Leydig cell volume, plasma testosterone levels were higher in SAMP than that in SAMR at 4 to 6 weeks old and lower in SAMP at 40 weeks old. These results indicate that both maturation and deterioration of testes occur at earlier ages in SAMP than SAMR.

# 50

**DETECTION OF PARTIAL ANDROGEN DEFICIENCY IN AGING MALES AND ITS IMPACT. PRELIMINAR REPORT.** Tapia Serrano R., *Salgado Echeverraa R., *Lezana V. J., Torres M. J., Moreno A. J., Andrologaa, Departamento de Urologaa, CMN Siglo XXI. IMSS. Mexico D.F. OBJECTIVE To detect the incidence of partial androgen deficiency in aging males (PADAM), and to determine if there are more frequency of sexual dysfunction, lower urinary tract symptoms (LUTS), depression. and decrease in muscular mass. MATERIALS AND METHODS We studied 41 male patients. The age was 40 years old and above, we performed in all patients a complete clinical study, laboratory tests, basal hormone levels LH, FSH, PRL, Testosterone (T), Estradiol (E2) Prostatic specific antigen (PSA) and Testicular Reserve test (TRT) with hCG. The patients were divided in two groups Group I: Twenty-eight patients (68.3%) without Partial deficiency of androgens, Group II: Thirteen patients (31.7%) with Partial androgens deficiency. We applied the International Index of Erectile Function (IIEF), the International Prostatic Symptoms Score (IPSS), The Beck's test to evaluate sexual function, LUTS and depression respectively. We measured the arm circumference and compared with standard values by age. RESULTS.There were not differences in andrological clinical study and hormone profile. The T was below 300 ng/dl in three patients. The TRT was significantly abnormal In Group II ($p= 0.0001$), The Group II showed that the frequency of morning erections was decreased ($p=0.032$), the scores of the IPSS was higher ($p= 0.003$). The plasmatic levels of total cholesterol ($p=0.013$) and triglycerides ($p= 0.009$) were increased. The IIEF and Beck's test were not statistically significant. CONCLUSIONS We concluded that to detect PADAM should practice a TRTand it does not only detect basal serum levels of androgens, The clinical implications were in the frequency of morning erections and lower urinay tract symptoms and abnormal plasmatic lipids profile.

# 51

**CALORIC RESTRICTION AFFECTS GENE EXPRESSION IN THE EPIDIDYMIS OF THE BROWN NORWAY RAT** Kathryn M. Jervis* and Bernard Robaire
Depts. Pharmacol. & Therap. and Ob/Gyn, McGill University, Montreal, Canada

The epididymis, the tissue where spermatozoa mature and are stored, undergoes profound, segment specific, biochemical and morphological changes with aging. While the mechanisms underlying aging of the epididymis are unclear, previous results suggest that oxidative stress may play a role. We used a caloric-restriction (CR) protocol to determine how manipulating oxidative stress levels affects aging of the epididymis. CR causes an extension of life span in many species and has been shown to attenuate both the age-related accrual of oxidative damage and the increased production of mitochondrial reactive oxygen species (ROS). Male Brown Norway rats were made caloric restricted; by 16 weeks onward they were sustained on 60% of caloric intake of ad libitum controls. At 12 months epididymides were collected (n=5), sectioned (initial segment, caput, corpus and cauda) and frozen. RNA was extracted, reversed transcribed, radiolabelled and used to probe cDNA arrays (n=5 arrays/segment, ClontechTM Rat-1.2 Atlas Array). Arrays were analyzed using GenespringTM software. The effect of CR was most pronounced in the corpus epididymidis; the expression of 15 genes decreased and one increased. Many of the genes that decreased by two fold or more are involved in oxidative stress defences; copper-zinc-containing superoxide dismutase 1 (Cu-Zn SOD1), glutathione S-transferase (GST) subunit 7 pi, GST subunit 8, and GST subunit 4 mu decreased by 65%, 56%, 53% and 51%, respectively. Therefore, CR results in a segment specific reduction in the expression of those genes involved in oxidative stress defences. We speculate that CR-mediated decreased production of ROS results in a reduction in the expression of genes involved in oxidative stress defences in the epididymis. Supported by NIH AG 08321.

# 52

**ESTIMATION OF ENDOGENOUS LUTEINIZING HORMONE-TESTOSTERONE SECRETORY COUPLING IN THREE MAMMALIAN SPECIES** J.D. Veldhuis, Division of Endocrinology, Dept Int Med, Gen Clin Res Ctr and Ctr for Biomathematical Technology, Univ Virginia, Charlottesville, Va, D.M. Keenan*, Dept Statistics, Univ Virginia, I. Clarke*, Prince Henry's Institute of Medical Research, Clayton, Australia, S. Alexander* and C.H.G. Irvine*, Equine Research Unit, Lincoln University, Christchurch, New Zealand

Neurohormonal ensembles maintain critical organismic functions, such as reproduction, growth, stress-adaptation and metabolism. Feedback and feedforward signaling among coupled glands mediates such homeostatic control, as transduced via putatively deterministic dose-response interfaces. Biological uncertainties (stochastic elements) arise in the form of pulsatile uni- and multicellular secretion into a turbulent (diffusive and advective) blood pool. In addition, whether unobserved but implicit in vivo dose-responsive linkages are inconstant over time is not known. The present analysis incorporates allowable stochastic variability of dose-responsive signaling within a deterministic construct of composite pulsatile secretion, molecular dispersion and irreversible elimination in the male reproductive axis. Validity is established by threefold simultaneous measurements of hypothalamic gonadotropin-releasing hormone (GnRH), pituitary luteinizing hormone (LH) and gonadal testosterone secretion monitored at 30-second or 5-min intervals in the awake, unrestrained horse and sheep. The ram, but not the stallion, exhibited statistically vivid (3-10 fold) within-animal variability in the predicted in vivo dose-dependent actions of LH on testosterone secretion. Likewise, inferred LH-testosterone feedforward coupling in normal men exhibited conspicuous within-subject variability over time, as assessed in peripheral and spermatic-vein blood. Accordingly, we infer that the in vivo LH-testosterone feedforward interface maintains time-varying adaptations in the sheep and human. Further investigations will be required to appraise the generality of such dynamic agonist/target-gland interactions and assess their possible disruption in pathophysiology.

# 53

**IN UTERO EXPOSURE TO PERSISTENT ORGANIC POLLUTANTS (POPS) AFFECTS GENE EXPRESSION IN THE FETAL TESTIS** Adedayo Adeeko, Daming Li, Jacquetta M. Trasler, Barbara F. Hales, Bernard Robaire, Depts. of Pharmacol. & Therap., Pediatrics, Human Genetics, and Ob/Gyn, McGill Uni., Montreal, Canada

POPS include both pesticides and polychlorinated biphenyls. Exposure during pregnancy to the high concentrations of POPS found in the diet of the Inuit is a public health concern. A mixture of 28 POPS was dissolved in corn oil and administered by gavage (at a dose representing 1000 times Inuit dietary levels) to pregnant SD rats from gestation day 0-19. On day 20 of gestation, fetal testes were dissected, RNA was isolated and 32P-labelled for expression profiling using Atlas Rat 1.2 Arrays; membranes (n=5) were scanned and data were analyzed (GeneSpring). The number of genes detected was 288 in control and 250 in treated. 43 genes were expressed exclusively in control testes; these included LH/HCG receptor, retinoid X receptor alpha, GTPases/GTP binding proteins, G1/S-specific cyclin E, and cyclin-dependent kinase 7. Only 5 genes were expressed exclusively after POPS exposure (P27Kip1; neuropilin; Sky proto-oncogene; CYP 2C23 and RalGDSB). POPS caused a downregulation (at least 1.5-fold) in 33 genes, including 14-3-3 protein zeta subtype; cyclin-dependent kinase 4 and dual-specificity-mitogen-activated protein kinase; only 3 genes (G1/S-specific cyclin D2, matrix metalloproteinase-14 precursor and carbonic anhydrase) were upregulated (1.5 fold). Our results identify novel genes expressed in the fetal rat testis and demonstrate that in utero exposure to POPS alters testicular gene expression. Supported by TSRI Canada.

# 54

**GESTATIONAL EXPOSURE TO ETHANE DIMETHANESULFONATE (EDS) ALTERS DEVELOPMENT OF THE MOUSE TESTIS.**

D.K. Tarka*1,2, J.D. Suarez*2, N.L. Roberts*2, J.M. Rogers*1,2, M.P. Hardy3, and G.R. Klinefelter1,2. 1University of North Carolina, Curriculum in Toxicology, Chapel Hill, NC; 2USEPA, NHEERL, RTD, RTP, NC, and 3Population Council, NY, NY.

Previously we found that administration of the well known Leydig cell (LC) toxicant, EDS, to pregnant dams (160 mg/kg, i.p.) during gestation days (GD) 11-17 compromised reproductive development in male progeny. On GD 16, EDS toxicity manifested as a diminished testosterone (T) peak, and on GD 18, males had reduced anogenital distance. We sought to determine whether this exposure results in persistent effects throughout reproductive development. Although puberty was delayed as evidenced by delayed preputial separation, hCG-stimulated T production by testis parenchyma was unchanged. However, light micrographs of prepubertal testes from EDS exposed males revealed seminiferous tubule (ST) cross sections containing only Sertoli cells, indicating a delay in the onset of spermatogenesis. As adults, EDS-exposed males had reduced epididymal sperm reserves, and females mated to these males had reduced fertility ratios and litter sizes. Serum LH levels increased as did hCG-stimulated T production per gram of testis. Additionally, the STs were observed to have incomplete germ cell associations. Morphometric analysis revealed an EDS-related increase in interstitial area (934 ± 357 vs. 3184 ± 793µm2) and a concomitant decrease in ST area (21952 ± 3700 vs. 14181 ± 1306 µm2), suggesting a relative increase in LCs. Clearly, gestational exposure to EDS can produce profound delays in puberty and spermatogenesis. Thus, while adult mouse LCs are known to be insensitive to EDS, EDS may have direct action on the fetal LC, resulting in abnormal development of the adult testis. (This abstract does not necessarily reflect EPA policy.) DKT: T901915

# 55

**IN UTERO EXPOSURE TO TRIBUTYLTIN ALTERS CELLULAR JUNCTIONS IN ADULT RAT VENTRAL PROSTATE.** J. Barthelemy*, D.G. Cyr. INRS-Institut Armand Frappier, Univ. Qu»bec, Pointe Claire, Canada.

Tributyltin (TBT) is an environmental contaminant used in anti-fouling agents for boats, and as a by-product of industrial processes. Little information exists on the effects of TBT on mammalian reproduction. Intercellular tight junctions are formed by transmembrane proteins such as claudins. The formation of tight junctions involves signalling components of adhering junctions which are formed by cadherins. The present objectives were to determine the effects of in utero exposure to TBT on epithelial cadherin (E-Cad) and claudin-1 (Cldn-1) in the rat ventral prostate. Pregnant Sprague-Dawley rats were given varying doses of TBT (2.5, 10 or 20mg/kg in corn oil by gavage) throughout gestation. Controls received vehicle alone. The pups were weaned on day 21 and sacrificed at day 91. Ventral prostate weights of TBT treated rats were significantly decreased in all treatment groups, but there were no effects on seminal vesicle weights. Serum levels of testosterone were decreased in the two highest dose groups. E-Cad mRNA levels were decreased in a dose-dependent manner. Immunoreactive E-Cad was localized between epithelial cells and the levels appeared to decrease with increasing TBT dose. Cldn-1 was immunolocalized to the apical lateral margins of adjacent prostatic epithelial cells. Cldn-1 immunolocalization was dispersed along the lateral plasma membrane with increasing TBT dose. This suggests that either the targeting of Cldn-1, or its localization in tight junctions is altered as a result of fetal TBT exposure. These data indicate that in utero TBT exposure results in permanent alterations in circulating testosterone, ventral prostate weight and that these are associated with a loss of cell adhesion and altered tight junctions. Supported by the TSRI (Health Canada).

# 56

**TESTOSTERONE LEVELS IN MALE PESTICIDE APPLICATORS AND THE SEX OF THEIR CHILDREN**

S.E. Holland*, V.F. Garry*, University of Minnesota, Minneapolis, MN
S.M. Schrader, J.S. Kesner*, E.A. Knecht*, NIOSH, Cincinnati, OH

In a study of pesticide applicators in the Red River Valley of northwest Minnesota, there appears to be a shift in sex ratio in offspring of some groups of applicators. Total serum testosterone concentrations were measured in 144 subjects in July and October, 1998, and a mean [(July + October)/2] value was computed for each individual. Of these subjects, 96 had fathered children and had performed some pesticide applications during their lives. These applicators were divided into quartiles according to their mean total serum testosterone concentrations. Within each quartile, the sex ratio of their offspring (male:female) was determined.

| Quartile | Testosterone Range (ng/ml)* | Age X ± SE | No. of Fathers | No. of Male Children | No. of Female Children | Sex Ratio |
|---|---|---|---|---|---|---|
| 1st | ≤ 3.73 | 46.0 ±1.7 | 24 | 29 | 38 | 0.76 |
| 2nd | 3.74-4.37 | 45.0 ±1.6 | 24 | 30 | 42 | 0.71 |
| 3rd | 4.38-5.63 | 49.4 ±2.2 | 24 | 33 | 34 | 0.97 |
| 4th | >5.64 | 44.0 ±2.3 | 24 | 43 | 27 | 1.59 |
| NHS** | | | | 2,026,854 | 1,932,563 | 1.049 |

*normal clinical range 3.00-12.00 ng/ml; assay coefficient of variation = 6.7%
** National Health Statistics

Significant differences exist (two-tailed Fisher's exact test) in the sex ratios observed between the 1st and 4th quartiles (P=0.04), 2nd and 4th(P=0.02), and combined 1st + 2nd compared to combined 3rd + 4th (P=0.04). These results indicate that the sex ratio of children borne to pesticide applicators is related to the applicators' total serum testosterone levels. Notably, there is a significant difference in the percentage of fungicide-exposed subjects across quartiles. During the 1998 season, 47.9% of subjects in the combined 1st + 2nd quartiles used fungicides vs. 27.1% in the combined 3rd + 4th (p=0.035). Historically, 72.9% of subjects in the 1st + 2nd quartiles had used fungicides during their lives vs. 45.8% in the 3rd +4th (p=0.007). The most commonly used fungicides in this region of Minnesota are of the organotin class. It has been suggested that testosterone levels of the father at the time of conception may influence the sex of offspring born. Whether this connection can be drawn from the results seen here is uncertain. First, it is unclear to what extent the mean total testosterone levels taken in this study may reflect the individuals' testosterone levels at the time of their children's conception. Furthermore, it is not known whether the association between lower testosterone levels and lower sex ratio reflects a cause and effect, or if they may be a parallel response to a common toxicant mechanism.

# 57

**SPERM MOTILITY IN HSF1 KNOCKOUT MICE AFTER HEAT SHOCK IS ASSOCIATED WITH FERTILITY DEFICITS.** LF Strader*. SD Perreault, JC Luft*, and DJ Dix*. US EPA/ORD, Reproductive Toxicology Div., Research Triangle Park, NC

Heat shock proteins (HSPs) protect cells from environmental exposures such as hyperthermia, oxygen radicals, and heavy metals. During the stress response in mouse testis, expression of inducible HSPs is regulated by activation of heat shock factor 1 (Hsf1). We hypothesized that Hsf1 gene knockout (KO) mice would be more susceptible than wild type (WT) to hyperthermia. Adult WT and KO males were exposed to 25 degrees C (control) or 43 degrees C (heat shock) for 20 minutes. Males were bred from 3-4 weeks after treatment to confirm fertility deficits in response to hyperthermia, and again from 12-13 weeks to examine recovery in WT and KO mice. Results of the first breeding period showed significant reduction in the number of litters for WT, and no litters for KO males exposed to heat. By 12 weeks the number of litters did not differ between heat shocked and control WT, while heat shocked KO mice continued to be significantly different. Sixteen weeks after treatment males were sacrificed for organ weights, histology, and epididymal sperm motility using CASA (Hamilton Thorne IVOS, version 12.0L). Sperm were tracked for 1 sec (60 frames/sec) with a minimum track length of 30 frames. Progressive sperm were defined by setting average path velocity (VAP)=80 and Straightness=50. CASA parameters were similar for WT males, both control and heat shock, indicating recovery of sperm motility. In contrast, Hsf1 KO males exhibited significant decrements in percent motile and percent progressive sperm, and mean VAP, straight line velocity, curvilinear velocity, straightness, and linearity - indicating a lack of recovery following heat shock. Thus sperm motility data could explain differences in breeding performance between WT and Hsf1 KO mice.

# 58

**ABNORMAL SPERM MORPHOLOGY AND OFFSPRING SEX RATIO IN MALE FUNGICIDE APPLICATORS** SM Schrader, TW Turner, NIOSH, Cincinnati, OH VF Garry, SE Holland, Univ of MN, Minneapolis, MN

An agricultural health survey of 670 families was conducted in 5 northwest Minnesota counties. Of these, 550 had children or a pregnancy, and 522 gave detailed information about pesticide use. Of the 522 men who indicated they applied pesticides, 520 applied herbicides. Many also applied insecticides and/or fungicides. Due to the extensive mixture of pesticide types used, and since in this rural setting, it was not possible to find a comparison group of families not exposed to agrochemicals, the men using herbicides only were the referent group compared to other groups using herbicides and other types of pesticides. The sex ratio (male:female) of the children (342 live births) of the 110 men applying herbicides only was 1.12 vs 1.11 in the children (209 live births) of the 72 applicators applying both herbicides and insecticides. These ratios are not statistically different from the U.S. national sex ratio of 1.047. In contrast, the offspring sex ratio (508 live births) of the 180 men applying herbicides, insecticides, and fungicides was 0.80 (p=0.02, compared to herbicide only applicators). A subsequent reproductive study of men applying pesticides, recruited from the families in the health survey, was conducted. The percentage of normal sperm morphology in pesticide applicators who had applied fungicides among other pesticides (n=31) was significantly lower compared to pesticide applicators who had never applied fungicides (n=59). The difference was significant using the traditional WHO classification (30.3% vs 21.0%; p=0.02) and the Strict Criteria classification (11.0% vs 3.8%; p=0.001). This research suggests that fungicides may alter sex ratios and sperm morphology. Are these effects related or independent health outcomes? Are the altered sex ratios due to changes in the sperm as suggested by the abnormal morphology, or is the sex ratio a result of maternal exposure during the pregnancy? This same group of herbicide, insecticide, and fungicide applicators had an excess pregnancy loss (11.15% vs 7.10% in herbicide only; OR 1.64; 1.009-2.673). Could the lower sex ratio be a result of a disproportionate number of male fetuses being lost?

# 59

**OFF-SITE SEMEN ANALYSIS IN MEN INVESTIGATED FOR REPRODUCTIVE TOXICITY: PRESERVATION OF SPERM SHAPE AND THE CHROMATIN ANILINE BLUE STAINING PATTERN.** Ciler Celik-Ozenci, Lynne Vigue and Gabor Huszar. The Sperm Phys. Lab., Department of OB/Gyn, Yale School of Medicine, New Haven CT 06510

**INTRODUCTION**: Spermiogenetic maturation, sperm shape and chromatin maturity are affected by reproductive toxicity. We have devised conditions that preserve the various sperm biochemical markers for 24-36 hours post ejaculation. These markers include the 70 kDa testis expressed chaperone, HspA2 (formerly CK-M), CK activity (represent cytoplasmic extrusion), % immature sperm with cytoplasmic retention, and proportion of viable sperm at ejaculation. We expect that home collection and semen transportation to a central laboratory would improve compliance and analysis quality. However, after overnight shipping, the sperm properties may change. We now report on a method that preserve sperm shape, and pattern of clear and dark chromatin aniline blue staining, the latter indicate the late persistence of histones.

**METHODS**: We added 20 µg phenylmethylsulphonylfluoride (PMSF)/ml. of neat semen, and kept the sample at 4°C for 24 hours, in order to simulate shipping conditions. Subsequently, we have compared the proportion of sperm with immature (dark) vs. mature (light) aniline blue staining, as well as the attributes of sperm morphometry in the initial and stored samples by the Metamorph program (Univ. Imaging Systems, Downingtown, PA).

**RESULTS**: The proportion of sperm with abnormal dark and light aniline blue staining was unchanged in the initial vs. 24 hour samples (21.1% and 78.9% vs. 22.5% and 77.5%, N=8 men, 1400 sperm evaluated in each). With respect to the morphometrical attributes, we studied 1000 sperm in the initial and 24 h storage samples of 8 men. The various parameters (initial values in parentheses): head area (17.7$\mu^2$), head perimeter (16.3µ), long head axis (6.1µ), short head axis (4.9µ), tail length (56µ) showed changes of <5%.

**CONCLUSIONS**: We have demonstrated that in PMSF/4°C semen storage conditions, both the proportion of sperm with immature histone staining, and shape of stored sperm remain unchanged for 24 hours (Supp. OH-04061).

# 60

**SPERM COUNT VS BLOOD LEAD GENERAL LINEAR MODEL: MULTIPLE STUDIES FROM WORKSITE EXPOSURES** W.J.Moorman*, P.B. Shaw*, T.W.Turner*and S.M. Schrader, National Institute for Occupational Safety and Health Division of Applied Research and Technology Cincinnati, Ohio

In the U.S. blood lead levels have decreased, yet worker exposures in some industries still have elevated blood leads as reported in the CDC Adult Blood Lead Epidemiologic Survey. With just 27 states reporting, there were 5,772 reports of blood lead between 25 and 39ug/dl and over 100 reports of blood lead over 60g/dl. Blood lead levels of concern to occupational safety and health professionals are defined as levels above 40ug/dl for adult workers per the OSHA standard however some health scientists have concern about the impact of very low blood lead levels in terms of impaired semen quality. We have pooled the relevant literature and extracted data to develop a general linear model relating sperm count and blood lead levels. While numerous published reports clearly demonstrate that lead adversely affects spermatogenesis in men, few of these reports present actual data for sperm counts and blood lead levels useful for a quantitative comparison. Seven reports however, do present enough data to obtain group means for sperm count and blood lead levels. These reports provided data from 415 workers. The ranges for means of blood lead and sperm count were 10-90 and 32-101, respectively. The model incorporating blood lead and study as explanatory variables showed an inverse relationship between blood lead and sperm count, accounting for 82.5% of the variance. For each 10 ug/dl increase in blood lead, a decrease of 6.2 million sperm/ml was indicated.

# 61

**POLYCHLORINATED BIPHENYLS INDUCED ALTERATIONS OF MORPHOLOGY AND CYTOSKELETON IN MCF-7 HUMAN BREAST CANCER CELLS** S.Raychoudhury and A.F.Flowers* Department of Biological and Physical Sciences, Benedict College, Columbia, SC 29204.

Polychlorinated biphenyls are ubiquitous and persistent environmental contaminants. Previously we demonstrated that PCBs are toxic to immature Sertoli cells in vitro. PCBs are also considered as environmental xenoestrogens. A variety of PCBs exhibited possible estrogenic effects on uterus and pituitary gland. Here we have examined in vitro effects of PCBs (Aroclor 1254 and Aroclor 1242) on estrogen responsive MCF-7 human breast cancer cells, and compared their effects with estradiol 17fl, and tamoxifen, another ligand for the estrogen receptor. Low concentrations (500 ng or 5 ug per ml)of PCBs, estradiol 17fl, and tamoxifen were added to the cultures of MCF-7 cells, and incubated for 72 hours. Morphological changes were observed under phase-contrast microscope. Concomitant cytosketal changes were documented with rhodamine-conjugated phalloidin (anti-F-actin) and monoclonal anti a-tubulin antibodies using indirect immunofluorescence microscopy. Tre atment with PCBs, and estradiol exerted striking changes in MCF-7 cell morphology. We have also observed shortened and disorganized actin fi laments in these treatment groups. MCF-7 cells also exhibited altered and scant microtubule staining with PCBs and estradiol treatments. These effects were also shared by tamoxifen. The results indicate that exposure of PCBs and estrogen to human breast cancer cells yielded changes in cytoskeleton. This may provide a useful model to study steroid hormone regulation of cytoskeleton in responsive human tumor cells, and to compare environmental pollutants that are xenoestrogens. Supported by NIH Grants P20RR11588 and S06GM08117.

# 62

**THE INFLUENCE OF ANTISPERM ANTIBODIES ON THE ACROSOME REACTION** C Bohring, J Skrzypek and W Krause Department of Andrology, Clinical Training Center of the European Academy of Andrology, University Hospital Marburg, Germany

In the present study the influence of ASA on the acrosome reaction was investigated using flow cytometry for discrimination of acrosome reacted and unreacted spermatozoa. The influence of antisperm antibodies (ASA) on the acrosome reaction (AR) were determined. Spermatozoa from a pool of healthy donors were incubated with 30 seminal plasma samples from infertile men containing ASA, in comparison to a control group of 10 samples without ASA and 5 samples with buffer only. The spontaneous acrosome reaction (SAR) and the induced acrosome reaction (IAR) by calcium ionophor A23187 were observed and determined by means of a flowcytometer. Doublestaining estimates of acrosomal integrity were determined by using a antibody against the inneracrosamale membrane and a secondary FITC labeled antibody. Cell viability was obtained by counterstaining with propidium iodide (PI). Therefore a discrimination between acrosome reacted ,unreacted and dead sperm is practicable. Only living sperms were included. The ASA treated spermatozoa showed significantly higher SAR and IAR results than the control group. ASA from patient seminal plasma samples are able to enhance the spontaneous and induced acrosome reaction in donor spermatozoa. This may be of clinical relevance, since usually the spermatozoa undergo the acrosome reaction nearby the egg cell when they start the zona pellucida penetration. Spermatozoa which underwent already the AR induced by ASA, will then not be able to fertilize any more.

# 63

HUMAN SPERM WITH SEVERELY DEFECTIVE MORPHOLOGY VARY WIDELY IN THEIR ABILITY TO PENETRATE ZONA-FREE HAMSTER EGGS.

Richard Bronson, Susan Bronson*, Lucilla Oula* Departments of Ob/Gyn & Pathology, Health Sciences Center, State University of New York at Stony Brook

A body of evidence suggests that morphologically abnormal sperm exhibit impaired ability to fertilize. Their ability to bind to zona pellucidae and undergo an acrosome reaction may be altered and increases in cytosolic calcium in response to progesterone diminished. Fertilization rates at IVF are lower for those ejaculates judged abnormal by "strict" criteria. While teratospermia may be seen in the presence of varicoceles or following environmental stresses, abnormalities of sperm shape may also be associated with single autosomal gene mutations. These varying mechanisms leading to teratospermia could have different consequences as regards sperm function. We studied the ability of human sperm to penetrate zona-free hamster eggs, as related to their morphology. Sperm were recovered by Isolate density centrifugation from 54 ejaculates containing less than 20% normal forms, and 20-25 zona-free hamster eggs inseminated with 1 million motile capacitated cells. Acridine orange-stained eggs were scored after 3 hours incubation. No correlation was observed between sperm morphology, as judged by 1999 WHO criteria, and egg penetration rates (mean $\pm$ s.d.).

| % normal sperm | % eggs pene. | # pene. sperm/egg | # adherent sperm/egg |
|---|---|---|---|
| $\leq$10% n= 27 | 42.0$\pm$36.1 | 0.64$\pm$0.72 | 23.8$\pm$35.3 |
| >10% n= 27 | 49.5$\pm$23.4 | 3.38$\pm$3.90 | 28.4$\pm$11.5 |
| Control n=20 | 96.8$\pm$5.80 | 3.40$\pm$1.40 | 56.8$\pm$18.6 |

When severely abnormal sperm (<10% normal) were tested, the % eggs penetrated ranged from 0% to 100%, # of penetrating sperm/egg - 0.00 to 3.3, and # adherent sperm/egg - 3.6 to 48.2. Sperm morphology, as judged in the clinical andrology lab, cannot predict the wide variation in sperm function observed between different teratospermic men.

# 64

**DIFFERENTIAL EFFECTS OF GLUCOSE AND FRUCTOSE ON THE LOCATION OF GLYCOGEN SYNTHESIS IN MATURE DOG SPERMATOZOA**

T. Rigau*, J. M. Fernandez-Novell, M.J. Palomo, J. Ballester, J.J. Guinovart and J.E. Rodriguez-Gil. Dep. Animal Medicine and Surgery. School of Veterinary Medicine. Autonomous University of Barcelona

Incubation of dog spermatozoa from fresh ejaculates resulted in a time-and concentration-dependent glycogen deposition. This effect was related with a simultaneous activation of glycogen synthase and an inactivation of glycogen phospohrylase. However, glycogen deposition suffered a lag time of 15 min after incubation with 10 mM glucose, that were not observed with incubation with 10 mM fructose. Moreover, glucose induced the accumulation of glycogen synthase, and hence glycogen in the post-acrosomal section of the head and the midpiece, whereas fructose induced an uniformlus deposition of glycogen in all of the spermatozoon. These differences suggest that glycogen metabolsim in dog spermatozoa is regulated by two simultaneous ways. First, the modulation of the activities which regulates glycogen synthesis. Second, by directing the glycogen deposition to concrete sperm zones, which were specific on the glycogen-stimulating sugars. Moreover, this complexe regulation indicates that glycogen metabolism is playing an important role in the management of energy levels of dog spermatozoa, and sugars are playing more roles than the purely energetic in these cells.

# 65

**THE PRODUCTION OF NITRIC OXIDE IN BULL AND FISH SPERM.**

Atherton1, R.W., Bohle2*, D.S., Montville1*, C., Cruickshank3*,C.S., and McBean1*, A.D. [1]Department of Zoology and Physiology, [2] Department of Chemistry, The University of Wyoming, Laramie; [3]Department of Health and Exercise Science, Colorado State University, Ft. Collins, CO. Support by NIH P20RR15553-01: RWA and DSB.

Our labs1,2 have reported that Electron Paramagnetic Resonance spectroscopy [EPR] detects Nitric Oxide in fish eggs but not sperm [Creech, et. al., 1998]. This report documents for the first time that cryopreserved Bull sperm produce Nitric Oxide [4.31 +/-1.5 nM/106/> sperm/ml] comparable to human sperm [Donnelly, et. al., 1997]. In a comparison of World Precision Instruments, Inc. sensors, the solid-state microchip temperature independent sensor provided more reproducible results than the 2 mm membrane probe. When Bull sperm was thawed, washed 3 times, and allowed to decline in motility to approximately 50% of the initial motility [Hamilton-Thorne CEROS CASA system], the addition of L-Arginine at mM, uM, and nM concentrations significantly increased the percent of motility [ANOVA, P=<0.05], but had no significant effect on progressive motility nor on 3 sperm velocity parameters [straight line, VSL; curvilinear, VCL; & average path length, VAP]. In similar experiments with fish sperm, Nitric Oxide oxidation products [nitrite and nitrate] were not detected by HPLC in sperm supernants, L-Arginine was ineffective in stimulating motility, and L-NAME did not reduce motility. Nevertheless, there was minimal Nitric Oxide produced by washed sperm [0.315 +/-0.215 nM/106 cells], which could be accounted for by residual somatic cell activity.

# 66

**A COMPARATIVE ANALYSIS OF ENDOGENOUS PLATELET-ACTIVATING FACTOR CONTENT IN MAMMALIAN SPERM.** W.E. Roudebush1, J.A. Cano1, J.B. Massey*1, C.W. Elsner*1, A.A. Toledo*1, and J.R. Diehl*2, Reproductive Biology Associates 1, Atlanta, GA, Department of Animal & Veterinary Sciences, Clemson University 1, Clemson, SC.

Platelet-activating factor [PAF] is a unique and novel signaling phospholipid that has been implicated in a number of biological activities (e.g. reproduction) in addition to platelet activation. While the presence of PAF in the sperm of a number of mammalian species has been demonstrated, no studies have compared content between the species. Therefore, this study compared endogenous PAF levels in sperm between seven different mammalian species: human, squirrel monkey, rhesus macaque, baboon, porcine, and bovine. Semen was collected from mature males by the standard methodology for each species. Endogenous lipids were extracted from washed sperm and assayed for PAF by a commercially available [125I]-radioimmunoassay. Data were analyzed by regression analysis. Endogenous PAF was found in all samples assayed. Sperm-derived PAF content ranged from a low of 1.4 pM/106 cells (bull) to a high of 4.9 pM/106 cells (boar). PAF levels for all species (from lowest to highest) were as follows: bull, 1.4 pM/106 cells; squirrel monkey, 2.1 pM/106 cells; baboon, 2.3 pM/106 cells; human, 4.3 pM/106 cells; rhesus, 4.6 pM/106 cells; and boar, 4.9 pM/106 cells. Regression analysis revealed no significant differences in the mean PAF values among the species. The data confirms PAF's presence in mammalian sperm and that PAF content among the different species is conserved. Additional studies will elucidate the role of PAF in sperm function and the significance PAF plays in mammalian reproduction.

# 67

**POSSIBLE ROLE OF INTRACELLULAR CALCIUM IN THE HEAD-TO-HEAD AGGLUTINATION OF BOAR SPERMATOZOA** H. Harayama, Graduate School of Science and Technology, Kobe University, Kobe 657-8501, JAPAN

When boar spermatozoa are incubated in a medium designed for IVF, many of them become agglutinated at the acrosomes. Our previous reports have suggested that this agglutination is associated with molecular changes on the sperm surface that are promoted by capacitation-supporting factors (e.g., extracellular calcium, bicarbonate and serum albumin). Moreover, the agglutination is regulated via a cAMP-mediated signaling cascade that is apparently stimulated by bicarbonate. In order to reveal a downstream part of this signaling cascade, we examined the effects of BAPTA-AM (a cell-permeable calcium chelator) and thapsigargin (a Ca2+-ATPase inhibitor) on the agglutination of boar spermatozoa. Spermatozoa were collected from three mature boars, washed and resuspended in a modified Krebs-Ringer HEPES (mKRH). The sperm suspension was incubated in a water bath for 1 h, and then used to determine the percentages of head-to-head agglutinated spermatozoa. Pre-loading of BAPTA-AM (25 μM) into washed spermatozoa significantly reduced the agglutination resulting from the incubation in mKRH supplemented with bicarbonate (5 mM) plus IBMX (25 μM) or with Sp-5,6-DC1-cBiMPS (a cAMP analogue; 10 μM), whereas treatment with BAPTA (a cell-impermeable calcium chelator; 25 μM) had hardly any effect on this event. Moreover, the supplementation with thapsigargin (4 μM) significantly raised the percentages of head-to-head agglutinated spermatozoa after the incubation in mKRH without any modulator of the cAMP-mediated signaling cascade. These findings suggest a possible role of intracellular calcium in the sperm head-to-head agglutination that is regulated via the cAMP-mediated signaling cascade.

# 68

**PROBABILITY OF SPERM WITH ACTIVATED KILLER ENZYMES TO HAVE DAMAGED MEMBRANES**

U Paasch*, S Grunewald*, H-J Glander*; EAA Center, University of Leipzig, Germany Introduction: Caspases, apoptotic key enzymes are present and become activated (aCP) in ejaculated spermatozoa preceding externalized phosphatidylserine (PS) at membranes especially after cryopreservation (CRP). Magnetic cell sorting (MACS) was intro-duced to enrich membrane intact (PS-) sperm. The probability of sperm with aCP having PS+ membranes in native samples and after CRP was computed.

Methods: Native and cryopreserved semen samples from healthy donors (n=40) were investigated in 2 fractions depending on Annexin microbead binding to PS followed by MACS. aCP were determined by carboxyfluorescein derivatives, which are cell permeable and non-cytotoxic. After covalent binding these inhibitors become fluorescent upon cleavage by the aCP 8, 9, 1 and 3, which was supervised by fluorescence microscopy and FACS.

Results: Following CRP the amount of spermatozoa containing aCP 8, 9, 1 & 3 raised significantly (native/ CRP, %, X±SEM): aCP8 16.0±3.8/ 46.5±9.6; aCP9 14.9±6.5/ 36.9±16.8; aCP1 13.8±6.3/ 29.3±5.8; aCP3: 18.1±7.5/ 50.7±5.9. Logarithmic odds ratio were estimate to calculate the multiplier of the chance of a aCP+ sperm to be PS+ with regard to aCP-cells (log2OR native / CRP): CP8 4.1±0.6 / 5.7±0.7; CP9 4.9±0.7 / 7.0±1.4; CP1 3.6±1.5 / 5.2±1.1; CP3 2.9±0.5 / 5.5±0.8 ($p<0.05$). Rank order log2OR of native was CP9>CP8>CP1>CP3 in comparison to CRP CP9>CP8>CP3>CP1. The prob-ability was highest for CP9 in native cells and after CRP. For CP3 log2OR showed the highest increase after CRP.

Discussion: Sperm with aCP are highly probable to have PS+ membranes and can thereby highly specific separated by MACS. After CRP the probability to extract PS+-sperm having active death executers (aCP3) increased more than for initiators of apoptosis (aCP 8, 9), indicating that the apoptotic point of no return is overcome after CRP in PS+ cells.

# 69

**REACTIVE OXYGEN SPECIES INDUCE DEOXYRIBONUCLEIC ACID (DNA) DAMAGE IN EQUINE SPERM.** J. Baumber*, B.A. Ball*. Department of Population Health and Reproduction, University of California, Davis, CA.

The objective of this study was to examine the influence of reactive oxygen species (ROS), generated by the xanthine-xanthine oxidase (X-XO) system, on DNA fragmentation of equine sperm as determined by the single cell gel electrophoresis (comet) assay. Motile equine spermatozoa were separated on a discontinuous (80/40 %) Percoll gradient, washed and resuspended at 25 x 106/ml in a modified Tyrodeìs medium supplemented with 0.1% polyvinyl alcohol (TALP-PVA). In Experiment One, spermatozoa were incubated (1-hr, 37oC) according to the following treatments: 1) Sperm alone, 2) Sperm + X (0.3 mM) -XO (0.025 U/mL), 3) Sperm + X (0.6 mM) -XO (0.05 U/mL) and 4) Sperm + X (1 mM) -XO (0.1 U/mL). In Experiment Two, spermatozoa were incubated (1-hr, 37oC) with the X (1 mM) -XO (0.1 U/mL) system and either catalase (200 U/mL), superoxide dismutase (SOD, 200 U/mL) or glutathione (GSH, 10 mM). Following the 1-hr incubation, DNA fragmentation was determined by the comet assay. The degree of damage was assessed by visual scoring of each sperm comet (at least 100 sperm/treatment) on a scale of 0 (no fragmentation) to 4 (extensive fragmentation). In Experiment One there was an increase in comet grade following incubation with the XÒXO system, with the most extensive damage observed with 1 mM X and 0.1 U/ml XO. In Experiment Two, the increase in comet grade associated with X-XO treatment was counteracted by the addition of catalase but not SOD; GSH had an intermediate effect in preserving DNA integrity. This study indicates that ROS promote DNA fragmentation in equine sperm, hydrogen peroxide appears to be the major ROS responsible for such damage and catalase can protect equine sperm in vitro from DNA damage induced by oxidative stress.

# 70

**ANDROLOGICAL PARAMETER DIFFERENCES BETWEEN IDENTICAL GENOMIC BULLS** C. Lessard*, JF Bilodeau*, JL Bailey**, P Leclerc*, and R Sullivan*

From the *Département d'Obstétrique-Gynécologie, Faculté de Médecine, and **the Département des Sciences Animales, Faculté des Sciences de Agriculture et de Alimentation, Universityé Laval, Saitne-Foy, Québec, G1K 7P4

Embryonic cloning technique offers the possibility to obtain several genomically identical subject. Few information are reported about andrological parameters of identical twins. In 1995, the production of four bulls generated by blastomere separation had been reported by Johnson et al, referred as Quad (Q1, Q2, Q3, and Q4). The semen produced by the Quad during the period of 1994 to 1996 have been cryopreserved. In this study, andrological parameters of fresh and cryopreserved semen from Quad have been evaluated. Andrological parameters analysis of fresh semen showed a lower motility and a higher percent of abnormal sperm for Q3 comparatively to his brothers ($p>0.05$). Androl ogical parameters analysis of cryopreserved spermatozoa have revealed that Q4 s spermatozoa displayed more cryodamages comparatively at his brothers, especially Q1. Q4 s cryopreserved spermatozoa showed a lower percentage of motility, of progression motility and of mean path velocity compared to Q1 s cryopreserved spermatozoa ($p>0.05$). Further -nore, the presence of P25b protein was lower on Q4 s cryopreserved spermatozoa than his twins ($p>0.05$). P25b is a sperm surface protein proposed as a bull fertility marker. Development of embryos produced by in vitro fertilization using cryopreserved semen confirmed the damages observed on Q4s cryopreserved spermatozoa, with a noticeable lower percentage of embryo development. The andrological parameters analysis of fresh and cryopreserved semen suggest an interindividual semen difference between genomically identical subjects.

# 71

BULL FERTILITY – COMMERCIAL OUTCOME DATA INAPPROPRIATE TO ESTABLISH UTILITY OF A DIAGNOSTIC TEST OF SPERM QUALITY

R.P. Amann and R.H. Hammerstedt, BioPore Inc., State College, PA.

Many consider dairy cattle ideal to study relationships among attributes of sperm quality and establishment of pregnancy after AI; >400 AIs/ejaculate are routine. Status as pregnant after AI informs that all sperm attributes (plus all female attributes) were working correctly in some sperm. However, status as non-pregnant is uninformative concerning what (if any) sperm attribute(s) was defective (J Androl 21:10, 2000), if failure was a female or management (e.g., faulty estrus detection) problem, or if some other factor was involved. Summaries based on many females per bull use statistical corrections (ignoring sperm/AI) to calculate bull fertility. Nevertheless, bull accounts for <1% of total variation and variation due to ejaculate within bull is 2/3 of that among bulls (P Christensen, personal communication; 18th NAAB Tech Conf, 50, 2001). USA AI industry "ERCR" values (deviations from mean; ~53% pregnant) range from -13 to +11. Values are between -2 and +2 for 77% of bulls. The 95% CI for most bulls is ≥2 units. Only 2% of bulls have a value below -4. Most subfertile bulls are culled from studs by <2 yr of age. Clearly, ERCR (or similar data) are useless for linkage with outcome in laboratory evaluations of sperm aimed at detecting subfertile bulls. Note that accurate prediction of subfertility is a reasonable goal, but accurate prediction of fertility is unlikely (J Androl 14:397, 1993). A larger problem with ERCR data is that number of sperm/AI differs among bulls, is excessive, and is near or above the asymptotic value for the change in pregnancy rate as a function of sperm/AI (to maximize pregnancy rate). This is contraindicated if the goal is measuring potential of sperm in a given sample to fertilize an oocyte and provide a viable embryo. To provide fertility outcome data in which bull (or treatment imposed on semen) accounts for a substantial proportion of the total variance, one should carefully control extraneous factors, use only nulliparous cattle of one breed (to minimize female factor), and inseminate a uniform low dose (i.e., 0.8 or 1.5 x $10^6$ frozen-thawed sperm). Treatment effects likely would be detected. To validate a diagnostic test, however, this is logistically difficult as data for >20 bulls (some subfertile) and >1000 AIs/bull would be desirable.

# 72

THE FATE OF BOVINE OOCYTES INTERACTING *IN VIVO* WITH 1, 2 OR ≥3 SPERM; IMPLICATIONS ON DATA INTERPRETATION

RP Amann[1] and RG Saacke[2], [1]BioPore Inc, State College, PA and [2]Virginia Polytechnic Institute & State University, Blacksburg, VA

It general is acknowledged that the more accessory sperm (AcS) associated with an ovum, the greater probability that ovum will form an embryo and fetus. This obfuscates the fate of ova associated with ≤1 AcS. We compiled data for 267 ova from non-lactating cows inseminated (AI) with 20, 25, or 100 x$10^6$ sperm from 1 of 6 fertile bulls. AI occurred 8-12 h after onset of a natural estrus (visual detection) or 0, 12 or 24 h after onset of an estrus induced with $PGF_2\alpha$ (automated detection). Ova were recovered 6 d after AI and AcS enumerated (J Anim Sci 70:484, 1992). After AI of 20-25 x$10^6$ sperm (232 ova), ova with 0-1 or ≥6 AcS had 1:4 vs 2:1 odds of forming an excellent/good (EG) embryo. Unexpected were 40 and 81% probabilities that fertilization (EG plus fair or degenerated (FD) embryo) had occurred in ova with 0 or 1 AcS. Hence, presence of 0 or 1 AcS is not a good predictor of fertilization failure. Implications will be presented. [funding: Select Sires Inc; Nat Assoc Animal Breeders]

| Estrus, Semen, and AI time | All ova | Number of ova: 0 AcS EG | 0 AcS FD | 0 AcS UF | 1 AcS EG | 1 AcS FD | 1 AcS UF | 2+ AcS EG | 2+ AcS FD | 2+ AcS UF | % fertilized: 0 AcS | 1 AcS | 2+ AcS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Natural; visual, AI at 8-12 h** | | | | | | | | | | | | | |
| Fresh --- 100 million | 35 | 2 | 5 | 4 | 2 | 2 | 0 | 14 | 6 | 0 | 64 | 100 | 100 |
| **Natural; visual, AI at 8-12 h** | | | | | | | | | | | | | |
| Fresh ---- 20 million | 21 | 2 | 0 | 5 | 1 | 1 | 0 | 6 | 5 | 1 | 29 | 100 | 92 |
| Frozen --- 25 million | 53 | 0 | 4 | 8 | 5 | 3 | 1 | 21 | 11 | 0 | 33 | 89 | 100 |
| **PGF+HeatWatch - 25 million** | | | | | | | | | | | | | |
| Frozen --- AI at 0 h | 60 | 8 | 4 | 18 | 3 | 0 | 4 | 14 | 8 | 1 | 40 | 43 | 96 |
| Frozen --- AI at 12 h | 59 | 4 | 7 | 15 | 3 | 1 | 0 | 17 | 11 | 1 | 42 | 100 | 97 |
| Frozen --- AI at 24 h | 39 | 2 | 4 | 7 | 3 | 1 | 0 | 10 | 12 | 0 | 46 | 100 | 100 |
| **All data 20 or 25 M sperm** | 232 | 16 | 19 | 53 | 15 | 6 | 5 | 68 | 47 | 3 | 40 | 81 | 97 |

# 73

**EFFECT OF WEIGHT AND AEROBIC TRAINING ON STEROID HORMONES AND SEMEN PROFILES** W.H. Park*, K.H. Rha*, K.J. Cho*, M.S. Lee, Yonsei and Hanyang University, Seoul, Korea

OBJECTIVE: To determine if weight and aerobic training affects hormones and semen profiles in healthy young men. DESIGN: 10-week radomized trial with 15 men assigned to 3 groups: 1) weight training (bench press, sit up, leg squat (3 sets/day; 70% of one-repetition maximum(1RM)) 2) aerobic training (30 mn/day; 70% of maximum heart rate(HRmax)) 3) control group.

SUBJECTS AND METHODS: Fifteen volunteers attending college (age 22.6(19-25) years) had their blood sampled in the morning for testosterone, LH, FSH, prolactine and cortisol, and had semen samples taken for computerized semen analysis for at the start of study, during(3 times) and after study. RESULTS: Testosterone(F=2.17), cortisol(F=1.00), FSH(F=1.61) and LH(3.53) decreased during exercise in the weight training group. Sperm concentration(F=1.84) is increased during exercise in the weight training group, and semen volume is increased in both the weight training(F=1.00) and the aerobic group(F=2.99). Such increases resumed to the normal range after cessation of exercise in the weight training group. There were no significant changes in other sperm motility/morphology and serum prolactin levels. CONCLUSIONS: Weight training seems to decrease demand for hormonal stimulus and increases semen volume and sperm concentration.

# 74

**SEMEN QUALITY SCORE - A NOVEL METHOD TO PREDICT SUCCESSFUL OUTCOME IN DONOR INSEMINATION FOR COUPLES WITH MALE FACTOR INFERTILITY** Pavithra Ranganathan*, Ilian Bandaranayake*, Jaswinder Chauhan*, Mehmet Oder*, David Nelson*, Ashok Agarwal. Cleveland Clinic Foundation

We aimed to establish semen quality scores (SQS) in a population of proven fertile sperm donors and its correlation with successful pregnancy rates in women undergoing artificial reproductive techniques (ART) with sperm from anonymous donors due to the infertility of their male partners. A review of the medical records of 111 women who were active in the donor insemination program between 1993-2001 was done. These women underwent 724 ART cycles with sperm from 27 anonymous semen donors. The semen analysis reports from the donors were analyzed after thawing and each sample was given an SQS. Out of 111 patients, 70 had at least one pregnancy and 60 had at least one live birth, with a mean 6.52 cycles per patient. Five significant risk factors for low pregnancy and live birth rates were identified: female infertility factor, positive laparoscopy, maternal age, low number of previous births, and lack of ovulatory stimulation. After adjusting for these factors, both pre-freeze SQS (p=0.03) and post-thaw SQS (p=0.04) were found to be significantly related to IUI live birth rates. With a post-freeze SQS of 110 or higher, 19.3% of the IUI cycles resulted in pregnancy, compared with a pregnancy rate of only 10.8% per cycle for post-freeze SQS scores below 105. SQS can be used as a predictor of successful pregnancy in infertile patients undergoing donor insemination. Significantly higher rates of pregnancy and live birth can occur with the use of semen samples from anonymous donors with an SQS greater than 110. Use of such semen samples may help not only decrease the cost of ART procedures to the patient, but can also significantly improve the outcome of these procedures.

# 75

**LONGITUDINAL STUDY OF SEMEN QUALITY AFTER INTERMITTENT EXPOSURE TO AIR POLLUTION.** J. Rubes*, D. Zudova*, Veterinary Research Institute, Brno, CR, S.G. Selevan*, US EPA/ORD/NCEA, Washington, DC, D.P. Evenson, South Dakota State University, Brookings, SD, and S.D. Perreault, US EPA/ORD/NHEERL, RTP, NC.

A previous study of semen quality in men providing one sample each, showed significant associations between exposure to high levels of air pollution and adverse effects on sperm morphology and sperm chromatin structure (Selevan et al., Environ Health Perspect 108:887, 2000). These positive findings prompted a follow-up study with a longitudinal design. Thirty-seven men from Teplice District, Czech Republic were surveyed on 7 occasions over 2 years time: during late summer when pollution was low and winter when pollution was high. Semen outcomes were analyzed for changes associated with levels of air pollution components (SO2, NOx, PM10 or PAH) or with season (late summer or winter) using a mixed model regression analysis (SAS) for repeated measures, and controlling for potential confounders. No significant associations were found between exposure and sperm concentration, percent motile sperm, or percent normal sperm heads. In contrast, the percent sperm with abnormal chromatin structure increased with exposure. Specifically, DNA Fragmentation Index (DFI), obtained using SCSATM, showed significant ($p<0.05$) positive associations with season of high air pollution (b=0.191, 95% CI: 0.018, 0.365), and with SO2 levels (b=0.026, CI: 0.001, 0.053). Correlations between DFI and either PM10 or PAH were of borderline significance ($p<0.066$ and $p<0.059$, respectively). These findings confirm our earlier results suggesting that intermittent exposures to high levels of air pollution may increase risks of infertility and male-mediated developmental toxicity. This abstract does not reflect EPA policy.

# 76

**SEMEN QUALITY OF SPERM DONORS IN LOS ANGELES AND PALO ALTO: EFFECTS OF TIME, TEMPERATURE, AND LOCATION** I.M. Fowler*, P. Kraft*, K.T. Berhane*, E. Kim*, R.Z. Sokol, Keck School of Medicine of the University of Southern California, and the California Cryobank, Los Angeles, CA

INTRODUCTION/OBJECTIVES: Although published data both support and refute that a significant decrease in sperm quality (SQ) has occurred worldwide during the past 30-50 years, these variable findings suggest that if a decline in SQ exists, there may be geographic differences. SQ has been noted to be compromised by high ambient temperature (AT). We hypothesized that changes in AT may explain the observed geographic variability noted in reports of declining sperm counts. We examined both semen analysis (SA) and AT data from two geographic locations to determine if (1) a temporal trend in SQ exists, (2) geographical differences in SQ exist, and (3) SQ is related to changes in AT. STUDY DESIGN: Samples from sperm donors located in 2 cities in CA who donated for at least ten consecutive months from 1/96-12/98 were studied. SA data, including donation date, donor age, average count, total motile, and donorís home address zip code, were accessed from one donor sperm bank with locations in Los Angeles (LA) (n=50) and Palo Alto (PA) (n=35). SA were performed using WHO criteria and standardized between the two locations. AT data for specific geographic grids was obtained from Sonoma Tech., Inc. (Petaluma, CA) for the same time period. Donors were matched to geographic grids by zip code and mixed model linear regression was used to model linear relationships between outcome and time or AT. RESULTS: Donors in LA had higher average counts and total motilities than those in PA (Count:87.1 ±39.1 vs. 79.1 ±34.6, $p<0.001$; TM: 191.9 ±95.0 vs. 158.4 ±83.8, $p<0.001$). Mixed model linear regression indicated a 5.9% decrease in average count per year [95% CI = (5.5%, 6.4%)]; a 2.5% decrease in total motile per year [95% CI = (1.8%, 3.2%)]; and a15.9% decrease in total motile from LA to PA [95% CI = (10.9%, 20.6%)]. Mixed model linear regression for AT vs. count and total motile showed no correlations. CONCLUSIONS: Our data suggest 1) an overall decline in semen quality over the time period of 1/96-12/98; 2) significant differences in semen quality between donors in Los Angeles and donors in Palo Alto. Although an average ambient temperature difference between Los Angeles and Palo Alto existed during the study period, this difference did not impact the disparity in semen quality parameters between Palo Alto and Los Angeles and likely does not explain the observed geographic differences. Supported by NIEHS F3 ES11535-01 award to RZS.

# 77

**SEASONAL VARIATION OF SEMEN PROFILES IN HEALTHY YOUNG MEN IN KOREA** K.H. Rha*, W.H. Park* J.Y. Noh*, S.W. Han*, M.S. Lee. Yonsei University, Seoul, Korea

OBJECTIVE: To determine whether there is a seasonal change in semen

profiles in healthy young men in their twenties

DESIGN: Repeated semen analysis - 3 times/season in 1 week interval andevery season (January, April, July, October); analyzed by computerized semen analysis (IVOS, Hamilton-Thorn, USA)

SUBJECTS: Twenty healthy young college men volunteered to give semen samples for academic purposes; Strict period of abstinence of 3-5 days was enforced. Semen analysis was performed according to the WHO recommendations.

RESULTS: Sperm concentration was highest in spring(88.8+-19.1 million/ml)and lowest in summer(51.2+-8.5 million/ml)(p=0.0235). Sperm motility was highest in spring (88.5+-9.2 %) and lowest in autumn(70.3+-8.3 %)(p=0.043). No difference was noted in other parameters including semen volume and morphology.

CONCLUSIONS: There seems to be a trend of increased sperm concentration in the spring time.

This study was supported by KFDA 2001 endocrine disruptor research and the BK 21 for medical sciences, Yonsei University, Korea.

# 78

**INTER-SAMPLE VARIABILITY IN POST-THAW MOTILITY AND MOTION KINETICS OF CRYOPRESERVED HUMAN SPERM** R.A. Saleh*, R.K. Sharma*, J. Chauhan*, M. Oder*, N. Esfandiari*, E. Nada*, R. Raina*, B. Paige*, D.R. Nelson*, A. Agarwal, Urological Institute, Cleveland Clinic Foundation, Cleveland, OH; South Valley University, Egypt

Human semen samples may show sample to sample (inter-sample) variability of sperm parameters due to multi-factorial nature of sperm production and transport through male reproductive tract. We reviewed charts of 109 men referred to the Sperm Bank at Cleveland Clinic Foundation. We compared inter-sample variability of pre-freeze and post-thaw (24 hours after freezing) sperm motility and motion kinetics [average path velocity (VAP), straight-line velocity (VSL), curvilinear velocity (VCL), linearity (LIN) & amplitude of lateral head displacement (ALH)] of cryopreserved semen collected at 2-3 days intervals. Patients were classified into 5 groups: infertile men (n =19), testicular cancer (n =22), Hodgkin's disease (n =15), lymphoma (n =18), and other cancers (n =19). Semen samples collected from 16 fertile men, prior to vasectomy, served as controls. Two to 4 samples were collected from each individual. The post-thaw variability of sperm motility, VSL, VAP, LIN & ALH were significantly higher than pre-freeze variability (P<0.0001, 0.02, 0.006, 0.009 & 0.0001; respectively). The increase in post-thaw variability was equally observed in fertile and infertile men, and in men with different testicular pathologies. Post-thaw variability was not correlated with individual's age, length of disease, history of smoking, or alcohol intake. In conclusion, semen samples collected for cryopreservation may show a high degree of post-thaw variability of sperm motility & motion kinetics despite low degree of pre-freeze variability. Further research is required to explain the exact mechanism(s) of such phenomenon, which may help select ideal samples for cryopreservation.

# 79

**ASSISTED REPRODUCTION USING HAMSTER SPERMATOZOA GENERATED INTO XENOGENEIC TESTES**

*S. Tsabalas*[*1], Ch. Mamoulakis[1], A. Kaponis*[*1], D. Baltogiannis*[*1], X. Giannakopoulos*[*1], I. Miyagawa[2], A. Tasos*[1], N. Sofikitis[1,2]*

*[1]Dept of Urology, Ioannina University, Ioannina, Greece*
*[2]Dept of Urology, Tottori University, Yonago, Japan*

**INTRODUCTION AND OBJECTIVE:** Transplantation of donor spermatogonia into the seminiferous tubuli of recipient animals has resulted in the completion of donor spermatogenesis within the recipient testis. However, the reproductive potential of donor spermatozoa generated into recipient xenogeneic testes has not been studied yet and is evaluated in the current study.

**MATERIALS AND METHODS:** A piece of testicular tissue (300 mg) was removed from the left testis of each of 15 nude rats. The latter piece of rat testicular tissue was replaced by a piece of testicular tissue collected from each of 15 cryptorchid hamsters with spermatogenic arrest at the primary spermatocyte stage. Postoperatively all rats were administered vascular endothelial growth factor three times a week (intrascrotally). Five months later the left testis of each rat was evaluated. When hamster spermatozoa and round spermatids (RSs) were found into the rat testes, they were injected into two different groups of hamster oocytes (groups A and B, respectively). Additional spermatozoa and RSs recovered from healthy hamster testes were injected into two different groups of hamster oocytes (groups C and D, respectively). Fertilized oocytes were cultured and transferred into the fallopian tubes of pseudo-pregnant recipients.

**RESULTS:** The fertilization rate (FR) and the pregnancy rate (PR) were significantly larger (P<0.05; Chi-square test-Yates correction) in group C (50% and 33%, respectively) than in group A (20% and 10%, respectively). In contrast, there were no significant differences in FR and PR between groups B and D. All the hamster offspring produced via ooplasmic injections of hamster spermatozoa or hamster RSs recovered from rat testes were healthy.

**CONCLUSIONS:** Ooplasmic injections of donor spermatozoa or RSs generated into recipient host testes can result in delivery of healthy offspring. Defects in donor species spermiogenesis (within the recipient testis) may be responsible for the smaller outcome of ICSI techniques using hamster spermatozoa generated into rat testes.

# 80

**MAPPING" PERCUTANEOUS NEEDLE TESTICULAR BIOPSY IN NON-OBSTRUCTIVE AZOOSPERMIA**

.K.Wolski(1,2),P.Kluge(3)*,B.Biarda(1)*,K.Koziol(1)*, P.Lewandowski(1)*,(1)Infertility Center"Novum",Warsaw,POLAND (2)Clinic of Urology & (3)Department of Pathology, The Children's Memorial Health Institute,Warsaw,POLAND

Aims: Fine needle aspiration map can detects sperm in non- obstructive azoospermia (NOA)in different regions of testis although first open biopsy showed no spermatozoa (Turek 97). Material & methods: During 10.99-10.01 in 22 azoospermic men aged 22-45(mean 32 yo) "mapping" percutaneous testicular biopsies (MPTB) were performed.This manipulation was second sampling for 17 NOA men with first diagnosis: maturation arrest(MA) after treatment-8;Sertoli Only Cell syndrome (SCOs)-6;testicular biopsy done long ago in another center for verification before ICSI-3.In 5 NOA men MPTB was done as a first sampling from solitary testis:orchidectomy due to atrophy-3 and neoplasm-2.All biopsies were done ambulatory, under general anesthesia(Propofol, Fentalnyl),using needle biopsy 1.6 from HepafixΔset,B.Braun,Germany.From each testis from 2 to 9 specimens were obtained.Every fragment of tissue was divided into two parts-smaller one was examine under microscope and the larger was frozen for future ICSI-TESA. MPTB mean time was about 30 minutes.After biopsy patients took antibiotic and oral analgesic drugs for 3 days. Results: Generally,sperm cells were found after MPTB in 3/22(59%) men with NOA: 4/8(50%)-MA;3/6(50%)-SCOs;2/3(67%)-verification before ICSI;1/2(50%)-neoplasm;3/3(100%)in atrophic patients.One complication (4.5%) was observed (testicular hematoma) and surgical intervention was done. Conclusions: 1."Mapping" percutaneous testicular biopsy is useful procedures as second step of sperm obtaining for ICSI-TESA in NOA men after first negative classic biopsy or as a verification for cases with positive biopsy performed in the past. 2.In men with one testis MPTB seems to be favorable as a first sampling. 3.This simple and safe manipulation is possible to perform as outpatient procedure.

# 81

**A COMPARISON OF TWO DENSITY GRADIENT PREPARATIONS FOR SPERM SEPARATION FOR MEDICALLY ASSISTED CONCEPTION: EFFECT ON RECOVERY, CLEAN-UP, MOTILITY, AND MOTION PARAMETERS.** W.E. Roudebush, J.A. Cano*, M.H. Durrance*, M.C. Holmes*, M.A. Witt*, S.M. Slayden*, and H.I. Kort*, Reproductive Biology Associates, Atlanta, GA, USA.

Objective: To compare two silane-coated silica bead sperm preparation methods (Enhance, Conception Technologies, San Diego, CA, USA; PureSperm, NidaCon Laboratories AB, Gothenburg, Sweden) with respect to recovery efficiency, clean-up (presence of debris or round cells), and CASA motion parameters. Design: Comparison of two commercial sperm silane-coated silica bead processing methods. Setting: Private medical center-based andrology laboratory and infertility program. Patients: One-hundred couples who presented for routine sperm washing for intrauterine insemination. Interventions: Semen specimens, randomly assigned to one of two silane-coated silica bead processing methods, were evaluated by CASA. Main Outcome Measures: Recovery efficiency, debris/round cells, and CASA motion parameters (including hyperactivation) before and after separation of the two different processing methods. Results: There was no significant difference between the two study populations with respect to their initial (raw) motion parameters. There was no significant difference between the two preps with respect to their ability to remove cellular debris and, or round cells. There was no significant difference in recovery efficiencies (motile sperm) between Enhance (37.4%) and PureSperm (40.5%) processed specimens. Whereas PureSperm and Enhance both significantly ($P<0.05$) improved all sperm motion parameters over the initial-raw values, PureSpermís improvement was significantly ($P<0.001$) greater than Enhance in all CASA categories. Additionally, the percent of hyperactivated (as defined by the CASA categories VCL, ALH, and linearity) sperm was significantly ($P<0.001$) greater in PureSperm (7.3%) than Enhance (1.2%). Conclusion: While both sperm washing products significantly improved sperm performance, PureSperm consistently had superior end products across every CASA category. Considering the substantial improvement of sperm motion characteristics, PureSperm is ideal for routine sperm washing for intrauterine insemination.

# 82

**CRYOPRESERVED SPERM FROM TESTICULAR SPERM OBTAINED POST-HUMOUSLY RESULTS IN SUCCESSFUL DELIVERY AFTER INTRACYTOPLASMIC SPERM INJECTION (ICSI).** ML Check*, JH Check, D Summers-Chase*, JK Choe*, UMDNJ, Robert Wood Johnson

Med. School at Camden, Cooper Hosp./Univ. Med. Cntr., Dept. OB/GYN, Div.Repro. Endo. & Infertility, Camden, NJ

There has been one published case in peer review literature of an early chemical pregnancy resulting from in vitro fertilization (IVF) and ICSI using cryopreserved-thawed sperm that had been aspirated from the testes of a man who had been dead for several hours. The case presented here describes a successful conception and delivery following IVF and ICSI with gametes from this same couple. With the first chemical pregnancy there were no motile sperm found to inseminate. However, in the successful case there were some live sperm on her fifth oocyte retrieval which resulted in the live delivery. The fifth IVF cycle, which still used the initial frozen sample aspirated from the testes many hours after death, was completed 2 1/4 years after the first retrieval at age 37. There were 14 mature oocytes produced. Fourteen motile sperm were found for ICSI. Four oocytes fertilized and a 9-cell, 8-cell, and two 6-cell embryos were transferred. She conceived and delivered a single full term healthy baby in June 2001. Though motile sperm were found for ICSI in cycles 4 and 5, all 5 retrieval cycles resulted in the transfer of embryos. Thus this is the first reported success of using frozen sperm aspirated from testes post-humously that has resulted in a live pregnancy. We are aware of one other case that only made the lay press.

# 83

**PREGNANCY RATES WITH FROZEN TESE SPERM ARE HIGHLY DEPENDENT ON AGE OF FEMALE PARTNER.** P. Studney*, M. Puccini*, L. Ray*, L. Nelson*, C. Niederberger, L. Ross, G.S. Prins, Departments of Urology and Obstetrics & Gynecology, University of Illinois at Chicago, Chicago, IL

Cryopreservation of testicular sperm retrieved by surgical extraction has been successful in achieving satisfactory numbers of post-thaw viable sperm for IVF-ICSI (Prins et al, 1999). In conjunction with a large, established IVF program, we have attained equivalent pregnancy and live birth rates with frozen TESE sperm as with fresh TESE sperm (Habermann, et al, 2000). With a new IVF program at UIC, we have used frozen TESE sperm exclusively for the past year and herein report that pregnancy success is highly dependent on age of the female partner. Fourteen azoospermic males underwent TESE with cryopreservation of isolated sperm as previously described. Sperm were assessed for pre-freeze count,viability and morphology and for post-thaw count and viability. A total of 16 cycles of IVF-ICSI were performed resulting in 7 pregnancies (44%). When pregnancy results were analyzed by age of the female partner, the IVF-ICSI pregnancy rate with frozen TESE sperm was 80% for patients under 35 yrs (n=5), 50% for patients between 35-37 (n=4) and 14% for patients > 38 yrs (n=7), Chi-squared test for trend, P=0.023. Importantly, analysis of sperm parameters pre-freeze and post-thaw did not vary between either the pregnant vs non-pregnant couples nor with the age of the female partner indicating that the female component is the key variable affecting the pregnancy outcome. These preliminary data show that 1) TESE sperm properly separated and frozen at the time of retrieval can be successfully used in a small, new IVF program at rates equivalent to a well-established, large program, and 2) that age of the female partner is a significant factor in pregnancy success when frozen TESE sperm are used for IVF-ICSI.

# 84

**CASE STUDY: PREGNANCY RESULTING FROM IVF FOLLOWING ICSI USING SPERM HEAD WITH DETACHED TAIL** B.R. Emery1,3, C. Thorp4, P. Kuneck4, L. Erickson4, D.T. Carrell1,2,3,4 1 University of Utah School of Medicine, Department of Andrology, 2 Obstetrics and Gynecology and 3 Physiology, Abbot Northwestern Hospital, 4 Center of Reproductive Medicine

Midpiece defects resulting in male factor infertility due to decreased motility are often treated using intracytoplasmic sperm injection (ICSI). Such midpiece defects include easily decapitated sperm defect, which has been described previously by Kamal, et al. (Hum. Reprod.; vol. 14 (11), 2791-2795, 1999). In the case presented here a couple was referred for assisted reproduction due to decreased ovarian reserve. The couple underwent an in vitro fertilization (IVF) cycle using donated oocytes. ICSI was attempted with standard technique and failed due to the inability to manipulate the sperm without breaking the tail from the head. ICSI proceeded by injecting, as close to each other as possible, a sperm head and the removed tail portion into each oocyte. This resulted in fourteen embryos out of twenty-two mature oocytes retrieved of varying quality on the third day after retrieval. Following IVF semen was evaluated by transmission electron microscopy (TEM). TEM revealed a severely abnormal morphology, including microcephalic sperm and sperm with a degraded/abnormal connecting piece and poor centrosome formation. Few sperm were seen with an attached tail, and a small percent of these contained normal head morphology. Two eight cell embryos were transferred back three days after ICSI. The couple achieved chemical pregnancy as seen by elevated serum hCG concentration on day fourteen post transfer. This case reveals that fertilization using ICSI with sperm from a patient with easily decapitated sperm defect is possible even if intact sperm are not available. Additionally, pregnancy may result in transfer of embryos created in this manner.

# 85

ICSI- AND ROSI-MEDIATED TRANSGENESIS IN THE MOUSE

H. Akutsu, H. Kusakabe, T. Osada and R. Yanagimachi, Institute for Biogenesis Research, University of Hawaii Medical School, Honolulu, HI 96822

We report here an improved ICSI-mediated transgenesis and ROSI-mediated transgenesis. A drop (~2 µl) of sperm mass squeezed out of the cauda epididymis was placed at the bottom of a 1.5 ml plastic tube, covered with 200 ul of EGTA medium (50 mM EGTA + 50 mM NaCl+ 10 mM Tris-HCl, pH 7.4 - 8.2). After standing for 10 min at 37°C, sperm diffused into the medium was collected. A 30 ul aliquot of the sperm suspension was transferred into another tube which was then plunged into liquid nitrogen for 10 sec. Upon defrosting by hand-warming, the aliquot was mixed with 10 µl of 4 mM dithiothreitol (DTT) in phosphate-buffered saline. Concentrations of sperm and DTT at this stage were ~$10^6$/ml and 1 mM, respectively. After standing for 30 min at 0-4°C, linearized pCX-EGFP DNA in TE buffer was added such that DNA concentration in the medium was ~6 ng/ml. One min later, sperm-DNA medium was mixed with 10% (w/v) polyvinyl pyrrolidone (PVP) in Hepes-buffered CZB medium such that the final PVP concentration was ~7%. ICSI was performed as described previously (Biol. Reprod. 52, 709-720, 1995) except that only sperm head was injected into each oocyte. The amount of DNA-containing medium injected with sperm head was ~1 pl. ICSI was completed within 2 hr after sperm isolation from the epididymis. Sperm treated in the same manner but without DTT treatment served as the control. Of 213 oocytes injected with DTT-treated sperm, 137 (64%) developed into morulae/blastocysts, 119 (87%) of which were EGFP-positive. 25 (27%) of 94 transferred morulae/blastocysts developed into live offspring, 10 (40%) of which being EGFP positive. All these 10 grow into fertile adults and their offspring were all EGFP-positive. In the control group, 135 (79%) of 170 ICSI oocytes developed into morulae/blastocysts, 103 (76%) of which were EGFP-positive. 18 (33%) of 54 trans ferred morulae/blastocysts became live offspring, 2 (11%) of which were EGFP-positive. EGFP-positive offspring were also obtained by the simultaneous injection of an oocyte with a freshly isolated nucleus of round spermatid and pCX-EGFP DNA. Two EGFP-positive offspring were obtained after transfer of 43 ROSI-derived morulae/blastocysts.

# 86

**Y CHROMOSOME DELETION (YP11.3) ASSOCIATED WITH COMPLETE GLOBOZOOSPERMIA - A STUDY OF 7 BROTHERS** Z. Kilani,1 R. Ismail,1 S. Ghunaim,1 F. Flesch,2 D. Bradley, 2 I. Brewis,2 C.Barratt, 2 & 1. 1Farah Hospital, Amman, PO Box 5323, Jordan; 2Assisted Conception Unit, Birmingham Women's Hospital, Birmingham, B15 2TG, UK.

Introduction Although fertilization and pregnancies can be achieved in men with globozoospermia, success rates are low (Kilani et al., 1998). Currently, the cause(s) of globozoospermia are unknown. In this study of 7 brothers we show that a deletion on the Y chromosome (p11.3) is strongly associated with globozoospermia. Materials & Methods In a family of 10 siblings, 2 sisters and 3 brothers were naturally fertile. 5 brothers were infertile and required ICSI. We examined 4 of the infertile (A,B,C,D) and 3 of the fertile brothers (E,F,G). Fine structure of the spermatozoa was examined using TEM. The presence of acrosomes was detected using 18.6 monoclonal antibody. Karyotype analysis was performed on PBLs. Results 3 brothers had complete globozoospermia assessed by TEM (A-C). No acrosomes were present. Brothers A-D had a 46 XY karyotype but a large deletion in the Yp11.3 region in 10-25% of cells. 17 cycles of ICSI were performed (A=3, B=4, C=6, D=4) and the average normal (2PN) fertilization rate was 37%. 2 cycles had no fertilization (B,C). 3 brothers (A-C) had 13 cycles of ICSI yet failed to achieve a pregnancy. One brother (D), also with a mosaic deletion (25% of cells) and globozoospermia (as diagnosed at the light microscope level, TEM results to be confirmed) has one on going pregnancy on the 4th ICSI attempt. Conclusion Deletion on the Y chromosome (Yp11.3), even in a small percentage of cells, is strongly associated with globozoospermia and subsequent infertility.

# 87

**GENE EXPRESSION PROFILES IN THE VENTRAL, DORSAL AND LATERAL LOBES OF THE BROWN NORWAY RAT PROSTATE** T.R. Brown,Y. Tian,* Div. Reproductive Biology, Dept. Biochemistry and Molecular Biology, Johns Hopkins School of Public Health, Baltimore, MD 21205

The rat prostate is an exocrine gland comprised of three anatomically and functionally independent lobes. The three lobes, ventral, dorsal and lateral, require androgen for differentiation and development and for maintenance of function. However, the level of androgen receptor expression differs between lobes and castration causes epithelial cell apoptosis in the ventral, but not in the dorsal and lateral lobes. Characterization of the lobe-specific pattern of gene expression will provide insight into the functional and molecular differences between the lobes and their responses to hormones. Microarrays containing 1176 different cDNAs were used to characterize lobe-specific differences in gene expression by hybridization with probes prepared from RNA of each lobe. Overall, the largest number of genes was expressed in the lateral lobe (325), relatively fewer in the ventral lobe (316) and the fewest in the dorsal lobe (265). A relatively large number (215) of the same transcripts were identified in all three lobes. The probasin gene exhibited the greatest difference in expression between lobes; expression in the lateral lobe was 10-fold higher than in the dorsal lobe and 100-fold higher than in the ventral lobe. Glutathione-S-transferase M2, copper-zinc superoxide dismutase, cathepsin L and clusterin were expressed at high levels in all three lobes, but the magnitude varied several fold between lobes. These studies demonstrate the power of gene expression array technology in identifying the specific pattern of transcript expression for each prostate lobe and provide the tools necessary to characterize changes in gene expression due to the effects of androgens and/or estrogens and aging. (Supported by P01 AG08321)

# 88

**DIFFERENTIAL EXPRESSION OF NITRIC OXIDE SYNTHASE ISOFORMS IN HUMAN PROSTATE (BPH, PROSTATIC CARCINOMA AND NORMAL) TISSUE** Y.S. Mehta, T.J. Bivalacqua, D. Sarma, K. Moparty, R. Davis, A.B. Abdel-Mageed, S.C. Sikka. Tulane Medical Center, New Orleans, LA

We examined the endogenous expression of nitric oxide synthase (NOS) in prostates containing a diverse and distinct array of diseased tissue. The objective is to investigate a potential role of NOS and nitric oxide (NO) in the pathogenesis of benign prostatic hyperplasia (BPH). We focused our direction to the fibromuscular stromal component of the prostate, a region widely thought to have an inductive effect on BPH. Immunoreactivity for all three isoforms of NOS (eNOS, nNOS, iNOS) was examined in tissue samples from five patients who underwent radical prostatectomies. Sections were stained with H&E to histologically verify regions of BPH, carcinoma and normal prostate tissue. Each of the three isoforms of NOS showed varying patterns of expression in these sections, using a semi-quantitative scale of 1-4 +. Neuronal NOS (nNOS) showed strong expression (++++) in the stroma of BPH and normal prostate areas, however it exhibited very little reactivity (+) in well-differentiated cancer regions and slightly stronger staining (++) in poorly differentiated cancer tissue. Endothelial NOS (eNOS) showed increased staining (++) in BPH stroma compared to weak staining (+) in normal tissue. In addition, eNOS showed a marked increase in expression in cancer (++++) areas versus BPH and normal areas. The inducible form of NOS (iNOS) stained BPH and cancer areas equally (++++). Our studies indicate distinct patterns of expression among the various NOS isoforms in tissue representative of multiple and distinct disease states. The above experiments provide the groundwork for further molecular research into the potential role of NOS and NO in the pathogenesis of prostatic diseases.

# 89

**PROTEIN PROFILING OF EXPRESSED PROSTATIC SECRETIONS.** C.H.Muller, D. Diamond* and R.E. Berger, *Ciphergen Biosystems, Fremont, CA and Dept. of Urology, University of Washington, Seattle WA 98195

Analysis of expressed prostatic fluid proteins may assist in the diagnosis of inflammatory (category IIIa), non-inflammatory (cat IIIb) chronic nonbacterial prostatitis/chronic pelvic pain syndrome (CPPS), and asymptomatic inflammatory prostatitis (cat IV). We use a novel mass spectroscopy approach utilizing selective adsorption chips to begin characterizing differences between men with CPPS and men without symptoms, and between men with and without inflammation.

METHODS Men with cat III CPPS (cases) and men without pelvic pain (controls) were enrolled after excluding men with bacterial prostatitis, urethritis or positive gu history. Prostatic secretions (EPS) were obtained by digital expression and quickly analyzed for inflammation by hemacytometer. EPS were centrifuged to obtain a cell-free supernatant which was diluted into protease inhibitor cocktail and frozen at ñ80C. Thawed aliquots were processed for pr otein determination by micro Lowry method, diluted to 1:20, and 2uL were p laced with acetonitrile onto reverse phase Ciphergen ProteinChip"! Arrays. Pr otein profiles were generated by Ciphergen SELDI (surface enhanced laser d esorption/ionization) technology for 17 controls and 34 cases. A second sample from seven men was also analyzed.

RESULTS EPS protein concentrations ranged from 30-50 mg/mL. Protein profiles optimized from 3 to 50 kDa resolved up to 43 peaks per sample. Major peaks corresponded to masses of PSA (28kDa), PAP (47.1) and PSP (10.8), and these were present in almost all samples. Other major peaks present in some samples had masses of 3.4, 5.4, 21.7, 29.3, 33.5, 38.7, and 47.1kDa. The 3.4kDa peaks were present only in samples with inflammation, whereas the 5 .4 and 10.8kDa peaks were missing or low in these same samples. CONCLU SION The ProteinChip"! Array technology was successfully and rapidly use d to analyze a range of proteins from 58 EPS samples. Our experience demonstrates the feasibility of this highly sensitive, rapid and adaptable technique which may help identify specific and different processes during the course of CPPS and inflammation. Supported by the Paul G. Allen Foundation for Medical Research.

# 90

**INCREASED TISSUE FACTOR (TF) EXPRESSION IN PROSTATE CANCER: ROLE AS NEOPLASTIC MARKER IN EXFOLIATED PROSTATE CELLS IN POST-PROSTATE MASSAGE URINE** M.J. Wilson1,2,3,7, T.J. Kappel*1,2, R. Bach*1,4, R. Haller*1,3, J.W. Slaton*1,3,7, N.F. Wasserman*1,5,7, B. Quast*6, A.A. Sinha*1,6,7 VA Medical Center1 and Departments Lab. Med. & Pathology2, Urol. Surgery3, Medicine4, Radiology5, and Gen., Cell Biol. & Devel.6, Cancer Center7, University Minnesota, 55417.

TF is a transmembrane glycoprotein that functions in intracellular signalling and angiogenesis, as well as its well characterized role in hemostasis. Its expression is increased in prostate cancer. Our goal was to evaluate TF as a neoplastic marker in exfoliated prostate cells in post prostate massage urine. We examined TF expression by immunoflurorescence microscopy (mouse monoclonal antibody) in frozen sections of radical prostatectomy tissues. TF was localized to ductal epithelial cells and in epithelial cells in some glands with stacked epithelium. Only scattered secretory and basal epithelial cells in nodular BPH expressed TF, whereas prostatic cancers were uniformly positive for TF. Immunofluorescent localization of TF, PSA, and their colocalization was examined in Cytospin preparations of cell suspensions prepared from post prostate massage urine. The percent cells positive for PSA was 24.3 and 24.8% in post prostate massage urine of men without (N = 9) and with (N = 10) prostate cancer upon biopsy. TF positive cells were 15.5 and 23.1% of total cells, and 6.4 and 10.4% of PSA positive cells respectively in men with and without cancer. These data show that TF is over expressed in prostate cancer and suggest that it may serve as a marker for malignant neoplasia in exfoliated prostatic cells.

# 91

**THE ASSOCIATION BETWEEN ANDROGEN RECEPTOR AND EGF RECEPTOR INHIBITS EGF-INDUCED INVASION IN PROSTATE CANCER CELL LINES.** L. Bonaccorsi, V. Carloni, M. Muratori, G. Forti and E. Baldi. Dept. of Clinical Physiopathology, Andrology Unit, University of Florence, Italy.

Emerging evidence indicate that androgen-sensitive prostate cancer is characterized by a lower malignant potential. We previously demonstrated that androgen receptor (AR) expression by transfection of PC3 cell line (Bonaccorsi et al, 2000) decreases laminin adhesion and Matrigel invasion of these cells through reduction of a6b4 expression. Treatment with androgens further reduced adhesion and invasion of PC3-AR cells without modifying a6b4 expression. We investigated here if the AR, upon activation by androgens, has a direct effect on a6b4-EGF receptor (EGF-R) interaction and intracellular signalling leading to invasion of these cells. Immunoconfocal microscopy demonstrated that in control cells (PC3-Neo), a6b4 and EGF-R co-localized a nd redistribute at the plasma membrane in response to EGF. In PC3-AR cells co-localization between a6b4-EGF-R was strongly reduced and abolished by pre-treatment with R1881. Co-immunoprecipitation studies confirmed the interaction between EGF-R and a6b4. Tyrosine phosphorylation of b4 subunit in response to EGF was strongly reduced in PC3-AR cells compared to PC3-Neo. To determine whether disruption of EGF-R-a6b4 interaction was due to a direct action of the AR on EGF-R, immunoconfocal and co-immunopre cipitation studies of EGF-R and AR were conducted. These studies showed co-localization at membrane level as well as co-immunoprecipitation of the two proteins, indicating an interaction of a6b4-EGF-R. PI3K activity, a key signaling pathway for invasion, was decreased both in basal conditions and in response to EGF and was further reduced by treatment with R1881. In conclusion, in androgen-sensitive prostate carcinoma cells, androgens and A R contribute to confer a less malignant phenotype both by reducing the expression of a6b4 and by interfering with EGF-R-a6b4 interaction and signal ing leading to invasion through a direct interaction between AR and EGF-R.

# 92

**LONGTERM EFFICACY OF LHRH AGONIST THERAPY AND ANTIANDROGEN MONOTHERAPY IN THE TREATMENT OF LOCALIZED (T1-3) PROSTATE CANCER**

Rupesh Raina*, Edward E Cherullo*, William Conrad*, David R Nelson*, Ashok Agarwal, Craig D Zippe*, Cleveland, OH

To review the efficacy, side effects, and compliance of LHRH agonist and anti-androgen monotherapy in the treatment of localized (T1-3) prostate cancer. Records of 97 patients with clinically localized prostate cancer who received either LHRH agonist monotherapy or anti-androgen monotherapy were reviewed. Patients were divided into two groups, group I (mean age 76 [plusminus] s.e.m. 1) consisted of those receiving LHRH agonist (n = 62) and group II (mean age 76 [plusminus] s.e.m. 1.2) consisted of patients treated with anti-androgen monotherapy (n = 35). In group II, 18 patients received bicalutamide (50mg), 13 nilutamide (150mg), and 4 flutamide (750mg). The PSA levels, Gleason scores, clinical stage, and side effects were recorded. The mean follow up period was 50.8 [plusminus] 8.5 months in group I compared to 43.1 [plusminus] 2.2 months in group II. Only 1 of the 62 patients (1.6%) in group I showed PSA progression, whereas 20 of the 35 patients (57.1%) in group II showed progression (see table). In group II, 10/20 (50%) showing PSA progression were treated with LHRH salvage therapy and 8/10 (80%) responded. Hot flashes (54.8%) and lethargy (41.9%) were the most common side effects in group I. In contrast, nipple-tenderness (40%) and light-dark adaptation (17.1%) were more often seen in group II. LHRH agonist therapy provides excellent long-term control of localized prostate cancer and can effectively salvage PSA failures on anti-androgen monotherapy. Anti-androgen monotherapy in the doses prescribed did not provide adequate long-term control in the majority of patients.

# 93

**EARLY USE OF VACUUM CONSTRICTION DEVICE (VCD) FOLLOWING RADICAL PROSTATECTOMY (RP) FACILITATES EARLY SEXUAL ACTIVITY AND POTENTIAL RETURN OF ERECTION** R. Raina*, R.A. Saleh*, J. Chauhan, A. Agarwal, C.D. Zippe, Urological Institute, Cleveland Clinic Foundation, Cleveland, OH The objective of this study was to examine the efficacy and compliance of early use of VCD following RP to potentially prevent corporeal fibrosis and expedite recovery of erectile function. Following RP and catheter removal, 74 patients (mean age, 59 yrs) were instructed to use the VCD daily (without constriction ring) to induce intracavernous pressure and tumescence. The constriction ring was applied if sexual intercourse was attempted. VCD use began an average of 3.9 wks (2-8 wks) after surgery and the mean follow-up was 8 months (6-9 mo). The treatment efficacy was analyzed by responses to abridged 5-item version of the International Index of Erectile function (IIEF) and stratified by nerve-sparing status. Patient outcome regarding compliance, return of natural erections, and ability for vaginal intercourse were assessed. All patients used VCD with a compliance rate of 81% (60/74). Patients successfully used VCD with constriction band for vaginal intercourse at a frequency of twice/week with spousal satisfaction rate of 33/60 (55%). Nineteen out of 60 (32%) reported return of natural erections at mean interval of 9 months with 10/19 (52%) patients having erections sufficient for vaginal intercourse. Fourteen out of the 74 (19%) discontinued VCD due to problem with the pump (discomfort 55%, unable to get air tight seal 8%, social inconvenience 17%, and penile bruising 20%). In conclusion, early use of VCD following RP facilitates early sexual intercourse, early patient/spousal sexual satisfaction, and potentially, early return of natural erections.

# 94

**DIETARY ISOFLAVONES INDUCE APOPTOSIS IN PROSTATE CARCINOMA**

Risbridger GP, Jarred RA, Keikha M, Dowling C McPherson S, Husband A, Pedesen J & Frydenberg MF. Centre for Urological Research, Monash Institute of Reproduction & Development, and Department of Urology, Monash University, 246 Clayton Road, Melbourne, Australia; Melbourne Pathology, Collingwood, Melbourne, Australia

Phytoestrogens are implicated as agents that contribute to the low incidence of prostate disease in Asian men. This study reports the effects of a combination of 4 main isoflavones found in the typical Asian diet (genistein, biochanin formononetin and diadzein) on prostate cell apoptosis. 40 men were recruited to the study upon diagnosis of prostate cancer (Gleason score 5 or more). Prior to surgery 20 men consumed 160mg/day of dietary isoflavones in the aglycone state with a ratio of biochanin+genistein:formononetin+diadzein of 2:1. A comparison of pre and post treatment parameters showed no significant change in serum PSA, testosterone or Gleason score in treated men and no adverse events were recorded in the treated group. Using unbiased methods of sampling, radical prostatectomy specimens from isoflavone treated men were compared to those from untreated control patients and the incidence of apoptosis determined using morpholgy and TUNEL staining. There was no significant difference in the incidence of apoptosis in regions of non-malignant or high grade tumor, but in regions of low grade cancer (Gleason grade 3 or less) there was a significant ($p<0.001$) increase in the number of cells in apoptosis. This study shows that the short-term consumption of dietary isoflavones induced significant apoptosis in low grade prostate cancer that may be of potential benefit. It also demonstrates that the valid assessment of therapeutics should be conducted in the absence of dietary isoflavones as their actions may confound the interpretation of results.

# 95

**IS TESTOSTERONE AND PSA RELATED IN ELDERLY POPULATION?** K.H. Rha*, J.W. Jung*, Y.D. Choi*, S.W. Han*, S.J. Hong*, M.S. Lee. Yonsei University, Seoul, Korea

Purpose : The serum levels of testosterone is known to be decreased in the elderly population, but most data is accumulated in large laboratories not actual community based population. Serum prostate specific antigen(PSA) has become routine serological screening test in Korea and its levels is known to be related to age. We analyzed the levels of serum testosterone and PSA in a rural population in Korea. Material and Methods : From March to September 1999, a total of 714 men over 50 were enrolled to take both serum PSA and testosterone. The data was analyzed by multiple regression analysis. Results : The mean age was 66.92∞Ê8.42years(50-89) and the distribution according to age 50-54 36 men, 55-59 70 men, 60-64 135 men, 65-69 169 men, 70-74 157 men, 75-79 105 men, 80-84 29 men, and 85-89 13 men. Their mean serum testosterone level was 4.85ug/dl, 4.68ug/dl, 4.83ug/dl, 4.71ug/dl, 4.67ug/dl, 4.41ug/dl, 4.41ug/dl and 4.92ug/dl, respectively with the mean value of 4.50ug/ml. The serum testosterone level was not correlated with age(r=0.0039) or serum PSA(r=0.093). Conclusion : Seryn testosterone levels did not change according to age, and also unrelated to levels of serum PSA.

# 96

**ACCUMULATION OF MACROPHAGES IN THE TESTIS, EFFERENT DUCTS AND EPIDIDYMIS OF CATHEPSIN A DEFICIENT MICE.**

**N.Korah[1], A. d'Azzo[2], L. Hermo[1]**

**Department of Anatomy, McGill University, Montreal[1], St. Jude Children's Research Hospital, Memphis, TN[2]**

The lysosomal enzyme cathepsin A (PPCA) functions as a protective protein and catalyst for α–neuraminidase and β–galactosidase in a multienzyme complex and is abundantly expressed in the testis, efferent ducts (EDs) and epididymis. In the present study, Bouin's- and glutaraldehyde-fixed material for LM immunocytochemistry and routine EM analysis, respectively, of 2, 6 and 10 month old PPCA-/- and +/+ mice were examined to determine the effects of PPCA deficiency on these tissues. While the Sertoli cells of the testis of PPCA -/- mice appeared normal as revealed by LM prosaposin immunostaining and routine EM analysis, and no changes were noted to germ cells at the various stages, a dramatic increase in the number of vacuolated interstitial cells was noted with age. The identity of these cells was confirmed with a monoclonal antibody F4/80 that recognizes macrophages. The latter are a normal component of the interstitial space of the testis, but at low numbers in wild type mice. Such cells were also noted in the intertubular spaces of the EDs and epididymis of PPCA-/- mice, but not in PPCA+/+ mice. Interestingly, the EDs showed no major abnormalities to the epithelium at various ages of PPCA-/- mice. This was in contrast to the epididymis, where gross abnormalities were noted with age to the epithelial cells lining this duct. Thus in PPCA-/- mice, macrophages appear to be recruited as a response to different factors in the case of the testis, EDs and epididymis. (Supported by CIHR).

# 97

**SHORT AND LONG-TERM EFFECTS OF THE ANTI-ESTROGEN ICI 182,780 ON THE TESTIS AND EFFERENT DUCTULES, WITHOUT CHANGES IN TESTOSTERONE 1**

Cleida A. Oliveira*2,3, Kay Carnes*2, Qing Zhou2, Rong Nie2, David E. Kuehl*2, Gary L. Jackson*2; Luiz R. França3, Masaaki Nakai2, Rex A. Hess2,42Dept of Vet Biosci, Univ of Illinois, 2001 S. Lincoln, Urbana, IL 61802; 3Depts of Morph & Physiol, Fed Univ of Minas Gerais, Belo Horizonte-MG- Brazil

Targeted disruption of mouse estrogen receptor-a (aERKO) has demonstrated that ERa is essential for fertility in males. An alternative model for the study of estrogen in the male was developed in the rat using the anti-estrogen ICI 182,780, which blocks both ERa and ERb and reproduces the male phenotype seen in aERKO, including testicular atrophy and infertility. The present study evaluates time-response effects of ICI to determine the sequence of events leading to infertility and testicular atrophy. Male rats, 30 days old, were treated with ICI 182,780 (10mg) or castor oil (control) for 3 to 150 days. There were no differences in body weights, nor in the wt of ventral prostate and seminal vesicle-coagulating glands. No effect was found on serum testosterone and LH concentrations, except for an LH increase on d 15. ICI induced dilation of efferent ductules on d 3 post-treatment and dilation increased over time (200% increase). Efferent ductule epithelium was reduced in height; however, microvilli increased in length up to d 73, but then decreased. A transient increase in lysosomes in nonciliated cells was seen from d 15 to 100. Testicular wt increased by d 45 and seminiferous tubules were dilated on d 52, effects on testis that persisted until d 100. On d 150, the testis wt decreased and severe atrophy was observed, suggesting back-pressure atrophy due to fluid accumulation. The expression of NHE3 (Na+/H+ exchanger) was down regulated in efferent ductules after treatment, providing an explanation for the accumulation of fluid. This study provides further evidence that estrogen is involved in the regulation of structure and function of the efferent ductules and that a functional ER is required for the expression of NHE3, as well as for maintenance of ion and fluid reabsorption, an essential function for long-term fertility in the rodent. Supported by NIH grant HD35126

# 98

**GERM CELL-SPECIFIC EXPRESSION OF A CYSTATIN-RELATED EPIDIDYMAL SPERMATOGENIC (CRES) PROMOTER-CAT TRANSGENE**

N. Hsia1, J.P. Brousal2*, S.R. Hann2*, and G.A. Cornwall1. 1Texas Tech University Health Sciences Center, Lubbock, TX and 2Vanderbilt University School of Medicine, Nashville, TN.

The cystatin-related epididymal spermatogenic (cres) gene encodes a protease inhibitor putatively involved in the regulation of specific proteolytic processing in reproduction. Cres mRNA expression is highly restricted to the round spermatids, proximal caput epididymidis, anterior pituitary gonadotrophs, and ovary. To begin to elucidate the molecular mechanisms controlling the tissue- and cell-specific expression of the cres gene, transgenic mice were generated containing 1.6-kb of cres promoter sequence fused to the chloramphenicol acetyltransferase (CAT) reporter gene. A CAT ELISA was used to examine CAT protein levels in different transgenic tissues extracts. High levels of CAT protein were detected only in testis, epididymal, and sperm extracts whereas lower levels of CAT protein were present in the pituitary and the female reproductive tract. RT-PCR analysis showed the presence of CAT mRNA only after day 22 of the developing mouse testis suggesting CAT mRNA is specifically expressed in round spermatids. However, CAT mRNA was not detected in the adult epididymis, suggesting epididymal CAT protein detected by ELISA is localized in spermatozoa. Preliminary RT-PCR analysis of the pituitary and ovary mRNA from transgenic mice showed no expression of the CAT transgene. Since low levels of CAT protein were detected in these tissues, this may be a consequence of extremely low and transient levels of CAT mRNA expression. Taken together, our studies suggest 1.6-kb of cres promoter is sufficient to drive strong and specific expression to the round spermatids of the testis.

# 99

**EFFECT OF 5 ALPHA-REDUCTASE (5A-R) INHIBITION ON GENE EXPRESSION IN THE EPIDIDYMIS** N. Henderson*, B. Robaire, Depts. Pharmacol. & Therap. and Ob/Gyn, McGill University, Montreal, QC, Canada

The epididymis is the site of sperm maturation and storage. 5a-Rs (type 1 and 2) are key factors in this tissue because of their role in converting testosterone to dihydrotestosterone (DHT), the main androgen regulating epididymal functions. To better understand 5a-R mediated regulation of gene expression in the epididymis, the effects of inhibiting both 5a-Rs were examined. Epididymides were collected from rats treated for 28d orally with 0 or 10 mg/kg/day of PNU157706, a dual 5a-R inhibitor. This dosing regimen decreases prostatic DHT by >90%. RNA was extracted, reversed transcribed, radiolabelled, and probed on cDNA arrays (Atlas Rat 1.2, n=5 arrays/treatment group) containing 1178 cDNAs and analyzed using GeneSpringTM; changes in gene expression of 2 fold or greater were further analyzed. Genes in the initial segment and caput epididymidis were most markedly affected by inhibition of 5a-Rs. Treatment resulted in an increased number of genes detected in the initial segment (control 353, treated 484), while a decreased number of genes was found to be expressed in the caput epididymidis (control 384, treated 313). In the initial segment, 58 genes increased in expression compared to 12 that decreased. In contrast, only 8 genes increased in the caput epididymidis while 18 decreased. Interestingly, several proto-oncogenes were affected. Fyn, c-N-ras and erbB4 increased in the initial segment, but remained unchanged (Fyn, c-N-ras) or decreased (erbB4) in the caput epididymidis. Conversely, c-met proto-oncogene decreased in the initial segment, while increasing in the caput region. Since proto-oncogenes regulate key components of signaling pathways, they may play a role in the segment-specific 5a-R mediated regulation of gene expression in the epididymis. Supported by CIHR Canada.

# 100

**ANALYSIS OF THE 5' UPSTREAM REGION OF RAT 5 ALPHA REDUCTASE (5A-R) TYPE 2** Shayesta Seenundun* and Bernard Robaire Depts. Pharmacol. & Therap. and Ob/Gyn, McGill University, Montreal, Canada

INTRODUCTION. 5a-R reduces testosterone to the more potent dihydrotestosterone (DHT), the main androgen responsible for maintaining epididymal structure and functions (sperm maturation and storage). Inhibiting the synthesis of DHT may be important in the development of male contraceptives. The mRNA of the type 2 isozyme is more abundant than the type 1 mRNA in the epididymis. The type 2 5a-R may play a role in endocrine disorders, such as benign prostatic hyperplasia, prostate cancer and alopecia. Although its 5Ì upstream region has been sequenced, there is no information regarding the promoter and enhancer sequences regulating the expression of the 5a-R type 2 gene. METHODS. Sequential deletion analysis was done to map the 5Ìupstream region of the 5a-R type 2 gene. Five sequentially smaller fragments were generated: SacI (2736 bp), DraI (2062 bp), insert 5 (1563 bp), EcoRI (1152 bp) and HinDIII (680 bp); these were subcloned into the pGL3B (basic) and pGL3P (SV40 promoter upstream of luciferase gene) vectors (Promega) in both the + and Ò orientations. The constructs were transfected into PC3 cells by the calcium phosphate method and then analyzed for luciferase and B-galactosidase expression. RESULTS. When inserted into the pGL3B vector, only the EcoRI fragment showed luciferase activity, indicating gene expression; this construct had activity in both orientations. When the same fragments were cloned into the pGL3P vector, smaller + constructs correlated with increased expression; the - constructs consistently showed less activity than their + counterparts. DISCUSSION. These data indicate the presence of at least two strong repressor sequences upstream of the EcoRI construct and a weak bidirectional minimal promoter sequence situated between insert5 and HinDIII. Supported by CIHR.

# 101

**FURTHER CHARACTERIZATION OF THE MURINE EPIDIDYMAL SPAM1** (PH-20). H. Zhang* and P. A. Martin-DeLeon, Department of Biological Sciences, University of Delaware, Newark, DE

Previously we demonstrated that the murine epididymal Sperm Adhesion Molecule1 (Spam1 or PH-20) is synthesized by the epithelium, preferentially in the distal tract and is released in the luminal fluid (LF). We also showed that while testicular (TS) and epididymal Spam1 (ES) have hyaluronidase activity at neutral pH, they are under different transcriptional regulation. The aim of this study is to further compare characteristics of the two forms of the GPI-linked protein and to determine if secreted ES is released with its lipid anchor. With GeneRacer amplification of the 3ì end of the cDNA we show that the polyA tails are shorter in the epididymis than in the testis. Two-dimensional PAGE with immunoblotting reveals a single isoform for ES with the isoelectric point (pI) ranging from 7.6 to 8.3, and four isoforms from 6.6 to 9.0 for TS. Lectin blotting analysis of testicular (TT) and epididymal tissues (ET) and caudal sperm (CS) shows that PHA-E, LEL, and STL, which bind to N-linked chains, recognize a 67 kDa band in epididymis and sperm only; while PNA, which preferentially binds to O-linked side chains, recognizes the band in both of the tissues and sperm. This suggests the presence of an O-linked glycan in the murine Spam1 which was confirmed by enzymatic O-linked deglycosylation of extracts from TT and ET and CS which all showed a shift in the MW. This suggests that the O-linked glycosylation site that we identified by computer analysis of Spam1 cDNA is functional. Ultracentrifugation of the LF revealed that ES is secreted preferentially as insoluble particles, likely of a vesicular nature. Treatment of the insoluble material with PI-PLC revealed that the majority of ES is released with its lipid anchor, a form in which it can be taken up by sperm.

# 102

**AN ANTIMICROBIAL ROLE FOR THE EP2/HE2 GENE IN THE EPIDIDYMIS.**

O. Froehlich, C. Po*, L.G. Young, Department of Physiology, Emory University, Atlanta, GA

In the primate epididymis, the EP2/HE2 gene is transcribed into at least 9 different message variants. All encode secreted peptides from 3 to 15 kDa in size. The message variants are transcribed off 2 promoters and make use of at least 8 exons. We have previously shown that the EP2/HE2 gene is derived from two ancestral b-defensin genes, a family of genes encoding small secreted peptides with antibacterial activities. In deed, several of the message variants encode secretory peptides containing a b-defensin-like module. However, as the major message variant, EP2A/HE2al, does not encode a beta-defensin, it is not clear what the main physiological role of the EP2 gene might be. In order to test whether the gene has retained an antimicrobial role, we tested one of the defensin-like peptides, human EP2E, for its ability to inhibit bacterial growth. The peptide was produced as an epitope-tagged fusion protein from the bacterial expression vector pET-15b, analogous to the approach used for human beta-defensin-3 (Harder J et al., J Biol Chem 276:5707,2001). After affinity purification and removal of the affinity tag, the peptide showed strong bactericidal activity against a laboratory strain of E. coli. However, this activity suffered rapid degradation within several days. During the same time, the recombinant peptide formed multimeric aggregates with no antibacterial activity. The reason for this rapid inactivation is not clear. However, these experiments demonstrate that at least one of the peptides encoded by the EP2/HE2 gene has antibacterial activity. They also point towards a role of the gene in the defense against retrograde bacterial invasions of the male reproductive tract and a newly recognized role of the epididymis as protector of the testis against microbial infections.

# 103

CHARACTERIZATION AND IDENTIFICATION OF CAPACITATION-ASSOCIATED PIG SPERM TYROSINE PHOSPHOPROTEINS. C. Reyes-Moreno*, C. Dubé, S. Tardif and J.L. Bailey, Centre de Recherche en Biologie de la Reproduction, Université Laval, Québec, QC (Canada)

Membrane fluidization and protein tyrosine phosphorylation are two concomitant events related to sperm capacitation. In boar sperm, we have previously targeted a tyrosine phosphoprotein, "p32" and a dual-specificity kinase both of about Mr of 32,000. The appearance of p32 and kinase activity are both calcium-dependent. The present work was conducted to further characterize and to identify these capacitation-related proteins. Freshly ejaculated sperm from sexually mature boars were washed and incubated conditions known to induce capacitation. Immunoprecipitation, immunoblotting and proteomic analysis revealed seven soluble tyrosine-phosphorylated proteins, all aligned in the range of Mr 30,000 but with different isoelectric pH values (pI). Three capacitation-related tyrosine phosphoproteins were identified as the acrosin-binding sp32 (pI 6.5), and two triosephosphate isomerase isoforms (pI 7.1 and pI 7.9). A protein of Mr 20,000 was also screened and identified as porcine phospholipide hydroperoxide glutathione peroxidase. These proteins are known to be present in other specialized cells and could play important roles in energy metabolism, regulation of intracellular ion fluxes and pH, and most particularly in exocytosis, acrosin-activation, acrosomal structure or zona pellucida binding. Their discovery provides new insights to the biochemical mechanisms underlying the the earlier and latter stages sperm capacitation. *This work was supported by FCAR and NSERC.*

# 104

**PHOSPHOPROTEOME ANALYSIS OF CAPACITATED HUMAN SPERM CELLS.** Visconti, PE, Ficarro, S2, Chertihin, O1, White, F.2, Westbrook, A.1, Jayes, F.1, Kalab P., Shabanowitz, J.2, Herr J.C.1, Hunt, D.2,3 1Dep. of Cell Biol., 2Chem., and 3Pathol., U. of Virginia, Charlottesville, VA 22908, USA.

Mammalian sperm must undergo a maturational process called capacitation before fertilization can take place. Capacitation requires activation of PKA and correlates with an increase in tyrosine (tyr) phosphorylation. Though a few tyr kinase substrates have been identified, specific sites of phosphorylation have not been reported in the literature. We have used two different approaches to identify proteins that are phosphorylated during capacitation. First, we have performed 2-D analysis of capacitated human sperm proteins and visualized them by silver stain and immunoblot using antiphosphotyr (aPY) antibodies. Proteins phosphorylated on tyr residues were then sequenced by mass spectrometry. Second, we used immobilized metal affinity chromatography (IMAC) to enrich for phosphopeptides. This is the first demonstration of IMAC enrichment and subsequent mass spectrometric analysis of phosphopeptides from digests of total mammalian cell protein extracts. Several novel phosphorylation sites were revealed, among them, valosin-containing protein(VCP)/p97, a homologue of NSF. This protein was localized in the neck of the sperm before capacitation and in the anterior head in capacitated sperm suggesting a role of VCP/p97 in sperm capacitation. We have also mapped precise sites of tyr and ser phosphorylation for AKAP3 and AKAP4, known to be present in the sperm tail. In addition, we have developed a method to compare the phosphorylation state of a particular peptide before and after the induction of capacitation. Although this work is focused on the sperm ability to fertilize, this methodology could be easily transferred to map and compare exact phosphorylation sites in multiple biological systems.

# 105

**DIFFERENT SIGNAL TRANSDUCTION PATHWAYS ARE INVOLVED DURING HUMAN SPERM CAPACITATION INDUCED BY BIOLOGICAL AND PHARMACOLOGICAL AGENTS** J. Thundathil, E. de Lamirande, C. Gagnon, Urology Research Laboratory, Royal Victoria Hospital and McGill University, Montreal, Quebec, Canada

Human sperm capacitation involves activation of the extracellular signal regulated kinase (ERK) pathway as well as a related increased double phosphorylation of the Thr-Glu-Tyr motif (P-Thr-Glu-Tyr-P) in some sperm proteins. We investigated the regulation of this process. Fetal cord serum ultrafiltrate (FCSu), follicular fluid ultrafiltrate (FFu), progesterone and a combination of dibutyryl cAMP (dbcAMP; a cell permeant analog of cAMP) and isobutylmethylxanthine (IBMX; a phosphodiesterase inhibitor) were used as inducers of capacitation alone or in combination with inhibitors of protein kinase A (H89), protein tyrosine kinase (Tyrphostin A47, PP2) and of the ERK pathway (PD98059). We focussed our study on sperm proteins of 80 and 105 kDa because the P-Thr-Glu-Tyr-P in these proteins are increased during capacitation. The level of P-Thr-Glu-Tyr-P during sperm capacitation induced by FCSu, FFu and progesterone was regulated by similar signal transduction pathway, and involved receptor type protein tyrosine kinase and the ERK pathway. However, the level of P-Thr-Glu-Tyr-P in sperm capacitation induced by dbcAMP and IBMX was mainly mediated through protein kinase A and Src family of protein tyrosine kinase. In conclusion, biological agents (FCSu, FFu and progesterone) and pharmacological agents (dbcAMP and IBMX) may induce capacitation through regulation of different signal transduction mechanisms (Supported by CIHR and NSERC).

# 106

**ZONA PELLUCIDA-INDUCED ACROSOME REACTION IN THE HUMAN: DEPENDENCY ON ACTIVATION OF PERTUSSIS TOXIN-SENSITIVE GI PROTEIN AND CALCIUM INFLUX, AND PRIMING EFFECT OF PROGESTERONE AND FOLLICULAR FLUID.**

M. Morshedi, A. Schuffner , H.S. Bastiaan, H. Duran, Z.Y. Lin Z.Y, D.R. Franken, S. Oehninger S. The Jones Institute for Reproductive Medicine, Norfolk, Virginia, USA

In these studies we aimed to characterize the effects of the physiological, homologous agonists of the acrosome reaction, i.e., the zona pellucida and progesterone/follicular fluid on human spermatozoa. The specific aims of our studies were: (1) to examine the dependency of the solubilized, human zona pellucida-induced acrosome reaction on Gi protein activation and calcium influx; and (2) to determine whether progesterone/human follicular fluid exert a priming or synergist effect on the solubilized zona pellucida-induced acrosome reaction. Purified motile spermatozoa from fertile donors were exposed to the agonists/antagonists in a micro assay (Franken et al, JARG, 2000) and acrosomal status of live spermatozoa was determined by indirect immunofluorescence using PSA-FITC/Hoechst 33258. Pre-treatment with pertussis-toxin (100 ng/mL) and EGTA (2.5 mM) inhibited, to a large proportion, the zona pellucida-induced acrosome reaction without affecting the spontaneous rate of exocytosis. Progesterone (1.25 µg/mL) and human follicular fluid (10%) exerted a priming, time-dependent effect on the zona pellucida-induced acrosome reaction. These studies demonstrated that: (i) acrosomal exocytosis of capacitated human spermatozoa triggered by homologous zona pellucida is dependent on both Gi-protein (pertussis toxin sensitive) and calcium influx-regulated mechanisms; and (ii) progesterone and follicular fluid exerted a priming role on the zona pellucida-induced acrosome reaction.

# 107

**ACROSOME REACTION IN HUMAN SPERM: ROLES OF CAPACITATION, TYROSINE PHOSPHORYLATION AND MEMBRANE EXTERNALIZATION OF PHOSPHATIDYLSERINE**

M. Morshedi, A. Schuffner, Z.Y. Lin, H. Duran, M. Hsiu, D.R. Franken, S. Oehninger. The Jones Institute for Reproductive Medicine, Norfolk, VA, USA.

The objective of these studies was to assess the roles of capacitation conditions, tyrosine phosphorylation and plasma membrane externalization of phosphatidylserine (PS) on the spontaneous and agonist-induced acrosome reaction of human spermatozoa. Motile sperm were purified by gradient centrifugation and examined under capacitating conditions in human tubal fluid supplemented with human serum albumin (HSA) at 0,3, 6 and 24 hours. Protein tyrosine phosphorylation was examined by immunoblotting with a monoclonal antibody against phosphotyrosine; annexin V binding was used to monitor PS translocation; and acrosome reaction was detected by immunofluorescence using FITC-PSA and Hoechst 33258. Tyrosine phosphorylation of various sperm proteins was time- and HSA-dependent increasing over time and with higher doses of HSA. On the other hand, PS externalization increased over time but decreased with HSA addition. The spontaneous and calcium ionophore- induced acrosome reaction increased with capacitation time. There was a positive significant correlation ($r = .67$, $p<0.005$) between spontaneous acrosome reaction and PS externalization over time; calcium ionophore-stimulated acrosome reaction did not correlate with PS translocation. Taken together, these results suggest that (i) the spontaneous acrosome reaction increased over time in association with increased tyrosine phosphorylation and changes in phospholipid transbilayer behavior in the plasma membrane, both underlying molecular events of capacitation; and (ii) calcium ionophore induced acrosomal exocytosis that was dependent on capacitation time but did not result in further changes in PS translocation.

# 108

**COMBINATION OF DETERGENT AND DTT CAUSES PATERNAL CHROMOSOME DAMAGE.** Monika A Szczygiel*, Ryuzo Yanagimachi, W Steven Ward*. Institute for Biogenesis Research, University of Hawaii Medical School, Honolulu, Hawaii 96822.

We explored the effect of dithiothreitol (DTT) on chromosome stability in mouse spermatozoa. Spermatozoa were treated with(1) the ionic detergent mixed alkyltrimethylammonium bromide (ATAB), (2) the non-ionic detergent Triton X-100, (3) membrane disruption by vigorous pipetting (confirmed by the Live/Dead assay), or (4) untreated swim-up controls. For each treatment, two samples were tested, with or without the addition of DTT during the treatment procedure. Sperm nuclei obtained after different treatments were injected into oocytes for cytogenetic analysis - paternal and maternal chromosomes of the zygote before the first cleavage were visualized and examined. For each experiment in which oocytes where injected, part of the sperm preparation was examined for the stability of the sperm nuclear matrix by the halo assay. It was found that treating sperm with either detergent combined with DTT caused severe chromosome breakage, whereas the controls without DTT had normal chromosomes. Only 40% (59/149)and 22% (23/103) of examined metaphases were scored as normal in ATAB+DTT or Triton+DTT treatments, respectively. Treatment with detergents only allowed us to obtain normal karyotypes (ATAB, 85%, 99/210; Triton X 100 82%, 116/153) with a similar frequency as in the swim-up controls (92%, 103/112). Sperm in which the membranes were disrupted by vigorous pipetting in the presence of DTT had a reduced frequency of normal chromosome (62%, 64/104) while those without DTT were normal (79%, 125/159). Interestingly, chromosomes of swim-up sperm exposed to DTT without pipetting or detergents remained normal in 87%, 153/176, of the metaphases examined. In these sperm, the DTT was still able to reduce the protamines, as determined by the halo assay. Our results indicate that simultaneous treatment with detergent and DTT induces extensive chromosome breakage, and that protamine disulfide reduction alone is insufficient to cause this chromosomal damage.

# 109

**MALE INFERTILITY IN NECTIN-2 DEFICIENT MICE**

S. Mueller *, T. A. Rosenquist *, R. A. Bronson, E. Wimmer *, School of Medicine, SUNY at Stony Brook, Stony Brook, NY

The members of the nectin/CD155 gene family represent a new class of cell-cell adhesion molecules of the immunoglobulin superfamily. nectin-2 and -3 are highly expressed within the seminiferous epithelium in a stage specific manner. In order to investigate the function of nectins, we disrupted the nectin-2 gene in mice (knockout). nectin-2 -/- mice are viable and do not show any overt defects in embryonal or postnatal development. However, while nectin-2 -/- females were fully fertile, their male counterparts were found to be completely sterile. Upon further analysis we determined, that nectin-2 -/- males produced normal amounts of spermatozoa with normal motility, but grossly aberrant head and midpiece morphology. nectin-2 -/- spermatozoa display a variety of irregular shapes due to the presence of mitochondria in the head, improperly condensed nuclei and "curling back" of the head. Although the mating behavior of nectin-2 -/- males appeared normal and they produced copulation plugs comparable to wild type controls, their sperm failed to fertilize oocytes in vivo. In sperm penetration assays, nectin-2 -/- spermatozoa bound well to zona free hamster eggs, but penetrated at less than 10% of wild type levels. Furthermore, binding to zona intact mouse oocytes was greatly reduced. We conclude that the sterility phenotype of nectin-2 -/- male mice is caused by a combination of reduced spermatozoa-zona binding and sperm-egg fusion. The nectin-2 knockout mouse provides a unique model to study at the molecular level the link between gene function, sperm morphology and sperm function. We suggest that mutations in human nectin-2 and other nectin family genes may be a cause for infertility in men presenting with abnormal sperm head morphology.

# 110

**FATTY ACID PROFILE OF SPERMATOZOA FROM TERATOSPERMIC DOMESTIC CATS.**

B.S. Pukazhenthi1, M. Ollero2, B. Teter3*, J.G. Howard1, J.G. Alvarez2 and D.E. Wildt1* 1Conservation & Research Center, Smithsonian National Zoological Park, Front Royal, VA; 2Department of Obstetrics/Gynecology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; 3Department of Biochemistry, University of Maryland, College Park, MD

Cholesterol (C), phospholipid (PL) and fatty acid (FA) content were analyzed in the sperm of teratospermic (<40% normally shaped sperm/ejaculate) domestic cats (n = 4). Electroejaculates were washed in Hamìs F10 medium without serum. Sperm lipids were extracted in chloroform-methanol, and FA were transmethylated using a BF3-methanol reagent and analyzed by gas chromatography. C and PL were analyzed by TLC, identified and quantified by spectrodensitometry. Mean (± SEM) sperm motility was 68.7 ± 2.4%, and normal sperm were 26.0 ± 7.7%. Mean C content was 0.4 ± 0.1 nmol/106 cells, and C/PL molar ratio was 0.3 ± 0.2. The neutral PL phosphatidylcholine (44.3 ± 7.5%), sphingomyelin (27.2 ± 2.0%) and phosphatidylethanolamine (15.2 ± 9.3%) were most abundant. The major saturated FA were palmitic (16:0; 30.7 ± 1.5%) and stearic (18:0; 24.3 ± 5.6%). Monounsaturated FA included palmitoleic (16:1n-7; 4.1 ± 3.4%) and oleic (18:1n-9; 2.6 ± 1.1%). Polyunsaturated FA (PUFA) included linoleic (18:2n-6; 6.0 ± 0.9%), arachidonic (20:4n-6; 3.3 ± 1.7%) and docosahexaenoic (DHA; 22:6n-3; 1.7 ± 1.1%) with the most abundant being docosapentaenoic (DPA; 22:5n-6; 25.7 ± 7.0%). Of the total FA, unsaturated FA accounted for 45.0% with 78.9% being n-6 acids, whereas, PUFA accounted for 38.3% with 92.9% n-6 acids. These data represent the first characterization of felid sperm lipids. In contrast to human and rhesus monkey with DHA being the most abundant PUFA, DPA is the most prevalent PUFA in felid sperm, which is similar to the rabbit and dog. (NIH KO1 RR 00135)

# 111

**SPERMATOGENIC TRAIT DIFFERENCES IN THE NORMOSPERMIC VERSUS TERATOSPERMIC DOMESTIC CAT.**

K. Stoebel1*, K. Jewgenow1*, S. Blottner1*, D.E. Wildt2*, B.S. Pukazhenthi2. 1Institute for Zoo Biology and Wildlife Research, Berlin, Germany; 2Conservation & Research Center, Smithsonian National Zoological Park, Front Royal, VA, USA Teratospermia (ejaculation of <40% normally shaped sperm) commonly occurs in ~70% of felid species/subspecies (n = 30) studied to date. Here, we compared spermatogenesis in the normospermic (N; >60% normal sperm/ejaculate) versus teratospermic (T; <40%) domestic cat. Ten T males (mean +/- SD, 9.3 +/- 7.0% normal sperm) and ten N males (66.1 +/- 6.8% normal sperm) were castrated and testes processed for histo-morphometrical and flow-cytometrical analysis of spermatogenesis. No differences (P>0.05) were found in tubule diameter (~199.7 µm), epithelium height (~61.6 µm), interstitium area (~17.3%), Leydig cell number (~89.4 cells) and blood vessel number per cross section (~10.8) between N and T donors. However, quantification of cell populations at the spermiation stage revealed a higher (P<0.03) total number of elongated spermatids (eSp) per counted tubuli in T (915.6 +/- 278.2) compared to N (647 +/- 193.9) cats. The percentage of eSp was increased (P<0.001), whereas proportions of Sertoli cells (Sc; P<0.05) and spermatogonia (Sg; P<0.001) were decreased in T compared to N males. Consequently, the ratios of eSp:Sc (P<0.001) and eSp:Sg (P<0.001) were consistently greater in T cats. DNA flow cytometry revealed a higher (PP<0.01) total spermatogenic transformation (ratio of haploid:diploid cells) and meiotic index (ratio of haploid:tetraploid cells) for T cats. These findings indicate that the teratospermic cat, compared to its normospermic counterpart, produces (1) more sperm/Sc at the expense of overall lower sperm quality and (2) an increased proportion of eSp which perhaps is caused by delayed and/or altered spermiation. (NIH KO1 RR00135, DFG Je163/4-1)

# 112

**TERATOZOOSPERMIA – MOLECULAR BACKGROUND OF SPERM DYSFUNCTION**

M. Szczygieł and M. Kurpisz, Unit of Reproductive Biology, Institute of Human Genetics, Pol. Acad. Sci., Poznan, Poland.

It is not clear whether in teratozoospermia the altered sperm morphology is critical for diminished fertilization potential or the other associated factor(s). In our studies we posed a question of correct sperm morphology assessment, its consequences on sperm functional assay (SPA) as well as we searched for possible mutations in 3 selected genes, coding for protein 4.1 (cytosceleton), pro-acrosin (sperm penetration) and p53 (pro-apoptotic).

Out of 140 infertile males, we have selected 38 individuals with teratozoospermia according to Kruger restricted criteria (<14% with correct sperm morphology) and subjected to sperm-penetration assay (SPA). Regression analysis was subsequently performed. Molecular analysis of pro-acrosin gene was performed by using PCR SSCP (single strand conformational polymorphism) and PCR HD (heteroduplexes). Mutation of gene coding for 4.1 protein was analyzed by using PCR and restriction enzyme digestion (mutation in codon for initiation of translation) while gene for p53 protein was analyzed by NIRCA (non-isotopic RN-ase cleavage assay).

Morphology of sperm seems to be not a decisive factor for altered sperm-oocyte interaction, however, in normozoospermia, SPA results depended both on sperm morphology and motility (regression analysis). We did not find a mutation in codon of initiation of translation (p4.1). In pro-acrosin gene we did not identify a mutation responsible for lack of oocyte penetration but only the increased amount of polymorphisms in infertile population (exon 5). There are sequenced 8 mutations /polymorphisms revealed in proximity or exon VII. Although some revealed mutations may suggest lack of immediate influence on sperm-oocyte interaction, it may not exclude, the induction of genetic errors in early embryo development.

# 113

**EXPRESSION ANALYSIS OF THE Y CHROMOSOME GENES IN IDIOPATHIC AZOOSPERMIA AND SEVERE OLIGOZOOSPERMIA** E Koh*, H Suzuki, and M Namiki, Department of Urology, Kanazawa University, Kanazawa, Japan

Introduction and objectives Microdeletions in azoospermia factor (AZF) region have approximately 10% of males with idiopathic azoospermia or oligozoospermia. Three regions of microdeleted in Yq11 (AZFa, b, and c) have been indicated. In each region, candidate spermatogenic genes have been proposed. The aim of this study was to analyzed the expression of 11 Y chromosome genes in azoospermic and oligozoospermic patients, namely USP9Y (ubiquitin-specific protease 9, Y chromosome), DBY(DEAD/H box polypeptide, Y chromosome), UTY(ubiquitously TPR motif Y ), CDY(chromodomain Y), XKRY(XK related Y), SMCY(selected mouse cDNA on the Y), eIF-1AY (translation initiation factor 1A), RBM(RNA binding motif), DAZ(deleted in azoospermia), PRY(PTP-BL related Y), BPY2(basic protein Y2). Methods The patients consisted of 66 azoospermia who underwent testicular biopsy for histologic diagnosis or IVF treatment. Among 16 patients with obstructive azoospermia and normal spermatogenesis served as control. A small piece of testicular tissue by biopsy was used. RNA was reverse transcribed and polymerase chain reaction amplified using specific primers. These primers were designed for each gene sequence obtained from the gene data bank. All patients were also performed the genome screening for fifty STS (sequence tagged site) markers. Results A X-Y homologous gene is express ubiquitously. The DBY gene was only expressed in 33 of 41 (80%) in azoo/oligospermia patients. The expression analysis of Y specific genes was showed as follows. The RBM , DAZ, BPY2, and PRY were express in 51%, 68%, 78%, respectively. Conclusion It is possible that Y-specific genes, RBM , DAZ, BPY2, and PRY and X-Y homologous gene as DBY are associated with azoo/oligospermia.

# 114

**CARNITINE TREATMENT OF OLIGOSPERMIC MEN IN IUI/IVF PROCEDURE**

S.Micic, N.Lalic*, N.Bojanic*, Clinical center of Serbia, Clinic of Urology, Belgrade, Yugoslavia

In the epididymis of many species the major constituents are organic solutes L-carnitine, myo-inositol, glutation, lactate, glycerophorylcholine and certai steroids such as dyhydrotestosterone. Carnitine is a component of both seminal plasma and spermatozoa and is cofactor of several enzymes involved in the transformation of fatty acids, but also required for sperm motility. We analyzed 125 infertile men, during 6 months, in procedure of IUI/IVF, who 3 months received 3 gr of carnitine and 3 months without carnitine. All were in IUI/IVF procedure. The group was divided: I sperm count below 10x10/ml and II over 10x10/ml, A:treated and B:non-treated groups.

*THERAPY*

| *Group* | *0* | *3 months* | *6 months* |
|---|---|---|---|
| I | 4.48±0.7 | A: 7.22±2.4*<br>B: 4.98±0.9 | B: 5.35±2.44<br>A: 7.11±3.02* |
| II | 13.44±2.44 | A: 24.16±5.54*<br>B: 14.06±6.22 | B: 15.03±1.04<br>A: 26.16±3.48* |
| *PROGRESSIVE MOTILITY* | | | |
| I | 0.09±0.01 | A: 0.19±0.06*<br>B: 0.10±0.07 | B:0.11±0.08<br>A:0.21±0.09* |
| II | 0.12±0.08 | A: 0.29±0.2*<br>B: 0.14±0.09 | B: 0.13±0.07<br>A: 0.26±0.1* |
| *SWIM UP* | | | |
| I | 0.06±0.01 | A: 0.10±0.08*<br>B: 0.07±0.03 | B: 0.06±0.07<br>A: 0.11±0.09* |
| II | 1.1±0.9 | A: 9.7±2.3*<br>B: 1.5±0.8 | B: 1.8±0.9<br>A: 8.9±1.6* |

**Significance: p >0.01*

In conclusion we can say that treated groups improved progressive motility and swim up spermogram.

# 115

**RELATIONSHIP BETWEEN AGE OF MAN, SPERM DNA FRAGMENTATION AND INFERTILITY**

D. Evenson, South Dakota State University, Brookings, S.D., M. Virro*, Markham Stouffville Health Centre, Markham, Ontario, C. de Jonge, University of Minnesota, Minneapolis, MN and J. Brannian, University of South Dakota, Sioux Falls,SD

Age of man has a direct relationship with the extent of sperm DNA fragmentation and this, in turn, has a relationship to fertility potential. Over seven hundred aliquots of semen used for ICSI/IVF procedures were frozen and shipped to South Dakota State University. Each sample was thawed and analyzed by the Sperm Chromatin Structure Assay (SCSATM). There was a significant increase in the DNA Fragmentation Index (DFI) with age. Of interest, the intersect point between age and our current threshold of 30% DFI for a <1% probability of a successful term pregnancy was age 47. This value is similar to the value found in the California AGES study (Wyrobek et al., submitted) where the DFI was correlated with healthy, unexposed men in each decade of life from the 20's to the 70's. Thus, both studies appear to indicate an age-related DNA fragmentation and age should be considered in both toxicology and fertility studies.

# 116

**THE DEGREE OF SPERM DNA FRAGMENTATION AND/OR STABILITY TO PREDICT THE OUTCOME OF THE INTRAUTERINE INSEMINATION.** M. Morshedi, H. Duran, S. Taylor, S. Oehninger;The Jones Institute for Reproductive Medicine, Eastern Virginia Medical School, Norfolk, VA, USA

We investigated whether the degree of DNA fragmentation and/or stability of ejaculated human sperm could predict the outcome of intrauterine insemination (IUI) therapy. This was a prospective cohort study carried out at a tertiary center for reproductive medicine. Seventy-seven patients underwent 102 consecutive cycles of IUI using washed semen. Demographic parameters, treatment cycle characteristics and sperm parameters of the sample used for IUI were evaluated. Sperm features that were analyzed included: concentration and motility parameters (CASA), morphology (strict criteria), DNA fragmentation (terminal deoxynucleotidyl transferase-mediated dUDP nick-end labeling, TUNEL) and stability (acridine orange staining under both acid and heat denaturing conditions). The main outcome measure was clinical pregnancy as defined by ultrasonographic visualization of intrauterine gestational sac(s). Results: Logistic regression analysis revealed that the following variables could predict IUI outcome: number of ovarian follicles on day of hCG, the age of the woman, sperm morphology, DNA fragmentation and average path velocity after semen preparation and sperm concentration in semen. No samples with >12% of spermatozoa having DNA fragmentation resulted in pregnancy. Only TUNEL and the age of woman exhibited significant receiver operator characteristic curves to predict IUI outcome. We conclude that the degree of sperm DNA fragmentation appears to be a major determinant of IUI outcome and that other variables such as the number of follicles, woman's age, morphology, average path velocity of spermatozoa after preparation and sperm concentration in semen can enter into a statistical model to predict pregnancy.

# 117

**DEVELOPMENT OF AN INEXPENSIVE ARTIFICAL VAGINA FOR SEMEN COLLECTION FROM RABBITS**

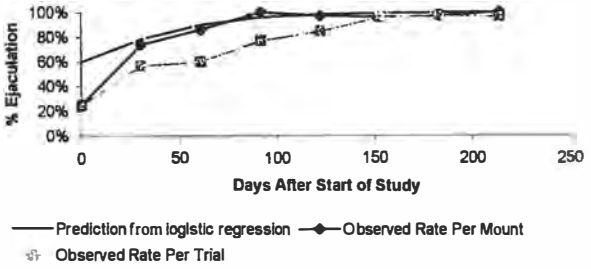
Cathy K. Naughton, David Nelson, Anthony J. Thomas, Jr., The Cleveland Clinic Foundation, Cleveland, OH

The rabbit is well suited for infertility research because of the animal's size, low cost and accessibility of the genitalia. There are a number of studies reporting sperm count and motility in rabbits; however, no easily reproducible or inexpensive device for semen collection has been reported. We herein report the construction of an inexpensive, effective artificial vagina assembled from easily obtainable products, which may be used to collect rabbit ejaculates.

The artificial vagina was assembled using a 3" T-connector polyvinyl chloride (PVC) pipe (3/4"x3/4"x1/2"openings), 2 rubber chair tips (25mm and 32mm inner diameter), a rubber band, and a silicone condom collection device (HDC; San Jose, CA). The 25mm rubber tip covers the ½" "T" opening of the pipe. A condom is placed through and through the ¾" openings of the PVC pipe stretched over one end and secured with a rubber band. The 32mm rubber tip covers the end opposite the opened condom. The device is hand-held beneath a sedated doe with the open end pointed in a caudal direction. As the buck begins to mount, the device is placed more posterior and inferior to allow penetration of the male rabbit into the artificial vagina. Ejaculation occurs rapidly and the device is removed.

Two hundred and forty-three ejaculation trials were attempted on 16 rabbits. Eighty-six percent (209/243) of the trials resulted in rabbit mounts. Overall, 91% (191/209) of mounts resulted in successful semen collection (97% by CKN). Observed ejaculate collection rate per trial, per mount and prediction from logistic regression are shown. After 60 mounts, a 98% successful ejaculate collection rate was achieved. The cost of the reusable device is $2.64, plus silicone condoms at $7.50 each.

This practical artificial vagina for semen collection is an inexpensive and effective means of obtaining sperm from rabbits for andrologic study.




# 118

**A NOVEL HOME-USE TEST DEVICE FOR MOTILE SPERM**

L. Bjorndahl, I. Brewis, E. Barratt, B. Ola, D. Bradley, K. Sharif, M. Afnan, C. Barratt.

Assisted Conception Unit, Birmingham Women's Hospital B15 2TG, England.

Introduction The concentration of motile spermatozoa is the most predictive parameter for estimating a man's natural fertility. Currently, this parameter can only be performed by professionally trained technicians in a laboratory setting. Our objective was to develop a simple and reliable test for motile sperm concentration that could be performed by a lay user in their home environment. Materials and Methods The development of the test device (Fertell) has been performed in conjunction with Genosis Ltd (UK) and has led to a single-use device that incorporates a specimen collection container and testing unit with integral temperature control. Progressively motile sperm separate from liquefied semen by a swim-up process through hylaronate. After this a fraction of the swim-up is reacted with colloidal gold-labelled anti-CD59 monoclonal antibody and the labelled sperm are trapped on a nitrocellulose lateral-flow test strip. The appearance of a red line (positive result) on the surface of this strip is indicative of £l 0 x 106 progressively motile sperm in the semen sample . Results Initially, 50 samples from unselected patients were examined. A comprehensive semen analysis incorporating CASA and a modified H yaluronate migration test (HMT) was performed in parallel to Fertell. Forty two samples showed a clear result (red line or no red line) with a correct classification of 97.6%. In 8 cases the results was borderline positive (faint red line). In 6 of these samples the HMT was normal and the mean concentration of progressively motile sperm was 8.7 x 106 [range 7.3-9.6]). The overall accuracy was 94%. Conclusion We have successfully developed a simple home-use test device which is completed within 40 minutes.

# 119

**A DOUBLE CHAMBER DEVICE FOR ADVANCED SPERM TESTING: SEMEN ANALYSIS AND AN ASSAY FOR SPERM MATURITY BY HYALURONIC ACID (HA) BINDING.** G. Huszar, C. Celik-Ozenci*, A. Jakab, L.Vigue*. Sperm Lab. Yale Sch. Med. New Haven, CT

**Introduction:** We have established that during human spermiogenesis, there is a remodeling of the sperm plasma membrane, which facilitates the formation of the zona pellucida-, and HA-binding sites. This occurs simultaneously with cytoplasmic extrusion and the major expression of the HspA2 chaperone protein. Immature sperm with cytoplasmic retention show diminished binding to the zona pellucida or to HA-coated surfaces. Other characteristics of immature sperm include abnormal chromatin, increased rates of chromosomal aneuploidies, and a shorter tail length. In this work we studied the diagnostic potential of HA binding for measuring sperm maturity.

**Methods:** We utilized Cell-VU™ double chamber slides. The A chamber was used for semen analysis, while the B chamber was coated with HA for the sperm binding assay (Biocoat Co., Glenside, PA). Drops of semen in the A and B chambers were video taped at 0-2, 4-6, 8-10, 12-14, 16-18, and 20 22 min. HA binding is expressed as % bound sperm/total motile sperm.

**Results:** 56 samples were tested. The % HA-bound sperm at the six time points were (mean±SEM): 66.2±4.2%, 75.6±4.3%, 77.4±4.1%, 77.9±4.1%, 78.3±3.9% and 78.6±4.3%. Thus, maximum binding was achieved within 10 min. This was true in samples with different low and high (<50% or >90% maximum sperm HA binding rates. As cytoplasmic extrusion and membrane remodeling are both late spermiogenetic events, sperm CK activity and HA binding were closely related ($r=-0.8$, $p<0.001$, $N=56$). We also verified that HA-bound sperm are of improved maturity by comparing sperm in semen vs. in HA-bound fractions. Following aniline blue staining, the respective proportion of clear sperm with normal chromatin increased from 63.3% to 92.4%, $p<0.001$ ($N=5$ men, 2000 sperm evaluated); the tail length/head long axis ratios were: 8.8 vs. 10.5, $p<0.001$ ($N= 5$ men, 600 sperm evaluated).

**Conclusions:** This device, on solid scientific basis, allows the analysis of semen and of the proportion of mature sperm. It will improve assessment of sperm maturity, fertility and reproductive toxicity (HD-19505, OH-04061).

# 120

HUMAN OR ANIMAL FERTILITY – EVALUATION OF OUTCOME TO ESTABLISH UTILITY OF A DIAGNOSTIC TEST OF SPERM QUALITY OR DETECT A CHANGE IN FERTILIZING POTENTIAL OF SPERM

R.P. Amann and R.H. Hammerstedt, BioPore Inc., State College, PA.

Many consider pregnant *vs* non-pregnant status after IUI or copulation the logical endpoint to establish predictive power of a laboratory diagnostic test of sperm quality. This is fallacious (J Androl 21:10, 2000). Success in conventional IVF (e.g., % fertilized ova) might be the best end point, but utility is problematic because excessive sperm are used. How clinically test a profertility molecule? IVF could be appropriate for molecules thought to increase sperm-zona binding or facilitate timely acrosome reactions. IUI is the rational approach for molecules thought to stabilize plasma membranes or enhance interaction of sperm with oviductal epithelium. Ultimately, IUI would be the primary use of profertility molecules. Valid measurement of outcome requires assay conditions placing results on the dose-responsive portion of the curve. This is true for fertility as for hormone concentration. Typically, number of sperm in an IVF droplet or IUI dose substantially exceeds the number needed to maximize "success". IVF co-incubation is long. These maximize fertility, but minimize male effect on the outcome. Conventional IVF almost precludes the right conclusion concerning a diagnostic assay or efficacy of a profertility molecule. Further, IVF samples usually are processed to exclude many sperm; the diagnostic assay must use identical samples. To validate a diagnostic assay: (1) use one standard and limiting number of sperm (e.g., 20,000 in 100 µl) for all IVFs; or (2) measure the outcome most biologically relevant to the sperm attribute measured, and not fertility. To reach the right conclusion concerning a profertility molecule, early fertility studies (either IVF or IUI) should include both "normal controls" and "low-number controls" (untreated), with appropriate doses of molecule added to low-number aliquots. The low-number should be ≤0.5-0.1X the normal value. Balancing goals of detecting differences and establishing pregnancies via IUI, one might use $0.8x10^6$ bull sperm or $8\text{-}10x10^6$ human donor sperm. For humans, alternating use of treated and control low-number insemination doses in 2 successive cycles, followed by IUI of the normal number in the 3rd cycle might be appropriate.

# 121

PRESENCE OF TIMP-2 ON SPERM CORRESPONDS TO FERTILITY OF RANGE BEEF BULLS G.R. Dawson*, M.E. Bellin*, J.N. Oyarzo*, H.E. Hawkins*, M.J. Arns, R.L. Ax, Department of Animal Sciences, University of Arizona, Tucson, AZ.

Several heparin-binding proteins (HBP) isolated from bovine seminal fluid capacitate bovine sperm *in vitro*. The 24 kDa protein has been identified as tissue inhibitor of metalloproteinases-type 2 (TIMP-2). TIMP-2 mRNA is expressed in bovine accessory sex glands and TIMP-2 binds to sperm at ejaculation. TIMP-2 is a non-glycosylated HBP recognized by a monoclonal antibody (M1). This study categorized range beef bulls as TIMP-2 positive or TIMP-2 negative in sperm extracts using Western blots or ELISA techniques with the M1 antibody. Fertility data was based on pregnancy rates.

Table I. Fertility of bulls categorized as sperm-associated protein TIMP-2 positive or TIMP-2 negative. Constant ratio of one bull per 25 cows over a 60 day breeding season.

| TIMP-2 Status | #Bulls | #Cows Bred | #Pregnant | %Pregnant |
|---|---|---|---|---|
| Positive | 180 | 3,985 | 3,431 | 86.1 |
| Negative | 67 | 1,225 | 894 | 72.9 |
| Total | 247 | 5,210 | 4,325 | 83.0 |

Data in Table I represent a 13% higher fertility rate in bulls which tested positive for TIMP-2 on sperm membranes versus TIMP-2 negative bulls. All breeding trials were conducted using a constant ratio of 1 bull:25 cows during a 60 d exposure period.

Sperm surface-associated TIMP-2 appears to have a direct relationship to fertility potential of range beef bulls. In addition, TIMP-2 presence or absence on sperm surface membranes offers the ability to segregate bulls and predict their fertility potential based on this specific HBP. Additional experimentation is needed to understand TIMP-2 sperm-associated interactions and transduction signal(s) which may contribute to differences in fertility. In conclusion, detection of sperm surface-associated TIMP-2 corresponds to increased fertility of range beef bulls.

# 122

**NONGENOMIC PROGESTERONE RECEPTORS IN HUMAN SPERMATOZOA** M.Luconi, L.Bonaccorsi*, G.Forti, E.Baldi*.Dept.Clinical Physiopathology,Andrology Unit,University of Florence,Italy

Previously results from our laboratory (Luconi et al, 1998) identified a protein of 57kDa on sperm surface as the putative membrane receptor mediating progesterone (P) rapid effects in human sperm. c262 antibody directed against the P binding domain of the genomic receptor identifies this 57kDa band also blocking the biological rapid effects induced by P, suggesting that the surface receptor contains a"conserved" region in the C-terminal. Identification of a third P receptor isoform (C-receptor) showing a weight of 50-60kDa (Wei et al, 1996) and sharing the C-terminal of A and B genomic isoforms, raises the chance that the sperm P receptor (PR) may be similar to the C isoform. To test this, we used two different strategies: RT-PCR amplification of different regions of the genomic receptor using human sperm RNA and isolation and sequencing of proteins by 2D gel electrophoresis after immunoprecipitation with c262. We amplified sequences using all the 5 pairs of primers we designed in 3 different regions of genomic receptor cDNA and performing RT-PCR on RNA from swim up-selected human spermatozoa. In particular, primers to the DNA-binding domain amplified in sperm a band higher than the corresponding one in placenta used as control. A new PR isoform with an expected weight of 57kDa has been reported (Hirata et al 2000) in sperm and contains an additional exon (exon S) close to the DNA-binding domain. We are performing additional RT-PCR experiments with primers in exon S to confirm this result. 2D gel analysis of sperm proteins immunoprecipitated with c262 reveals the presence of 2 spots of 57 and 29kDa with a pH range of 6-7. Microsequencing of these protein spots reveals the presence of peptide fragments with no matching with any sequences in database, suggesting the presence of a new receptor.

# 123

**REDUCTION OF SEMINAL ANTIOXIDANT CAPACITY IN INFERTILE MEN WITH LEUKOCYTOSPERMIA: A PROSPECTIVE STUDY** R.A. Saleh*, R.K. Sharma*, J. Chauhan*, M. Oder*, N. Esfandiari*, D.R. Nelson*, E.A. Nada*, R. Raina*, A.J. Thomas, Jr., A. Agarwal, Urological Institute, Cleveland Clinic Foundation, Cleveland, OH; South Valley University, Egypt

Seminal oxidative stress (OS) is an imbalance between levels of reactive oxygen species (ROS) and total antioxidant capacity (TAC). Leukocytospermia is associated with excessive ROS production. The objective of this study was to examine TAC levels in infertile men with leukocytospermia. Semen specimens from 48 infertile men were examined according to the World Health Organization (WHO) guidelines (WHO, 1999). Patient samples were classified into: leukocytospermic (>1X106 leukocytes/mL semen; n=16) and non-leukocytospermic (<1X106 leukocytes/mL semen; n=32); and 13 normal donors served as controls. Seminal TAC was measured by an enhanced chemiluminescence assay and results expressed as Trolox equivalent. Sperm motility was significantly reduced in leukocytospermic group compared to non-leukocytospermic group (P=0.04) and donors (P<0.001). Sperm concentration and normal forms were significantly reduced in leukocytospermic group compared to donors (P=0.008 and 0.0003, respectively). Levels of TAC [median (25TH & 75TH percentiles)] in leukocytospermic group was 636 (437, 982) compared to 986 (847, 1199) in non-leukocytospermic group (P=0.04) and 989 (863, 1534) in donors (P=0.01). Seminal leukocyte concentrations were negatively correlated with TAC levels (r=-0.34, P=0.007). In conclusion, sperm from infertile men with leukocytospermia may be at higher risk of OS due to reduction of ROS scavenging capacity in semen. Low seminal TAC in leukocytospermic patients suggests the potential benefit of antioxidant supplementation in lowering OS status and, in turn, improving sperm quality in these patients.

# 124

**MEIOTIC ABNORMALITIES IN AZOOSPERMIA AND BILATERAL VARICOCELE.**

**G. TRITTO, M.-O. NORTH*, Service of Urology, Saint-Louis Hospital, *Service Histologie-Embryologie-Cytogenetique, Saint Vincent de Paul – Cochin, Paris, France**

**International Association of Sciences and Technologies in Andrology – IASTA - e-mail: gtritto@magic.fr**

**On 127 patients with bilateral varicocele and severe male infertility factor, azoospermia is associated in 23% of cases. Testicular biopsies realized during the microsurgical correction show an incidence of ~ 10% of Maturation Arrest (MA): 44.5% of cases express a bilateral MA.**

**In the azoospermic population with bilateral varicocele, 39.3% do not present any spermatocyte and 32.1% have a normal meiotic behaviour.**

**Meiotic abnormalities, classified in pure genetic and pure toxic, evaluated for each bioptic sample and for each testis in the azoospermic cases with or without MA, show an incidence of 28.6%. Meiotic abnormalities are classified in four groups: I – Abnormal DNA coiling pattern type; II – Asynaptic type, distributed in IIA: association between lack of SV formation and abnormal pairing in all stages (Hulten et oth., 1974); IIB: complete asynapsis resulting in late-zygotene arrest (Navarro et oth., 1980); IIC: meiotic impairment secondary to unfavourable physiological background (Speed and Chandley, 1990); III – Desynaptic type (Templado et oth., 1978); IV – Chiasmatic abnormalities (Navarro et oth., 1980).**

**21.5% of cases present pure genetic abnormalities, distributed in: Class I – absent; Class IIA – 7%; Class IIB – 7%; Class III – absent; Class IV – 7%.**

**In class II, no spermatids or spermatozoa are present; in the other classes, a complete spermatogenesis is represented: some risk of meiotic gene mutation transmission (Hulten et oth., 1974; Templado et oth., 1986) and high risk of aneuploidy in Class II and IV (Templado et oth., 1986) are detected and described.**

**7% of cases present pure toxic meiotic abnormalities (Class IIC) as partial asynapsis, high rate of sex vesicle-autosome association (more than 4.8%), chromosome breaks.**

# 125

**INFLUENCE ON THE PREGNANCY OF MOTILITY OF FRESH AND FROZEN-THAWED TESTICULAR SPERMATOZOA IN OBSTRUCTIVE AZOOSPERMIA**

J.T.Seo*,J.M.Yun*, Y.S.Lee*, Y.S.Park*1, H.S.Lee*1, S.H.Lee*1, H.K.Byun*1, H.W.Youm*1, S.J.Song*1, I.S.Kang*2, Dept of Uro,Lab.of Reprod.Biol&Infert1, Dept of Ob&Gyn2, Samsung Cheil Hospital & Women's Healthcare Center, Seoul, Korea

Objective: Testicular sperm using ICSI could achieved optimal fertilization and pregnancy. We performed to observed the influence on fertilization and pregnancy of motility of fresh testicular sperm and sperm extracted from thawed tubule in obstructive azoospermia. Materials and Methods: We analyzed results of ICSI using fresh testicular sperm and sperm extracted from thawed tubule. The presence of motility were compared to determine the influencing factor for optimal fertilization and pregnancy rates. Results: In 316 cases TESE-ICSI, ICSI using fresh testicular sperm (fresh sperm group) were 163 cases and ICSI using sperm testicular sperm extracted from frozen-thawed tubule (thawed sperm group) were 153 cases. In fresh sperm group, fertilization rates were 71.3% and pregnancy rates were 32.5%. In thawed sperm group, fertilization rates were 65.1% and pregnancy rates were 33.3%. The fertilization and pregnancy rates of motile and non-motile testicular sperm were 72.9% and 33.6%, 50.0% and 18.2%, respectively ($p<0.05$). The fertilization and pregnancy rates of motile and non-motile sperm extracted from the thawed tubule were 67.8% and 34.7%, 55.1% and 28.1%, respectively ($p<0.05$). A comparison of the results of ICSI using motile fresh testicular sperm and motile sperm extracted from thawed tubule, fertilization and pregnancy rates were not significantly different (72.9% and 33.6%, 67.8% and 34.7%, respectively). Conclusion: These results suggest that successful pregnancy in TESE-ICSI treatment is influenced by motility of fresh testicular sperm and sperm extracted from thawed tubule in obstructive azoospermic patients.

# 126

**EFFECTS OF TWO TECHNIQUES OF LEFT VARICOCELECTOMY (VRCL) ON FERTILIZATION AND EMBRYONIC CAPACITY FOR IMPLANTATION**

A. Chatzikyriakidou, *1 Ch. Mamoulakis,1 A. Kaponis,*1 X. Giannakopoulos,* 1I. Miyagawa,2* D. Tsalikis,*1 D. Yiannakis,*1 N. Sofikitis 1,2, ; 1Dept of Urology, Ioannina University, Ioannina, Greece, 2 Dept of Urology, Tottori University, Yonago, Japan

We evaluated the effects of two techniques of left VRCL on and beyond fertilization. 22 infertile men with left varicocele were divided into two equal groups A and B. The standard parameters of semen analysis did not differ significantly between groups. Spermatozoa were collected and processed for ICSI techniques into donor oocytes. Fertilized oocytes were cultured. Fibronectin secretion (FS; marker of embryonic capacity for implantation) from fertilized oocytes was measured. There were no significant differences in FS between groups. Groups A and B were treated with retroperitoneal and subinguinal microsurgical VRCL, respectively. 12-15 months postoperatively, spermatozoa were collected and processed for ICSI techniques into donor oocytes. 12-15 months postoperatively, group B showed significantly larger ($p<0.05$, Wilcoxonìs test) mean values of sperm concentration (SC), % motile spermatozoa (MS), fertilization rate (FR), % injected oocytes processed up to the blastocyst stage, and embryonic FS (2220±124 IU/ml vs 1682±247 IU/ml) than group A. Within groups, SC, % MS, FR, and embryonic FS were significantly larger postoperatively than preoperatively. Both retroperitoneal and microsurgical VRCL have a beneficial effect on spermatogenesis and epididymal sperm maturation process. Both techniques result in the production of spermatozoa that a) have higher potential for fertilization and b) generate embryos of higher potential for implantation. Microsurgical VRCL may be the treatment of choice.

# 127

**PREVALENCE OF VARICOCELE IN CHILDREN AND ADOLESCENTS IN NAMHAE, KOREA; 3-YEAR FOLLOW-UP STUDY** K.H. Rha*, J.H. Kim*, B.H. Kim, D.H. Kwon, D.Y. Lee, S.W. Han*, M.S. Lee. Yonsei University, Seoul, Korea

Purpose : Whether there is a definite influence of varicocele upon future fertility is still dabated. We analyzed the results of physical examinations of children and adolescents and compared with the exam records performed 3 years previously to find out the natural course of the varicocele. Materials and Methods : The study population consisted 3271 school aged boys in Namhae County, Korea. A total of 2871(97.8%) boys aged between 7 to 18 years underwent evaluations., All examinations were carried out by urologists in May 1998 and June 2001 as part of annual school physical exam. Results : The mean age was 12.77 years. The prevalence of varicocele was 6.38%(237/2871). There were a peak varicocele prevalence from aged 14 to 16 (13,52%), 15 to 17 (17.8%). The varicocele incidence of adolescents (13-18 years) was 13.7%(210/1529). The prevalences according to age were 7-10 years 0.6%, 11 years 3.2%, 12 years 6.6%, 13 years 9.2%, 14 years 10.4%, 15 years 9.5%, 16 years 16.1%, 17 years 17.8%, and 18 years 12.8%. While the varicocele was unobserved almost in elemenatary student fewer than 11 years old, showed the highest incidence at 15-17 years old, and decreased in 18 years old. The proportion of varicocele grades were grade I 43.75%(104/237), II 34.17%(82/237), and grade III 22.08%(52/237). Eighty-nine boys out of 161 was followed for 3 years and found out that 68(76.4%) had either dissapearance or decrease in varicocele. Conclusion: As reported previously(J Androl Suppl 112, 2001), The prevalence of varicocele was evident in 13-17 year group and tend to decrease afterwards. The observation that many varicoceles decrease after 3 years should allude medical personnel for more prudent clinical decision.

# 128

**THE USE OF CYANOACRYLATE IN NEW TECHNIQUE OF VASOVASOSTOMY.** Chavarrìa-Guevara J; Sanchez G M; Garcìa J; Foyo N E; Moreno A J; Tapia-Serrano R. Andrologìa Urologìa- Hospital de Especialidades del CMN Siglo XXI, IMSS. MËxico, D.F.

Introduction.The microsurgical technique for vasovasostomy has been performed exclusively to reverse vasectomy. However the microsurgical technique needs a special training and its performing require long time and high cost. Objective. To show a new technique of vasovasostomy with ethyl-cyanoacrylate adhesive and to show the patency of vas deferens and its implications. Material and Methods. It was selected 6 male rabbits White New Zeland. The materials were 10-0 nylon sutures and ethyl-cyanoacrylate adhesive. Semen analysis were practiced in before they underwent surgical procedure. The ethyl-cyanoacrylate was incubated during 72 hrs. in culture mediums. The group underwent section and vasovasostomy by microscopic vision. The each end of the vas was approximated by two transmural sutures, followed by the application of cyanoacrylate circunferentially.Three months later we recollected semen and then they were killed; the vas deferens were recovery and studied their patency by macroscopic tests and histopathology study. Results. The cultures of cyanoacrylate were negative. The average surgical time was 55.6 min. One of 6 animals dead 2 days after surgery for respiratory complications. In the rest of the group patency was showed in nine vas deferens and obstruction was showed in only one. The semen were founded in all rabbits and the suture and methilen blue passed through vas lumen. The histopathology studies show thickeness of the muscular layer and inflammation in the site of sutures.The mucosal layer and the lumen were normal. Conclusions.This technique is a good alternative for vasovasostomy, and the surgical time is shorter.

# 129

FERTILITY AFTER CANCER: A PROSPECTIVE REVIEW OF ASSISTED REPRODUCTIVE OUTCOME WITH BANKED SEMEN SPECIMENS Pavithra Ranganathan, Jaswinder Chauhan, Mehmet Oder, Fabio F. Pasqualotto, Jorge Hallak,, Hiroshi Kobayashi, Anthony J. Thomas, Ashok Agarwal, The Cleveland Clinic Foundation

Our study aimed to determine the need for semen banking in-patients with cancer by evaluating their fertility outcomes with assisted reproductive techniques (ART) using pre-treatment semen specimens. Our sperm bank records were used to identify individuals who withdrew semen specimens for assisted reproduction. Study subjects were men recently diagnosed with cancer that sperm banked prior to undergoing therapy. All patients (n = 25) had their specimens transferred to various assisted reproduction programs of their choice. Information on fertility potential indices was obtained from medical records and by direct interviews. Patients were stratified by their cancer, testicular cancer (n = 9), Hodgkin's disease (n = 10), and others (n = 6). The pregnancy and live birth rates (LBR) were 27.3% and 33.3% for IUI (n= 13 cycles); 33.3% and 100% for IVF (n= 12 cycles); 46.1% and 66.6% for ICSI (n= 12 cycles); and 35.6% and 66.6% for overall ART (n= 37). The pregnancy and LBR by cancer were 66.6% and 66.6% for testicular cancer (n= 9); 40% and 75% for Hodgkin's disease (n= 10); 50% and 100% for other cancers (n= 6); and 52.2% and 80.5% for overall cancers (n= 25). See the table below for details of the assisted reproduction outcome by malignancy. Ours is the first large series documenting the fertility of sperm from cancer patients by ART. These patients showed significantly high rates of pregnancy and live births irrespective of the type of ART or malignancy. Babies born in our series had no birth defects. Our findings emphasize the need for physicians to encourage all reproductive age males to cryobank semen prior to antineoplastic.

# 130

**TADALAFIL PROVIDES PROMPT RESPONSE AND EXTENDED PERIOD OF RESPONSIVENESS FOR THE TREATMENT OF MEN WITH ERECTILE DYSFUNCTION**

H. Padma-Nathan[*1], K. Saikali[*2], V. Watkins[*3], A. Yu[*2], and S.J. Whitaker[*2] for the International Tadalafil Study Group. [1]University of Southern California, Los Angeles; [2]ICOS Corporation, Bothell; [3]Eli Lilly and Company, Indianapolis, USA.

**Objectives:** Tadalafil is a potent and selective phosphodiesterase type 5 inhibitor under review for marketing approval for the treatment of erectile dysfunction (ED). The time to onset (TTO: elapsed time from dosing to attainment of first erection resulting in successful intercourse) and period of responsiveness to tadalafil in men with erectile dysfunction ED were assessed in two phase 2 studies. **Methods:** In a double-blind, crossover, RigiScan™ study, 61 men received tadalafil 10 mg or placebo. Immediately after dosing and 24 hours after dosing, patients underwent RigiScan evaluations during visual sexual stimulation. Endpoints included TTO of response (≥55% penile rigidity for ≥3 consecutive minutes), percent responders, and cumulative time of rigidity ≥55%. In an at-home study, 223 men received tadalafil 10 or 20 mg or placebo with instructions to take study drug every 8–10 days (4 doses total) when ready to engage in sexual activity. No instructions restricting food or alcohol consumption were given. TTO (measured by stopwatch) was recorded in diaries. **Results:** In the RigiScan study, tadalafil provided a trend within 30 minutes and a statistically significant response vs placebo at 45 minutes ($P = 0.034$) and 24 hours ($P < 0.001$). In the at-home study, response to tadalafil 20 mg was significantly greater than placebo as early as 16 minutes after dosing ($P = 0.012$). The mean time for achievement of erection among responders was 17 minutes. In both studies, tadalafil was well tolerated and there were no treatment-related serious adverse events. **Conclusion:** In this research, tadalafil 20 mg enabled the onset of erection sufficient for completion of intercourse as early as 16 minutes after dosing and the period of responsiveness of tadalafil extended to at least 24 hours after dosing.

# 131

**TADALAFIL SIGNIFICANTLY IMPROVES ERECTILE FUNCTION IN MEN WITH ED, INTEGRATED ANALYSIS OF REGISTRATION TRIALS**

H. Padma-Nathan[*1], S. Wei[*2], A. Yu[*3], and S.J. Whitaker[*3] for the International Tadalafil Study Group. [1]University of Southern California, Los Angeles; [2]Eli Lilly and Company, Indianapolis; [3]ICOS Corporation, Bothell, USA.

**Introduction:** Tadalafil (under review for marketing approval for the treatment of erectile dysfunction [ED]) is a selective and potent inhibitor of phosphodiesterase type 5. In the presence of sexual stimulation, tadalafil enables men with ED to achieve and maintain erectile function (EF) sufficient for successful intercourse. The purpose of this multistudy, integrated analysis was to assess the efficacy and safety of tadalafil. **Methods:** Tadalafil doses of 2.5–20 mg were evaluated in 5 randomized, double-blind, placebo (PBO)-controlled, primary efficacy studies. Tadalafil was taken (without regard to food and alcohol intake) as needed up to once daily and patients were free to choose the time interval between dose administration and time of sexual attempts. Sexual Encounter Profile (SEP) diaries and the International Index of Erectile Function (IIEF) were used to evaluate its effect on EF. **Results:** In men with ED (N=1112; mean age, 59 y), tadalafil demonstrated consistent and statistically significant improvement compared with PBO on all endpoints evaluated and increased the percentage of successful intercourse attempts to up to 75% (SEP Question 3). Overall, tadalafil consistently showed efficacy in populations of men from a variety of ethnic backgrounds (age: 21 to 82 y) with ED of various severities and etiologies and allowed up to 59% of patients to return to a normal level of EF. Men reported consistent success for attempts 22–24 hours postdose (SEP Question 3: up to 80% success). Headache and dyspepsia were the most commonly reported adverse events (AEs) across studies. The discontinuation rate due to AEs in tadalafil-treated patients was low and not significantly different from that in PBO-treated patients. The AEs reported with tadalafil were generally mild or moderate, transient, and their frequency decreased with continued dosing. **Conclusions:** In these studies, tadalafil increased the percentage of successful sexual attempts in men with ED and was well tolerated. These data suggest that tadalafil may be an important treatment option for men with ED.

# 132

**TRANSPENILE DELIVERY OF MICROVASCULOKINETIC DRUGS ( TRAPS ) IN ERECTILE DYSFUNCTIONS. LASER DOPPLER FLOWMETRY STUDY.**

**G. TRITTO, Service of Urology, Saint-Louis Hospital, Paris, France - International Association of Sciences and Technologies in Andrology – IASTA - e-mail: gtritto@magic.fr**

**Microvasculokinetics drugs belonging to the Rutosides group, specifically complexed for transpenile barrier system delivery (TRAPS), are daily applied for 3 months on the penile skin of patients affected by erectile dysfunction with (micro)-vascular impairment.**

**Laser Doppler Flowmetry (LDF) recordings are collected at T0, T1, T2, T3 and the Δ score of the microvasculokinetic test is calculated with and without application of the drug ( trihydroxy-aethyl-rutoside or troxerutine Tr) versus placebo (P).**

**On Placebo group the Δ score remains unchanged at the low baseline values: PT0=6.99; PT30=7.77; PT60=5.81; PT90=8.41. On Tr group the Δ score increases progressively: TrT0=48.76; TrT30=63.12; TrT60=74.72; TrT90=81.77. The statistical difference between P and Tr groups is highly significative (P<0.001 for T0, T30, T60, T90).**

**The Laser Doppler Flowmetry evaluation of the (micro)vasculokinetic response in parallel on the penile skin and into the corpora cavernosa represents a new objective method to analyse the efficacy of new pro-erectogenic drugs with microvascular activity on the penile vascular compartments for penile rehabilitation.**

# 133

**ARE COMPLICATIONS NECESSARILY MORE COMMON WITH COMPLEX PENILE PROSTHESIS IMPLANTATION?**

R. Wang, B. Kay*, V.A. Cancellaro*, K.W. Lennox*, R.W. Lewis, Medical College of Georgia, Augusta, GA

INTRODUCTION: We compared the surgical approaches, types of penile prostheses used, success and complications involved in simple and complex penile prosthesis implantation. METHODS: We reviewed the charts of 103 patients who underwent penile prostheses implantation from 9/1995 to 9/2000. 74 patients with complete records and documented follow-up were included in this study. Simple penile prosthesis implantation is defined as the first implantation with no corporal fibrosis. Complex penile prosthesis implantation is for patients with significant corporal fibrosis, previous infection, history of corporal surgery or injury, and simultaneous implantation with artificial urinary sphincter. RESULTS: Fifty-eight percent (43/74) of patients had simple penile prosthesis implantation. 41.9% (31/74) of patients underwent complex penile prosthesis implantation. Simple penile prosthesis implantation was successfully performed though a single incision in all patients. 22.6% of complex penile prosthesis implantation surgeries required combination incisions ($P<0.05$). Inflatable penile prostheses were used in 93% (40/43) and 90% (28/31) of patients for simple and complex penile prosthesis implantation, respectively ($P>0.05$). The mean follow up was 27.5 months with functional prostheses in place in 97.7% and 96.8% of patients with simple and complex penile prosthesis implantation, respectively ($P>0.05$). Prosthesis infection, malposition, prolonged pain, and hematoma were not significant in the two groups. CONCLUSIONS: Complex penile prosthesis implantation can be successfully performed with minimal complications compared to simple penile prosthesis implantation.

# 134

**PROSPECTIVE STUDY OF PREOPERATIVE AND POSTOPERATIVE NOCTURNAL PENILE TUMESCENCE (NPT) IN MEN UNDERGOING BILATERAL NERVE SPARING RADICAL PROSTATECTOMY (BNSRRP)**

Andrew McCullough[1], Larry Levine[2], Harin Padma, Nathan[3]

New York University[1],NY, Rush University[2], IL,University of Southern California[3],CA

Introduction: A previous study has shown a dramatic loss of NPT in impotent men after BNSRRP. Preoperative scans were lacking in this population of previously potent men. We undertook a study to prospectively evaluate pre and post operative NPT in men undergoing NSRRP.

Methods: Between June 1999 and September 2000 54 men were selected to undergo pre and post operative NPT testing with RIGICAN PLUS®. After informed consent, they were screened for ED by IIEF. Excellent natural function by IIEF was required for inclusion in the study. All men had their surgery performed by three high volume radical prostatectomists. In order to qualify for a postoperative scan they had to have clear documentation of BNSRRP and have organ confined disease (PT2 or less).
Results: 54 men completed the study. The average age was 58

| | Duration of rigidity>55% Base (minutes) | Duration of rigidity>55% Tip (minutes) | Area under curve (AUC) rigidity Base | AUC rigidity Tip\| | AUC tumescence Base \| |
|---|---|---|---|---|---|
| Preop | 69 | 50 | 68 | 55 | 52 |
| Postop | 3 | 5 | 5 | 4 | 7 |
| P value | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |

Conclusion: Potent men undergoing BNSRRP experience a profound loss of nocturnal penile activity at one month. The loss of NPT is undoubtedly related to a neuropraxia, as it has previously been shown that arterial damage rarely occurs after BNSRRP. This loss of NPT if prolonged may predispose to chronic corporal hypoxia and corporal smooth muscle fibrosis. Longitudinal studies of erectile function are currently being concluded.

# 135

**LONG-TERM INTRACAVERNOUS (IC) THERAPY RESPONDERS CAN POTENTIALLY SWITCH TO SILDENAFIL CITRATE AFTER RADICAL PROSTATECTOMY (RP)**

Rupesh Raina*, RA Saleh*, Jaswinder Chauhan*, Sandra Ausmundson*, Milton M Lakin*, Ashok Agarwal, Craig D Zippe*, Urological Institute, Cleveland, OH

To assess whether long-term users of IC injections after RP can switch to oral therapy with sildenafil citrate. Thirty-six patients (mean age 60.9 yrs) with erectile dysfunction (ED) following RP were identified as long-term users of IC injection (mean 2.5 yrs). Prostaglandin (PG) E1 was used in 61% and Trimex solution (PGE1, papavarine, phentolamine) in the remaining 39%. The abridged 5-item International Index of Erectile Function (IIEF) questionnaire was used to assess the outcome with IC. These 36 patients received open lable sildenafil orally (50-100mg) for a minimum of 5 attempts. Following sildenafil use, patients were assessed with the abridged IIEF, partner satisfaction, and compliance to therapy. Of the 36 patients, 41% (15/36) successfully switched to sildenafil and discontinued IC injection. Thirty-eight percent (14/36) found sildenafil ineffective and remained on IC injection. Nineteen percent (7/36) found sildenafil alone to be suboptimal but continued using it, enhancing the efficacy of IC injections alone. Long-term users of IC injection therapy can potentially switch to sildenafil citrate with acceptable sexual satisfaction. Some long-term IC users (20%) can enhance sexual satisfaction with the addition of sildenafil citrate.

# 136

**COMBINATION THERAPY: SILDENAFIL CITRATE ENHANCES SEXUAL SATISFACTION IN VACUUM CONSTRICTION DEVICE (VCD) FAILURES FOLLOWING RADICAL PROSTATECTOMY (RRP)**

R Raina*, RA Saleh*, J Chauhan*, A Agarwal, MM Lakin*, CD Zippe*, Cleveland, OH

To assess the effectiveness of combining sildenafil citrate with VCD in men unsatisfied with the results of VCD alone. Thirty-one patients unsatisfied with the early use of VCD alone following RP (mean follow-up of 4.5 months) were instructed to take 100mg of sildenafil 1-2 hours prior to VCD use for sexual intercourse. Patients used combination therapy for a minimum of 5 attempts prior to assessment with the abridged International Index of Erectile Function (IIEF) questionnaire and a visual analogue scale to gauge rigidity. The effect of combination therapy on total IIEF-5 score and penile rigidity score were assessed. Of the 31 patients, 7 (22%) had no improvement with the addition of sildenafil with VCD and discontinued the drug, while 24 (77%) reported improved penile rigidity and sexual satisfaction. In these 24 patients, the IIEF-5 score showed significant improvement in each domain and patients reported that sildenafil enhanced their erections 100% of the time. Rigidity scores on a scale of 0-100 with VCD alone averaged 55% (23-85) for men and 59% (26-90) for their partners. With the addition of sildenafil, it increased to 76% for men and 82% for their partners. Thirty percent (7/24) reported the return of natural erections at 18 months using combination therapy with 5/7 sufficient for vaginal penetration. The addition of sildenafil with VCD improved sexual satisfaction and penile rigidity in patients unsatisfied with VCD alone.

# 137

**SILDENAFIL PLUS SERTRALINE IN THE TREATMENT OF PREMATURE EJACULATION.** Dr.A.Fernandez Lozano M.D.Urologist; Catalonian Health Institute.Barcelona. Spain.

INTRODUCTION. Premature ejaculation (PE) is a very common sexual dysfunction, which is beleived to affect a 35% of men. Defined as the inability to exert an adequate voluntary control on ejaculation after reaching a high degree of sexual arousal. Organic etiology is not frequent. Many of our patients are reluctant to follow psychotherapy for several reasons (economic,cultural,social).MATERIAL AND METHODS. Delayed ejaculation is one of the most common side effects of a wide number of antidepressant agents and sildenafil is well known as a producer of a better quality of erection. We report a study to asses the efficacy of sertraline and sertraline plus sildenafil in patients complaining of PE. A total of 62 patients with PE in different grades of involvement, ages ranging 23 to 65 years were studied. Of them, 46 had stable partner, while the others were single, divorced and widowers. Almost two thirds said had always suffered Primary Premature Ejaculation and the rest of them from 6 months to 8 years, occurring most of the times they had sexual relations.Sertraline Hidrochloride 50 mgrs/day was prescribed to all patients for two months, and to a group of 14 which never had experienced primary premature ejaculation we added sildenafil citrate (25-100mgrs) one hour before sexual intercourse. RESULTS. Both drugs were well tolerated. Drowsiness and gastrointestinal upset occurred in 7 patients, 2 anejaculations, 5 flushes, 4 headaches and 7 did not follow the study for unknown reasons. On the group treated with sertraline alone, in 25 patients the ejaculatory latency time increased from 0.9 minutes to a mean of 4.6, while in the sildenafil group increased from a baseline of 0.5 to a mean of 5.2. The patients with stable partner were asked to rank their sexual life from 0 to 5 (being 5 the sexual maximum satisfaction). This parameter also increased in both groups from 0.5 to a mean of 3 and 4.1 in the sildenafil group. CONCLUSIONS. Sildenafil citrate helps obtain a better erection and combined with sertraline affects positively to those patients whom either for age or because they¥ve started a new couple relation, associate PE and erectil disfunction. An increased satisfaction of their sexual life was the†response on about a 70% of the patients in our study.

# 138

**PEDIATRIC HERNIORRHAPHY AS AN UNUSUAL CAUSE OF TESTICULAR PAIN: MECHANISM AND PATHOPHYSIOLOGY** JI Sandlow, MD and CG Christiansen*. University of Iowa Hospitals and Clinics, Iowa City, IA

Pediatric operative procedures potentially involving the vas deferens are extremely common, comprising over 30% of pediatric surgery. Although rare, damage to the vas deferens is a major concern, with an estimated rate of iatrogenic injury between 0.8-2.0%. Most often, however, the injury is unilateral and goes unnoticed unless pathology develops on the contralateral side, thus resulting in infertility. Testicular pain following vasectomy is also a relatively rare phenomenon, with chronic pain occurring in less than 0.1% of procedures. The mechanism of pain is not well-understood, but is thought to be due to pressure increases within the epididymis secondary to the obstruction. We present a case of a young man who suffered an unrecognized transection of the vas deferens as an infant secondary to a herniorrhaphy, now presenting with testicular pain with ejaculation. Exploration revealed a markedly distended vas and epididymis, and although his defect was not amenable to repair, his pain resolved with ÏventingÓ of the testicular end of the vas deferens. We also review the pathophysiology of post-vasectomy pain syndrome, discuss the proposed mechanism of action, and how it relates to this specific case.

# 139

**IS THE YACHIA CORPORAL PLICATION FOR PEYRONIE'S DISEASE STILL EFFICACIOUS?** Buffi G. Boyd*, Peter A. Ruff*, Matthew Zelinski*, Run Wang, Ronald W. Lewis, Medical College of Georgia, Augusta, GA

INTRODUCTION: Yachia initially described his modification of the Nesbit procedure for the surgical treatment of Peyronie's Disease in 1990. We describe a single surgeon's experience with the Yachia procedure over a seven year period to determine if the procedure remains an effective intervention with adequate patient satisfaction. METHODS: A single surgeon's results at two institutions are presented. Previously published data (1994-1996) were compared to the surgeon's current series. The most recent data consisted of a retrospective chart review of 22 Yachia procedures performed from 12/1997 to 8/2001 and a phone survey consisting of the Sexual Health Inventory for Men plus five questions specific to the Yachia. Fourteen cases had complete records and phone survey and are included for analysis. RESULTS: The surgeon's initial series (Group 1) consisted of 30 patients with the mean age of 51 years. Our most recent series (Group 2) includes 14 patients with a mean age of 49 years. Average preoperative curvature in Group 1 was 58 degrees and in Group 2 was 55 degrees. Other preoperative perimeters including previous treatment, erectile dysfunction and trauma were comparable. Penile curvature was eliminated or significantly improved in 93% in both groups. Patient satisfaction was 83% in Group 1 and 79% in Group 2. The major complaint in both groups was penile shortening - 67% in Group 1 and 86% in Group 2. CONCLUSION: The Yachia procedure remains an efficacious surgical treatment for Peyronie's disease. The majority of patients are satisfied with their outcomes, however counseling for penile shortening should be performed prior to intervention.

# 140

**THE EFFECT OF TREATMENT OF SPERM WITH SUBNORMAL HYPOOSMOTIC SWELLING TEST (HOST) SCORES WITH THE PROTEIN DIGESTIVE ENZYME CHYMOTRYPSIN ON SUBSEQUENT VIABLE PREGNANCY RATES PRS) AFTER INTRAUTERINE INSEMINATION (IUI)** M.L. Check*, D. Kiefer*, J.H. Check, W. Hourani, A. Bollendorf UMDNJ, Robert Wood Johnson Med. School at Camden, Cooper Hosp./Univ. Med. Cntr., Dept. OB/GYN, Div. Repro. Endo. & Infertility, Camden, NJ

A prospective observational study was conducted to determine the PR after insemination of sperm with subnormal HOST scores (<50%) after correcting the scores (>50%) following treatment with the protein digestive enzyme, chymotrypsin. Infertile couples where the male partner was found to have a minimum of 5x10S6/mL sperm motile density and a HOST score <50% were given the option of immediately proceeding to in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) or trying some IUI treatment cycles with chymotrypsin-galactose treated sperm. Only those males where the HOST score improved to >50% were given the option of IUI. There were 90 IUI cycles and there were only 3 viable pregnancies (3.3% viable PR/treatment cycle). The most IUI cycles were in cycle 1 (n=39) and 2 (5.1%) achieved viable pregnancies. During this same time the viable PR following IVF with ICSI was 33.3%. These data confirm previous studies that sperm with HOST scores <50% rarely achieve pregnancies. Treatment of sperm with subnormal HOST scores allowed 3 more cases of viable pregnancies added to the 4 previously reported ones. However with expansion of the number of cycles to 90 compared to the previous 12 cycles evaluated, it is now clear that treatment of sperm with chymotrypsin, even when correcting the HOST scores, pales in comparison to success with IVF with ICSI. Sperm with low HOST scores are hypothesized to transfer a toxic factor (possibly proteinaceous) from the sperm to the zona pellucida by the supernumerary sperm which is bypassed by ICSI.

# 141

**EFFECT ON PSA LEVELS OF THE NOVEL, DUAL 5A REDUCTASE INHIBITOR, DUTASTERIDE WITH MARKED SUPPRESSION OF IHYDROTESTOSTERONE**

RV Clark1, TH Wilson*1, CG Roehrborn*2, G Andriole*3, 1Glaxo S mith Kline, Research & Development, Research Triangle Park NC, 2De pt of Urology, Univ of Texas Southwestern Med Center, Dallas, TX, 3 Dept of Urology, Washington Univ Med Center, St Louis, MO

Introduction: Dutasteride is a novel 5areductase inhibitor (5ARI) being evaluated for the treatment of BPH. Dutasteride acts on both type 1 and type 2 isozymes and suppresses serum DHT >90% from baseline. PSA levels are also suppressed by 5ARI, and type 1 inhibition suppresses total PSA @ 50%. This study evaluated the effect of dual 5ARI on total and free PSA levels. Methods: The study population was 674 subjects drawn from a Phase III trial of 24 months comparing dutasteride 0.5mg vs placebo for treatment of BPH associated symptoms and complications. The subset was based on availability of total and free PSA measures at baseline and month 12 of the study. Results: In the placebo group, total PSA, free PSA, and the F/T PSA ratio changed little from baseline to month 12 (median increases were 4.0%, 5.3% and 2.0% respectively. Dutasteride treatment resulted in median decreases of 52.5% for tPSA and 61.0% for fPSA. The F/T PSA ratio decreased from 0.22 to 0.18 (14.7% decrease). DHT decreased in dutasteride group > 92%. Conclusions: Potent dual 5AR inhibition by dutasteride lowers total PSA by about 50% preserving the utility of the 2X correction factor usually applied to 5AR inhibition despite marked DHT suppression. The F/T PSA ratio is decreased slightly, but its utility is maintained for detection of prostate cancer.

# 142

**DIFFERENTIAL CELLULAR PROLIFERATION AND APOPTOSIS OF HUMAN CAVERNOSAL CELLS IN RESPONSE TO OXIDATIVE STRESS AND ANTIOXIDANTS.** Suresh C. Sikka, Hong Wang*, and Wayne J.G. Hellstorm. Department of Urology, Tulane University Health Sciences Center, New Orleans, LA.

Introduction and Objective: Increased free radical generation and oxidative stress following penile trauma is responsible for the formation of the Peyronie's plaque. High doses of vitamin E have been used to treat Peyronie's plaque but with limited success. We have earlier shown that tissue fibrosis and activation of nuclear factor NF-kB and TGF-beta in response to oxidative stress are involved in the pathogenesis of such plaques. The present study evaluates cavernosal cell proliferation due to oxidative stress in absence and presence of vitamin E in order to understand the molecular/cellular basis of Peyronie's disease progression and prevention. Methods: We used our established cavernosal cell-culture model for monitoring cell growth and apoptosis. Primary culture cells (passage 2-3) from various patients were subjected to oxidative insult for 24 to 48 hours by hydrogen peroxide [0.3nM to 3mM] in absence and presence of various doses of vitamin E [10-100μM]. Kinetics of cell growth/mitochondrial activity was monitored by WST-8 assay and expressed as percent of control. Apoptosis was monitored by DNA fragmentation assay. Results: These results indicate significant differential cell-growth/inhibition pattern by different primary cells in response to various doses of H2O2. To our surprise low doses of both H2O2 and vitamin E were cell proliferative, while higher doses inhibited cell growth/mitochondrial activity. However, higher H2O2 concentrations [>30μM] showed significant apoptotic response that was reversed by vitamin E [50μM]. Conclusion: These findings indicate a differential response pattern by various cavernosal cells to oxidative stress and regulation in presence of vitamin E. Also, this may explain limited success in early plaque prevention by antioxidants, and by other treatment modalities.

# 143

**INDEXING ATYPICAL CYTOLOGY TO NMP22 DECREASES FALSE-POSITIVE RESULTS**

Rupesh Raina*, Heather Klepacz, Jaswinder Chauhan, Lee Ponsky, Shashikala Sharma, David R Nelson, Ashok Agarwal, Craig D Zippe, Urological Institute, Cleveland, OH

To assess atypical cytology as a positive bladder tumor marker and to determine if indexing atypical cytology to NMP22 can decrease false-positive results [or increase the positive predictive value (PPV)]. One hundred ninety-seven patients at risk for bladder cancers were identified as having atypical urine cytology. One hundred twenty six were incident (screening) cases and 71 were prevalent (monitoring) cases of bladder cancer. Office cystoscopy was performed on all patients with atypical cytology. All cancers were histologically confirmed and patients had a negative upper tract study within a 12-month interval. The atypical cytologies were then indexed to NMP22 values in an effort to decrease false-positive results. In the screening cases, the 126 atypical cytologies detected 17 cancers for a positive predictive value (PPV) of 13% (17/126). When stratified by NMP22, cutoff value of >10U/ML, PPV increased to 71% (15/21). In the monitoring cases, the 71 atypical cytologies detected 43 cancers for a PPV of 61% (43/71). When stratified by NMP22 cutoff >6U/ML, PPV increased to 92% (35/38). The clinical utility of atypical cytology was significantly enhanced in both screening and monitoring for bladder cancer when indexed to NMP22.

# 144

**TARGETED MULTICOLOR FLUORESCENE IN SITU HYBRIDIZATION (FISH) DETECTS TRANSITIONAL CELL CARCINOMA (TCC) IN PATIENTS WITH ATYPICAL TRANSITIONAL CELLS BY URINARY CYTOLOGY**

R Raina*, M Skacel*, L Liou*, A Agarwal, RR Tubbs*, CD Zippe*, Cleveland, OH

A multitarget FISH probe set containing probes to the centromeres of chromosomes 3, 7, 17 and to the 9p21 band has been recently shown to have high sensitivity (81%) and specificity (96%) for the detection of TCC. Using our modified method for ThinPrep slides, we retrospectively tested 80 cases diagnosed as atypical transitional cells by urinary cytology. We evaluated the ability of FISH to identify malignant cells in cytologically equivocal cases where morphology alone does not allow definitive diagnosis. Archived slides from 80 voided or instrumented urine specimens from 63 patients with concurrent bladder biopsy data were pretreated and subjected to hybridization with the multicolor FISH probe Urovysion (Vysis). The cohort included patients with biopsy proven grade I (n=11), II (n=26), III (n=18) TCC, CIS (n=5), stage pTa (n=48), T1 (n=5), T2 (n=3), T4 (n=2) and negative histology (n=20). Positive FISH result was defined as 5 or more transitional cells with alteration of 2 or more chromosomes or 20 cells with 9p21 deletion. All except for 5 (all pTa tumors) TCC cases were positive by FISH (92% sensitivity). 9 patients positive by FISH had negative concurrent biopsy, however, 8 of them developed recurrent TCC (including one invasive T2 tumor) within a 6-month period. The only Ïfalse positiveÓ patient had previously documented CIS also present in the immediately consecutive biopsy 15 months later. None of the 8 patients with negative biopsy and negative 6-month follow-up were FISH positive. FISH provides high sensitivity (92%) and specificity (91%) in targeted evaluation of cytologically equivocal transitional cells. The results suggest close follow-up of patients with a negative cystoscopy/biopsy but positive FISH results.

# GET IN THE SWIM

**If you know a child with muscular dystrophy who can benefit from a special getaway, tell him or her about MDA summer camps. They're fun and free!**

MDA

Muscular Dystrophy Association
Jerry Lewis, National Chairman
1-800-572-1717 • www.mdausa.org

*People Help MDA ... Because MDA Helps People*

PHOTO PARADE / EDDIE ADAMS