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Supplement

Journal of ANDROLOGY

American Society of Andrology

25th Annual Meeting

April 7–11, 2000
Boston, Massachusetts

Program and Abstracts

Published by THE AMERICAN SOCIETY OF ANDROLOGY

Schedule At A Glance

Friday, April 7, 2000

8:00 am — Andrology Laboratory Workshop (concludes at 5:00 pm)

Saturday, April 8, 2000

8:00 am — Postgraduate Course (concludes at 5:00 pm)

5:30 pm — Welcome Reception

7:30 pm — Welcome & Opening Remarks
Ernst Schering Research Foundation Lecture
• Clinical Investigative and Genetic Approaches to the Male Reproductive System
William Crowley, Jr, MD

Sunday, April 9, 2000

8:00 am — Serono Lecture
• Co-activators: The Power Boosters for Steroid Hormone Action
Bert O'Malley, MD

9:00 am — Distinguished Andrologist Award Presentation

9:15 am — Oral Session I: Germ Cells in the Testis

10:30 am — Coffee Break in the Exhibit Halls

11:00 am — Reproductive Sciences and the Public
• Reporting on Human Reproduction in the Third Millennium Theater
Gina Kolata
• Human Reproduction: The Third Millennium Theater
Michael E. McClure, PhD

12:15 pm — Women in Andrology Luncheon Meeting
• Declining Male Reproductive Health
Dolores J. Lamb, PhD

1:30 pm — Symposium I: Cell Fate
• Pluripotent Stem Cells, Germ Cells and Testicular Cancer
Peter Donovan, PhD
• Telomeres, Telomerase and Testes
Ronald DePinho, MD
• Consequences of Excessive and Deficient Testicular Germ Cell Apoptosis
Kim Boekelheide, MD, PhD

3:00 pm — Refreshment Break in the Exhibit Halls

3:30 pm — Women in Andrology Lecture
• Molecular Insights into Male Reproductive Development
Holly A. Ingraham, PhD

4:30 pm — Poster Session I

7:00 PM — Annual Banquet
Boston Museum of Science
The Museum is within easy walking distance of the headquarters hotel

Monday, April 10, 2000

7:00 am — Past Presidents' Breakfast

8:00 am — Buckeye State-of-the-Art Lecture
• LH and FSH Receptors: Insights into Reproductive Disorders and Signaling by G-Protein-Coupled Receptors
Deborah Segaloff, PhD

9:00 am — Young Andrologist Award Presentation
Distinguished Service Award Presentation

9:15 am — American Urological Association Lecture
• Tissue Engineering in the Genitourinary System
Anthony Atala, MD

10:15 am — Coffee Break in the Exhibit Halls

10:45 am — Oral Session II: Gene Expression in the Male Reproductive Tract (Simultaneous Session)

10:45 am — Oral Session III: Sperm Function and Fertility (Simultaneous Session)

12:15 pm — Laboratory Science Forum Luncheon Meeting
• Development and Evaluation of Vaginal Antimicrobial Contraceptive Compounds
Lourens J.D. Zaneveld, DVD, PhD

1:15 pm — Symposium II: Prostate Cancer
• Novel Aspects of the IGF-IGFBP Axis in Prostate Cancer
Pinchas Cohen, MD
• Finding Genes that Contribute to Prostate Cancer Susceptibility
Kathleen A. Cooney, MD
• Gene Expression Profiling of the Human Prostate Androgen-Response Program
Peter Nelson, MD

2:45 pm — ASA ANNUAL BUSINESS MEETING AND AWARDS PRESENTATION
• New Investigator Award
Sponsored by West Michigan Reproductive Institute, P.C.
• Outstanding Original Research Awards
• Research Excellence Award to a Female Trainee/Fellow
Established by Anna Steinberger, PhD and Supported by Women in Andrology
• Trainee Merit Awards
• Thomas S.K. Chang Trainee Travel Fund Awards
• Burroughs-Wellcome Fund Award for Outstanding Trainee Research

3:30 pm — Refreshment Break and Raffle Drawing in Exhibit Hall

4:00 pm — Pharmacia & Upjohn Current Clinical Perspectives: Gender Assignment and Gender Identity
• Moderator: Claude J. Migeon, MD
• Peter A. Lee, MD
• Kenneth J. Zucker, PhD

5:30 pm — Poster Session II

7:00 pm — Trainee Colloquium
• Can Male Reproductive Tissues/Cells be Utilized Outside of the Reproductive System: Andrology Not Just for Andrologists
Paul R. Sanberg, PhD

8:30 pm — Trainee Soirée

Tuesday, April 11, 2000

8:00 am — Latin American Lecture
• Calcium Channels and Sperm Physiology
Alberto Darszon, PhD

9:00 am — Symposium III: Male Germ Cells
• Potential and Limitations of Spermatogonial Transplantation
Michael D. Griswold, PhD
• Inhibition of Spermatogonial Differentiation by Androgens
Marvin L. Meistrich, PhD
• DNA Methylation in Germ Cells: Implications for Genomic Imprinting
Jacquetta M. Trasler, MD, PhD

10:30 AM — Conclusion of Annual Meeting

President's Welcome

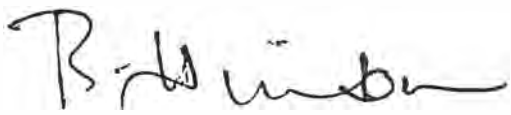
Welcome to the 25th Annual Meeting of the American Society of Andrology. We are all looking forward to having a terrific meeting in Boston. Organizing a program that meets the interests of such a diverse group of andrologists is one of the most challenging tasks for the Program Committee. Dr. Terry Brown and members of his Program Committee have met this challenge by planning an outstanding program. Several nationally and internationally-known speakers have been invited to present their latest findings. Dr. Lisa Tenover and members of the Postgraduate Committee have also planned an exciting Postgraduate Course on the timely topic of androgens. Dr. Erol Onel and Dr. Bob Newton from the Local Arrangements Committee have worked extremely hard to provide an excellent social program. Dr. Jean Fourcroy has spent a good number of hours in archiving the Society's ventures, and has played a major role in the celebration of the Society's 25th birthday in Boston. Cherokee Melton and our new Executive Director, Kathleen Matikonis, from Holland-Parlette Associates, have both worked hard in the organization of the Annual Meeting as well as the day-to-day running of the Society. My sincere thanks go to you all for your dedication to the Society.

A record number of abstracts were submitted to this meeting, and through the efforts of many members of the Society, an excellent series of presentations have been planned including: the Laboratory Science Forum, Trainee Colloquium and Soirée, Women in Andrology Luncheon and the Andrology Laboratory Workshop. Contributions such as these are vital to the success of the Society's Annual Meeting.

A special thanks go to all our sponsors who have generously supported our meeting, and these are listed throughout the program booklet. Without their support we simply could not invite first-class speakers nor provide many of the awards that are presented at our Annual Meeting. I encourage you to visit the exhibitors to learn about their new products and to express your appreciation for their presence.

I have thoroughly enjoyed working with the members of the Executive Council and the chairs of individual committees, and I thank you all for your advice and support this past year. Your contributions have made a real difference to the vitality of the Society. Your input has allowed the Society to consider electronic publication of the *Journal of Andrology,* the redesign of a new web page and the support of the International Congress of Andrology to be held in 2001 in Montreal.

Finally, I would like to thank the membership of the American Society of Andrology for the opportunity to serve as your president. It has been a distinct honor to work with so many members dedicated to the well-being of our Society. Thank you. Enjoy the meeting!



Barry T. Hinton, PhD, President

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Notice to Readers

All events will be held at the Royal Sonesta Boston Hotel in Cambridge unless otherwise noted. 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 25th Annual Meeting, contact the ASA staff by phone (415-764-4823), by fax (415-764-4915), or email (asa@hp-assoc.com), or visit ASA's home page at www.cvm.uiuc.edu/~r-hess/asa/front.html on the Internet.

Airport

Logan International Airport is approximately three miles from the Royal Sonesta Hotel (about a $15 cab ride).

Exhibits

Two extensive exhibit halls featuring equipment and information pertinent to andrologists will be open from 10:00 am to 6:00 pm on Sunday and from 10:00 am to 7:00 pm on Monday. Refreshment breaks will be held in the exhibit hall, and at 3:30 on Monday there will also be a raffle drawing in the exhibit hall.

Hotel Accommodations

The Royal Sonesta Hotel Boston is the headquarters and preferred hotel for the meeting. The hotel is located at 5 Cambridge Parkway, Cambridge, Massachusetts 02142.

A discounted room rate of $165 (single/double) is available for ASA meeting attendees. We can only guarantee a room at this rate until March 9, so we urge you to make your reservation as soon as possible. Reservations may be made by calling the hotel directly at 617-491-3600. Be sure to mention that you are attending the ASA meeting to receive the reduced rate.

Laboratory Science Forum

The 2000 Laboratory Science Forum will be held at Noon on Monday, April 10, in the Somerset Room. This year's topic deals with Development and Evaluation of Vaginal Microbial Contraceptive Compounds, presented by Lourens J.D. Zaneveld, DVM, PhD. Lunch tickets are available for $22.

Poster Sessions

Poster sessions will be held Sunday (4:30 pm to 7:00 pm) and Monday (5:30 pm to 7:00 pm) in Grand Ballroom B and the Skyline Suites. 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 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

Attendees are encouraged to register in advance if possible. Registration forms are available by contacting ASA:

74 New Montgomery Street, Suite 230
San Francisco, CA 94105
Phone: (415) 764-4823 • Fax: (415) 764-4915
Email: asa@hp-assoc.com

All registrations received after Thursday, March 9, 2000 (including on-site registrations) will be assessed a $45 fee. Cancellations must be made in writing and are subject to a $50 cancellation fee prior to March 9, 2000. After that date, no refunds will be given.

Participants who wish to attend only the afternoon session of the Postgraduate Course or Laboratory Workshop may register at half the usual fee for the course.

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

Friday, April 7	7:00 am - 6:00 pm
Saturday, April 8	7:00 am - 6:00 pm
Sunday, April 9	7:00 am - 6:00 pm
Monday, April 10	7:00 am - 5:00 pm
Tuesday, April 11	7:00 am - 10:00 am

Slide Preview Room

The West Tower coat room, located on the second floor of the hotel, will be available from 7:00 am until 11:00 pm, Friday through Tuesday for previewing slides and preparing presentations. A limited number of slide carousels will be available — presenters are encouraged to bring their own.

Trainee Colloquium & Soirée

The 2000 Trainee Colloquium will feature Paul Sandberg who will answer the question, "Can Male Reproductive Tissues/Cells be Utilized Outside of the Reproductive System?" The Colloquium will be held Monday, April 10, from 7:00 pm to 8:30 pm in the Somerset Room, followed by the Trainee Soirée. Both events are free of charge and open to everyone.

Women in Andrology

The Women in Andrology group will hold its annual business meeting on Sunday at 12:15 in University B and C (lobby level, East Tower). The meeting will include a presentation by Dolores J. Lamb, PhD on "Declining Male Reproductive Health." Lunch tickets are available for $25.

Welcome to Boston!

From the Local Arrangements Chairmen

Welcome to the 25th birthday party for the American Society of Andrology! It has been 15 years since we last met in Boston for the Third International Society of Andrology meeting. One of us fondly remembers that meeting (RN, who served as both the local arrangements and program chair), while the other (EO) was not yet out of high school at the time. We do share something in common, though: a commitment to make sure that all of you remember this meeting as a truly special event for the ASA. We are convinced that you will find Boston in Springtime to be an exciting and stimulating place to celebrate.

Just as our Society has continued to grow and prosper over these years, redevelopment continues in Boston with the "Big Dig." You can avoid most of the traffic, though, because our meeting site (the Royal Sonesta Hotel) is just across the Charles River in Cambridge — a short walk to downtown Boston and only a 15-minute cab ride from Logan Airport. With beautiful rooms overlooking the Charles River, the hotel offers convenience and comfort in a central location. You will find that the presentations, symposia and conference activities maintain the high level of quality you have come to expect. In addition, both the Andrology Laboratory Workshop and Postgraduate Course have been expanded in response to your requests.

But please, find some time to enjoy the unique history, museums, shopping, educational institutions, restaurants, and general culture that gives Boston its own special character. You may want to walk the Freedom Trail, taking in the site of the Boston Massacre, the Boston Tea Party, the birthplace of Paul Revere, the Old North Church, and other locations that helped to shape our nation. If you are feeling energetic, you can walk up to the Bunker Hill monument, not far from the hotel, where the American colonists only fired when they saw "the whites of their eyes." But remember, this isn't the only walking tour of Boston. The Black Heritage Trail will take you past the highlights of Massachusetts' early anti-slavery movement, as well as into the oldest African-American Church still standing in the United States. You can also find the oldest continuously commissioned ship in the world, the USS Constitution or "Old Ironsides," open for your inspection at Pier 1.

It's not just history though. Boston is home to some of the finest museums in the world. Our annual banquet will be a 25th birthday tribute to the ASA held at the Museum of Science, just a short walk from the convention hotel. For a more interactive experience, visit the Discovery Center section and learn more about computers and the brain and lightning and shadows. Fans of more traditional museums can enjoy the largest collection of Monet's works outside of France, as well as masterpieces by Van Gogh, Renoir, Homer and Rembrandt at the Museum of Fine Arts. Of special interest is the unique Isabella Gardner Museum on the Fenway with its Italianate gardens, concerts and paintings by Degas, Raphael, Titian and Matisse. Other museums of note are the John F. Kennedy Library and the newer Computer Museum (don't miss the walk-through two-story computer model of how a desk-top computer works).

After all that culture, it may be worth a leisurely walk through Boston Commons and the Public Gardens. In the latter, take a ride on the famous Swan Boats for a sense of calm unparalleled in a major city. Or you can take an amphibious "duck tour" through both the streets of Boston and the Charles River, recreating the trip of Robert McCloskey's classic children's book, *Make Way for Ducklings;* make sure you take the time to find the adorable duckling statues back in the Public Gardens. Then head across the street to the Bull and Finch pub where everybody knows your name — better known as the basis of the long-running TVshow, "Cheers." From there you can walk to the State House and Beacon Hill or take the "T," as Boston's efficient public transportation system is known, to explore some of Boston's neighborhoods: Little Italy in the North End, Chinatown, or the eclectic and trendy South End. You can finish off the day by grabbing some souvenirs at Faneuil Hall, or doing some more upscale shopping on fashionable Newbury Street.

Finally, don't ignore the sites just outside Boston itself. Cambridge, with its mix of world-class educational institutions and restaurants (and the highest number of bookstores per capita in the world) is worth a look. A trip to Lexington and Concord and the newly created Battle Road National Park, Thoreau's Walden Pond, and the Museum of Our National Heritage are well worth it if you have the time. Traveling up towards Salem lets you explore the site of the famous witch trials. And for relaxation along the majestic New England Coastline, nothing beats the rugged beauty of Cape Cod and Martha's Vineyard.

A few final words of thanks. New England Medical Center has graciously donated some of its meeting space for additional or impromptu lectures to be held off-site; keep your eyes posted for more details about these throughout the meeting. Our sponsors and exhibitors have provided funding for many of the events you will be enjoying — drop by their booths to say, "Thanks." And last but not least, Barry Hinton has been working tirelessly to ensure this will be the best birthday bash the ASA has ever had. Make sure to give him a pat on the back if you see him. If you have any questions, we are available at all times to help. So enjoy the conference, and once again, "Happy 25th Birthday, ASA!"

Robert Newton, MD, Co-Chair
Erol Onel, MD, Co-Chair

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	* *Deceased*	

Message from the Program Chair

A large number of members of the America Society of Andrology, those who are formal members of the Annual Meeting Program Committee mentioned below as well as many others, have contributed their input and suggestions for the content of our Silver Anniversary program. The development of a scientific program that encompasses the wide spectrum of clinical and scientific interests represented within our Society can be a daunting task. The Committee has arranged a balanced program that represents the many and varied interests of our Society with an emphasis on cutting edge science and technology presented by the premier investigators in their respective areas of expertise. Society members have responded with a record number of abstract submissions, and the future of our Society is represented among the large number of trainees who will be presenting their work orally and as posters. The scientific program is tightly packaged into our traditional two-and-a-half-day-meeting format and features lectureships, state-of-the-art lectures, symposia, and oral and poster sessions.

The Serono Lecturer for this year is Dr. Bert O'Malley, Chairman and Professor of Molecular and Cellular Biology at Baylor College of Medicine, who is widely respected as a scientific founder in the field of molecular endocrinology. Dr. O'Malley has devoted his career to investigations of nuclear hormone receptors and will present his latest work in a lecture entitled "Co-activators: The Power Boosters for Steroid Hormone Action". The annual American Urological Association Lecture will be presented by Dr. Anthony Atala, a urologist at Boston Children's Hospital of the Harvard Medical School, who will discuss his exciting work in the biotechnology of tissue engineering in a presentation entitled "Tissue Engineering in the Genitourinary System." Our newest sponsored lectureship, The Ernst Schering Lecture, will be presented by Dr. William Crowley, Jr. from Massachusetts General Hospital on the topic, "Clinical Investigative and Genetic Approaches to the Male Reproductive System." The second annual Latin American Lecture, entitled "Calcium Channels and Sperm Physiology" will be presented by Dr. Alberto Darzson from the Universidad Nacional Autonoma de Mexico. The first American Society of Andrology/Women in Andrology Lecture will feature a presentation by Dr. Holly Ingraham on "Molecular Insights into Male Reproductive Development." The Buckeye Lecture will be presented by Dr. Deborah Segaloff who will discuss "LH and FSH Receptors: Insights into Reproductive Disorders and Signaling by G-Protein-Coupled Receptors."

A new feature of the Annual Meeting is a symposium discussion that highlights the impact of advances in the reproductive sciences on the public sector and focuses on communication involving scientists, the lay press and the public domain. Ms. Gina Kolata, Science Reporter for the New York Times, and Dr. Michael McClure, Program Director from the National Institute of Environmental Health Science, will discuss how the translation and assimilation of discovery and advances in science impact upon our society now and in the future. The Pharmacia & Upjohn Current Clinical Perspectives symposium on "Gender Assignment and Identity" continues the tradition of presenting expert opinions on provocative topics of current interest. A discussion of current opinions on clinical and psychological aspects surrounding this topic will be presented by Doctors Peter Lee and Kenneth Zucker with the expert synthesis and commentary provided by Dr. Claude Migeon.

Three different symposia on the topics of Cell Fate, Prostate Cancer and Male Germ Cells will be featured on each day of the meeting. The first symposium on Cell Fate will feature Dr. Peter Donovan speaking on germ cells as pluripotent stem cells, Dr. Ronald DePinho addressing telomeres and telomerase activity in the testes and Dr. Kim Boekelheide discussing the role of apoptosis in spermatogenesis. The second symposium focuses on prostate cancer with Drs. Pinchas Cohen discussing novel aspects of the IGF-IGFBP axis, Kathleen Cooney describing how specific genetic loci may determine the genetic susceptibility of individuals to prostate cancer and Peter Nelson presenting the new technology of DNA arrays that can identify androgen regulation of genes in prostate carcinogenesis. The third symposium features three members of our Society who investigate novel aspects of male germ cell development. Dr. Michael Griswold will describe recent experiences with the technique of spermatogonial transplantation. Dr. Marvin Meistrich will discuss how androgens can be used to control spermatogonial differentiation and preserve germ cell viability during cancer therapy, and Dr. Jacquetta Trasler will present her studies on the role of DNA methylation for genomic imprinting in germ cells.

Members, trainees and guests of the Society will participate in three separate 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. We encourage all to take advantage of these opportunities to participate in the vigorous exchange of ideas which typifies our scientific community.

The Annual Meeting is preceded on Friday by the Andrology Laboratory Workshop on the topic of "Media and Techniques for the Gamete Labs" and on Saturday by the Postgraduate Course which ponders the question, "What's It All About? Androgens!" Both programs feature an excellent group of expert speakers.

Terry R. Brown, PhD
Chair, 2000 Program Committee

Program and Abstract Review Committee: Shalender Bhasin, Robert Chapin, Paul Cooke, Gail Cornwall, Janice Evans, Marc Goldstein, Michael Griswold, Norman Hecht, Jonathan Jarow, Dolores Lamb, Patricia Morris, Gail Prins, Bernard Robaire, Kenneth Roberts, Barbara Sanborn, Donald Tindall, and Barry Zirkin.

Serono Award Lectureship

Bert W. O'Malley, MD, is currently the Tom Thompson Distinguished Service Professor of Molecular and Cellular Biology at Baylor College of Medicine. He graduated from the University of Pittsburgh School of Medicine and did his clinical residency at Duke University Medical Center. He spent four years at the NIH where he was a Molecular Biology Section Head at NICHD. After four years at Vanderbilt University as the Lucious Birch Professor and Director of the Reproductive Biology Center, he moved to Baylor College of Medicine as Chairman of Molecular and Cellular Biology where he built one of the finest departments in the country over the next twenty-three years. This department was one of the first of a new generation of departments which combined the merging disciplines of cell structure, molecular biology, and developmental biology into a unified Department of Molecular and Cellular Biology.

Dr. O'Malley's laboratory has been a leader in uncovering the mode of action of the female sex steroids (progesterone and estrogen). Using the chick oviduct system originally, he uncovered the pathway for steroid hormone action and defined the mechanism by which steroid receptors act at the level of DNA to stimulate the initiation of gene transcription. This work has led to our molecular understanding of how hormonal antagonists work and has had major importance to the fields of endocrinology, reproduction, genetic disease, and endocrine cancers of the breast and prostate.

Dr. O'Malley was a founder of the field of Molecular Endocrinology and has trained numerous current leaders in this and related fields. He served as president of the Endocrine Society in 1985. Dr. O'Malley is a member of the National Academy of Sciences and the Institute of Medicine, and he is a fellow of the American Academy of Arts and Sciences and the American Academy of Microbiology. He has published more than 400 papers and holds 16 patents in the fields of Gene Regulation, Molecular Endocrinology and Steroid Receptor Action. He has served on the editorial boards of 14 scientific publications and numerous national committees. Dr. O'Malley has received honorary doctorate degrees from the Karolinska Institute, New York University and the National University of Ireland. Among his many awards and honors he has received the Ernst Oppenheimer Award, the Gregory Pincus Medal, the Lila Gruber Award, the Borden Award, the Dickson Prize, the Axel Munthe Award, the Bicentennial Medallion of Distinction (University of Pittsburgh), the Kodak Award, the Fred Conrad Koch Medal, the D.R. Edwards Medal, and the Doisy Lecture in Biochemistry.

Serono Lectureship Recipients

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

The Serono Lectureship is sponsored by Serono Laboratories, Inc.

Sponsors

The American Society of Andrology wishes to thank the following organizations for their generous support of the 2000 Annual Meeting, Postgraduate Course and Laboratory Workshop.

Gold Club

A minimum $10,000 contribution to an ASA endowment fund

Buckeye Urology and Andrology, Inc

Silver Club

A minimum $5,000 contribution to an ASA endowment fund

West Michigan Reproductive Institute, P.C.

Sustaining Sponsor

A minimum $500 contribution to ASA for five or more years

American Urological Association
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Pharmacia & Uphohn
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GlaxoWellcome Research & Development
Humangen
Mentor
National Institutes of Child Health and Human Development
Grant # R13HD/DK 38750
Pfizer
sigma-tau Consumer Products, a division of
sigma-tau Pharmaceuticals, Inc.
TIMM Medical
US Surgical
Wyeth-Ayerst

2000 Distinguished Andrologist Award

The American Society of Andrology has chosen Dr. Bayard T. Storey as the year 2000 Distinguished Andrologist.
Dr. Storey received a BA in Chemistry from Harvard University in 1952.

After a year at the Technische Hoschschule in Germany, he returned to the U.S. and completed an MS in Chemical Engineering at MIT. From 1955-1958, Dr. Storey attended Harvard once more and obtained an MA and PhD in Chemistry. After completing his training, Dr. Storey entered the work force, becoming an industrial polymer chemist. His work resulted in a patent for weakly basic anion exchange resins. He left industry in 1965 to assume the position of Special Fellow, National Institute of General Medical Studies, Department of Biophysics and Physical Biochemistry at the University of Pennsylvania. In 1967, after completing his fellowship, he joined the faculty of the University of Pennsylvania where he remains to the present. His tenure at the University of Pennsylvania has been noteworthy for appointments to a number of academic departments including Biophysics and Biochemistry, Obstetrics and Gynecology, and Physiology. Most recently, in 1996, he was elevated to the rank of Professor Emeritus of Reproductive Biology and Physiology. He also participates as a member of the Center for Research on Reproduction and Women's Health.

Over the years, Dr. Storey's research interests have been extensive and varied. Initially, he studied oxidative metabolism in plants. Subsequently, his interest in bioenergetics prompted him to investigate mitochondrial metabolism in sperm, and his career has since been dedicated to the study of numerous aspects of sperm physiology. Dr. Storey cites four areas of research in which he feels he has made notable contributions: 1) mammalian sperm energy metabolism; (2) induction of the acrosome reaction by the zona pellucida; (3) mechanisms and effects of lipid peroxidation in mammalian sperm; and (4) sperm cryopreservation. Although much of his work has been in the realm of basic science, the processes he has elucidated have had direct relevance to issues and practices in andrology such as the production of media, contraception development, and sperm function assessment. In recognition of his prolific and fundamental contributions to the field of Reproductive Biology, Dr. Storey was invited to participate as a member of the NIH Reproductive Biology Study Section from 1986-1990 and, over the years, he has served on the editorial boards of *Biology of Reproduction, Journal of Andrology,* and *Molecular Human Reproduction*. In 1991, the esteem of his peers was reflected in his selection as chairman of the prestigious Gordon Research Conference on Fertilization and Activation of Development, a position he continues to hold.

Dr. Storey has long been a supporter of the American Society of Andrology having attended and participated in meetings on a regular basis and having served on both the Executive Council and on the Awards Committee. He is admired as a promoter of young investigators in general and an advocate of female scientists in particular. Indeed, he observes that "The Society has been fortunate in having women as a solid percentage of its membership. The field of reproductive biology, in general, has attracted many women, such that the field gets the full use of the intellectual talents of both sexes." He further suggests that "a little more recognition could be given to the women in our Society in the years of the next century. One could hope that the Society would recognize its good fortune and seek to obtain higher visibility for its women members."

In an academic career that has spanned over thirty years, Dr. Storey has produced a prodigious body of literature elucidating numerous aspects of mammalian sperm physiology, and he has launched the careers of many accomplished investigators who have already moved research in andrology into the next century. This legacy alone would justify an award, but Dr. Storey's colleagues and former fellows and students are quick to point out that he is an exemplary individual who also deserves recognition for his "encyclopedic" knowledge of diverse disciplines, his integrity, his lack of scientific jealousy, and his *joie de vivre.* For all of these attributes, for his contributions as mentor and scientist, and for his lifelong dedication to the field of andrology, the American Society of Andrology is delighted to honor Dr. Bayard Storey with the 2000 Distinguished Andrologist Award.

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

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

2000 Distinguished Service Award

Dr. Bernard Robaire is the recipient of the 2000 Distinguished Service Award. It is perhaps Dr. Robaire's multicultural background that has made him so successful as a consensus builder and leader within the andrology community. Born in Tunisia, Dr. Robaire trained in both the U.S. and Canada. He received a BA in Bacteriology from UCLA in 1970, then, only four years later, completed his PhD in Pharmacology and Therapeutics at McGill University in Montreal. Returning to the U.S., Dr. Robaire became an NIH postdoctoral fellow in the Department of Population Dynamics, School of Hygiene and Public Health at Johns Hopkins University. Following a brief tenure (1977-1979) as Associate Scientist at the Royal Victoria Hospital in Montreal, he joined the faculty at McGill University as assistant professor in both the Department of Pharmacology and Therapeutics and the Department of Obstetrics and Gynecology. Over the ensuing 21 years, he ascended to the rank of associate professor and professor, titles he currently holds. Along the way, Dr. Robaire served as director of the Centre for the Study of Reproduction and executive director of the Centre for Medicine, Ethics and Law at McGill. Since 1980, he has also had an appointment to the Royal Victoria Hospital as medical scientist. From 1993-1998, Dr. Robaire held the office of Associate Vice-Principal for Research at McGill. Throughout his distinguished career, he has mentored a number of graduate students and postdoctoral fellows which, he feels, is his most significant accomplishment and whose success is a great source of pride.

As a scientist, Dr. Robaire has made significant contributions to our knowledge of epididymal physiology, sperm-egg interactions, and reproductive toxicology. Most recently, he has explored age-related changes in the seminiferous epithelium and epididymal tubules of the rat.

Dr. Robaire has been instrumental in maintaining ASA's viability as an organization. Over the past 23 years, he has served the Society in a number of capacities as both a leader and an ambassador. He has been a member of the Awards Committee (1980-1982), Executive Council member (1983-1986), chairman of the Nominating Committee (1987-1988), member of the Nominations Committee (1988-1989), chairman of the Local Arrangements Committee (1990-1991), and member of the Long-Range Planning Committee (1994-1996). He held the post of vice-president of ASA from 1992-1993, then was president from 1993-1994. During his tenure as president, he instituted a course for primary care physicians, created and developed The Andrology Handbook, accumulated a large surplus in operating funds, and established an association between the Testis Workshop and the ASA Annual Meeting. Most recently, he has chaired the Future Meetings Committee, and he was instrumental in bringing the upcoming International Society of Andrology meeting to Montreal. It will be held in conjunction with the ASA Annual Meeting in 2001.

Dr. Robaire envisions the ASA having a pivotal role in the science and politics of andrology in the future. In his view, the organization must continue to promote andrology research, to educate the government, the press, and the public regarding key issues in andrology, and to help establish ethical systems to govern human reproduction.

For his dedicated service to the Society, exemplary leadership and vision, and devotion to scientific endeavor and the training of young scientists, the ASA is pleased to bestow on Dr. Bernard Robaire the 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

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

2000 Young Andrologist Award

Matthew P. Hardy, the recipient of the 2000 Young Andrologist Award, currently holds a dual appointment as scientist, Center for Biomedical Research at The Population Council and as assistant professor, Rockefeller University, both in New York City. A graduate of Oberlin College where he received his degree in two disciplines, biology and philosophy, Dr. Hardy went on to complete his PhD in biology at the University of Virginia. His dissertation research earned him the James Norman Denton Award in 1986. He then embarked on a postdoctoral fellowship in reproductive biology with Dr. Larry Ewing at Johns Hopkins University. It was there that his focus on Leydig cell research began. Perhaps his greatest accomplishment during his postdoctoral years was developing, in collaboration with Dr. Gary Klinefelter, novel techniques for the isolation and culture of Leydig cells. This methodology revolutionized the study of Leydig cells because it allowed them to maintain their steroidogenic properties in vitro.

After a brief tenure as research associate in the Department of Population Dynamics, School of Hygiene and Public Health at Johns Hopkins, Dr. Hardy joined The Population Council as staff scientist in 1991. According to his colleague, Dr. Gary Klinefelter, he rapidly established himself as a leader in the field of Leydig cell biology, carrying out investigations into "the ontogeny, physiology, and, more recently, toxicology of the Leydig cell." During the six years he has been at The Population Council, Dr. Hardy has mentored seven postdoctoral fellows and has been awarded three NIH grants, and in his relatively brief career, he has published 50 papers and has been honored with numerous invited lectureships. His accomplishments have earned him the respect and admiration of his fellow scientists resulting in multiple productive collaborations and positions on the editorial boards of three prestigious journals. It is a particular tribute to Dr. Hardy's expertise that he was asked to co-edit the text, *The Leydig Cell,* which is considered the definitive reference on the subject.

Throughout his career, Dr. Hardy has been a member and active supporter of the American Society of Andrology, having chaired the Bylaws Committee, served on the Program Committee, and been elected to the Executive Council. He cites the ASA for contributing to his professional growth: "The small size of the Society, I believe, facilitates the interactions between its clinical and basic science members. These interactions serve to enhance and perpetuate research in male reproductive biology."

Because Dr. Matthew Hardy has made extensive and significant contributions in the field of Leydig cell biology and has provided dedicated service to the ASA, it is the distinct pleasure of the Society to award him the 2000 Young Andrologist Award.

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

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

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 2000 New Investigator Award will be announced at the awards ceremony at 2:45 pm on Monday, April 10, 2000.

New Investigator Award Recipients

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

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

Course Objectives & CME Credit Information

Annual Meeting

Following this program the participant should be able to:

- Recognize the role of nuclear hormone receptors in reproduction and development
- Relate the technological advances in tissue engineering to their importance in the clinic
- Describe the role of gonadotropins in spermatogenesis and steroidogenesis in the testis
- Describe the role of genetics, growth factors and specific genes in the progression of prostate cancer
- Understand the role of developmentally important genes and the mechanisms of transcriptional control during spermatogenesis and fertilization

This program has 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.

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

Postgraduate Course

Following this program the participant should be able to:

- Describe the role of androgens in the development and promotion of prostate disease, both at the molecular level and clinically
- Describe new medical therapies for erectile dysfunction
- Understand the molecular action of androgens in regulating the expression of certain genes
- Describe the functions, regulations, and roles of the androgen receptor and the steroid 5 alpha reductase isozymes
- Describe the current knowledge concerning the process and control of steroidogenesis by the Leydig cell
- Describe the current knowledge about the use of androgens in women

This program has 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.

The University of Minnesota designates this continuing medical education activity for 6.5 hours in category I credit toward the AMA Physician's Recognition Award. Each physician should claim only those hours of credit actually spent in the educational activity.

Andrology Laboratory Workshop

After attending this course the participant will be able to:

- Identify and use different media available for the gamete lab
- Find the best strategy to cryopreserve ejaculated, epididymal and testicular sperm
- Understand ways to improve ICSI results
- Get an update on egg freezing
- Re-evaluate egg/sperm contribution to embryo quality

Continuing education units (CEUs) will be granted pending review by the American Board of Bioanalysis (ABB).

Postgraduate Course: What's It All About? Androgens!

Course Description

The 2000 Postgraduate Course will include presentations on the action of androgens and estrogens in prostate diseases, androgen regulation of gene expression, androgen receptor regulation, function and dysfunction, among others.

Saturday, April 8, 2000

Grand Ballroom A

8:00 am	INTRODUCTION *J. Lisa Tenover, MD, PhD, 2000 Postgraduate Course Committee Chair*
8:10 am	ACTION OF ANDROGENS AND ESTROGENS IN PROSTATE DISEASES *Leland W. K. Chung, PhD, University of Virginia*
9:00 am	TESTOSTERONE AND PROSTATE DISEASE: WHAT'S THE REAL DEAL? *Abraham Morgentaler, MD, Harvard Medical School*
9:50 am	COFFEE BREAK
10:10 am	ANDROGEN REGULATION OF GENE EXPRESSION *Diane M. Robins, PhD, University of Michigan Medical School*
11:00 am	ERECTILE DYSFUNCTION — CURRENT AND FUTURE THERAPIES *Ronald W. Lewis, MD, Medical College of Georgia*
11:50 am	LUNCH (ON YOUR OWN)
1:00 pm	ANDROGEN RECEPTOR — REGULATION, FUNCTION AND DYSFUNCTION *Arun K. Roy, PhD, University of Texas*
1:50 pm	ANDROGEN USE IN WOMEN *Susan R. Davis, MBBS, PhD, Jean Hailes Foundation*
2:40 pm	REFRESHMENT BREAK
3:00 pm	LEYDIG CELL STEROIDOGENESIS *Matthew P. Hardy, PhD, Population Council*
3:50 pm	PHYSIOLOGICAL ROLES OF STEROID 5-ALPHA REDUCTASE ISOZYMES *David W. Russell, PhD, University of Texas Southwestern Medical Center*
5:00 pm	CONCLUSION

Saturday, April 8, 2000

5:30 pm WELCOME RECEPTION
Riverfront

7:30 pm WELCOME & OPENING REMARKS
Grand Ballroom A
Barry T. Hinton, PhD, President, and Robert A. Newton, MD, Local Arrangements Co-Chair

ERNST SCHERING RESEARCH FOUNDATION LECTURE
Chair: Barry T. Hinton, PhD
Clinical Investigative and Genetic Approaches to the Male Reproductive System
William Crowley, Jr., MD, Harvard Medical School

Sunday, April 9, 2000

8:00 am SERONO LECTURE
Grand Ballroom A
Chair: Dolores J. Lamb, PhD
Co-activators: The Power Boosters for Steroid Hormone Action
Bert O'Malley, MD, Baylor College of Medicine

9:00 am PRESENTATION OF THE DISTINGUISHED ANDROLOGIST AWARD TO BAYARD STOREY, PhD
Grand Ballroom A
Sponsored by the American Society of Andrology

9:15 am ORAL SESSION I: Germ Cells in the Testis
Park View East Tower
Chairs: Patricia Morris, PhD and Wael Salameh, PhD

1 Characterization of the Spermatogenic Cell Cycle Defects in the A-MYB Knockout Mice
T.B. Guo, W.A. Salameh, P.N. Huynh, A.P. Sinha-Hikim, X. Chen, C. Wang, A. Toscani, E.P. Reddy, R.S. Swerdloff

2 Sertoli Cell Mitochondrial Pro- and Anti- Apoptotic Proteins are Regulated by Steroid Hormones and Interferon-Gamma
H. Abdel-Haq, J.F. Catterall, P.L. Morris

3 Redistribution of Bax is an Early Step in an Apoptotic Pathway Leading to Germ Cell Death Triggered by Mild Testicular Hyperthermia
C.M. Yamamoto, A.P. Sinha-Hikim, B. Shapiro, P.N. Huynh, Y. Lue, W.A. Salameh, C. Wang, R.S. Swerdloff

4 Molecular Pathway of Germ Cell Apoptosis Following Acute Ischemia in the Rodent Testis
J.J. Lysiak, S.D. Turner, T.T. Turner

5 Progressive Loss of Germ Cells by Apoptosis in XXY Male Mouse: An Excellent Experimental Model for Klinefelter Syndrome
Y.H. Lue, P.N. Rao, A.P. Sinha Hikim, M. Im, W.A. Salameh, P. Yen, C. Wang, R.S. Swerdloff

10:30 am COFFEE BREAK IN THE EXHIBIT HALLS
Grand Ballroom B and Skyline Suites

11:00 am REPRODUCTIVE SCIENCES AND THE PUBLIC
Grand Ballroom A
Chair: Barry Zirkin, PhD
Reporting on Human Reproduction in the Third Millennium Theater
Gina Kolata, The New York Times

Human Reproduction: The Third Millennium Theater
Michael E. McClure, PhD, NIEHS

12:15 pm WOMEN IN ANDROLOGY LUNCHEON
University B & C
Declining Male Reproductive Health
Dolores J. Lamb, PhD

1:30 pm SYMPOSIUM I: CELL FATE
Grand Ballroom A
Chairs: Christina Wang, MD, and Martin Dym, PhD
Pluripotent Stem Cells, Germ Cells and Testicular Cancer
Peter Donovan, PhD, Kimmel Cancer Institute, Thomas Jefferson University

Telomeres, Telomerase and Testes
Ronald DePinho, MD, Harvard Medical School

Consequences of Excessive and Deficient Testicular Germ Cell Apoptosis
Kim Boekelheide, MD, PhD, Brown University

3:00 pm REFRESHMENT BREAK IN THE EXHIBIT HALLS
Grand Ballroom B and Skyline Suites

3:30 pm WOMEN IN ANDROLOGY LECTURE
Grand Ballroom A
Molecular Insights into Male Reproductive Development
Holly A. Ingraham, PhD, University of California, San Francisco

4:30 pm POSTER SESSION I
Grand Ballroom B and Skyline Suites
Wine and Cheese Reception sponsored by Hamilton Thorne Research

7:00 PM ANNUAL BANQUET
Boston Museum of Science
The Museum is within easy walking distance of the headquarters hotel

Monday, April 10, 2000

7:00 am PAST PRESIDENTS' BREAKFAST
Somerset Room

8:00 am BUCKEYE STATE-OF-THE-ART LECTURE
Grand Ballroom A
Chair: Matthew Hardy, PhD
LH and FSH Receptors: Insights into Reproductive Disorders and Signaling by G-Protein-Coupled Receptors
Deborah Segaloff, PhD, University of Iowa

9:00 am PRESENTATION OF THE YOUNG ANDROLOGIST AWARD TO MATTHEW P. HARDY, PhD
Grand Ballroom A
Sponsored by the Texas Institute for Reproductive Medicine and Endocrinology, P.A.

PRESENTATION OF THE DISTINGUISHED SERVICE AWARD TO BERNARD ROBAIRE, PhD
Grand Ballroom A
Sponsored by Genetics and IVF Institute

9:15 am AMERICAN UROLOGICAL ASSOCIATION LECTURE
Grand Ballroom A
Chair: Arnold Belker, MD
Tissue Engineering in the Genitourinary System
Anthony Atala, MD, Harvard Medical School

10:15 am COFFEE BREAK IN THE EXHIBIT HALLS
Grand Ballroom B and Skyline Suites

10:45 am ORAL SESSION II: Gene Expression in the Male Reproductive Tract (SIMULTANEOUS SESSION)
Grand Ballroom A
Chairs: Gail Cornwall, PhD, and Kenneth Roberts, PhD

82 Osteotesticular Protein Tyrosine Phosphatase (OST-PTP) Expression in Testis
M.R. Maduro, E.A. Olmsted, A.R. Davis, D.J. Lamb

83 Cloning of the Rat GATA-4 Gene Promoter and Characterization of 5' Regulatory Elements Required for Sertoli Cell-Specific Expression
E. Legault, R.S. Viger

84 Developmental Regulation of Leydig Cell Androgens by MIS
M.M. Lee, R.D. Tien, E. Niu, C.M. Sottas, M.P. Hardy

85 Connexin 43 (α1) and Connexin 32 (β1) Ontogeny in the Rat Prostate and Effects of Neonatal Estrogen Exposure
H. Habermann, P. Mehta, G.S. Prins

86 Increased Androgen Receptor Expression Correlates With Development of Age-Dependent, Lobe-Specific Spontaneous Hyperplasia of the Brown Norway Rat Prostate
P.P. Banerjee, S. Banerjee, T.R. Brown

87 Identification of Fibroblast Growth Factor Receptors in the Rat Epididymis: Possible Initiators of Signaling Pathways Involved in Regulation of GGT mRNA IV Expression
J.L. Kirby, R.J. Lye, J.C. Labus, B.T. Hinton

10:45 am ORAL SESSION III: Sperm Function and Fertility (SIMULTANEOUS SESSION)
Park View East Tower
Chairs: Erol Onel, MD and Patricia Olds-Clarke, PhD

88 Analysis of Infertility in Male Mice Deficient in Inositol Polyphosphate 5-Phosphatase (INPP5B): Results From Assays for Sperm Function
J.P. Evans, E. Hellsten, R.L. Nussbaum

89 Murine Germ Cells Do Not Require Functional Androgen Receptors to Undergo Meiosis Following Spermatogonial Stem Cell Transplantation into Normal Mouse Testes
D.S. Johnston, M.D. Griswold

90 Identification of mRNA Transcripts Associated With Human Spermatogenesis: Feasibility of Differential Display Polymerase Chain Reaction (ddPCR) Technique
A. Mielnik, P.T.K. Chan, P.N. Schlegel

91 Evidence for the Presence of L-Type Ca^{2+} Channels in Human Spermatozoa
J.C.K. Brown, C. Bray, C.L.R. Barratt, S.J. Publicover

92 Nitric Oxide Regulates the cAMP Pathway of Human Spermatozoa During Capacitation
M.B. Herrero, S. Chatterjee, L. Lefièvre, C. Gagnon

93 The cGMP-Specific Phosphodiesterase Inhibitor, Sildenafil, Stimulates Sperm Motility, Hyper-Activation and Capacitation
L. Lefièvre, E. de Lamirande, C. Gagnon

12:15 pm LUNCH Editorial Board (Univ. A)

LABORATORY SCIENCE FORUM LUNCHEON MEETING
Somerset Room
Development and Evaluation of Vaginal Antimicrobial Contraceptive Compounds
Lourens J.D. Zaneveld, DVD, PhD, Rush University

call Ryan + answer email

1:15 pm SYMPOSIUM II: PROSTATE CANCER
Grand Ballroom A
Chairs: Gail Prins, PhD, and Glenn Cunningham, MD
Novel Aspects of the IGF-IGFBP Axis in Prostate Cancer
Pinchas Cohen, MD, Mattel Children's Hospital - UCLA

Finding Genes that Contribute to Prostate Cancer Susceptibility
Kathleen A. Cooney, MD, University of Michigan

Gene Expression Profiling of the Human Prostate Androgen-Response Program
Peter Nelson, MD, Fred Hutchinson Cancer Research Center

2:45 pm ASA ANNUAL BUSINESS MEETING AND AWARDS PRESENTATION
Grand Ballroom A
New Investigator Award
Sponsored by the West Michigan Reproductive Institute, P.C.
Outstanding Original Research Awards
Research Excellence Award for Female Trainee/Fellow
Established by Anna Steinberger, PhD, supported by Women in Andrology
Trainee Merit Awards
Thomas S.K. Chang Trainee Travel Fund Awards
Burroughs-Wellcome Fund Award for Outstanding Trainee Research

3:15 Archives
3:30 pm REFRESHMENT BREAK AND RAFFLE DRAWING IN EXHIBIT HALLS
Grand Ballroom B and Skyline Suites

4:00 pm PHARMACIA & UPJOHN CURRENT CLINICAL PERSPECTIVES
Grand Ballroom A
Gender Assignment and Gender Identity
Moderator: Claude J. Migeon, MD, Johns Hopkins University School of Medicine
Peter A. Lee, MD, PhD, The Milton S. Hershey Medical Center
Kenneth J. Zucker, PhD, Center for Addiction and Mental Health - Clarke Division

5:15 Archives
5:30 pm POSTER SESSION II
Grand Ballroom B and Skyline Suites

7:00 pm TRAINEE COLLOQUIUM
Somerset Room
Can Male Reproductive Tissues/Cells be Utilized Outside of the Reproductive System: Andrology Not Just for Andrologists
Paul R. Sanberg, PhD, DSc, University of South Florida College of Medicine

8:30 pm TRAINEE SOIRÉE
Somerset Room

Tuesday, April 11, 2000

8:00 am LATIN AMERICAN LECTURE
Grand Ballroom A
Chair: Carlos Suarez-Quian, PhD
Calcium Channels and Sperm Physiology
Alberto Darszon, PhD, Universidad Nacional Autonoma de Mexico

9:00 am SYMPOSIUM III: MALE GERM CELLS
Grand Ballroom A
Chairs: Norman Hecht, PhD, and Janice Bailey, PhD
Potential and Limitations of Spermatogonial Transplantation
Michael D. Griswold, PhD, Washington State University

Inhibition of Spermatogonial Differentiation by Androgens
Marvin L. Meistrich, PhD, University of Texas, MD Anderson Cancer Center

DNA Methylation in Germ Cells: Implications for Genomic Imprinting
Jacquetta M. Trasler, MD, PhD, McGill University

10:30 AM CONCLUSION

Future Meetings

2001: Montréal, Québec, Canada
VII International Congress of Andrology,
hosted by the American Society of Andrology
June 15-19

2002: Seattle, Washington
Laboratory Workshop: April 24
Annual Meeting: April 25 - 28
Postgraduate Course: April 29

2003: New York, New York

For additional information on all meetings, contact:
American Society of Andrology
74 New Montgomery, Suite 230
San Francisco, CA 94105
Phone: (415) 764-4823 Fax (415) 764-4915
Email: asa@hp-assoc.com
URL: www.cvm.uiuc.edu/~r-hess/asa/front/html

Laboratory Workshop: Media & Techniques for the Gamete Labs

Course Description

This year's Laboratory Workshop continues our practice of presenting practical information on modern andrology laboratory testing. Focusing on "Media and Techniques for the Gamete Labs," the sessions consist of a review of media used in the ART laboratories and sperm preparation for IUI, IVF and ICSI. Other sessions will deal with sperm cryodamage and strategies to improve outcome, epididymal and testicular sperm cryopreservation, human egg and embryo freezing and poor embryo development.

This course is specifically designed for andrologists, whether clinician, laboratory director, biologist, technician, postgraduate researcher or student.

Friday, April 7, 2000

Grand Ballroom A

8:00 am	INTRODUCTION *Pasquale Patrizio, MD, 2000 Andrology Laboratories Committee Chair*
8:15 am	REVIEW OF MEDIA USED IN THE ART LABORATORIES *Patrick Quinn, PhD, HCLD, SAGE BioPharma*
9:10 am	SPERM PREPARATION FOR IUI AND IVF *David Mortimer, PhD, Genesis Fertility Center*
10:00 am	COFFEE BREAK
10:20 am	SPERM PREPARATION FOR ICSI AND TECHNICAL DETAILS *Gianpiero Palermo, MD, Cornell University*
11:15 am	SPERM CRYODAMAGE AND STRATEGIES TO IMPROVE OUTCOME *Sergio C. Oehninger, MD, Jones Institute for Reproductive Medicine*
12:00 Noon	LUNCH (ON YOUR OWN)
1:30 pm	EPIDIDYMAL AND TESTICULAR SPERM CRYOPRESERVATION *Pasquale Patrizio, MD, University of Pennsylvania*
2:10 pm	HUMAN EGG AND EMBRYO FREEZING *Michael John Tucker, PhD, FI Biol., Georgia Reproductive Specialists*
3:15 pm	REFRESHMENT BREAK
3:35 pm	POOR EMBRYO DEVELOPMENT: WHERE DOES THE SPERM FIT IN? *Denny Sakkas, PhD, Birmingham Women's Hospital*
4:30 pm	PANEL DISCUSSION

Poster Session 1

6 Expression of P26h Transcript, an Acrosomal Sperm Protein, in Germ Cells of Post Pubertal Hamster / *C. Gaudreault, C. Légaré, R. Sullivan*

7 Identification and Characterization of the P31m, A Monkey Sperm Protein / *N. Lamontagne, C. Légaré, C. Gaudreault, R. Sullivan*

8 Immunolocalization of Aquaporins 1, 8 and 9 in the Testis and Epididymis of Adult Rats / *H. Badran, L. Hermo*

9 Ultrastructure of the Vas Deferens (VD) From Healthy Non-Vasectomized and Vasectomized Patients / *S. Andonian, P. Durie, V. Mak, A. Zini, K. Jarvi, L. Hermo*

10 Localization of Estrogen Receptor α in the Reproductive Tract of Adult Male Dogs and Cats: An Immunohistochemistry Study / *R. Nie, Q. Zhou, E. Jassim, R.A. Hess*

11 Immunolocalization of Several Steroid Hormone Receptors in Efferent Ductules of Adult Rat / *Q. Zhou, R. Nie, R.A. Hess*

12 Promoter and Splicing mRNA Variants of the Epididymis-Specific Gene EP2 in Rhesus Monkey (*M. mulatta*) / *O. Fröhlich, C. Po, L. Young*

13 Genomic Structure of the Human Epididymal EP2 Gene / *O. Fröhlich, C. Po, N. Ibrahim, L. Young*

14 Effects of Inhibin on Androgen-Stimulated Caput Epididymal Gene Expression in the Cynomolgus Monkey / *M. Mahony, B. Billeter*

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CHARACTERIZATION OF THE SPERMATOGENIC CELL CYCLE DEFECTS IN THE A-MYB KNOCKOUT MICE. T.B. Guo*[1], W.A. Salameh[1], P.N. Huynh[1], A.P. Sinha-Hikim[1], X. Chen*[1], C. Wang[1], A. Toscani*[2], E.P. Reddy*[2], and R.S. Swerdloff[1]. [1]Div. Of Endocrinology, Harbor-UCLA Medical Center, Torrance, CA and [2]Fels Institute for Cancer Research, Temple University School of Medicine, Philadelphia, PA.

A-myb, a member of the Myb family of proto-oncogenes, is a transcription factor that regulates cell cycle events during G1/S-transition and S phase progression in somatic cells and is regulated by cyclin A2/cyclin dependent kinase 2 complex. A-myb is abundantly expressed in the spermatogonia (SPGs), preleptotene (PL) and pachytene (P) spermatocytes. Previous work on A-myb knockout (KO) mice uncovered a critical role for A-myb during spermatogenesis, in that the KO mice were azoospermic due to an apparent spermatogenic arrest at the P stage. Light microscopy of KO mice testes confirms the block at mid G2 of meiosis 1 and shows vacuolization of Sertoli cells and apoptotic germ cells. Electron microscopy further revealed that while the somatic cells, SPGs and PL cells were normal ultrastructurally, P cells displayed features typical of germ cell apoptosis. Notably, the synaptonemal complex could occasionally be observed in the apoptotic P cells. Flow cytometry cells stained with propidium iodide showed an aberrant cell-cycle distribution of testicular cells in the KO mice (n=4). A dramatic increase of signals to the left of the 1N gating was consistently observed. This likely reflected cells in various stages of apoptosis as seen in the LM and EM. A residual 1N peak was detected in the KO mouse samples, although no haploid cells could be discerned through microscopy. There were no other significant alterations in the relative abundance of 2N, 4N cell populations and S phase cells. To assess the possible role of A-myb in the G1/S-transition of in SPGs and PL cells, we are performing formal comparative morphometric analysis in adult KO mice and wild type controls. In addition, we are studying testis sections of KO mice sacrificed on days 6, 8, 10, 15 and 18 postpartum. Preliminary results show evidence of increased incidence of P cell apoptosis at day 18 when the first wave of P cells reach the diplotene stage and then undergo meiotic divisions. Thus, in addition to its role in G1/S-transition in somatic cells, A-myb plays a critical role in G2 or G2/M-transition of meiosis I akin to that of cyclin A1 in mouse testes. A functional relationship between these two proteins is under investigation.

2

SERTOLI CELL MITOCHONDRIAL PRO- AND ANTI- APOPTOTIC PROTEINS ARE REGULATED BY STEROID HORMONES AND INTERFERON-GAMMA. H. Abdel-Haq*, J. F. Catterall and P. L. Morris, Center for Biomedical Research, Population Council and The Rockefeller University. NY, NY 10021.

Previously we showed that interferon gamma (IFNγ) regulates the expression of interleukin 1-beta converting enzyme (ICE, Caspase-1) gene expression in Sertoli cells (Kanzaki and Morris, 1998). In particular cell types, Caspase-1 can process pro-IL1β to its active form favoring either cell death or survival. Caspases are cysteine proteases that mediate apoptosis by proteolysis of specific substrates in sensitive cells. Caspase-3 is involved in cleavage of the androgen receptor (AR). By itself, IFNγ does not increase programmed cell death of primary Sertoli cells, findings suggestive that this testicular cell has mechanisms to counterbalance certain "pro-death" signals. Potential survival components include those that mediate testicular androgen regulation. As mitochondria are critical elements of the apoptotic cell death machinery, we determined the sub-cellular distribution of the anti-apoptotic protein Bcl-xL and the pro-death protein BAX following treatment (0-12 h) of Sertoli cells with androgen (T), IFNγ, or glucocorticoid (DEX). Proteins from control and treated Sertoli cells were fractionated into cytoplasmic, nuclear and mitochondrial extracts for Western analyses. As expected, T increased cytoplasmic and nuclear levels of AR but not GR. Both Dexamethasone (DEX) and IFNγ transiently increased mitochondrial BAX and reduced mitochondrial Bcl-xL. T alone did not affect mitochondrial levels of Bcl-xL but abrogated the effects of IFNγ. DEX increased both cytoplasmic Caspase-1 and -3 levels. In all groups, we failed to detect Caspase-3 in mitochondria. Taken together, our findings suggest that both the sub-cellular localization and the ratios of the proteins contributing to Sertoli cell survival are regulated by steroid hormones as well as cytokines. These studies were supported by NICHD/NIH U54-13541 as part of the Specialized Cooperative Centers Program in Reproduction Research.

3

REDISTRIBUTION OF BAX IS AN EARLY STEP IN AN APOPTOTIC PATHWAY LEADING TO GERM CELL DEATH TRIGGERED BY MILD TESTICULAR HYPERTHERMIA. C.M. Yamamoto*, A.P. Sinha Hikim, B. Shapiro*, P.N. Huynh, Y. Lue, W.A. Salameh, C. Wang, and R.S. Swerdloff, Department of Medicine, Harbor-UCLA Medical Center, Torrance, CA.

Programmed cell death occurs spontaneously during spermatogenesis and is induced in a stage- and cell-specific manner by mild testicular hyperthermia. Studies with transgenic and knockout mice suggest that members of the Bcl-2 family are important regulators of germ cell apoptosis. The molecular mechanisms by which heat induces germ cell apoptosis, however, remain poorly understood. To delineate the apoptotic pathways involved, we examined the temporal changes in the pro- and anti-apoptotic Bcl-2 family members (Bax and Bcl-2, respectively) in rat testes after transient exposure to heat. Testes of adult male rats were exposed to 22°C (control) or 43°C (experimental) for 15 minutes. Animals were sacrificed 0.5, 2, 6 and 24 hours after heat exposure. As previously reported, local testicular heating resulted in stage-specific activation of germ cell apoptosis within 6h, involving early (I-IV) and late (XII-XIV) stages. Initiation of germ cell apoptosis was preceded by a redistribution of Bax from a cytoplasmic to perinuclear or nuclear localization as assessed by immunohistochemistry. In contrast, Bcl-2 subcellular localization remained diffusely cytoplasmic in those cell types susceptible to heat-induced apoptosis. Despite the striking redistribution, Bax levels remained unchanged as determined by Western analysis; Bcl-2 levels increased significantly by 6h after heat exposure. Northern blot and RT-PCR analyses indicated no change in either Bax or Bcl-2 mRNA levels in response to heat suggesting the involvement of post-transcriptional rather than transcriptional mechanisms mediating their activity. The marked subcellular redistribution of Bax prior to activation of apoptosis and the possible compensatory increase in Bcl-2 suggest an involvement of Bcl-2 family members in heat-induced apoptotic death of germ cells.

4

MOLECULAR PATHWAY OF GERM CELL APOPTOSIS FOLLOWING ACUTE ISCHEMIA IN THE RODENT TESTIS
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Testicular ischemia induced by torsion of the spermatic cord results in aspermatogenesis. Previous studies from this laboratory employing the TUNEL technique and DNA laddering have demonstrated this aspermatogenesis is due to germ cell-specific apoptosis (Biol. Reprod. 1997; 57, 1267-1274). The present study was designed to investigate the molecular apoptotic pathway in germ cells as well as the potential role of polymorphonuclear cells (PMNs) in germ cell apoptosis following acute ischemia in the murine and rat testis. Animals were subjected to bilateral 1h (rat) or 2h (mouse) ischemia-inducing torsion and testes harvested at 1, 2, 4, and 24h after reperfusion. RNA from rat seminiferous tubules was examined for the presence and regulation of specific apoptotic molecules by RNase protection assay. In addition, immunostaining for apoptotic cells and evaluation of PMN recruitment was performed on mouse and rat tissue sections. Results revealed Bcl-X_L was the most prominent mRNA species detected. Caspases 1, 2, 3, and Bax mRNA were consistently upregulated when compared to sham controls; however, the time of upregulation after torsion was variable. Bcl-X_L and Bcl-X_S were less consistently upregulated. Immunostaining of both rat and mouse testis sections with F7-26, an antibody to ssDNA (Apostain), revealed an increase in germ cell apoptosis reaching a maximum at 24h after repair of torsion. Germ cells of stages II-III were the predominant early responders to the apoptotic stimuli. An increase in PMN recruitment to subtunical venules was also noted 4h after torsion. Mice deficient for E-Selectin, a glycoprotein important for PMN adhesion to endothelial cells, showed decreased PMN margination compared to wild-type animals at 4h post torsion. E-Selectin knockout mice also demonstrated decreased germ cell-specifc apoptosis at 4 and 24h post torsion. Results from these studies suggest a potential role for PMNs in torsion induced germ cell-specific apoptosis, and that the molecular apoptotic pathway involves the proapoptotic mitochondrial associated molecule Bax and the caspases 1, 2, and 3. This work was sponsored by NIH grant DK53072.

5

PROGRESSIVE LOSS OF GERM CELLS BY APOPTOSIS IN XXY MALE MOUSE: AN EXCELLENT EXPERIMENTAL MODEL FOR KLINEFELTER SYNDROME. YH Lue, *PN Rao, AP Sinha Hikim, M Im, WA Salameh, P Yen, C Wang and RS Swerdloff, Dept. of Medicine, Harbor-UCLA Medical Center, Research and Education Institute, Torrance, CA. *Pathology and Lab Medicine, UCLA School of Medicine, CA.

It has been reported that injecting male embryonic stem cells into the blastocele of female embryos produces female chimeras capable of transmitting the embryonic stem cell genome resulting in a high incidence of XXY male mice in their offspring (PNAS, 1995;92:3120-3123). The objective of the present study was to characterize testicular phenotype of the XXY mice. Using the breeding scheme described above, 4 adult, 1 three-day, 1 ten-day old XXY mice and 1 adult XYY mouse and their age matched littermate male mice (XY, normal control) were studied. Standard cytogenetic analyses were performed on culture fibroblasts obtained from earclips which were further confirmed by southern blotting and X chromosome painting using FISH technique. The apoptosis was detected by TUNEL assay. The results showed that the testis weight in adult XXY mice (0.02±0.01 g) was dramatically decreased than control (0.11±0.01 g). No significant differences were apparent in serum testosterone levels. Testes from 3d- and 10d-old mice exhibited fewer prespermatogonia and spermatogonia, as a result of accelerated apoptosis. Histology from adult XXY mice showed small seminiferous tubules with varying degrees of intraepithelial vacuolization and complete absence of germ cells. Hyperplasia of Leydig cells was noted in interstitium. Electron microscopic examination showed Sertoli cells containing scanty amounts of cytoplasm and an irregular nucleus with prominent nucleolus. Junctional regions between Sertoli cells appeared normal. In some tubules, a "nest" of apparently degenerating Sertoli cells was found. Histology from adult XYY mouse showed overall normal spermatogenesis with patchy distribution of disrupted tubules containing apoptotic germ cells. We conclude that progressive loss of germ cells via apoptosis occurs from postnatal to adult resulting in complete absence of germ cells in the adult XXY mice. The XXY mouse is an excellent experimental model for its human XXY counterpart Klinefelter syndrome.

6

EXPRESSION OF P26h TRANSCRIPT, AN ACROSOMAL SPERM PROTEIN, IN GERM CELLS OF POST PUBERTAL HAMSTER .

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We have previously described P26h, a hamster sperm protein, proposed to be involved in gamete interactions. P26h is acquired by sperm during epididymal transit under androgen control. Moreover we have shown that the P26h transcript (1081 bp) is mainly expressed in the testis and encodes for a 244 amino acids protein. In this study, we underwent the determination of the age at which the P26h transcript appears in hamster and which testicular cell type express it. Northern blot analysis were performed on total RNA extracted from testis of hamsters of different ages. Results show that the P26h transcript appears at three weeks of age, corresponding to the age of onset of hamster puberty, reaches a maximum of expression at five weeks of age and remains constant therafter. To determine which cells express the P26h transcript, testicular tissues were digested and cells were separated by FACS (Fluorescence-Activated Cell Sorting), using autofluorescence and propidium iodide staining. Total RNA from testicular cell populations were extracted and RT-PCRs were performed using specific P26h oligonucleotids. Preliminary results show that the P26h transcript is expressed in leptotene, zygotene, pachytene, round and elongated spermatids. Supported by MRC-CANADA.

7

IDENTIFICATION AND CHARACTERIZATION OF THE P31m, A MONKEY SPERM PROTEIN

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In order to acquire its fertilizing ability, the spermatozoon must undergo a series of physiological and biochemical modifications which take place during the epididymal transit. These include the addition or the modification of surface proteins involved in sperm-egg interactions allowing consequently a successful fertilization. We have previously identified and characterised P34H, a 34-kDa human epididymal sperm protein, which has been shown to be involved in the sperm-zona pellucida interaction. The aim of this study was to determine if there was a protein showing functional, biochemical and immunogenic similarities with the P34H in the monkey (*Macaca fascicularis*). Western blot analyses were performed on monkey sperm protein extracts with an antiserum specific for P34H. A 31-kDa protein (P31m) was detected in proteins extracted from cauda epididymidal spermatozoa. Immunostaining techniques using the same antiserum were performed on monkey testis and epididymis. These revealed the presence of the protein on the acrosomal cap of the spermatozoa recovered from the caput epididymidis with an increasing intensity to the cauda. Northern blot analyses has shown the presence of a single P31m messenger RNA (mRNA) of about 1kb in the testis and the epididymis, principally in the cauda region. Cloning and sequencing of a 400bp nucleotidic segment revealed 92% identity with the human P34H cDNA. *In situ* hybridization are in progress to determine the cell type of the monkey reproductive tract responsible for P31m transcriptional activity. The work was supported by MRC-Canada.

8

IMMUNOLOCALIZATION OF AQUAPORINS 1, 8 AND 9 IN THE TESTIS AND EPIDIDYMIS OF ADULT RATS.

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Aquaporins (AQPs) are membrane protein channels that allow rapid passage of water through an epithelium containing tight junctions. In the present study, immunocytochemistry was performed to localize several of the 9 members of this family in the testis and epididymis of adult rats. In the efferent ducts (EDs), intense reactivity for AQP-1 was present on microvilli (Mv) and outlining endosomes of the apical region of the nonciliated epithelial cells. A linear reaction was also noted on the basolateral plasma membranes of these cells and in several small spherical structures of their basal cytoplasm. In the epididymis, an intense reaction appeared in association with myoid cells but only in tubules of the initial segment. Endothelial cells of blood vessels of the epididymis were reactive, but there was no staining of any structures in the testis. AQP-8 was expressed exclusively in the testis outlining zygotene and pachytene spermatocytes as well as all spermatids. AQP-9 showed a web-like reaction in the interstitial space of the testis outlining Leydig cells. AQP-9 also appeared as a dense band of reaction product on the Mv of nonciliated cells of the EDs, as well as those of principal cells of all epididymal regions except the intermediate zone. Clear cells were mainly reactive in the cauda region. Taken together these data reveal tissue as well as cell and region specificity for the distribution of various AQPs. The data also indicate differences in the subcellular distribution of a given AQP within the same cell type as well as overlapping of some AQPs suggesting that AQPs may not only be involved in reabsorption of water but other substances as well. (Supported by MRC of Canada).

9

ULTRASTRUCTURE OF THE VAS DEFERENS (VD) FROM HEALTHY NON-VASECTOMIZED AND VASECTOMIZED PATIENTS. Andonian S, Durie P*, Mak,V, Zini A*, Jarvi K* and Hermo L; Dept of Anatomy and Cell Biology, McGill University and * Mount Sinai Hospital, Canada.

Despite more than 30 million vasectomies, the structure and function of the VD is not fully understood. In the present study, the ultrastructural features of the scrotal VD from healthy and vasectomized males were examined by light and electron microscopy. In healthy males, the VD epithelium (E) consisted of columnar principal cells whose major feature was apical blebs (ABs) emanating from their surface. ABs contained ER elements, numerous free ribosomes and membranous profiles, unlike the apical cytoplasm of principal cells that contained Golgi apparatus and lipofuscin granules. Many ABs appeared to reside free in the lumen, and the presence of numerous membranous profiles therein suggested that ABs undergo degeneration and liberate their contents into the lumen representing apocrine secretion. In vasectomized males, the features of the site proximal (P) to the incision of the VD differed from that of the distal (D) site. In the case of the P site, although the E was reduced in size and appeared fimbriated, principal cells still had ABs and showed numerous lysosomes suggesting an active role in endocytosis. In contrast, the tubule diameter of the D site was reduced in size, the lumen was occluded and the E was represented as a thin layer of cells showing a paucity of organelles. These data suggest that the morphological integrity of principal cells is regulated by luminal factors derived from the testis or epididymis and that these features could account for the low success rate of vasectomy reversal. (Supported by Am Ped Soc and MRC).

10

LOCALIZATION OF ESTROGEN RECEPTOR α IN THE REPRODUCTIVE TRACT OF ADULT MALE DOGS AND CATS: AN IMMUNOHISTOCHEMISTRY STUDY

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The expression of estrogen receptorα (ERα) in the male reproductive system has been described in many species. However, the distribution of ERα in adult male dog and cat reproductive tracts has not been reported. In the present study, ERα was localized by immunohistochemistry using mouse anti-humam antibody(NCL-ER-LH2), which is ERα specific. The expression of ERα in dog was found in the nuclei of epithelium cells of rete testis and efferent ducts, including ciliated and non ciliated cells, but not in the epididymis and vas deferens. In testis, Leydig cells and other intertubular cells showed weakly positive for ERα. From rete testis to vas deferens, some stromal cells were weak to moderately positive. In the cat, ERα was seen in testis, epithelium cells of efferent ductules and in epididymis and vas deferens, but not in rete testis. Nuclei of ciliated cells in the efferent ductules stained less intensely than did the nonciliated cells. In the epididymis, epithelial cells of initial segment were negative for ERα, while other regions were moderately positive. We also observed that some nuclei of smooth muscles cells and connective tissue cells were ERα immunopositive from efferent ductules to vas deferens. These results revealed differences between dogs and cats in the localization of ERα in the male reproductive tract. However, as in other mammals, the efferent ductules remain the region of highest concentration for ERα.

11

IMMUNOLOCALIZATION OF SEVERAL STEROID HORMONE RECEPTORS IN EFFERENT DUCTULES OF ADULT RAT Qing Zhou*, Rong Nie*, and Rex A. Hess. Dept. of Veterinary Biosciences, University of Illinois, Urbana, IL 61802.

Efferent ductules, which connect the lumen of the rete testis to the head of the epididymis, reabsorb most of the fluids secreted in the seminiferous tubules. It has been demonstrated that estrogen is involved in the regulation of this function through estrogen receptorα. However, the involvement of other steroid hormones is unknown. The purpose of this study is to determine the cellular localization of estrogen receptorα (ERα),β(ERβ), androgen receptor (AR), progesterone receptor (PR) and vitamin D receptor (VDR) in the efferent ductules. Adult Sprague Dawley rats (120 days) were perfused with 10% cold neutral buffered formalin (NBF) and immunohistochemical studies were done in efferent ductules. Results: ERα, ERβ, PR, AR and VDR were localized in both ciliated and non-ciliated epithelial cells of efferent ductules with strong positive staining. These receptors were also localized in stromal cells; however, ERα showed a relatively weak positive staining in the connective tissues. This is the first immunohistochemical study to show that the expression of ERβ and VDR in efferent ductules of the male reproductive system. These results indicate that the efferent ductules may utilize numerous steroid hormones in the regulation of its function.

12

PROMOTER AND SPLICING mRNA VARIANTS OF THE EPIDIDYMIS-SPECIFIC GENE EP2 IN RHESUS MONKEY (*M. mulatta*)

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Message variants produced by the EP2 gene in the rhesus monkey epididymis were characterized using PCR. Using 5'- and 3'-RACE, we demonstrated that the EP2 variants EP2A through EP2E, previously identified in chimpanzee epididymis, also exist in rhesus epididymis. The chimpanzee and rhesus sequences are highly homologous with approximately 90% sequence identity on both the DNA and the amino acid level.

Using RT-PCR to amplify cDNA segments between the start and the end of the open reading frame of EP2A variant, we isolated 9 additional splicing variants. These 9 variants use the first 2 and the last 2 exons of the EP2A variant and 1 exon of the EP2C variant. In addition, they contain 3 exons not previously identified in EP2A-EP2E. These 3 exons are, however, present in the human EP2 genomic sequence. They are located in the large intron containing the B promoter.

Several exons of the EP2 variants contain nucleotide sequences that are not divisible by 3. Inclusion or omission of these exons results in a shift in the open reading frame. For example, one exon, which is used in 4 message variants, codes for 3 different amino acid sequences.

As each of the 9 new rhesus EP2 variants contain the same the 5'-end as variant EP2A, they code for a leader sequence and, therefore, for putative secretory proteins. After removal of the leader sequence, the 9 mature proteins range between 3 kDa to 15 kDa in size.

As with the chimpanzee, some of the rhesus EP2 variants code for peptides whose sequence is similar to β-defensins, whereas others code for proteins which contain no recognizable homology with any known proteins.

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13

GENOMIC STRUCTURE OF THE HUMAN EPIDIDYMAL EP2 GENE
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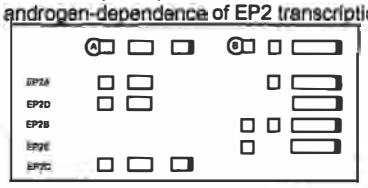
The EP2 gene gives rise to at least 5 message variants (EP2A-EP2E) having different 5'-ends, 3'-ends and alternative internal exon usage. The human EP2 gene is about 16 kb long. It contains two promoter regions and accounts for all known cDNAs and ESTs of the databases. Both promoters, A and B, contain what appear to be androgen response elements (AREs) and ARE half-sites, consistent with the observed androgen-dependence of EP2 transcription.



The EP2 variants EP2C, EP2D and EP2E code for peptides that have a distribution of cysteines characteristic for β-defensins. Sequence alignments of DNA show that the location of the splicing site of β-defensins is conserved in the EP2 gene. However, EP2 includes an intron/exon/intron combination, whereas defensins have only a single intron. We suggest that the EP2 gene is composed of two ancestral β-defensin genes, one producing EP2C and the other producing EP2E. This is supported by the existence of the two promoters and the large intronic gap of 10 kb upstream of promoter B.

To map the location of the EP2 gene, we used PCR to test yeast artificial chromosomes (YACs) of known location . The EP2 gene locus is on human chromosome 8p22-23 close to that of DefB2 (β-defensin-2) at the centromeric end of the gene cluster region that contains all of the known defensins.

Moreover, the exon arrangement in variants EP2A and EP2D shows that the EP2 gene is more than a tandem arrangement of two defensin genes in that the two defensin genes have fused into a new gene. This fusion, in combination with the evolution of an additional exon in each half-gene, one of which results in a shift in the open reading frame, gives rise to the novel non-defensin like proteins EP2A and EP2B.

(Supported by NIH Grant RR05994)

14

EFFECTS OF INHIBIN ON ANDROGEN-STIMULATED CAPUT EPIDIDYMAL GENE EXPRESSION IN THE CYNOMOLGUS MONKEY. M. Mahony, M. Billeter*, Dept OB/GYN, Eastern Virginia Medical School, Norfolk, VA.

The mammalian epididymis is under androgen control presumably by the 5α-reduced metabolites of testosterone (T) including dihydrotestosterone (DHT). Two genes encode two isozymes, types 1 and 2, of 4-ene-steroid 5α-reductase that converts T to DHT. The specific aim was to measure the action of inhibin, present in primate epididymis (EP), on androgen-modulated 5α-reductase types 1 and 2 gene expression in isolated caput EP tubules from cynomolgus monkeys *(Macaca fascicularis)* (n=5). Individual tubules were enzymatically isolated with collagenase (0.5%) and elastase (0.01%), minced and rinsed of luminal contents. EP tubules (100mg) were treated with inhibin (porcine, 100ng/mL) versus medium (MEM w/ 0.1%BSA) control for 6 hr at 33°C and 5%CO_2 in air, alone or in combination with T (10μM) or DHT (10μM). RT-PCR was performed on mRNA extracted from control or treated EP tubules. Relative abundance of 5α-reductase types 1 and 2 and β-actin mRNA was measured by scintillation spectroscopy. Results indicated in medium control, DHT but not T significantly increased 5α-reductase type 1 ($p < 0.03$) and type 2 ($p < 0.002$) mRNA levels with no alteration in β-actin mRNA levels. Inhibin had no effect on either types 1 and 2 or β-actin mRNA levels without androgen-stimulation. However, inhibin significantly inhibited DHT-stimulation of type 2 ($p < 0.0001$) and type 1 ($p < 0.02$) and β-actin ($p < 0.05$) mRNA levels. Conversely, inhibin had no effect on T-stimulated types 1 and 2 or β-actin mRNA levels. Our preliminary results suggest that inhibin may be a physiologic modulator of DHT-stimulated mRNA expression and caput epididymal function. Supported by NIH HD35250.

15

GLUTATHIONE-S-TRANSFERASE EXPRESSION IN THE AGING BROWN NORWAY RAT EPIDIDYMIS
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It is well established that the male reproductive tract is compromised with age. In the epididymis, advancing age results in morphological and biochemical changes that are qualitatively different in the proximal part of the tubule (inital segment) as compared to more distal parts of the tubule (cauda). One of the hypotheses proposed to account for the changes observed in the epididymis with age is increased oxidative stress load. In order to better understand the role of oxidative stress in the aging epididymis, the expression of glutathione-S-transferases (GST, alpha, micro, mu, pi, theta), enzymes known to play a role in cellular anti-oxidant defenses, was examined. Tissues from young (3mo) and old (20-21mo) male Brown Norway (BN) rats (n=3) were sectioned, RNA was extracted from the initial segment and cauda epididymidis, and used to probe 3 different sets of cDNA Atlas arrays. Of the GSTs examined, two (alpha and theta) were not detectable. The expression of the other GSTs (micro, mu, pi) changed in a segment specific manner. In the initial segment, GSTmu remained unchanged, the expression of GSTmicro was repressed by more than 25%, and the expression of GSTpi was induced to more than 210%. In contrast, in the cauda epididymidis, both GSTmu and micro were repressed by 25%, while the expression of GSTpi was unchanged with age. These results indicate that the initial segment and cauda epididymidis are differentially able to respond to oxidative stress with age; oxidative stress may contribute to the changes observed in these segments with age. Supported by NIA-AG08321

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IS CLAUDIN-1 AN ADHESION MOLECULE IN THE RAT EPIDIDYMIS?
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Claudins are a family of transmembrane proteins which have been localized to tight junctions. In the present study, the immunolocalization of claudin-1 (Cl-1) was undertaken to determine its cell and region specific distribution in the epididymis and to determine if Cl-1 is androgen-regulated. In the light microscope, Cl-1 expression appeared as a linear reaction product along the entire lateral plasma membranes between adjacent principal cells as well as between principal, narrow and clear cell interfaces. Immunostaining was also present between principal and basal cells as well as along the basal plasma membrane of epithelial cells throughout the epididymis. The most intense reaction appeared in the distal cauda epididymidis. No staining was observed in efferent ducts or testis. In the electron microscope, the most conspicuous site of gold labeling was between the basal plasma membrane of the various epithelial cells and the basement membrane. Postnatal development studies indicated that by day 7, Cl-1 was already localized between principal cells and that by day 28, immunostaining in the epididymis was similar to that of adult animals. Examination of tissues from orchidectomized rats with or without testosterone replacement up to day 21 revealed no significant changes to Cl-1 expression, suggesting that Cl-1 is not androgen-regulated. Together these studies demonstrate that Cl-1 in the epididymis may serve as an adhesion molecule binding the different cell types to each other at sites other than tight junctions exclusively as well as to the basement membrane, and that it is not regulated by androgens.

17

ISOLATION OF A DNA FRAGMENT ENCODING THE 5'-UPSTREAM REGION OF THE MEP 17 GENE

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It is well known that the epididymis provides a unique intraluminal environment that allows sperm maturation to occur. Our laboratory has previously found that a murine epididymal retinoic acid-binding protein (mE-RABP), belonging to the lipocalin superfamily, is secreted by the epithelium of the mid/distal caput epididymidis. Functional studies, using transgenic mouse, revealed that a 5 kb DNA fragment of the 5'-flanking region of the mE-RABP gene contains all the information required for the hormonal regulation and the spatial and temporal expression of the mE-RABP gene in the epididymis. We have recently identified within the 5 kb DNA fragment a novel gene, named MEP 17, highly homologous to the mE-RABP gene. Preliminary studies using *in situ* hybridization analysis revealed that MEP 17 mRNA was localized only in the principal cells of the initial segment, whereas mE-RABP mRNA was expressed in the distal caput epididymidis. In order to characterize the 5'-flanking region of the gene, a 2.5 kb EcoRI and a 7 kb EcoRV restriction fragments overlapping with the 5'-flanking region of the mE-RABP gene were isolated from the genomic BAC clone used to isolate the mE-RABP. A computer based analysis of the DNA sequence of the 2.5 kb DNA fragment revealed the presence of a putative androgen receptor binding site (ARBS), several putative AP-1 and c-Ets cis-DNA regulatory elements as well as several potential Sry-related transcription factors binding sites. These data provide a framework for further analysis of the regulation of MEP 17 expression and structure-function of the protein. (Supported by NIH grant HD36900 and a grant from the Rockfeller Foundation/Ernst Schering Foundation.)

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LOCALIZATION AND REGULATION OF INDOLEAMINE 2,3-DIOXYGENASE EXPRESSION IN THE EPIDIDYMIS.

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Indoleamine 2,3-dioxygenase (IDO) is the initial and rate-limiting enzyme that catabolizes tryptophan and other indole derivatives to kynurenine. It's activity is found in various mouse tissues with the highest enzyme activity in the epididymis. However, the pattern of its expression and its regulation in epididymis is currently unknown. In the present study, we investigated IDO expression in the epididymis and vas deferens at different ages, and the hormonal regulation of its expression in epididymis.. We found that IDO expression in the epididymis starts at 2 weeks post partum and reaches its peak at week 8, and is kept at moderate levels the rest of life. Localization of IDO by *in situ* hybridization showed that it is absent in testis; highest expression in distal part of the caput; weak expression in the cauda and vas deferens; and very little expression in the corpus. IDO expression is confined to the epididymal epithelial cells. Our data indicates that testosterone regulates IDO expression in the epididymis, as when testosterone is removed from the hormone supply by castration, there is a large decline of IDO expression. Significantly replacement of the hormone can rescue IDO expression. Sperm and other testicular factors have no effect on IDO expression within the epididymis. In conclusion, IDO is differentially distributed within the epididymis, its expression in the epididymis is age-dependent and androgen-dependent.

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OXIDATIVE STRESS AND IL-6 LEVELS IN PATIENTS UNDERGOING VASECTOMY REVERSAL

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Oxidative stress as a result of elevated levels of reactive oxygen species (ROS) and depressed levels of antioxidants is associated with male infertility. Cytokines are also involved in the production of reactive oxygen species. It is known that IL-6 levels are increased in the seminal plasma of infertile men. The role of oxidative stress in men following vasectomy reversal is unclear. The purpose of our study was to determine whether men undergoing vasectomy reversal have high seminal oxidative stress using three measures of oxidative stress: reactive oxygen species (ROS), total antioxidant capacity (TAC), and a composite ROS-TAC score. In addition we also measured the IL-6 concentration in the seminal plasma. Semen and seminal plasma samples were obtained from 23 men following 6 to 8 months after vasectomy reversal and 11 normal donors. Vasectomy reversal patients were further divided into infertile (n = 13) and fertile (n = 11). ROS and TAC production in the semen specimens was measured by the chemiluminescence assay. A composite ROS-TAC score was generated. IL-6 concentration in the seminal plasma was measured by the enzyme-linked immunoassay. Vasectomy reversal infertile patients had higher ROS levels (mean log [ROS + 1] 2.46 ± 0.28) compared to vasectomy reversal fertile patients (1.84 ± 0.29; P = 0.2), and controls (1.25 ± 0.31; P = 0.006). TAC levels were comparable in the three groups. The ROS-TAC scores in infertile men were reduced (46.8 ± 4.28) compared to those in the controls (50.0 ± 3.02). IL-6 levels were significantly elevated in both vasectomy reversal infertile group (1.89 ± 0.37; p <0.01) and fertile vasectomy reversal group (1.76 ± 0.24; p <0.02) compared to controls (0.83 ± 0.31). Infertility following vasectomy reversal is associated with increased level of ROS and elevated IL-6. Oxidative stress in these patients may decrease over time following increased interval after their reversal. [Supported by a research grant from The Cleveland Clinic Foundation.]

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EXPRESSION OF P34H, A SPERM PROTEIN, ALONG THE EPIDIDYMIS OF VASECTOMIZED MEN AND IN VAS DEFERENS

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We have previously described P34H, a human epididymal sperm protein, proposed to be involved in sperm-zona pellucida interactions. P34H mRNA (912bp) has been shown to be expressed by the principal cells of the human corpus epididymis. Recovery of fertility following anastomosis of the vas deferens with testicular tubules, vas efferens or along the epididymis has challenged the physiological significance of the epididymal transit in humans. In order to understand sperm maturation in these pathological situations, we have studied the expression of P34H in the vas deferens and along the epididymis of vasectomized men. Northern blot analysis revealed a single 912bp P34H mRNA expressed at similar levels in the human vas deferens as well as in the corpus epididymidis. *In situ* hybridization experiments showed high labelling of epithelial cells bordering the vas deferens lumen. P34H expression was also studied on epididymal segments from vasectomized man. Hybridization signal was detectable in proximal caput of vasectomized tissues at a much higher intensity than the one characterizing tissues from normal men. Some histological modifications of the vasectomized men epididymis have also been observed. The epididymal lumen diameter, as an example, increases from the distal caput to the distal cauda while the bordering principal cells were smaller in height. Thus, obstruction of vas deferens may alter the expression along the human epididymis of mRNA encoding for proteins necessary for sperm maturation. Furthermore, our results suggest that the vas deferens may play an important function in these processes. This work was supported by MRC-Canada.

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THE SPECTRUM O F CYSTIC FIBROSIS (CF) GENE ANOMALIES IN A LARGE COHORT OF MEN WITH CONGENITAL BILATERAL ABSENCE OF THE VAS DEFERENS (CBAVD). TJ McCallum*, A Milunsky*, J Milunsky*, and RD Oates, Boston Medical Center, Boston, MA

Introduction and Objectives: The CF gene encodes a protein termed CFTR. Mutations and/or abnormalities in the CF gene are responsible for the manifestations of CF and CBAVD, depending upon whether the mutations are "severe" or "mild". The 5T polymorphism variant in intron 8 leads to reduced output of normal CFTR from that gene. It is not uncommon to detect a 5T variant in combination with an opposite allele mutation in CBAVD men. Surgical sperm retrieval coupled with ICSI helps these men to become biologic fathers. It is imperative to identify the patient and partner's genotype prior to ICSI so the potential risk of CBAVD or CF to their offspring can be determined. The objective of this study was to define the number and type of CF genotype mutations in our population of CBAVD men.

Materials and Methods: 153 men had CF genotype analysis, with 105 also having intron 8 poly T testing.

Results: The table shows the actual numbers, as well as percentages of the total, that were compound heterozygotes (two mutations detected), simple heterozygotes (only one mutation detected) with or without the 5T variant, and no mutations identified. 68% of men had at least one identifiable anomaly, their CF gene abnormalities being the probable genetic basis of their CBAVD.

	Actual Number	% of Total
Compound Heterozygotes	21	14
Simple Heterozygotes	83	54
With 5T Variant	28	
Without 5T Variant	31	
Poly T tract not tested	24	
No mutation identified	49	32

Conclusions: This data demonstrates the association of CBAVD and CF gene mutations. CFTR has a pivotal role in vasal development although the mechanism of action is not yet known. All males with CBAVD and their spouses need CF mutation analysis with intron 8 poly T assessment prior to sperm retrieval. This allows for proper genetic counseling to occur. If the spouse is a carrier of a CF mutation, assisted reproductive therapies may help create a child with CF.

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AFFECT OF ENDOTOXIN AND INTERLEUKIN ON THE ARRANGEMENT OF MICROFILAMENTS IN CULTURED SERTOLI CELLS

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Alterations in male fertility can develop from multiple causes. It has been reported that disease states which increase endotoxin levels can result in decreased sperm production and thus male fertility. The reason for this decreased fertility, whether from the endotoxin itself, the subsequent release of interleukin, or other factors is unclear. We have previously shown that alterations in the Sertoli cell cytoskeleton - resulting from hormonal changes - lead to decreased spermatid binding. It has also been shown that changes in the cytoskeleton alters the processing and movement at the developing germ cell through the testicular epithelium. The current study was developed to determine if endotoxin and/or interleukin, when applied directly to cultured Sertoli cells, altered the actin component of the cytoskeleton. Cultured Sertoli cells from 40 d.o. rats were cultured with and without FSH and testosterone. The cells were subsequently exposed to endotoxin and/or interleukin for 48 hrs. After culture cells were fixed and stained with rhodamine phalloidin to reveal the arrangement of filamentous actin. We found that the endotoxin and interleukin do alter the arrangement of microfilaments resulting in an increase in the stress fiber appearance vs. the more peripheral localization seen in control cultures. If this direct affect of these compounds alters spermatid bonding is not yet known.

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THE LIPOPHILIC FACTOR PRODUCED BY MACROPHAGES THAT STIMULATES STEROIDOGENESIS IS 25-HYDROXYCHOLESTEROL. [1]Y.O. Lukyanenko*, [2]W.D. Nes*, [2]Z. Jia*, [3]S. Quideau*, [4]W. N. Howald*, [4]T. Pratum*, [5]R. West*, and [1]J. C. Hutson. [1]Texas Tech University Health Sciences Center and [2]Texas Tech University, Lubbock, TX, [3]University of Bordeaux, Talence, France, [4]University of Washington and [5]Zymogenetics, Seattle, WA. The purpose was to identify the lipophilic factor (MDF) made by macrophages that stimulates steroidogenesis in Leydig cells, adrenal cortical cells and granulosa cells, and to determine if it is present in the testis and/or acts when injected intratesticularly. Media from rat testicular and peritoneal macrophages were extracted with ether and the lipophilic extracts purified sequentially on C_{18}, silica, and cyano high performance liquid chromatography (HPLC) columns, monitoring activity with a Leydig cell bioassay. Purified MDF was analyzed by gas chromatography/mass spectrometry (GC/MS) and nuclear magnetic resonance spectroscopy (NMR). The MS fragmentation pattern of MDF was identical to that of 25-hydroxycholesterol (25-HC). When a reference preparation of 25-HC was subjected to the same purification scheme as MDF, it had the same retention times as MDF on all HPLC and GC columns. Analysis by NMR supported identification of MDF as 25-HC. Control medium (not exposed to macrophages) did not have biological. Extracts of whole testis contained 25-HC, and when 25-HC was injected into the testes, intratesticular testosterone levels increased as predicted. These data indicate that the lipophilic factor produced by macrophages that stimulates steroidogenesis is 25-HC. Supported by the NIH (HD34708) to JCH, and the Welch Foundation (D1276) to WDN.

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HYPERTHYROIDISM ON TESTICULAR INTERSTITIAL CELLS AND ANDROGEN SECRETORY CAPACITY OF THE PREPUBERTAL RAT.

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Effects of hyperthyroidism on the testis interstitium during prepuberty was studied using Sprague Dawley rats. Six control groups of male rats (received daily SC injections of saline from postnatal day 1) and six T3 groups of rats (received daily SC injections of triiodothyronine/T_3, 50 µg/kg b.wt from postnatal day 1) were used. Rats were killed at days 5, 7, 9, 12, 16 and 21. One testis of each rat was used to determine LH-stimulated (100ng/ml) testicular androgen secretory capacity *in vitro*. The other testis was either used for morphometric studies (n=5) or for immunolocalization of 3β hydroxysteroid dehydrogenase (3βHSD, n=3). T_3 resulted in significant reductions in body and testis weights. Morphometric analysis reveled that lower testis weight in T_3 treated rats were mainly due to the reduction of total volume of seminiferous cords/tubules. The number of interstitial mesenchymal cells (MC) was lower ($p<0.05$) in T3 rats compared to age matched controls. The number of fetal Leydig cells (FLC) was not different between the two groups, however, FLC hypotrophy was detected in T_3 rats at day 16 in contrast to day 21 in control rats. In both treatment groups, morphologically identifiable adult Leydig cells (ALC) were observed at day 12 and thereafter, however, the ALC number per testis in T3 rats was twice as much as those of controls. Positive immunolabeling for 3β-HSD was first detected in MC/progenitor cells in day 9 rats in the T3 group and in day 12 rats in the control group. Testicular testosterone production *in-vitro* was lower ($p<0.05$) in T3 rats compared to controls at each age tested and further reductions to very low levels were observed in T3 rats at days 16 and 21. Testicular androstenedione production was also lower ($p<0.05$) in T3 rats at days 5 and 7, but increased ($p<0.05$) thereafter than in control rats. These results demonstrate that hyperthyroidism stimulates premature hypotrophy of FLC and early differentiation of increased numbers of MC to ALC in the prepubertal rat testis further supporting the view that thyroid hormone is essential for the MC to differentiate into ALC in the prepubertal rat testis.

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MORPHOLOGICAL AND FINE STRUCTURAL FEATURES OF THE ZEBRAFISH TESTIS. C.G. Schrepferman*, M.J. Bateman*, P.M. Heidger*, A.P. Sandra*, M.B. Cohen*, J.I. Sandlow. University of Iowa Departments of Urology, Anatomy and Cell Biology, and Pathology, Iowa City, Iowa, USA.

Objectives: Zebrafish (*Danio rerio*) have been identified as an excellent animal system for the study of vertebrate development and genetics. Techniques to induce genomic and germ-line mutations are well-described and reproducible. To our knowledge, a comprehensive investigation of the morphology of the zebrafish testis has not been undertaken. The testis of the adult zebrafish was characterized histologically and fine structurally utilizing light and electron microscopy.

Methods: For histological studies, adult male zebrafish were fixed for a minimum of 24 hours in Bouin's solution. Following alcohol dehydration, the middle third of the fish was embedded into paraffin, sectioned at 3µm, stained with hematoxylin and eosin, and observed under bright field optics at low and high power. For electron microscopy, similar adult testicular tissue was harvested and immediately fixed in 2.5% glutaraldehyde followed by post-fixation in osmium. Tissue was then dehydrated with acetone, embedded in epon, and sectioned for routine electron microscopic examination.

Results: The testes were identified as paired, cylindrical structures arching ventrolaterally along the swim bladder, extending craniad to the liver and terminating caudad near the cloaca. A distinct tubular architecture, similar to that observed in mammalian and certain other teleost testes, was demonstrated. Primary and secondary spermatogonia, primary and secondary spermatocytes, spermatids, and spermatozoa were identified. In addition, individual tubules contained supportive cells (analogous to Sertoli cells) that were joined by intercellular junctions. Germ cells were segregated into small cystic packets along the lining of the tubule, each cyst containing multiple cells at similar stages of differentiation. Mature spermatozoa were flagellated and packed the tubular lumina in the unbred fish. Interstitial cells containing abundant smooth and rough endoplasmic reticulum were identified.

Conclusions: The zebrafish testis exhibits many critical morphological features in common with other teleost and mammalian testicular tissue. These studies of normal adult tissue provide a basis upon which to evaluate the results of future studies designed to potentially alter testicular or germ cell development in this animal model system.

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GALECTIN-3 EXPRESSION IN MOUSE TESTIS

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Galectin-3 is a β-galactoside-binding animal lectin, studied extensively in the blood vascular system. Since galectin-3 exhibits a wide variety of biological functions including cell-adhesion in many systems, our aim was to examine the expression of galectin-3 in the testis. Immunohistochemical staining with the monoclonal rat IgG_{2a} (M3/38.1.2.8.HL.2, anti-MAC-2 from ATCC), demonstrated the presence of galectin-3 in sections of mouse testis, predominantly in Leydig cells. Advanced germ cells including spermatids were galectin-3 positive. Sertoli cells in some tubules were also immunoreactive against anti-galectin-3. Western blot analysis using anti-galectin-3 was carried out with HT-29 cells (Human colon cancer cells), MSC-1 (mouse Sertoli cell line) and MA-10 cells (mouse Leydig cell line). Positive control HT-29 cells reacted with anti-galectin-3 as expected at 30-kDa. MSC-1 cells showed weak galectin-3 like immunoreactivity. One major band of approximately at 34 kDa was detected in extracts of MA-10 cells. We then employed indirect immunofluorescence microscopy to examine localization of galectin-3 on HT-29 (positive control), MSC-1 and MA-10 cells. All three cells reacted positively with anti-galectin-3, and MA-10 cells were strongly positive. We suggest that galectin-3 may be involved in multiple interactions between testicular cells, and/or between cells and galectin-3 reactive extracellular matrix components like laminin. (Supported by NIH grants P20RR11588 and S06GM08117).

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UP-REGULATION OF STEROIDOGENIC FACTOR-1 GENE BY INSULIN-LIKE GROWTH FACTOR-1 IN RAT LEYDIG CELLS.

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Steroidogenic factor-1 (SF-1) is an orphan nuclear receptor that plays a critical role in the tissue specific regulation of genes encoding the cytochrome P450 steroid hydroxylases. SF-1 also regulates the genes for Müllerian inhibitory substance, glycoprotein α-subunit, LHβ, DAX-1, and the receptors for GnRH, prolactin and ACTH. Although SF-1 has been identified in testicular Leydig cells, little is currently known about its hormonal regulation. Previously we have reported that Leydig cells express insulin-like growth factor-1 (IGF-I) receptors and that IGF-I enhances hCG-stimulated testosterone formation. IGF-I also enhances hCG-induced P450scc and steroidogenic acute regulatory protein (StAR) gene expression. The StAR 5'-flanking region was found to contain a binding site for the SF-1 that is critical for basal promoter activity, thus implicating SF-1 as a possible regulator of StAR expression. In the present study, we investigated the role of IGF-I in the regulation of SF-1. Adult male Sprague-Dawley rats (60-day-old) were sacrificed and testes were removed. Leydig cells were isolated by arterial perfusion, centrifugal elutriation and Percoll density centrifugation. Cells were cultured with or without IGF-1 (10, 50 or 100 ng/ml) for 24 h. Total RNA was extracted for Northern blot analyses. IGF-I increased SF-1 mRNA levels in a dose-dependent manner. IGF-1 in a concentration of 100 ng/ml increased SF-1 mRNA levels by 2.3-fold. This is further confirmed by in site hybridization of cultured Leydig cells. In conclusion, IGF-1 up-regulates SF-1 gene in rat Leydig cells. Effects of IGF-1 on P450scc and StAR genes may be mediated by up-regulation of SF-1.

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CONTROL OF THE MEIOTIC CELL CYCLE BY CDK2ß

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It is well known that control of the mitotic cell cycle relies on the accurate coordination and activity of the cyclin dependent kinases. Less well known is the role these key regulators play during the meiotic cell cycle. We have been studying the cyclins and Cdks during meiosis in order to understand the control of this unique cell division. We have identified a splice variant of the mitotic Cdk2 gene, called Cdk2ß, that is highly enriched during meiosis. To test the hypothesis that Cdk2ß functions during meiosis in the mouse, we have studied 1) its expression using RT-PCR, immunoblot, and immunohistochemical analyses, 2) its activity using immunoprecipitation and kinase assays, and 3) its binding partners using immunoprecipitation and two-hybrid analyses. Our data indicate that Cdk2ß is expressed exclusively during late pachytene to the end of MI of meiosis at both the mRNA and protein levels. The somatic form of Cdk2, Cdk2α, is expressed only in spermatogonia and pre-leptotene cells. The expression of Cdk2ß very closely overlaps that of the meiosis-specific cyclin, cyclin A1. Interestingly, Cdk2ß appears to bind Cyclin A1, but not Cyclin A2, and furthermore Cdk2ß immunoprecipitates (cyclin A1 positive) possess Histone H1 kinase activity. These data suggest that cyclin A1/Cdk2ß functions during meiosis at a time to effect meiotic DNA repair and/or chromosome segregation. [Supported in part by the South Plains Foundation and the March of Dimes (#1FY-99-601) to SER and by a grant from the Howard Hughes Medical Institute through the Undergraduate Biological Sciences Education Program to DMS.]

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INHIBITION OF TESTOSTERONE PRODUCTION IN ADULT RAT LEYDIG CELLS AFTER ADMINISTRATION OF MÜLLERIAN INHIBITING SUBSTANCE *IN VIVO*

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Müllerian inhibiting substance (MIS) is a 140 KDa glycoprotein belonging to the TGF-β family of growth factors. Rat Sertoli cells secrete MIS by day 13 of gestation causing regression of the analage of female reproductive tract structures in the male. It has been proposed that continued secretion of MIS after birth inhibits proliferation and development of Leydig cells. However, few studies have addressed whether MIS influences Leydig cell function in sexually mature animals. Therefore the goal of the present study was to determine if MIS inhibits testosterone production by adult Leydig cells *in vivo*. Adult Sprague-Dawley rats (60-65 day old) received 75 µg MIS in 50 µl of Dulbecco's phosphate buffered saline solution by intratesticular (IT) or intraperitoneal (IP) injection. A control group (VEH) received an intratesticular injection of the vehicle. Blood was collected at 2, 4, 6, 8, 12, and 24 hours to assay serum testosterone concentration. In addition, interstitial fluid was collected at 4, 8, 12 and 24 hrs to estimate MIS levels in the testis. IT administration of MIS resulted in high levels of MIS in the testis (574 ± 60 ng/ml vs VEH, 0 ng/ml, $p<0.001$) at 4 hrs. The uninjected contralateral counterpart of the IT testis had an intermediate concentration of MIS 41.8 ± 9.6 ng/ml at 4 hrs. Intraperitoneal administration of MIS raised intratesticular MIS levels to a far lower extent (21.6 ± 14 ng/ml.). Serum testosterone declined in the IT group, compared to IP and VEH, after 4 hours (IT, 0.7 ± 0.05 ng/ml, IP, 1.1 ± 0 2 ng/ml, VEH, 1.6 ± 0.13 ng/ml, $p<0.001$). The observed reduction in serum testosterone was transient and testosterone levels were restored back to normal levels at 6 hrs. Leydig cells that were purified from testes four hours after IT administration of MIS had lower rates of testosterone production *in vitro* relative to VEH controls upon incubation with 100 ng/ml ovine LH (IT, 176 ± 20.55 vs VEH, 302.6 ± 60.18 ng/10^6cells•3hrs, $p=0.002$). In conclusion, administration of MIS intratesticularly resulted in a transient decline in serum testosterone and the rate of testosterone production by purified adult Leydig cells. This indicates that MIS can decrease testosterone production by adult rat Leydig cells and suggests a functional role for MIS in adult rat testes. Supported by NIH R29 HD36768 (M.M.L.) and R01 HD32588 (M.P.H.).

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ENZYMATIC ACTIVITY OF HUMAN SPERMATOGENIC CELL-SPECIFIC GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE (GAPD2)

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Spermatogenic cells contain isozymes of a number of glycolytic enzymes. We have recently reported the sequence of the cDNA for the human spermatogenic cell-specific isozyme of glyceraldehyde 3-phosphate dehydrogenase (*GAPD2*), a homologue of mouse *Gapd-s* (Welch et al., J Androl, in press). Both human GAPD2 and mouse GAPD-S contain an N-terminal proline-rich domain not found in somatic GAPDH. We hypothesize that GAPD2 and GAPD-S regulate glycolysis and energy production required for sperm motility and fertilization. GAPD2 is localized to the principal piece of the human sperm flagellum, as in mouse and rat. GAPD2 protein and enzyme activity remain associated with human spermatozoa following permeabilization with CHAPS detergent, but are released by treatment of spermatozoa with trypsin, chymotrypsin and elastase. After protease treatment, the apparent molecular weight of released GAPD2 is reduced to 45,000, almost 20,000 less than the native protein which migrates at 62,000. This reduced apparent molecular weight is slightly less than a recombinant form of mouse GAPD-S that lacks the proline-rich domain and migrates at 48,000. A recombinant form of human GAPD2 that lacks the proline-rich domain also has been expressed. This truncated protein demonstrates enzyme activity and migrates at a molecular weight similar to GAPD2 released from spermatozoa by protease treatment. We predict that, as in mouse, the N-terminal proline-rich domain of human GAPD2 anchors this glycolytic enzyme to the fibrous sheath. These data are consistent with the hypothesis that human GAPD2, like mouse GAPD-S, is tightly associated with the fibrous sheath of the sperm flagellum and may play a key role in regulating sperm motility. (This research was supported in part by NICHD/NIH through cooperative agreement U54HD35041 as part of the Specialized Cooperative Centers Program in Reproduction Research.)

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PROTEIN KINASE C INCREASES 11β-HYDROXYSTEROID DEHYDROGENASE OXIDATION AND INHIBITS REDUCTION IN RAT LEYDIG CELLS

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Glucocorticoid hormones control Leydig cell function through a receptor-mediated mechanism. The enzyme 11β-hydroxysteroid dehydrogenase (11βHSD) plays an important role in Leydig cells by metabolizing glucocorticoids, catalyzing the interconversion of corticosterone (the active form in rodents) and 11-dehydrocorticosterone (the biologically inert form). The net direction of this interconversion determines the amount of biologically active ligand, corticosterone, available for glucocorticoid receptor binding. We hypothesized that 11βHSD oxidative and reductive activities are controlled separately in Leydig cells, and that shifts in the favored direction of 11βHSD calalysis are a mechanism by which corticosterone levels are controlled. Therefore, in the present study, we tested the dependency of 11βHSD oxidative and reductive activities on protein kinase C (PKC) and calcium dependent signaling pathways. 11βHSD oxidative and reductive activities were measured in freshly isolated intact rat Leydig cells using 25 nM radiolabeled substrates after treatment with protein kinase modulators. We found that PKC and calcium dependent signaling had opposing effects on 11βHSD oxidative and reductive activities. Stimulation of PKC using the PKC activator, 6-[N-decylamino]-4-hydroxymethy-linole (DHI), increased 11βHSD oxidative activity from 5.08% (conversion rate, mean ± SE) to 48.23% with an EC50 of 1.70 ± 0.44 µM and inhibited reductive activity from 26.90% to 3.66% with an IC50 of 0.22 ± 0.5 µM. This indicated that PKC activation in Leydig cells favors 11βHSD oxidation and lowered levels of corticosterone. In contrast, addition of calcium increased 11βHSD reductive activities and decreased oxidative activities, favoring reduction and conversion of inert 11-dehydrocorticosterone into active corticosterone. The opposite effect was seen after elimination of calcium-dependent signaling, including removal of calcium by EGTA or addition of the calmodulin (calcium binding protein) inhibitor SKF7171A. We conclude that 11βHSD oxidative and reductive activities are separately regulated and that, in contrast to calcium-dependent signaling, PKC stimulated 11βHSD oxidation and inhibited reduction. Maintenance of a predominantly oxidative 11βHSD could serve to eliminate adverse glucocorticoid-induced action in Leydig cells. Supported by NIH HD 33000.

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PRENATAL EXPOSURE TO DEXAMETHASONE LEADS TO SUSTAINED REDUCTIONS IN SERUM TESTOSTERONE LEVELS.

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Recent studies have underscored the importance of the early life environment by demonstrating that treatment of pregnant rats with dexamethasone (DEX), a synthetic glucocorticoid, results in small birth weights, glucose intolerance, reduced hippocampal glucocorticoid receptor (GR) numbers, and a markedly higher systolic blood pressure at maturity. Since it is known that excessive cortisol levels in human males are associated with low testosterone (T) and decreased reproductive function, the goal of the present study was to determine whether high levels of maternal glucocorticoids result in postnatal changes in T biosynthesis. Pregnant rats were treated with DEX (100 µg/kg/day, i.p.) or vehicle (4% ethanol-0.9% saline) during the last seven days of gestation. Adult male offspring of DEX-treated pregnant females had increased levels of circulating corticosterone (DEX, 72.38 ± 12.27 vs Control, 38.01 ± 7.29 ng/ml $p \leq 0.05$), markedly decreased serum T levels (DEX, 1.53 ± 0.12 vs Control, 2.17 ± 0.28 ng/ml, $p \leq 0.05$) and increased serum luteinizing hormone (LH) levels (DEX, 0.43 ± .04 vs Control, 0.25 ± .03 ng/ml $p \leq 0.001$). In order to determine whether steroidogenic capacity was affected in these adult offspring after exposure to DEX *in utero*, T concentrations were evaluated in spent culture medium following three-hour incubations of freshly-isolated Leydig cells. Cells isolated from the DEX-treated group had increased rates of T production under both basal (DEX, 29.68 ± 2.08 vs Control, 17.23 ± 1.05 ng/10^6cells/3h, $p \leq .001$) and LH-stimulated (DEX, 266.65 ± 12.51 vs Control, 170.21 ± 10.56 ng/10^6 cells/3h, $p < 0.001$) conditions. Since Leydig cells isolated from the low serum T group exhibit an increased capacity for T biosynthesis, it is apparent that compensatory mechanisms established *in vivo* to counteract the inhibitory effects of high serum corticosterone (CORT) are maintained *in vitro* despite the absence of CORT. In conclusion, adult offspring exposed to DEX *in utero* exhibit a persistent increase in serum CORT that suppresses T biosynthesis *in vivo*. These data support the hypothesis that testicular function in adult male rats exposed to high levels of glucocorticoid *in utero* is significantly compromised during postnatal life. This work was generously supported by NSF (IBN9806035) and NIH (F33-HD08451-01).

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CHARACTERIZATION OF A NEW LYSOSOMAL TARGETING SIGNAL AND INVOLVEMENT OF SPHINGOMYELIN FOR THE TRANSPORT OF PROSAPOSIN TO THE LYSOSOMES.

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Prosaposin (SGP-1) was isolated, characterized and cloned from Sertoli cells in culture (Collard et al., 1987). Prosaposin is secreted or targeted to the lysosomes where it is processed into four smaller saposins (A-D) required for the hydrolysis of sphingolipids. The deficiency of saposins B and C results in variant forms of metachromatic leukodystrophy and Gaucher's disease, which are characterized by lysosomal storage of non degraded sphingolipids. The first objective of this investigation was to identify the targeting signal responsible for the transport of prosaposin to the lysosomes. To achieve this goal, we performed a selective deletion of domains A-D, and the flanking N-terminal and C-terminal domains of this protein. The mutated forms of the protein were subcloned into the expression vector pcDNA3.1B and subsequently transfected to COS cells. Confocal microscopy revealed that the C-terminus of this protein is sufficient and necessary to target prosaposin to the lysosomes. Recently, we demonstrated that targeting of prosaposin is also mediated by its interaction with sphingolipids. Thus, the second objective of this investigation was to characterize the lipid involved in its transport to the lysosomes. CHO cells overexpressing human prosaposin were incubated with the inhibitor of sphingomyelin synthesis D609 and with the inhibitor of glycosphingolipid synthesis PDMP. Immunofluorescence confocal microscopy demonstrated that only D609 blocked the transport of prosaposin to the lysosomes. When D609 treated cells were co-incubated with sphingomyelin, the transport of prosaposin to the lysosomes was restored. In conclusion, we have demonstrated that the C-terminus contains the lysosomal targeting sequence of prosaposin and that the transport of this protein to the lysosomes is mediated by its interaction with sphingomyelin. Supported by MRC and FRSQ.

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MULTIPLE CADHERIN SUPERFAMILY MEMBERS WITH UNIQUE EXPRESSION PROFILES ARE PRODUCED IN RAT TESTIS Kamin J. Johnson*, Sutchin Patel* and Kim Boekelheide, Department of Pathology and Laboratory Medicine, Brown University, Providence, RI

Adhesion between germ and Sertoli cells is thought to be crucial for spermatogenesis. Cadherin superfamily proteins, including classic cadherins and protocadherins, are important mediators of cell-cell adhesion. Using a degenerate PCR cloning strategy, we surveyed the expression of cadherin superfamily members in rat testis. Similar to brain, testis expressed a large number of cadherin superfamily members: 7 classic cadherins of both types I and II, 16 protocadherins, and 2 cadherin-related receptor protein sequences. All three protocadherin families (α, β, and γ) were found in testis. Using a semi-quantitative RT-PCR assay, mRNA expression was determined for each cadherin superfamily member during a postnatal developmental time-course and following ablation of specific testis cell types by ethanedimethanesulfonate, methoxyacetic acid, and 2,5-hexanedione. Diverse expression patterns were observed among the cadherins, suggesting that cadherin expression is cell type-specific in testis. The large number and variety of cadherin superfamily members found in testis supports a critical function for cadherin-mediated cell-cell adhesion in spermatogenesis.

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FURTHER CHARACTERIZATION OF DETERGENT INSOLUBLE GLYCOSPHINGOLIPID ENRICHED MEMBRANE MICRODOMAINS (DIGS) OBTAINED FROM RAT SERTOLI CELL CULTURES. W.E. Evans IV*, P.M. Gill*, C.A. Grieco* and S.E. Nyquist, Department of Biology, Bucknell University, Lewisburg, PA.

Recently we reported the presence of GPI-anchored ceruloplasmin on the Sertoli cell apical surface and observed that this GPI-anchored protein is highly concentrated in DIGs. Here we report a further characterization of these Sertoli cell fractions. DIGs, reported in numerous cell lines, form caveolae in the presence of caveolin, but are also present in multiple cell lines in the absence of caveolin and/or caveolae. In both cases, these microdomains are thought to represent centers of cell signaling. These microdomains, due to their hydrophobic character, are insoluble in cold 1% Triton X-100, and based on their high cholesterol & glycosphingolipid content (low density), can be isolated via density gradient centrifugation. DIG fractions yield 0.45 % (n=6) of total harvested Sertoli cell protein. A harvest of seven 75 cm^2 flasks yields about 150 µg protein. Following SDS-PAGE, western blotting, immunostaining using polyclonal anti-caveolin antibody (Transduction), and ECL (Amersham), data were collected demonstrating that Sertoli DIG lack detectable caveolin. Use of a second polyclonal antibody (Upstate), and immunofluorescent microscopy of tissue sections produced similar results. Following gentle biotinylation (rigorous biotinylation destroys yield) with the cell-impermeant biotinylating reagent, sulfosuccinimidyl-6-(biotinamido) hexanoate (Pierce), DIG fractions were analyzed for proteins exposed on the extracellular surface. Eleven major bands were observed, and, with the exception of ceruloplasmin, they all appeared to be glycoproteins based on their broad electrophoretic bands and their susceptibility to N-Glycanase digestion. If these DIG fractions are cell signaling centers, then both receptors and G-proteins should be present. Studies to date suggest that insulin and IGF-1 receptors, although clearly seen in Sertoli cell lysates, do not appear in the DIGs obtained from unstimulated cell cultures. By use of the photoaffinity label, 8-azido-[γ^{32}P]-GTP, to label G-proteins, major bands were observed in the region of 20-22 kD and 45 kD. This suggests the presence of members of both low molecular weight and heterotrimeric G-protein families. Work continues to identify signaling functions associated with these DIG fractions.

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ASSOCIATION OF SELENOPROTEIN P WITH TESTOSTERONE PRODUCTION IN CULTURED LEYDIG CELLS

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Selenoprotein P is a plasma selenoprotein and is thought to be as antioxidant defense agent such as glutathione peroxidase in the testis. We have recently reported that selenoprotein P mRNA was predominantly expressed in Leydig cells (Koga et al, Biol Reprod 58:261, 1998), and suggested to involved in testosterone production. On the other hand, the testosterone production was also reported to be associated with the O_2 toxicity in cultured Leydig cells (Georgiou et al, Endocrinology 121:1390, 1987). To assess the association of selenoprotein P with testosterone production, we examined the changes of the expression of selenoprotein P mRNA following the stimulation of cAMP in cultured Leydig cells under the condition of normal O_2 concentration. MLTC-1 (Mouse Leydig cell tumor) was used for this study. The expression of selenoprotein P mRNA was analysed by Northern blotting using total RNA eluted from each cultured cell, and testosterone concentration in each culture medium was measured by radio immunoassay. When cAMP was added at 0, 0.01, and 0.1 mM in the cell cultures, the expression of selenoprotein P was stimulated dose-dependently. cAMP was added at 0.1 mM in the cell cultures, and the expression of selenoprotein P and testosterone concentration was evaluated after the incubation time of 2, 5, 9, 15, or 24 hours (h). The expression of selenoprotein P was the maximal value at 9 h. The testosterone concentration in the medium also increased and was the maximal value at 15 h. Our data suggest that the expression of selenoprotein P in the Leydig cells increases following cAMP stimulation and that associates with O_2 toxicity induced by cAMP-mediated testosterone production as an antioxidant agent.

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TESTIS HISTOMETRY, SEMINIFEROUS EPITHELIUM CYCLE AND DAILY SPERM PRODUCTION IN SEXUALLY MATURE CATS (*Felis domestica*)

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In the present investigation, nine sexually mature cats were sacrificed and had their testis perfused-fixed through the left ventricle and abdominal aorta with 4% glutaraldehyde. Testis tissue were embedded in plastic (glycol methacrilate) and 3µm thick sections were stained with toluidine blue for light microscopy analysis. The testis weight in cats was 1.2 grams and testes mass related to the body mass (gonadosomatic index) was 0.08%. The tunica albuginea in these animals comprised about 19% of the testis. Seminiferous tubule and Leydig cell volume density (%) in cats were approximately 90% and 6%, respectively. The mean tubular diameter was 220µm, while 23 meters of seminiferous tubule were found per testis and per gram of testis. The frequencies of the eight stages of the cycle, characterized according to the tubular morphology system, were as follows: stage 1, 24.9%; stage 2, 12.9%; stage 3, 7.7%; stage 4, 17.6%; stage 5, 7.2%; stage 6, 11.9%; stage 7, 6.8% and stage 8, 11 %. The number of round spermatids for each pachytene (meiotic index) was 2.8, meaning that significant cell loss occurred during the two meiotic divisions. The total number of germ cells and the number of round spermatids per each Sertoli cell nucleolus at stage 1 of the cycle was 9.8 and 5.1, respectively. The Leydig cell volume and the nucleus volume were about 2000µm^3 and 260µm^3, respectively. The number of Leydig cells and Sertoli cells per gram of testis in cats were approximately 30 millions and 32 millions, respectively. The daily sperm production per gram of testis in cats (efficiency of spermatogenesis) was about 16 x 10^6. This data was obtained based on previous studies from our laboratory which showed that the spermatogenic cycle length in cats is 10.4 days (França et al., 1999). To our knowledge, this is the first investigation to perform a more detailed and comprehensive study of the testis structure in cats. Also, this is the first report in the literature showing the population of Sertoli cell and Leydig cell per gram of testis and the daily sperm production in any kind of Felidae species.

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MORPHOMETRIC AND ULTRASTRUCTURAL EVALUATION OF THE TESTIS IN ADULT WISTAR RATS TREATED WITH CIMETIDINE

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Cimetidine is a potent histaminic H2-receptor antagonist and is still widely prescribed in the treatment of gastric and duodenal ulcer. Due to its ability to competitively block dihydrotestosterone binding to androgen cytosol receptors, many reports in the literature have shown that cimetidine administration significantly reduces seminal vesicle gland weight and prostate gland weight. However, few and controversial results related to its possible effects on testis structure and function are available. The aim of the present investigation was to evaluate histometrically the testis, utilizing both light and TEM microscopy, in adult rats treated for about 8 weeks with Cimetidine (Tagamet, SmithKline Beechman). Three groups of 8 randomly-selected adult Wistar rats were utilized: Group I, control; Group II, Cim-treated (50mg/kg/BW); Group III, Cim-treated (250mg/kg/BW). Treated animals received i.p. daily injections of Cimetidine for 59 days, while control rats received 0.9% saline injections. At the end of the experiment rats were perfused with 4% glutaraldehyde. Before sacrifice, blood samples were taken to measure FSH and T serum levels. Compared with control, animals treated with the high dose had the testis weight decreased by 16% ($p>0.05$). However, epididimys weight, seminal vesicle weight and ventral prostate gland weight were decreased by approximately 40% in the same animals ($p<0.05$). Although there was a trend toward increasing T serum levels in treated animals, the difference observed was not significant ($p>0.05$). However, rats treated with the high dose had significantly higher FSH serum levels ($p<0.05$). Large individual variation was observed concerning the testis size and seminiferous tubule morphology in rats from both treated groups. Seminiferous epithelium height and tunica propria volume were significantly decreased ($p<0,05$) in high dose treated rats. TEM analysis showed that, in treated animals, basal lamina was morphologically altered in seminiferous tubule that were showing an apparently normal spermatogenesis. Apoptotic peritubular myoid cells were also observed. As myoid cells are androgen dependent, their perturbation could be the inicial mechanism responsible for the changes observed in seminiferous epithelium in treated animals.

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DAILY SPERM PRODUCTION AND SERTOLI CELL NUMBER PER TESTIS IN HYPOTHYROIDIC RATS TREATED WITH GNRH ANTAGONIST (ANTIDE), DURING TESTIS DEVELOPMENT

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Sertoli cell (SC) number per testis, established during the perinatal period, determines the final testis size and the number of sperm produced in adult animals. Studies with the goitrogen PTU (6-propyl-2-thiouracil) have shown that transient hypothyroidism during neonatal period leads to a highly significant increase in testis size, SC population and daily sperm production (DSP) in rats. Paradoxically, serum FSH levels, the hormone considered as the major mitogenic factor for SC proliferation, was low during all the experimental period. This report investigates the effects of the GnRH antagonist (Antide) (Bachem, CA, USA) in the testis of neonatally hypothyroidic rats sacrificed at 20 days of age and 100 days of age. Four groups with 16 randomly selected newborn Wistar rats were utilized: Group I, control; Group II, PTU-treated; Group III, Antide-treated; Group IV, Antide + PTU. All treatments were from 0-20 days of age. Pups treated with GnRH antagonist received daily s.c. injection containing 10µg of Antide in 0.1mL solution of distilled water, while the remaining groups received only the vehicle. At the end of the treatment with PTU and Antide, and at 100 days of age, 8 rats from each group had their testis fixed with 4% glutaraldehyde in order to perform testis histometrical analysis. Before sacrifice, blood samples were taken to measure FSH and testosterone serum levels in 100 days old animals. Rats treated with Antide had higher FSH levels ($p<0.05$) compared with control. However, testosterone serum levels and Leydig cell volume compartment were similar in all groups. Compared with PTU treated animals, adult rats treated with Antide and Antide + PTU showed significantly ($p<0,05$) decreased testis weight, seminiferous tubule volume, total tubular length, number of SC per testis and DSP per testis. No significant ($p>0,05$) differences were observed for the same parameters between Antide + PTU treated animals and control. These results indicate that, although present in low concentration, FSH is still a very important mitogenic factor for SC mitosis in PTU-treated animals. Also, other factors than FSH are possibly involved in SC proliferation.

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EFFECTS OF NEONATAL HYPERTHYROIDISM INDUCED BY ADMINISTRATION OF TRIIDOTHYRONINE (T3), ON SERTOLI CELL AND LEYDIG CELL NUMBERS IN LANDRACE BOARS

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The hormone triiodothyronine (T3) secreted by the thyroid gland is considered as an inhibitor of Sertoli cell mitosis and a promoter of Sertoli cell differentiation that occurs before puberty. Transient neonatal hypothyroidism caused by the treatment with PTU (6-propyl-2-thiouracil) significantly increases testis size, Sertoli cell and Leydig cell population, and daily sperm production in rodents. Paradoxically, experiments in our laboratory showed the opposite results for pigs, suggesting that the hormones secreted by the thyroid gland might not have the same effects in pig testis as observed for rodents. So, the objective of the present study is to investigate the testis structure and function in post-pubertal Landrace boars that were made hyperthyroidic through the administration of T3 during the neonatal period. Three groups of randomly selected newborn Landrace piglets were utilized as follows: Group I, control (n = 11); Group II, T3-treated, 50µg/kg/BW/day for 29 days (n = 8); Group III, T3-treated, 100µg/kg/BW/day for 21 days (n = 8). All treated animals received daily s.c. injection of T3, while the controls received only the vehicle. At approximately 160 days of age, testes were removed and fixed through the testicular artery with 4% glutaraldehyde. Testis samples were embedded in plastic and 4µm thick sections were stained with toluidine blue and analyzed. The number of Sertoli cells per testis was significantly decreased ($p<0.05$) in T3-100 animals and significantly increased ($p<0.05$) in T3-50 animals, compared with control. Although similar trend was observed for Leydig cell population, the differences found were not significant ($p>0.05$). The tubular diameter and the number of round spermatids per seminiferous tubule cross-section at stage 1 of the cycle were significantly higher ($p<0.05$) in T3-100 animals, indicating that spermatogenesis was more advanced in this group. In conclusion, the paradoxical results found in the present experiment show that the mechanisms of control of Sertoli cell and Leydig cell proliferation in pigs, concerning the role of thyroid hormones, apparently does not follow that described for the rodents so far investigated.

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TESTIS MORPHOMETRY, SEMINIFEROUS EPITHELIUM CYCLE AND SPERM PRODUCTION IN PECCARIES (*Tayassu tajacu*).

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The collared peccary is the only wild ungulate of the Western Hemisphere and has a yearlong breeding season. The peccary is becoming a species of economical interest due to the good flavor and low cholesterol content of its meat. The reproductive biology of the male collared peccary remains largely unexploited. Indeed, the data related to the testis histology and function for this species is very scarce. The aims of the present report were to characterize the testis structure, the seminiferous epithelium cycle, and to estimate sperm production in sexually mature peccaries. Six sexually mature animals from a commercial abattoir were utilized. Immediately after sacrifice, testes were removed and fixed by immersion with 4% glutaraldehyde. From each animal, the right testis was utilized for histological and histometrical evaluation, while the contralateral testis was frozen to estimate sperm production by homogenization technique. Testis tissue was cut and embedded and 4µm thick sections were stained with toluidine blue and analyzed. The volume density of Leydig cells and seminiferous tubule was 12% and 76%, respectively. The mean seminiferous tubule diameter was 240µm, while the total length of seminiferous tubule per testis was 283 meters. Leydig cells in peccaries have a peculiar morphology and organization in the intertubular compartment, and its individual volume was 1200µm^3. The number of Leydig cells, Sertoli cells and spermatids per gram of testis and per testis was 118 x 10^6, 21 x 10^6 and 92 x 10^6, and 21 x 10^8, 3.8 x 10^8 and 16 x 10^8, respectively. Staging in peccaries was based on the tubular morphology, where 8 stages of the cycle are yielded for all species. 6.5 spermatids were found for each Sertoli cell at stage 1 of the cycle of seminiferous epithelium. This support capacity, which is considered as an index of Sertoli cell efficiency and function, is situated in an intermediate to low level among the mammalian species investigated up to date. The meiotic index, which is the number of young spermatids for each pachytene primary spermatocyte, was 3.4. It means that, from the theoretical number of spermatids expected, more than 10% of cell loss occurred during the two meiotic divisions that happens at stage 4. Based on many parameters investigated, such as cell population and characterization of the seminiferous epithelium cycle, spermatogenesis in peccaries is very similar to that described for the domestic pigs.

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CORRELATION BETWEEN SEMEN VOLUME AND BLOOD TYPE (ABO) IN FERTILE INDIVIDUALS

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Human assisted reproduction has been widely used during the last decade, mainly because of recent developments in diagnosis and treatment of infertility. Among several factors that are able to cause infertility, 40% of them are due to male problems, while 40% to female problems and about 20% are related to both factors. Although new methodologies and approaches, such as intracytoplasmic sperm injection technique (ICSI), are now available to solve many infertility problems, anonymous sperm donation still plays a fundamental role in reproductive medicine, particularly for untreatable azoospermic patients. In the present investigation, semen characteristics from 110 fertile individuals who donated semen to a sperm bank were evaluated. The selection criteria for donors was according to the American Society of Fertility (ASF). Correlations with all data available about the donors were also performed. Donation of sperm took place between 1993 and 1995, for a sperm bank in the Human Institute of Reproduction of Belo Horizonte, Minas Gerais, Brazil. The donors' period of abstinence ranged from 3 to 5 days, but no difference was observed in semen volume concerning this parameter. The age of virtually all donors, with the exception of two, ranged from 22 to 32 years. All parameters related to sperm quality were according to what is considered normal by the World Health Organization (WHO). Significant correlation ($p < 0,05$) was found between semen volume and donors's blood type ($r = 0,22$). Analysis of variance showed that individuals with "O" and "B" blood type had semen volume significantly higher than those presenting "A" and "AB" blood type (3,1mL; 3,2mL; 2,7mL; 2,3mL, respectively, $p < 0,05$). The blood type distribution among donors followed the same pattern observed in general population (O = 44%; A = 42%, B = 11%; AB = 3%). To our knowledge, this is the first report in the literature showing that blood type correlates with semen volume in fertile men.

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AGE-RELATED INCREASE IN GERM CELL APOPTOSIS IN MALE BROWN-NORWAY (BN) RATS IS ASSOCIATED WITH INCREASED EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS).

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Aging in the male is associated with an impaired reproductive function due to alterations in the hypothalamic-pituitary-gonadal axis, which lead to an accelerated germ cell apoptosis, impaired spermatogenesis, and decrease in serum testosterone levels. We have proposed the BN rat as a model for male reproductive aging, showing testicular alterations indicative of a combined primary and secondary testicular disorder resulting in a reduction of total sperm production. However, the molecular events underlying germ cell death remain unclear. Because of the putative role of iNOS in NO mediated toxicity in various tissues, we now examined its role in age-related germ cell loss in testis. We speculate that increased apoptosis of germ cells in aging may be mediated by a spontaneous induction of iNOS that leads to increased NO formation and apoptosis. Testes from male adult (3 months) and old (24 months) BN rats were excised and western blots (WB) performed using antibodies against iNOS and nNOS. NOS activity was assessed by nitrite levels and synthesis of ^{3}H-L-citrulline from ^{3}H-L-arginine. The expression of iNOS protein (WB) was detectable at 3 months of age and increased by 2.5 fold in the normal-looking testis from the old rats, and by 4-fold in the regressed testis from these animals. No significant change occurred in nNOS expression in the old rats. NOS activity in the post-mitochondrial supernatant was increased by 26% (nitrites) and 32% (citrulline) in the old animals. iNOS was detectable in adult rats only in Leydig cells, and was intensified in the normal-looking testes of the old animals who also showed many positive Sertoli cells. The immuno-reactivity was localized in most Sertoli cells in the regressed testis from the old animals. nNOS was detectable only in the Leydig cells in both the adult and the old animals, but no age-related changes were apparent. Our results suggest that aging in the BN rat is accompanied by a spontaneous induction of iNOS and that this leads to an increase in NO formation and apoptosis.

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CASPASE-7 INCREASES IN GERM CELL UNDERGOING APOPTOSIS FOLLOWING HEAT STRESS

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We have previously shown that mild testicular hyperthermia in the rat (43 °C for 15 min) causes germ cell apoptosis, within 24 h, in a stage and cell-specific manner (Endocrinology 140: 1709-1717, 1999). The cell types affected by heat stress are pachytene spermatocytes (P) and early spermatids at stages I-IV, and pachytene, diplotene, dividing spermatocytes at stages XII-XIV. However, little is known about the complex mechanisms by which testicular hyperthermia induces germ cell apoptosis. Caspase-7, also known as Mch3/ICE-LAP3/CMH-1, is a member of the caspase-3 subfamily of cysteine protease and has been postulated to be the central executioner of cell death in non-germ cells. In this study, we test the hypothesis that caspase-7 may play a role in germ cell apoptosis following heat stress. Scrota of adult male SD rats were exposed to a temperature of 22 °C (control) or 43 °C for 15 minutes, and sacrificed at 1/2, 2, 6, and 24 h after heat treatment. In the control animals, caspase-7 immunostaining was detected mainly in a few germ cells undergoing spontaneous apoptosis, whereas viable germ cells, Sertoli cells and Leydig cells exhibited weak cytosolic caspase-7 immunostaining. Following heat treatment, caspase-7 immunoreactivity increased as early as 2 h, peaked at 6 h and decreased by 24 h post heat stress. The increase in caspase-7 immunospecificity was cell-specific, occurring only in those germ cells that are susceptible to heat induced apoptosis. These data indicate that caspase-7 is the down-stream executioner of germ cell apoptosis.

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GLUCOCORTICOID-MEDIATED INDUCTION OF APOPTOSIS IN RAT LEYDIG CELLS

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The occurrence of glucocorticoid receptor-mediated reductions in the expression of testosterone biosynthetic enzymes is well established. While it is known that glucocorticoids initiate the process of apoptosis in other cell types, there are few data with respect to Leydig cells. Since diminished numbers of Leydig cells per testis are associated with lower levels of circulating testosterone, the aim of the present study was to investigate whether increased Leydig cell apoptosis may contribute to glucocorticoid-mediated declines in testosterone production. Mid-pubertal, 35-day-old rats were bilaterally adrenalectomized. On day four after surgery, the animals were treated with two intraperitoneal injections (AM and PM) of CORT (2.5mg/kg b. w.) using dimethylsulfoxide as the vehicle. Leydig cells were isolated, after treatment in vivo, by a multistep procedure involving dispersion with collagenase and Percoll density gradient centrifugation. Apoptosis of isolated Leydig cells stained by propidium iodide (PI) or TUNEL labeling of cleaved DNA was evaluated with flow cytometry (FACS) or confocal laser scanning microscopy (CLSM), respectively. PI staining with FACS analysis revealed a significant increase in Leydig cell apoptosis after treatment with CORT (20.47% incidence of apoptosis compared to 4.98% in vehicle-treated controls, $P<0.01$). CLSM analysis of TUNEL labeling showed that isolated Leydig cells from CORT-treated rats also had increased frequencies of apoptosis compared to control. Further evidence of CORT-mediated induction of Leydig cell apoptosis was seen after treatment in vitro. Leydig cells purified from intact mid-pubertal day 35 postpartum rats were treated with CORT in vitro for 24 hours at 25, 50, 75, 100 and 125 nM, respectively, and then stained with PI for CLSM analysis. Leydig cells exposed to 25 nM CORT had low but detectable levels of apoptosis, as defined by the presence of intracellular apoptotic bodies. Membrane-bound, smooth-surfaced apoptotic bodies, containing a variety of intact cytoplasmic organelles and nuclear fragments, were observed with 50 nM CORT treatment after 24 hours. Starting at the higher dose of 75 nM, necrotic changes occurred in Leydig cells, as defined by loss of membrane integrity and random digestion of DNA. Increased Leydig cell apoptosis was also detected in PI stained Leydig cell analyzed by FACS: an incidence of 35% apoptosis in Leydig cells exposed to 50 nM CORT compared to that of 9.54% in the control ($P<0.01$). These results provide the first evidence for involvement of the endogenous glucocorticoid, CORT, in Leydig cell apoptosis, and indicate that declines in testosterone production may be due in part to reductions in Leydig cell number.

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UPREGULATION OF APOPTOTIC PROTEINS IN THE EJACULATED SPERMATOZOA OF OLIGOZOOSPERMIC MALES

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Numerous studies have shown that mature ejaculated spermatozoa possess endogenous nuclear DNA strand breaks[1,2]. Furthermore, men with abnormal sperm parameters are more likely to possess strand breaks[3]. It is thought that apoptotic mechanisms are responsible for the presence of spermatozoa with nuclear DNA damage, and a major participant is believed to be the Fas mediated pathway[4]. Our aim is to further characterize the role of apoptosis during sperm production by examining the role of other apoptotic markers. In this study we report the presence of a member of the Bcl-2 family of proteins in ejaculated spermatozoa, Bcl-x.

Ejaculated spermatozoa (29 samples) were labeled with the anti-Bcl-x antibody and the number of spermatozoa labeled positive were counted using a Fluorescence Activated Cell Sorter. In addition, spermatozoa from the same samples were labeled to examine the percentage of spermatozoa with nuclear DNA damage, using the terminal deoxynucleotidyl transferase (TUNEL) assay. The mean ($\pm$ SD) percentage of sperm positive for Bcl-x in 11 oligozoospermic men with less than 20 million sperm per ml was 29.2 ± 17.0 which was significantly higher ($P<0.05$) than in the 18 normal men with greater than 20 million sperm per ml (13.7 ± 11.6). Interestingly, the same pattern was observed when comparing the ratio of Bcl-x / TUNEL in each patient, whereby oligozoospermic men had a significantly ($P<0.05$) higher ratio of 1.8 ± 1.5 compared to 0.8 ± 1.0 in normal males.

The results indicate that Bcl-x is upregulated in men with decreased sperm numbers. Bcl-x can exist in the anti-apoptotic Bcl-xL and pro-apoptotic Bcl-xS forms, therefore further studies are needed to distinguish which form is present in ejaculated spermatozoa. It is interesting to hypothesize that in oligozoospermic men, the level of the anti-apoptotic inhibitor, Bcl-xL, might be increased during spermatogenesis in order to preserve sperm numbers.

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INVOLVEMENT OF C-SRC IN EGME-INDUCED RAT TESTICULAR APOPTOSIS

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Kinase activities were previously proposed to be central to germ cell apoptosis induced by ethylene glycol monomethyl ether (EGME) and its active metabolite methoxyacetic acid (MAA). We evaluated the role of tyrosine kinase c-Src in this lesion *in vivo*, as well as in *in vitro* cultured adult rat seminiferous tubules treated with MAA. In normal testicular tissue, immunoreactivity of Src was mostly detected in Sertoli cell cytoplasm, and reached the maximum level adluminally at spermiation. At the EM level, reaction product was noted in Sertoli cells and at the Sertoli / germ cell junctions, in both Sertoli and germ cells. A single dose of 200 mg/kg EGME induced an increase of Src immunoexpression in both epithelium and interstitium in rat testis. Eight hours after treatment, an intensive immunostaining of Src was observed specifically in the cytoplasm of the dying spermatocytes. As in previous studies, the apoptotic changes were replicated by exposure of adult rat seminiferous tubules to 5 mM MAA *in vitro*. Furthermore, spermatocyte degeneration was significantly prevented by co-treatment with 0.1 µM geldanamycin, 10 µM herbimycin A or 10 µM PP2, which are inhibitors of Src activity. These data collectively suggest that c-Src may mediate Sertoli-germ cell interaction in physiological events, and appears to play an important role in EGME/MAA-induced germ cell apoptosis.

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MOLECULAR GENETIC ANALYSIS OF TWO FIBROUS SHEATH PROTEINS, AKAP4 AND AKAP3, IN MEN WITH DYSPLASIA OF THE FIBROUS SHEATH.

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Dysplasia of the Fibrous Sheath (DFS) is characterized by male infertility associated with asthenozoospermia and morphologically abnormal sperm flagella. Typically, sperm from affected individuals possess short, thick, irregular tails. The most striking ultrastructural feature of sperm from men with DFS is a malformed fibrous sheath. In approximately 20% of the reported cases of DFS, the disease shows a familial incidence, strongly suggesting a genetic component. Human AKAP4 (formerly hAKAP82) and AKAP3 (formerly AKAP95T/AKAP110) are structural proteins of the FS that also function to anchor protein kinase-A (PK-A) to the FS via the regulatory subunit of the kinase. We hypothesized that defects in either AKAP4 or AKAP3 might result in a phenotype similar to that seen in DFS. Polyacrylamide gel electrophoretic and immunoblot analysis of sperm proteins using anti-AKAP4 and anti-AKAP3 did not detect any differences between either of these proteins in three DFS patiems compared to normal controls. Additionally, AKAP4 and AKAP3 in sperm from three DFS patients retained the ability to bind the regulatory subunit of PK-A. Sperm from five DFS patients were examined by light microscopic immunocytochemistry, and it was shown that AKAP4 localized to the abnormal flagella. Additionally, sperm from one DFS patient were examined by immunogold electron microscopy, and AKAP4 was identified in the FS of the amorphous flagella. Partial sequence analysis of the AKAP4 and AKAP3 genes in DFS patients did not detect mutations. Our results did not identify abnormalities in either gene or protein which might be responsible for DFS in humans; however, there are large regions of both AKAP4 and AKAP3 which have not yet been examined in detail. Further studies will be required to rule out defects in either of these genes or proteins as the cause of DFS. (Supported in part by National Institutes of Health Grants HD01 189 (to R.M.T.) and HD06274 (to G.L.G. and S.B.M.) and grants from CONICET (PICT0090) and ANPCyT (PICT0450) to H.E.C.).

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ACQUISITION OF EPIDIDYMAL SPERM MOTILITY IS ALTERED IN AGING BROWN NORWAY RAT. P. Syntin* and B. Robaire. McGill University, Montréal, Québec, Canada.

In the Brown Norway (BN) rat, aging of the male reproductive tract occurs at a time when pathological changes are not taking place in other systems. In the epididymis, some striking segment-specific changes occur in the histological and biochemical characteristics of the tissue. We hypothesized that epididymal maturation of spermatozoa, and hence sperm motility, might be altered during aging. Motility parameters of caput and cauda epididymal spermatozoa obtained from young (3-4 months) and old (20-22 months) BN rats were compared using computer-assisted sperm motility analysis. In the caput epididymidis, the percentage of motile sperm was similar between young (Y) and old rats, on either the normal (O-N) or regressed-testis (O-R) side (Y:16%, O-N: 14.5%, O-R:10%). No significant differences were observed for any of the seven motility parameters analyzed. In contrast, the motility of spermatozoa from the cauda epididymidis was significantly decreased (Y:76%, O-N:62%) on the normal testis-side of aged animals but the kinematic parameters were unchanged. However, on the regressed-testis side, the motility was decreased remarkably (Y:76%, O-R:25%); all 3 velocity parameters (average pathway, straight-line, and curvilinear) were significantly reduced ($P<0.02$), while those reflecting beat characteristics were not. Thus, in old animals, the low motility observed in caput spermatozoa was changed neither quantitatively nor qualitatively; the percent of motile spermatozoa from the cauda epididymidis was decreased, and spermatozoa from the regressed-testis side had altered motility characteristics. This altered sperm motility acquisition during aging may result from the major changes found in cellular epididymal architecture and function. Supported by NIA-AG08321.

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DIFFERENTIAL EXPRESSION OF DNA REPAIR GENES DURING SPERMATOGENESIS
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DNA damage of the male germ cell, if not repaired, can lead to altered progeny outcome. To assess the DNA repair capability of the different cell types arising during spermatogenesis, we examined the expression of genes involved in different classes of DNA repair. These were: PARP, APEX, and TDG, which participate in base excision repair (BER); PCNA, a transcription and nucleotide excision repair (NER) protein; and RAD51, a homologous recombination repair gene. Pachytene spermatocytes, round spermatids, and elongated spermatids were isolated from adult rat testes; RNA was extracted and probed on cDNA Atlas Array membranes (n=3). The pattern of expression obtained differed significantly among cell types for a number of genes. PARP was highly expressed in pachytene, with levels significantly decreasing in round (70%) and further in elongated spermatids (72% of round). APEX was present in pachytene, increased 3.5 fold in round spermatids, and decreased by 50% in elongated spermatids. The pattern of PARP and APEX expression was consistent with previous findings. The other member of BER, TDG, was only detected in pachytene spermatocytes. PCNA was highly expressed at different levels in the three cell types. There was a 2.5 fold increase between pachytene and round spermatids, while the levels decreased by almost 95% in the elongated spermatids. RAD51 was present in pachytene spermatocytes, but not detected in round or elongated spermatids. These results demonstrate that key components of the DNA repair machinery are differentially expressed during male germ cell differentiation. Supported by the MRC of Canada.

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IN VITRO CAPACITATION AND ACROSOME REACTION OF BOVINE SPERM FACILITATED BY NOREPINEPHRINE (NE). A.L. Way and G.J. Killian, J.O. Almquist Research Center, Penn State University, University Park, PA.

Previous studies have determined that epinephrine and NE induce the acrosome reaction in sperm. The discovery of β-adrenergic receptors on bovine oviductal epithelial cells (Endocrinol. 1999;140:2679-2684) suggests that catecholamines may be present in oviductal fluid and may influence oviductal or gamete function. To determine if NE affects bovine sperm capacitation and the acrosome reaction, semen from three Holstein bulls was pooled and washed twice (500 g, 10 min) in modified Tyrode's medium (MTM). In experiment 1, sperm (5×10^7/ml) were incubated in MTM containing 0, 0.01, 0.1, 1.0, 10, 100 and 1000 ng/ml NE for 6 h (39°C, 5% CO_2/air). Every 2 h an aliquot of sperm suspension was incubated with 100 μg/ml lysophosphatidylcholine (LPC) to induce the acrosome reaction in capacitated sperm (10 min, 39°C). Samples were evaluated for motility, and for viability and acrosomal integrity using eosin B/aniline blue staining and DIC microscopy. To determine if NE induced the acrosome reaction in capacitated sperm (Experiment 2), 5×10^7 sperm/ml were incubated in capacitating medium (10 μg/ml heparin in MTM), or MTM alone (negative control). Every 2 h an aliquot of sperm suspension was challenged with NE and evaluated as described. In both experiments the only differences observed were at 2 h. Sperm incubated with 10 ng/ml NE for 2 h followed by LPC (Experiment 1), or capacitated with heparin for 2 h and challenged with 10 ng/ml NE (Experiment 2) had significantly more acrosome-reacted live sperm than sperm incubated in other concentrations of NE or controls. At 2 h the percentage of acrosome-reacted live sperm incubated in 10 ng/ml NE and induced to acrosome react with LPC (23.33 ± 3.67) and sperm incubated in heparin and induced to acrosome react with NE (19.33 ± 4.63) were comparable to sperm capacitated with heparin for 6 h and induced to acrosome react with LPC (18.33 ± 5.67). Because either LPC or heparin were required in both experiments to observe the effect at 2 h with 10 ng/ml NE, we propose that physiological concentrations of NE accelerated bovine sperm capacitation or the acrosome reaction by potentiating the membrane destabilizing ability of these agents. (USDA grant #96-3523-3428)

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CELLULAR DISTRIBUTION OF BULL SPERM CALMODULIN-BINDING PROTEINS
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Previous studies from our laboratory have shown that a decrease in the intracellular concentrations of calmodulin occurs during the heparin-induced bull sperm capacitation process. In addition, this effect was associated with a decrease in the calmodulin binding properties of few sperm proteins. However, there was indication on the localization of these capacitation-affected proteins. This was recently investigated in 2 different ways: 1) biotinylated-calmodulin nitrocellulose overlay assay on protein extracts of subcellular fractions of bull spermatozoa and 2) biotinylated-calmodulin binding to fixed permeabilised spermatozoa and revealed by fluorescein-conjugated streptavidin. The nitrocellulose overlay assay revealed that one of these proteins (p32) is detected in the flagellar-enriched fractions whereas p30 is found in the fraction enriched with sperm heads. This latter calmodulin binding, p30, appears to be associated with the perinuclear theca. None of these binding proteins was solubilized by nonionic detergents. SDS was effective solubilizing p32, whereas p30 was extracted only in conditions reported to isolate the perinuclear theca. Using the fluorescence microscopy procedure, it was found that calmodulin binds to the sub- and post-acrosomal areas of the sperm head along with the midpiece in the presence of Ca^{2+}. Only a sharp band of fluorescence at the sub-acrosomal area was observed when this procedure was performed in the absence of Ca^{2+}, in the presence of EGTA. The pattern of cellular calmodulin binding was highly decreased when spermatozoa were incubated under capacitating conditions, in the presence of heparin, in agreement with the published effect of capacitation on calmodulin binding proteins. (supported by the Natural Science and Engineering Research Council of Canada and L' Aliance SEMEX Inc)

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CHARACTERIZATION OF A BULL SPERM PROTEIN RECOGNIZED BY A p60src SPECIFIC MONOCLONAL ANTIBODY

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The process of capacitation, is characterized by membranous and biochemical changes in the spermatozoa. At the molecular level, a number of studies have observed a change in the tyrosine phosphorylation of specific sperm proteins during capacitation. Studies utilizing various tyrosine kinase inhibitors as well as preliminary experiments done in our laboratory suggest that the Src family tyrosine kinase are present in the increase in sperm protein phosphotyrosyl content. The aim of this project is to identify the protein tyrosine kinase(s) implicated in the tyrosyl phosphorylation associated with capacitation of bull spermatozoa. Using Western blot assays, we have detected a 80kDa protein recognized by a p60src specific monoclonal antibody. This protein was found in the soluble fraction of bull sperm protein extract. Analysis by non-equilibrium 2D electrophoresis suggest that the p80 src-like protein has at least 3 isoforms with pI ranging from 7.4 to 8.2. By FPLC chromatography, we have purified the src-like protein and the amino acid sequence of a tryptic fragment will shortly help us to identify the protein. Furthermore, this protein could be part of a complex, as suggested by gel electrophoresis performed under non-reducing conditions and western blot. By indirect immunofluorescence, we have localized the protein in the post-acrosomal region. This project will help to provide a better understanding of the molecular mechanisms involved in mammalian sperm capacitation. This project is supported by NSERC and ALLIANCE SEMEX.

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IN VIVO INCORPORATION OF DOCOSAHEXAENOIC ACID INTO GERM CELLS AND EPIDIDYMAL MOUSE SPERMATOZOA.

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Docosahexaenoic acid (DHA) plays an important role in the regulation of normal sperm function. Although mature sperm incorporate radiolabeled DHA into membrane glycerolipids, the extent of this incorporation is <1% of total fatty acid content. In this study, DHA levels in germ cells, epididymal sperm, plasma, and pancreas were determined in mice following administration of either DHA or other n-3 fatty acids known to interfere with DHA biosynthesis and/or incorporation into cell membranes. C57 adult, weight-matched male mice were fed 40mg/day of either DHA, eicosapentaenoic acid (EPA), or α-linolenic acid (LNA) as an stable emulsion in Peptamen (Nestle, Inc.) for up to 5wks which is the duration of the spermatogenic cycle in mouse sperm. At sacrifice, blood was obtained by cardiac puncture, pancreas removed and homogenized, and germ cells and epididymal sperm obtained from the testes. Lipids were extracted with chloroform-methanol, fatty acids methylated, and analyzed by gas chromatography. Germ cell and epididymal sperm concentration was determined by phase-contrast microscopy and processed for histochemical analysis. The results are shown in the table below. Values are DHA levels in μg/ml (plasma), nmoles/mg of protein (pancreas), nmoles/million cells (germ cells and epididymal sperm), after 5wks of treatment, and represent the X±SD of 3 separate experiments.

	Plasma	Pancreas	Germ cells	Epididymal
control	0.050±0.006	0.62±0.07	1.80±0.18	0.47±0.06
LNA	0.035±0.004	1.80±0.31	1.40±0.16	0.27±0.03
PA	0.034±0.005	1.66±0.28	1.35±0.35	0.25±0.03
DHA	0.25±0.032	1.69±0.22	6.30±0.71	0.86±0.09

DHA levels were significantly increased in germ cells and epididymal sperm compared to untreated mice ($p=0.001$). In contrast, administration of LNA and EPA resulted in a significant decrease in DHA levels in germ cells and epididymal sperm ($p<0.05$). Therefore, DHA levels in human sperm glycerolipids can be significantly modified following oral administration of exogenous fatty acids. DHA content in epididymal sperm was significantly lower than in germ cells in all groups tested ($p<0.001$). Although DHA levels in plasma and pancreas reached maximal levels following 1 wk of DHA administration (data not shown), DHA levels in germ cells and epididymal sperm were not significantly different from those seen in control mice ($p=0.62$). These results suggest that (i) DHA is primarily incorporated into germ cells during spermatogenesis and (ii) that there is a net loss of DHA as germ cells differentiate into epididymal sperm. No significant differences in sperm concentration or morphology were found between the different groups.

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IDENTIFICATION OF A 32-kDa CAPACITATION-RELATED PHOSPHOPROTEIN WITH TYROSINE KINASE ACTIVITY IN PORCINE SPERM.

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The objectives of this study were to determine whether tyrosine phosphorylation of sperm proteins is associated with capacitation of pig sperm, which factors affect tyrosine phosphorylation and to demonstrate that a 32-kDa tyrosine-phosphoprotein possesses tyrosine kinase activity concomitant with capacitation. Sperm were incubated in non-capacitating or capacitating media (CM) for various times at which proteins were extracted, subject to SDS-PAGE then immunoblotted with an antiphosphotyrosine antibody. A 32-kDa tyrosine-phosphorylated protein (32PP) appeared only when the sperm were incubated in CM and concomitant with capacitation ($n>10$). The 32PP also appeared in CM-$NaHCO_3$, although the sperm did not capacitate ($p=0.0012$, $n=4$). Omission of BSA in CM did not affect the appearance of the 32PP, however, capacitation was also unaffected ($p=0.87$, $n=2$). Sperm incubated in CM Ca^{++} did not display the 32PP or undergo capacitation ($p=0.04$, $n=2$). To test the hypothesis that a protein tyrosine kinase (PTK) is activated during capacitation, enzyme renaturation of sperm proteins was performed on gels containing either poly glu:tyr (PTK substrate) or kemptide (protein kinase A substrate). A 32-kDa enzyme with kinase behavior was observed with both substrates, but was preferentially phosphorylated on tyrosine residues ($p=0.02$). Thus, the appearance of the 32PP is associated with capacitation in pig sperm in a $NaHCO_3$- and Ca^{++}-dependent manner but is not sufficient for its completion. As well, pig capacitation seems to be regulated by a PTK and is currently under investigation in our lab.

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FULL LENGTH LOW VOLTAGE-ACTIVATED ("T-TYPE") CALCIUM (Ca^{2+}) CHANNEL α_{1G} mRNA IS NOT DETECTED IN MAMMALIAN TESTIS AND SPERM.

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We searched for expression of mRNA transcripts encoding the α_{1G} ion pore-forming subunit of T-type Ca^{2+} channels in mammalian testis and sperm in an attempt to resolve the controversy whether the Ca^{2+} channels which regulate acrosome reactions (AR) are T-type or high voltage-activated ("HVA", e.g., "L-type"). Electrophysiological and optical studies with ion-selective fluorescent probes suggest that immature spermatogenic cells and ejaculated sperm express T-type Ca^{2+} currents. In contrast, Northern blots, reverse transcription-polymerase chain reaction (RT-PCR) analyses and immunohistochemical staining indicate expression only of HVA Ca^{2+} channel α_1 subunits (α_{1A}, α_{1C} and α_{1E}). An α_1 subunit has cytoplasmic amino and carboxy (C)-termini separated by 4 repetitive domains (I-IV). In vitro, HVA α_1 subunits produce T-type Ca^{2+} currents. Zona pellucida-induced Ca^{2+} entry sites and α_{1C} protein co-localize on mammalian sperm heads. Deletions in α_{1C} sequences may be associated with reduced AR.

In the current study, RNA from rat testis, rat brain and ejaculated human sperm from individual known fertile donors served as template for RT-PCR using 8 sets of PCR primers designed to amplify α_{1G} sequences beginning at domain I through the C-terminus. Control primers included those for testis-specific α_{1C} subunits and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). All primer pairs were designed to span a region containing at least 1 intron within the respective genes. All α_{1G} primer pairs amplified a product of the expected size from rat brain cDNA, confirming the expression of full length transcripts in this tissue. In contrast, no PCR product was detected using DNase-treated rat testis mRNA as template with α_{1G} primer pairs to domains I-III. Nevertheless, α_{1C} primers amplified sequences from rat testis template encoding domain IV and the C-terminus. Full length α_{1C} transcripts were identified in the same template. No PCR products were obtained when RT was omitted. None of the α_{1G} primer pairs tested, including those for domain IV, produced a product with sperm RNA template whereas α_{1C} and GAPDH gene-specific products were readily detected.

Full length α_{1G} mRNAs, homologous to those detected in brain, are not found in mammalian testis and sperm suggesting that a functional α_{1G} Ca^{2+} channel is not present in these tissues. The partial transcripts detected in testis may be derived from another gene or from alternative splicing of the same gene which produces brain α_{1G} subunits. In contrast, complete α_{1C} subunit transcripts and protein are expressed in mammalian testis and sperm. These data support the hypothesis that HVA L-type α_{1C} Ca^{2+} channels regulate sperm Ca^{2+} influx and AR. (Supported by NIEHS/NIH Grant No. ES 06100 to S.B.)

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SPERM FROM MICE CARRYING TWO *t* HAPLOTYPES ARE DEFECTIVE IN SEVERAL STEPS IN OOLEMMA PENETRATION.

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The *t* haplotypes are naturally occurring variant homologs of the proximal third of Chr17 (~30–40mbp DNA) and contain four large, non-overlapping inversions (*In1-4*) relative to the corresponding region (the *t* complex) of the wildtype homolog. Males carrying two *t* haplotypes (*t/t*) are sterile with sperm demonstrating poor motility and an inability to penetrate zona pellucida-free oocytes. The absence of sperm-oolemma penetration could be due to a defect in sperm binding and/or fusion with the oolemma. To determine whether sperm-oolemma binding was affected, an improved sperm-oocyte plasmalemma binding assay was used (Redkar & Olds-Clarke, J Androl 20, 500, 1999). In control +/+ mice, sperm binding was maximal (29±4 sperm/oocyte) and all eggs were polyspermic (fertilization index,FI,=4±0.3) by 30 min coincubation. Sperm from *t/t* mice showed negligible (1±1) binding and 0% oocyte penetration even after 180 min. To locate the genes responsible for the binding defect, sperm from mice carrying only a portion of a *t* haplopype were also tested. Sperm from mice homozygous only for the proximal inversions *In1-2* (fertile) exhibited a slow rate of binding (5±1 sperm/oocyte at 30 min) and a very low FI (0.01±0.01 at 30 min). However, by 120 min, sperm binding had increased (24±2) and all the oocytes were penetrated. Sperm from mice homozygous for the distal inversion *In4* demonstrated a rate of binding similar to the control but penetrated oocytes more slowly than the control (FI=0.7±0.2 at 30 min). These data suggest that genes important for sperm-oolemma binding are separate from genes involved in later stages of sperm-oolemma penetration and are located in different regions of the *t* complex. Though the molecular bases of these defects are yet to be understood, the significantly reduced capacitation observed in *t/t* sperm (Si and Olds-Clarke, BOR 61, 305, 1999) could be one of the contributing factors.

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MOLECULAR CHARACTERIZATION OF *Dnahc8*, A GENE CONTROLLING SPERM TAIL ASSEMBLY AND SPERM MOTILITY.

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Dnahc8, an axonemal dynein heavy chain, has been mapped to the *Hst6* locus in the 4th inversion of the mouse *t* complex on proximal Chr 17 (Samant et al., 1999 *Mamm. Genome* 10: 88-94; Fossella et al., In Press, *Mamm. Genome*). Two mutations in this gene, *Dnahc8t* and *Dnahc8s*, both resulting in male sterility when homozygous, cause aberrant sperm motility ("curlicue" phenotype) and failed sperm tail development ("whipless" phenotype), respectively. Compound heterozygous males (*Dnahc8t/Dnahc8s*; also sterile) exhibit "curlicue", implying that *Dnahc8s* is a loss-of-function allele while *Dnahc8t* is an altered-function allele. Previous northern analyses have shown that *Dnahc8* is testis-expressed, and have demonstrated that *Dnahc8s* is a null allele in the testis (Fossella et al., In Press, *Mamm. Genome*).

To fully characterize the roles of *Dnahc8* in male fertility, we have conducted an in-depth study of its gene expression. Analyses of an extensive range of tissues by northern, RNA dot-blot, and *in situ* hybridization have proven that *Dnahc8* is testis-specifically expressed. *In situ* hybridization experiments have also shown that expression takes place from middle to late stages of the spermatogenic cycle, and is exclusive to mid-pachytene to secondary spermatocytes, suggesting that *Dnahc8* is translated prior to spermiogenesis for distribution to all of the products of meiosis. To further understand the critical nature of *Dnahc8* expression to sperm tail development and function, we have sequenced a 160,000 bp genomic isolate of *Dnahc8+*, and are currently isolating and analyzing a cDNA copy (~15,000 bp) of *Dnahc8t* (altered-function allele), as well as the transcriptional control region upstream of the *Dnahc8s* (null allele) structural gene.

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SINGLE CELL IMAGING OF PROGESTERONE-INDUCED, BIPHASIC ELEVATION OF $[Ca^{2+}]_i$ IN HUMAN SPERMATOZOA

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Exposure of human spermatozoa to µM levels of progesterone evokes a biphasic increase in $[Ca^{2+}]_i$. An initial transient, which peaks and decays within 3-4 min, has been described in many laboratories (Aitken, 1997). A secondary, sustained $[Ca^{2+}]_i$ response has also been detected by the use of spectrofluorimetry (Baldi et al, 1998). We have used confocal imaging of Calcium Green-1 loaded human spermatozoa to investigate the charactersitics of progesterone-activated $[Ca^{2+}]_i$ signalling, particularly the secondary response, at the single-cell level.

Capacitated spermatozoa labelled with Calcium Green-1 AM were adhered to coverslips in a perfusion chamber and imaged on a confocal microscope. Dye retention and movement of the flagellum confirmed sperm viability.

Upon exposure to 3.2µM progesterone most spermatozoa (70.3±6.4%; mean±SEM n=8) showed a significant increase in fluorescence from the head that peaked within 1-2 min and decayed with a similar time-course. The modal increase in fluorescence (in responsive cells) was 35% but small numbers (1-2%) of cells showed increases of up to 200%. 31.2±7.2% (mean±SEM; n=8) of spermatozoa showed a significant, sustained increase in $[Ca^{2+}]_i$ which began 3-5 min after progesterone application and persisted until the end of the recording period (15-20 min). The amplitude of these responses (recorded 15 min after progesterone application) was typically 35% above control fluorescence but in a minority of responsive cells (4-5%) the amplitude was ≥200% above control level. The sustained response was not confined to cells that had undergone an initial transient response and there was no significant association between expression of the primary and the secondary responses.

Initial studies suggest that pre-treatment of preparations with nifedipine (10µM) has no significant effect on the transient response of $[Ca^{2+}]_i$ but reduces the number of cells in which the secondary response is seen (10.9±4.1%; mean±SEM; n=4).

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ENVIRONMENTAL LEAD (Pb2+) NEGATIVELY IMPACTS ON ARTIFICIAL INSEMINATION OUTCOMES.

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We performed a prospective double blinded, repeated analysis of acrosome function of motile sperm and of Pb2+ levels in seminal plasma (SP) from known fertile semen donors (n=10) and men being screened as potential semen donors (n=11) who were not occupationally exposed to Pb2+ and did not smoke cigarettes. This extends a previous study of infertility patients, showing a very broad range (<100 µg/L to >1200 µg/L) of Pb2+ levels. SP Pb2+ levels were negatively correlated with fertilization rates in vitro and directly with decreased progesterone (P)-induced acrosome reactions (AR). In vitro modeling studies indicate that sperm voltage-gated potassium channels (VGKC) regulated P-AR and that Pb2+ entered human sperm heads through VGKC. P-AR inhibitor sensitivities and cDNA sequencing implied existence of multiple sperm VGKC isoforms and suggested that each man's ejaculated sperm expresses a single VGKC isoform. This association of Pb2+ with a human sperm AR insufficiency needed confirmation in an independent population.

In the current analysis, all men studied were between ages 19-39 and semen analyses were within normal WHO limits. Three to seven SP specimens from each man were assayed for Pb2+ in triplicate by atomic absorption, with <5% intra-specimen variation between Pb2+ measurements and <9.5% intra-male variation. P-AR and P-AR inhibition by VGKC inhibitors (tetraethylammonium ion, charybdotoxin [CBTx] and 4-aminopyridine) were assessed in a subset (n=14). Histories of intra-uterine or cervical insemination cycle fecundity (f) were obtained for 5 of the known fertile donors. SP Pb2+ levels ranged from 150 to 1600 µg/L. Subjects were divided into two groups based on CBTx sensitivity ($CBTx^S$) or resistance ($CBTx^R$) of P-AR. $CBTx^S$ men exhibited significantly higher levels of P-AR (41.3±2.2%) and lower SP Pb2+ levels (199.6±36.2 µg/L) than $CBTx^R$ men (respectively, 29.7±4.8% and 745.3±55.1 µg/L) ($P<0.01$ and $P<0.0001$). The f value for $CBTx^S$ donors was 16.9±1.2% (2/31; 1/6; 3/19) while that for $CBTx^R$ donors was only 2.2±3.1% (10/230; 0/11) ($P<0.004$).

These similar results from a geographically separate population confirm the initial study and show the utilities of these assays. They also indicate that environmental Pb2+ exposure may have a greater impact upon human male fertility than currently appreciated. They further suggest that men expressing $CBTx^R$ VGKC represent a susceptibility subgroup more sensitive to environmental Pb2+ exposures than the general population. Sperm K+ channel isoforms may be developed into biomarkers to predict the effects of Pb2+ on human sperm function.

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SEMEN QUALITY OF MEN APPLYING PESTICIDES IN NORTHWEST MINNESOTA

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NIOSH and the University of Minnesota conducted a reproductive health study of men applying pesticides in northwest Minnesota. Two semen samples were collected from each of 90 study participants. The first semen sample was collected in July 1998 at the end of the herbicide application season and the second in late October 1998 at the end of the fungicide application season. A complete semen analysis was conducted on each semen sample including computerized semen analysis (HTM-IVOS, Hamilton-Thorn, Beverly, MA). The semen data were stratified by pesticide application history and analyzed for the effects of pesticides using generalized estimating equations (for discrete outcome variables) and the mixed model repeated measures approach (for continuous outcome variables). The percent normal sperm morphology (WHO) was significantly lower in the fungicide applicators (non-fungicide 29.8 % ± 2.6; fungicide 20.8% ± 3.4; $p = 0.016$). Fungicide applicators also had a lower sperm straight line velocity than the men not applying fungicides (non-fungicide 55.7 μm/sec ± 1.3; fungicide 48.4 μm ± 2.6; $p = 0.02$). A decrease in straightness of swimming path (VSL/VCL) was associated with both herbicide and fungicide application (non-herbicide 0.60 ± 0.03, herbicide 0.52 ± 0.01, $p = 0.02$; non-fungicide 0.58 ± 0.01, fungicide 0.54 ± 0.02, $p = 0.05$). The associations found in these analyses generate the need to separate the broad categories of herbicides and fungicides and study the association between specific pesticide chemicals and human semen quality. Further data analyses are underway detailing specific pesticide use based on current pesticide application records.

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DEVELOPMENT OF PIGS FOLLOWING *IN UTERO* AND LACTATIONAL EXPOSURE TO ORGANOCHLORINES: EFFECTS ON MALE REPRODUCTIVE FUNCTION. B. Bérubé*, J.P. Laforest*, R. Sullivan, M.A. Sirard*, P. Ayotte* and J.L. Bailey, Centre de Recherche en Biologie de la Reproduction, Dépt des sciences animales, Université Laval, Québec, QC G1K 7P4.

The development and maintenance of reproductive tissues is to a large extent controlled by steroid hormones. Some environmental chemicals like organochlorines mimic, while others antagonize natural hormone activity when tested with *in vitro* assays or in whole animal models (Fry 1981; Jobling 1995). Typical organochlorine mixtures found in highly exposed human populations contain a large variety of organochlorine compounds, including substances with estrogenic, anti-estrogenic or anti-androgenic capacities (Moore 1997; Safe 1990). The objective of this study is to assess the impact of pre- and postnatal exposure to organochlorine mixtures found in the Arctic on the development and function of the male reproductive system, using the pig as the model. We present here the results of the first part of this study. Sixteen sows were randomly distributed to 4 treatment groups and administered various levels of a polychorinated biphenyl (PCB) cocktail composed of 15 organochlorine products (control; 1μg/kg PCB; 10 μg/kg PCB and 100 μg/kg PCB) from 4 months of age until their first litter (~34 weeks). Treatment had no effect on the weight gain of the sows (269 ± 5 kg) and no anatomical or fertility effects were observed. The PCB concentration in the serum of the 100 μg/ml group was 14.4 ± 0.6 μg/L after 30 weeks of treatment. Prepubertal piglet development appeared unaffected by the treatments. Semen analyses will be performed on the pigs as reach puberty. At this time of the experiment, we cannot conclude that this organochlorine mixture affects the growth rate and fonctional development of the reproductive system of the pig. *This study is supported by the Arctic Environmental Strategy Northen Contaminant Program Branch of Health Canada.*

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THE CONTROL OF SPERMIATION IN THE RAT R.E. Chapin, M.W. Harris*, J.K. Haseman* and R.N. Wine*, Reproductive Toxicology Group and Biostatistics Group, NIEHS/NTP, RTP, NC

Because most testicular toxicants inhibit spermiation during the pathogenesis of a testicular lesion and little is known about this process, we have developed an *in vitro* model to explore the control of late spermatid release. When incubated overnight, short segments of stage VII-VIII tubules from adult SD rats evert at both ends, exposing the epithelium to the medium. With gentle rocking, progression can be observed histologically (spermiation, elongation of round spermatids, basal movement of residual bodies), and sperm in the medium can be counted to quantify release. Although not all sperm are released in control tubules, this provides an initial model with which to begin the study of this process. For control tubules in DME/F12 medium with ITS+®, EGF, and Testosterone, the degree of release was found to be highest in stage VIII tubules (50-60%), and lowest in stage VI tubule fragments (14%), while stage VII tubules gave intermediate values (≈27%). Sodium azide (1.3 – 2.6 mM) and sodium cyanide (1.7 mM) inhibited release by ≈ 40%. The omission of EGF from the medium, or the inclusion of the EGF kinase blocker PD168393 (30 nM), both lowered release, suggesting the involvement of EGF and protein phosphorylation. Indeed, previous immunohistology suggested that protein phosphorylation might play a key role in spermiation. In support of this hypothesis, the phosphatase inhibitors okadaic acid (30-100 nM) and peroxovanadate (1.25 -3.8 μM) produced dose-related increases in sperm release. This model provides a tool for the study of both the hormonal control of spermiation, and mechanisms of toxicant-induced disruptions. The data gathered to date are consistent with the concept that protein phosphorylation is involved in controlling sperm release.

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EFFECT OF A MIXTURE OF ENVIRONMENTAL CONTAMINANTS ON THE MALE RAT

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Our current understanding of the toxicity of persistent chemical pollutants to the human population is derived, to a large extent, from studies in rodents exposed to high doses of a single toxic agent. Little is known about their impact at low concentrations, such as exist in the environment. We assessed the reproductive toxicity of a complex mixture of organochlorine pollutants in the adult male rat. The mixture contained persistent organochlorine pesticides and industrial chemicals to which the general population in North America are chronically exposed. It included dioxin, PCBs, DDT metabolites, dieldrin, methoxychlor, mirex, polychlorinated benzenes, lead and cadmium.

Adult male rats were gavaged with the mixture daily for 72 days at doses equivalent to 1X, 10X, 100X and 1000X the MRL (minimum risk level) for each component. Liver and kidney weights were elevated at the highest dose. Adrenal, thymus, reproductive organs, sperm production or circulating and pituitary levels of LH, FSH, prolactin and testosterone were not affected. The T4/TSH ratio was suppressed three-fold. Hepatic microsomal UDP glucuronyl transferase (1000X MRL), EROD (10X) and BROD (100X) were significantly elevated. Liver from the 1000X treatment group had many lesions. Serum lactate dehydrogenase and urea nitrogen were depressed 50% at 100X MRL. In summary, low dose exposure of a mixture of persistent pollutants had little effect on male rat reproductive processes but at high levels had adverse impacts on thyroid hormone levels, and liver and kidney. *(Supported by the Canadian Chlorine Co-ordinating Committee)*

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LEYDIG CELLS MEDIATE THE REPOPULATION OF SEMINIFEROUS TUBULES BY LEUPROLIDE FOLLOWING 2,5-HEXANEDIONE-INDUCED TESTICULAR ATROPHY. H.A. Schoenfeld*, S.J. Hall*, K. Boekelheide, Dept. of Pathology and Laboratory Medicine, Brown University, Providence, RI.

2,5-Hexanedione (2,5-HD), a reactive metabolite of the environmental toxicant n-Hexane and Sertoli cell toxicant, produces a persistent testicular atrophy in rats when administered in drinking water (1-% v/v) for 3.2 weeks. Previous studies in our laboratory indicate that the expression of stem cell factor (SCF), a Sertoli cell-derived growth factor, changes following 2,5-HD treatment, resulting in an increase in the expression of soluble relative to the membrane forms of SCF. Leuprolide, a GnRH agonist, reverses 2,5-HD-induced testicular atrophy, resulting in a repopulation of atrophic tubules and normalized stem cell factor expression. The membrane form of SCF interacts with the c-kit receptor expressed on spermatogonia to stimulate germ cell differentiation. The role of the soluble form of SCF has not been well characterized, although it may interact with distant Leydig cells. To examine the role of the soluble form of SCF and the Leydig cell in the stimulation of spermatogenesis by leuprolide, rats which had been treated with 2,5-HD were administered a single i.p. injection of the Leydig cell toxin ethane dimethane sulfonate (EDS) (80 mg/kg), followed by 3 s.c. injections of leuprolide, 25 days apart. Treatment with EDS caused an ablation of testosterone-producing Leydig cells, which was sustained for the duration of leuprolide treatment. The combination of leuprolide and EDS increased the membrane form of SCF to control levels however failed to stimulate a repopulation of seminiferous tubules. These results indicate that paracrine- acting factors produced by Leydig cells are also necessary for the stimulation of spermatogenesis by Leuprolide.

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SPERM ANEUPLOIDY NOT ASSOCIATED WITH 2,4-D HERBICIDE, SMOKING, OR CAFFEINE EXPOSURE IN CANADIAN FARM FAMILIES AT INCREASED RISK FOR PREGNANCY LOSS

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An earlier epidemiologic study of 2000 Ontario farm families showed an increased risk for miscarriage and pre-term delivery in couples where the males had been exposed to certain pesticides (Savitz et al., 1997, AJE, 146:1025-1036). To investigate if male-mediated pregnancy loss in this group of farm families might be due to herbicide induced sperm aneuploidy, we measured 2,4-D residues and sperm aneuploidy in semen from a subset of the men enrolled in the original study.

Ninety-seven farmers donated semen. 2,4-D phenoxy herbicide residues were detected in 50% of the samples (range 1 ppb to 640 ppb). Aneuploidy for chromosomes X, Y, 18 was measured in 40 samples using fluorescence in situ hybridization. Questionnaire data on lifestyle behaviors including smoking, alcohol and caffeine use was also evaluated.

For sperm aneuploidy, 15 samples positive for 2,4-D residue were compared to 25 2,4-D negative samples. Disomy frequencies per 10,000 scored cells were: XX18 1.4, YY18 1.9, XY18 9.0, 18-18 4.3, and diploidy XY1818 6.1. All are solidly within the range reported for healthy men studied previously in our lab. No significant associations between any of the aneuploidy outcomes and 2,4-D exposure were found. Proportion of X:Y bearing sperm did not deviate from expected 50:50. Smoking and caffeine consumption were not associated with sperm aneuploidy for chromosomes X, Y or 18 in this data set but there were very few smokers.

Conclusions: The increased risk for spontaneous abortion associated with 2,4-D exposure in this group of family farmers does not appear to be mediated through sperm aneuploidy for X, Y, or 18. Further study with other pesticides and sampling periods is indicated.

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THE RESULTS OF INTRA CYTOPLASMIC SPERM INJECTION (ICSI) WITH NON MOTILE SPERM SELECTED BY TAIL TO HEAD RATIO (T-H). Murphy D, Marmar JL, Corson SL, Gibbs M, Huszar G. Division of Urology, Robert Wood Johnson Medical School at Camden and Fertility Testing Laboratory, Philadelphia, PA

Introduction: Frozen testicular sperm may be used for ICSI, but after the thaw, these sperm are usually non motile. Live-dead stains may identify viable sperm, but the dyes may be toxic to the eggs. Hypo osmotic swelling tests may determine viability, but sperm may be lost during the testing process. Recently, T-H measurements of 10:1 identified mature sperm based upon CK values, and viable sperm in whole semen based on motility. In this report, we applied T-H ratio to select non motile testicular sperm after the thaw. These sperm were used for ICSI in 8 cases.

Methods: First, a droplet of whole semen was stained with SYBR-14 and propidium iodide to determine viability (alive-green, dead-red). Despite these dyes, the sperm remained motile and 100% of the motile sperm stained green. The T-H ratio was measured for the motile sperm with a calibrated eye piece. Second, five frozen testicular biopsies were thawed and stained. The T-H ratio was measured on 116 non motile sperm; 51 viable 65 non viable. Third, a group of 8 patients with non-obstructive azoospermia had ICSI with non motile thawed testicular sperm selected by T-H.

Results: The 900 motile and viable sperm in whole semen after percoll all had T-H of 10:1. The 51 viable non motile testicular sperm from the thawed testicular specimens were measured and 82.3% viable sperm had a T-H > 10, 24.6% had a T-H <10. The summary statistics for T-H > 10 and viable were: sensitivity 82.3%, specificity 75.3%, pos. predictive value 72.4% and likelihood ratio 3.32%. Eight cases had ICSI including three with hypospermatogenesis, two with incomplete sertoli cell only, and three with maturation arrest. The median age of the wives was 33 years (25-40). There were a total of 43 mature eggs and 20 fertilized with non-motile sperm having a T-H >10 (46.5%). There were three pregnancies and two live births.

Conclusions: Non motile testicular sperm have 72.4% likelihood of viability based on T-H ratio > 10. These types of sperm may be selected for ICSI with fertilization rates of 46.5%. Thus, the use of T-H ratio is a reasonable, simple and safe method for selecting non motile thawed sperm for ICSI leading to term pregnancies.

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THE ROLE OF CREATINE KINASE AND HYPOOSMOTIC SWELLING IN FRESH AND CRYOPRESERVED SAMPLES FROM INFERTILE PATIENTS

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Damage to the sperm membrane occurs during the freeze-thaw process resulting in poor sperm motion and functional characteristics. The purpose of this study was to determine whether sperm cell damage and changes in functional characteristics that occur during the freeze-thaw process are related to a reduction in sperm cell metabolism as indicated by creatine kinase (CK) level. Also, we assessed the value of the hypoosmotic swelling test (HOS) as a test of sperm viability in frozen samples. Semen specimens were obtained from 10 normal healthy volunteers and 19 infertile men. Each ejaculate was divided into two aliquots. One aliquot of fresh sample was used to measure CK activity using a kit and sperm viability with the HOS test. The second aliquot was cryopreserved with TEST-yolk buffer. After 24 hours, samples were thawed and cryoprotective medium was removed. Levels of CK and sperm viability were assessed in thawed specimens and compared with prefreeze values. In the pre-freeze samples, CK levels (median and interquartile values) in donors was 0.01 (0.01 to 0.05) units/10^8 sperm and 0.06 (0.04 to 0.24) units/10^8 sperm in patients, respectively, ($P = 0.003$). In the post-thaw specimens, donors and infertile men had a CK levels of 0.01 (0.01 to 0.04) units/10^8 sperm and 0.06 (0.03 to 0.15) units/10^8 sperm, respectively, ($P = 0.002$). Percentage change from pre-freeze to post-thaw in the donors was not significantly different. However, the percentage change from pre-freeze to post-thaw was significant in the patient group (median and interquartile values) (-7.32 [-15 to 0.0]) ($P = 0.01$). The HOS test was not significantly different between donors and patients both in pre-freeze and post-thaw specimens. HOS is not a good indicator of sperm viability in cryopreserved samples. Spermatozoa from infertile men may be more susceptible to cryopreservation-induced damage as indicated by a reduced CK level. This may be due to the loss of cytoplasm during removal of cryoprotectant before processing the specimen for CK activity. [Supported by a research grant from the Cleveland Clinic Foundation.]

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KINEMATICS OF CRYOPRESERVED-THAWED HUMAN SPERMATOZOA: EFFECTS OF SPERM WASHING, PROCESSING AND IN VITRO INCUBATION

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Recovery of the optimal number of functionally intact spermatozoa from thawed samples has always been a goal of semen cryopreservation programs. This study evaluated the kinematics of spermatozoa after two sperm preparation procedures used to process cryopreserved-thawed samples, and how motion characteristics and viability were maintained hours post-manipulation. Donor semen samples (n = 15) were diluted 1:1 with TEST yolk-buffer freezing medium and then cryopreserved. After thawing, each sample underwent the following steps: (1) removal of the cryoprotectant; (2) processing using the swim-up method; and (3) incubation with BWW containing 3% BSA at 37°C under 5% CO_2 in air for 3 hours. Percent motility and motion characteristics were determined after each step using a computer-assisted semen analyzer equipped with hyperactivation module software. Sperm viability was also assessed after each step. Dilution and centrifugation of cryopreserved-thawed specimens resulted in a 30% decrease in the sperm count. Compared with simple sperm washing, samples processed by swim-up had higher viability ($P = 0.02$), percentage motility ($P < 0.05$), curvilinear, straight-line, and average path velocities ($P < 0.001$), and amplitude of lateral head displacement ($P < 0.05$). Incubation of cryopreserved-thawed spermatozoa under capacitating conditions for 3 hours maintained motion characteristics while decreased percentage motility ($P < 0.05$) and viability ($P < 0.001$). Cryopreserved specimens processed by swim-up resulted in the recovery of spermatozoa with higher viability and superior quality as assessed by motion analysis. Appropriate timing in using these cells for artificial insemination or other assisted reproductive procedures seems to be crucial to enhance fertilization and pregnancy outcome, since freeze-thawed spermatozoa have reduced longevity. [Supported by a research grant from The Cleveland Clinic Foundation].

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OVERALL SEMEN QUALITY SCORES DEVELOPED BY PRINCIPAL COMPONENTS ANALYSIS OF SEMEN CHARACTERISTICS

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Semen analyses typically produce a wide variety and number of semen characteristics that are correlated, indicating that underlying measures of semen quality can be used to reduce the number of variables evaluated. We examined sperm motion characteristics of a cross section of men to determine whether nine characteristics of sperm specimen can be reduced to one or two measures of semen quality. A cross-sectional sample of 250 men who provided semen samples was used to examine the variability among and correlation of patient semen characteristics. A separate sample of 19 donor males was used to determine normal ranges of the semen scores. Principal component analysis indicated that the semen characteristics could be summarized as two semen scores that account for 80.3% of the all of the variability among original semen characteristics. The first principal component was a weighted sum of all of the semen characteristics and accounted for 64.7% of the overall variability. This component was named "SQ" (semen quality and quantity). The second component was a weighted sum of eight of the characteristics minus concentration. This was considered a measure of relative quality (e.g., the morphology and function, given the concentration) and was thus named "RQ." As a reference standard, the distributions of SQ and RQ among the healthy were calculated (100 ± 10). Among the sample of patients, the average SQ and RQ scores were 89.9 (min 25.1, max 130.4) and 106.1 (min 45.2, max 165.9), respectively. Semen characteristics can be reduced to two semen quality scores, which account for over 80% of the variability expressed by all of the semen characteristics individually. We believe that reducing the nine semen characteristics to the two scores will be more efficient by allowing quick comparisons of semen quality. In addition, the semen scores may provide improved assessments of male fertility. [Supported by a research grant from the Cleveland Clinic Foundation.]

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SPERM CRYOPRESERVATION IN SOME SYSTEMIC DISEASES

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Cytotoxic drug therapy is indicated in resistant cases of some systemic diseases. Some of these patients may wish to have children in the future, and are referred for sperm banking before they start therapy. This study compared the prefreeze and postthaw semen analyses in a group of 14 patients with the following systemic diseases: Wegner's granulomatosis (n = 1), vasculitis (n = 2), kidney transplant (n = 1), rheumatoid arthritis (n = 2), systemic lupus erythematosus (n = 3), systemic sclerosis (n = 2), nephrotic syndrome (n = 2), and psoriatic arthritis (n = 1) with those of a group of healthy donors (n = 50). Patients did not differ from donors in age ($P = 0.76$) or ejaculate volume (0.86). Total motile sperm count, motility, curvilinear velocity, and linearity was significantly lower in patients than in donors for the prefreeze ($P < 0.01$) and postthaw specimens ($P < 0.01$). However, the percentage change from prefreeze to postthaw analyses for any of the sperm motion characteristics showed no significant difference between patients and donors. Our results indicated that fresh and frozen-thawed semen specimens in the patients with systemic diseases is of poor quality. As chemotherapy may further impair semen quality in these patients, they should be offered the chance to preserve their semen before the start of their therapy. This will allow them the chance to establish pregnancy in the future with assisted reproductive techniques. [Supported by a research grant from the Cleveland Clinic Foundation.]

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RELATIONSHIP BETWEEN CREATINE KINASE LEVELS AND ABNORMAL SPERM MORPHOLOGY IN PATIENTS WITH VARICOCELE

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Varicocele is one of the most common conditions associated with male infertility. The incidence of varicocele is 10 - 20% in the general population and 30 - 40% in men with infertility. Infertile men with varicocele exhibit impaired semen characteristics. Elevated levels of creatine kinase are associated with excessive residual cytoplasm, an indicator of the degree of sperm maturity. The purpose of our study was to determine if creatine kinase (CK) could be used as a marker of sperm quality and study its correlation with morphology in patients with clinical varicocele. Semen samples from of 31 infertile patients with clinical varicocele attending our infertility clinic and 19 donors were examined. Semen parameters were assessed according to the World Health Organization (WHO) guidelines. WHO and Kruger's strict criteria was used to assess sperm morphology. Patients with leukocytospermia were excluded from the study. Creatine kinase levels were measured after extraction with Triton-X using a creatine kinase kit. The results were expressed as $U/10^8$ sperm. Varicocele patients had lower sperm concentration, motility and morphology compared to donors ($P < 0.05$). Creatine kinase levels were higher in infertile varicocele patients (0.06 $U/10^8$ sperm) than donors (0.01 $U/10^8$ sperm) ($P < 0.05$). Creatine kinase levels were inversely correlated with sperm morphology both according to the WHO ($r = -0.27$; $P = 0.02$) and Kruger's strict criteria ($r = -0.33$; $P = 0.01$). In conclusion, elevated creatine kinase levels are associated with abnormal sperm morphology. Creatine kinase may be a good predictor of semen quality in infertile men with varicocele. [Supported by a research grant from the Cleveland Clinic Foundation.]

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RELATIONSHIP BETWEEN REACTIVE OXYGEN SPECIES AND SPERM VIABILITY IN INFERTILE PATIENTS.

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Although reactive oxygen species (ROS) has an important role on sperm physiologic functions, high levels of ROS can overwhelm the antioxidant capacity of the seminal plasma and disrupt sperm function by inducing peroxidative damage to the sperm plasma membrane and DNA. The hypoosmotic swelling (HOS) test assesses sperm viability and is an indicator of membrane integrity. It is based on the ability of live spermatozoa to withstand moderate hypoosmotic stress. The purpose of our study was to compare the levels of ROS with sperm parameters and sperm viability as assessed by the HOS test in infertile men. Semen specimens from 238 men attending our infertility clinic between 1997 to 1998 were examined for sperm concentration, sperm motility and morphology according to the WHO criteria and for sperm viability with the HOS test. ROS production was measured by the chemiluminescence assay. The results were expressed as [Log (ROS + 1) X 10^4 counted photons/minute/20 X 10^6 sperm]. ROS levels were negatively related to sperm concentration ($r = -0.36$; $P = 0.0001$), sperm motility ($r = -0.14$; $P = 0.02$), and sperm morphology ($r = -0.27$; $P = 0.0001$). Also, sperm viability was inversely correlated with ROS levels ($r = -0.14$; $P = 0.03$), demonstrating that higher ROS levels in infertile men leads to lower sperm viability. In conclusion, poor sperm viability is associated with higher levels of ROS in infertile men. Treatment of these patients should include strategies to reduce seminal oxidative stress. [Supported by a research grant from the Cleveland Clinic Foundation.]

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ANTIOXIDANT ACTIVITY IN THE SEMEN OF FERTILE AND INFERTILE MEN

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Introduction: It is reported that high levels of oxidants are detected in the semen of 25% of infertile men. However, the primary defect responsible for elevated semen oxidants (primary oxidant excess or antioxidant deficiency) remains controversial. To address this, we evaluated catalase- and superoxide dismutase (SOD)-like activities, two key antioxidant activities, in the seminal plasma of fertile and infertile men.

Methods: Semen samples were obtained from consecutive men presenting for vasectomy (n=12) and infertility evaluation (n=105) at our institution. Catalase-like activity was measured by the decrease in hydrogen peroxide after incubation with seminal plasma. SOD-like activity was measured as the inhibition of nitroblue tetrazolium reduction due to superoxide anion generation by xanthine plus xanthine oxidase.

Results: Mean seminal catalase-like activity (± SEM) in fertile men was not significantly different from that of infertile men (369 ± 49 vs. 326 ± 17 U/mL, respectively). However, mean SOD-like activity in the semen of infertile men was significantly greater than in semen of fertile controls (46.7 ± 1.5 vs. 37.0 ± 2.8 U/ml, respectively, $p<0.05$).

Conclusions: Our data show that infertile men do not have deficient seminal plasma SOD- and/or catalase-like activity. These findings suggest that the high semen ROS levels in some infertile men are likely due to excessive generation of ROS rather than deficient ROS scavenging activity in semen.

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INFLUENCE OF SEMEN PROCESSING TECHNIQUE ON HUMAN SPERM DNA INTEGRITY

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Introduction: There is growing concern regarding the potential detrimental effect of semen processing on sperm DNA integrity. We sought to compare the effects of density-gradient centrifugation and swim-up on sperm motility and DNA integrity.

Methods: Semen samples (n=22) were obtained from consecutive normospermic and oligospermic men presenting for infertility evaluation. Aliquots of the same sample were simultaneously processed using 2-layer Percoll density-gradient centrifugation and swim-up. Sperm motility and chromatin structure (evaluated by flow cytometry analysis of acridine orange-treated spermatozoa) were monitored before and after semen processing.

Results: Mean sperm motility (± SEM) improved significantly after processing with 2-layer Percoll gradient and swim-up compared to whole semen (65.6 ± 4.0 and 73.0 ± 3.0% vs. 52.0 ± 3.6%, respectively, $p<0.005$), with no significant difference in motility between Percoll- and swim-up-treated spermatozoa. However, the percentage of spermatozoa with denatured DNA was reduced significantly in swim-up-treated but not in Percoll-treated spermatozoa compared to whole semen (4.8 ± 1.2% and 13.6 ± 3.6 vs. 10.1 ± 2.3 %, respectively, $p<0.05$).

Conclusions: Although Percoll density-gradient centrifugation is comparable to swim-up technique in recovering spermatozoa with enhanced motility, spermatozoa recovered after swim-up possess higher DNA integrity. These data urge us to re-examine our current sperm processing techniques in order to minimize sperm DNA damage and the potential transmission of genetic mutations in assisted reproduction cycles.

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MICROSURGICAL VARICOCELECTOMY IN THE ADULT DOES NOT INCREASE TESTICULAR VOLUME

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Background: It has been reported that total testicular volume (assessed by physical examination) increases after adult varicocele ligation (Gentile&Cockett, *Fertil Steril* 58:209-211, 1992). Using scrotal ultrasonography, we have re-examined the potential influence of varicocelectomy on testicular volume.

Methods: A retrospective review of testicular volumes and semen parameters of 65 men who underwent microsurgical varicocelectomy between 1996 and 1998 was performed. Ultrasound assessed testicular volumes and total motile sperm counts were compared prior to varicocelectomy and at a mean of 6.9 months post-operatively

Results: Bilateral varicocelectomy was performed in 23 men, while 42 men underwent left varicocelectomy. There was no significant change in the mean total testicular volume at 6.9 months post procedure compared to pre-operatively (23.2 cc vs. 24.2 cc, p=0.48, 95%CI ΔVol= 0.23 to 2.4 cc). Similarly, mean left and mean right testicular volumes did not change significantly pre and post varicocelectomy ($p > 0.05$). The total motile sperm count improved in 62% of men.

Conclusions: This is the first study to examine the effect of adult varicocelectomy on testicular volume using ultrasound derived measurements of volume. Unlike previous reports, our data suggests that while adult varicocelectomy improves semen quality in the majority of infertile men, it does not result in a significant increase in testicular volume.

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THE EFFECT OF INTERFERON ALPHA 2B ON A PEYRONIE'S-LIKE CONDITION IN THE RAT

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Peyronie's disease is a connective tissue disorder of the tunica albuginea of the penis. Approximately 20% of Peyronie's patients suffer from erectile dysfunction which may be a direct physiologic effect correlated with both histological and morphological changes which are occurring in the penis. A new animal model for Peyronie's disease has been proposed by El-Sakka and colleagues which uses transforming growth factor beta 1 (TGF-β1) to induce a Peyronie's-like condition in the rat. In lieu of surgery, intralesional therapy is a less invasive method for the treatment of Peyronie's disease. This study evaluated intralesional injections of interferon alpha 2B (IFN-α-2B) in a Peyronie's-like condition in the rat.

Twenty CD rats received transforming growth factor beta 1 (TGF-β1) (0.5 μg) injected into the tunica albuginea and were divided into two groups (n=10). One week after injection of TGF-β1, one group received weekly injections of IFN-α-2B ($1X10^6$ units in 0.1 ml of saline) into the tunica albuginea. The other group received weekly injections of saline (0.1 ml) into the tunica. The rats were sacrificed at six weeks. The penises were cut in cross sections and fixed in 10% formalin, paraffin-embedded, and stained with trichrome and Verhoff's van Giesen for collagen and elastic fibers.

The rats receiving only saline injections showed significant fragmentation of the tunica albuginea, increased collagen deposition, and compression of the deep dorsal vein. However, those rats which received weekly injections of IFN-α-2B demonstrated less elastic fiber fragmentation in the tunica albuginea, reduced collagen deposition, and less packed collagen fibers around the deep dorsal vein and neurovascular bundle.

Weekly injections of IFN-α-2B into the tunica albuginea of the Peyronie's rat resulted in significant improvement of histological and morphological changes in the tissue architecture of the penis when compared to those experimental rats which received only saline-injection. These results suggest that early intralesional treatment with IFN-α-2B suppresses a Peyronie's-like condition in the rat.

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AN ASSOCIATION BETWEEN ANDROGENS AND HEMISPHERIC LATERALIZATION IN 46,XXY MEN

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Our group has previously reported an association between patterns of functional cerebral hemispheric lateralization and hypogonadal status in 46,XY men seeking treatment for erectile dysfunction (Wisniewski, Nguyen, Flannery & Dobs, 1999). Specifically, hypogonadal men failed to exhibit normal patterns of right hemisphere dominance compared to controls. In a new group of men with Klinefelter Syndrome (46,XXY), a similar failure to produce normal patterns of right hemisphere dominance was observed in 4 of the 5 men tested. These results indicate that the relationship between low testosterone concentration and atypical patterns of hemisphere lateralization generalize to men with Klinefelter Syndrome.

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EFFECT OF TESTOSTERONE AND ESTROGEN ON LIPOPROTEINS IN POST-MENOPAUSAL WOMEN.

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Concentrations of LDL have shown to be in a heterogeneous population varying in size, density and composition, which may be influenced by sex hormones. Debate exists on whether testosterone (T) is a cardiovascular risk or preventive agent. In this double-blinded randomized clinical trial, we enrolled 40 post-menopausal women (age 56.48 ± 8.10 years on a stable 0.625 mg estrogen dose) to 1.25 mg esterified estrogen (E-group) or 2.5 mg methyl-testosterone and 1.25 mg estrogen (MT-group) to determine the effects of testosterone and estrogen on lipid profile. Both the E and MT-group started out with comparable hormone profiles. After 4 months, both groups had similar elevations in estradiol. The MT group had an androgen effect as evidenced by a decrease in leutinizing hormone by 9.8 IU/L ($p = 0.0025$) while no such change was observed with the E-group. Both groups at baseline had compatible lipid profile. The E-group had no change in cholesterol, HDL, and triglyceride; however, the level of LDL fell (135.85 ± 23.71 to 122.11 ± 19.70 mg/dl, $p < 0.03$) as did the LDL size (274.32 ± 10.97 A to 264.74 ± 12.66 A, $p < 0.02$). The MT-group decreased in cholesterol (233.40 ± 44.28 to 213.68 ± 39.75 mg/dl, $p < 0.01$), triglycerides (168.50 ± 80.44 to 113.79 ± 61.56 mg/dl, $p = 0.001$), and HDL (44.20 ± 10.49 to 32.58 ± 6.34 mg/dl, $p < 0.0001$). No change was observed with LDL or its size. The E-group changed 4 patients from phenotype A to B and no one changed from B to A. MT-group had one conversion from A to B, one B to A, and two AB to A. In summary, the increased estrogen had no affect on the lipid profile, but did decrease LDL size. The addition of T resulted in a decrease in HDL, but did reverse estrogen effects on LDL size and phenotype.

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DECREASED QUALITY OF LIFE AND SEXUAL FUNCTION IN MEN WITH PROSTATE CANCER WHO HAVE BEEN ANDROGEN DEPRIVED. J Lieb II, R Walters, M Eisenberger, M Carducci, T DeWeese, A Tang, A Dobs. From the Johns Hopkins University School of Medicine, Baltimore, MD

Androgen deprivation therapy (ADT) is a common treatment for men with metastatic prostate cancer or rising serum prostate specific antigen levels. Although the complications of male hypogonadism are well-recognized, they have not been fully described in this growing population of men with ADT. We enrolled 20 men with prostate cancer and ADT (ADT-group) and compared them to two sets of age-matched controls - 18 men with carcinoma, but no ADT (non-ADT) and 20 men without cancers. The serum testosterone levels were castrate levels in the ADT men, 326±103 ng/dl in the non-ADT group, and 507±162 ng/dl in the normal in the controls. Men in the ADT-group had poorer physical function ($p=0.003$) and more role limitations (SF-36) ($p=0.003$). Using Watts Sexual Function Questionnaire, ADT resulted in decreased arousal and libido ($p<0.0001$). Other outcome measures included body composition, bone mineral density, and muscle strength. In summary, ADT in men with prostate cancer was associated with decreased functional status, diminished sense of well-being, and decreased sexual function. In conclusion, the risks and benefits of ADT in men with prostate cancer need to be clearly discussed with patients.

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REGULATION OF STEROID LEVELS WITHIN THE FETO-PLACENTAL-MATERNAL UNIT BY THE ENDOTHELIAL CELLS OF THE VILLOUS MICROARTERIAL SYSTEM

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Placental steroids are important for the maintenance of pregnancy, the adequate timing of parturition and for the maturation of fetal organs. Therefore, regulatory mechanisms must be in place to control the production and the metabolism of these steroids. Among those mechanisms, the 17ß-hydroxysteroid dehydrogenase (HSD) type I and 2 enzymes represent reliable candidates to control the amounts of active and inactive steroids produced by the placenta. The type 1 17ß-HSD exclusively reduces estrone into estradiol *in vivo*. Conversely, the type 2 17ß-HSD oxidizes estradiol, testosterone and dihydrotestosterone with equal reactivity. We previously showed that isolated trophoblasts, cultured long enough for syncytialization *in vitro*, contain high levels of expressed 17ß-HSD-1 but undetectable type 2 17ß-HSD expression. By contrast, term villi from which trophoblasts are isolated contain high levels of 17ß-HSD-2. Using *in situ* hybridization with 17ß-HSD RNA probes coupled to the immunodetection of specific markers (vimentin for endothelial cells and fibroblasts or human placental lactogen for trophoblasts), we showed that 17ß-HSD-2 mRNA and activity are both specifically associated with endothelial cells of the fetal microarterial component of the villi. localization of the 17β-HSD-2 in the fetal microarterial system highlights the key role of this enzyme in the control of steroid output from the fetus.

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OSTEOTESTICULAR PROTEIN TYROSINE PHOSPHATASE (OST-PTP) EXPRESSION IN TESTIS

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Phosphorylation is known to be a key chemical modification for most of the decisions a cell has to make. Protein tyrosine kinases (PTKs), responsible for the tyrosine phosphorylation of other signaling proteins, have been extensively characterized. Although the dynamic regulation of this reversible reaction requires the interplay of another class of proteins, the tyrosine phosphatases (PTPs), less is understood about how these proteins down-regulate signaling pathways activated by PTKs. PTPs may possess at least one catalytic domain containing the active site motif. Both transmembrane or receptor-like and intracellular PTPs have been identified.

A novel receptor-like PTP was identified and termed osteotesticular (OST)-PTP, due to its restricted expression in bone and testis. OST-PTP expression was regulated during osteoblast differentiation and showed stage-specific expression in the testis. The exclusive expression in rat spermatogonia led to the suggestion that it could be used as a marker for sperm stem cell identification and isolation. We report the identification in the testis of a truncated form of OST-PTP (tOST-PTP) that doesn't contain the catalytic domain of OST-PTP. This tOST-PTP shows, by *in situ* hybridization, a different pattern of expression. Contrary to OST-PTP, tOSTPTP is widely expressed during spermatogenesis in all testicular cell types. The level of expression of tOSP-PTP is less than the one shown by OST-PTP in spermatogonia. This finding implies a tight control over OST-PTP in the testis, which in turn suggests an important role for OST-PTP in male germ cells differentiation. Future research is necessary to understand how these two proteins are regulated and regulate PTKs signalling in the testis.

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CLONING OF THE RAT GATA-4 GENE PROMOTER AND CHARACTERIZATION OF 5' REGULATORY ELEMENTS REQUIRED FOR SERTOLI CELL-SPECIFIC EXPRESSION.

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The GATA transcription factors regulate gene expression by binding to the consensus DNA sequence (A/T)GATA(A/G) in the promoter region of target genes. The *GATA-4* gene is abundantly expressed in the testis and our laboratory has recently identified the promoter for Müllerian inhibiting substance, an essential hormone required for male sex differentiation, as the first natural target for GATA-4 in Sertoli cells of the fetal testis. In order to better understand the molecular mechanisms that control GATA-4 gene expression, we have cloned the rat GATA-4 promoter. We isolated a 13 kb genomic clone, over 10 kb of which corresponded to the 5'-flanking region of the rat *GATA-4* gene. Unlike the other GATA genes described to date, the 5'-untranslated region of the *GATA-4* gene is subdivided into two exons: exon 1a and exon 1b. The proximal GATA-4 promoter is GC rich and does not contain a consensus TATA box. We have mapped the major transcription initiation sites by primer extension assays. The *GATA-4* gene has several transcription start sites, the two major ones are present at -593 bp and -572 bp upstream of the ATG. We have generated several deletion constructs (from -2100 to -38 bp) to begin mapping promoter elements required for the cell-specific expression of the *GATA-4* gene. The deletion constructs were transfected in neonate (5-day-old) primary rat Sertoli cell cultures. Interestingly, full promoter activity was maintained between -2100 and -221 bp. However, deletions to -94, -73, and -38 bp decreased promoter activity to 15%, 2%, and 0% of the -2100 bp construct, respectively. Sequence analysis of the proximal GATA-4 promoter revealed the presence of potential binding elements for several regulatory transcription factors such as Sp1 (-157 and -51 bp), Egr-1/WT-1 (-104 bp), and an E-box for the binding of bHLH factors (-90 bp). The Egr-1/WT-1 element is of immediate interest to us because the WT-1 factor (Wilms' tumor-1) is known to be a critical regulator of both gonadal development and sex differentiation, two processes that we have proposed to be GATA-4-dependent. Supported by the MRC of Canada.

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DEVELOPMENTAL REGULATION OF LEYDIG CELL ANDROGENS BY MIS

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Adequate androgen production by testicular Leydig cells is essential for the differentiation of masculine genital structures during male embryonic development and for acquisition of secondary sexual characteristics and reproductive competence at puberty. During pubertal maturation, Leydig cells undergo three defined stages of differentiation characterized by decreasing proliferative rates and increasing steroidogenic capacity. The predominant androgen produced during these maturational stages is also developmentally regulated in that androsterone is produced in the peri-pubertal rodent, while androstanediol is produced during puberty and testosterone is the major androgen in the adult. We reported that Mullerian inhibiting substance (MIS), a key hormone for sexual differentiation, has an integral paracrine role in regulation of Leydig cell proliferation during testicular maturation. These studies were designed to determine whether MIS also plays a role in regulation of Leydig cell steroidogenesis during development. We compared the effects of 30 nM MIS on androgen synthesis in primary cultures of **progenitor** Leydig cells isolated from 21d prepubertal Sprague-Dawley rats and **immature** Leydig cells isolated from 35d pubertal rats. In **progenitor** Leydig cells, treatment with 30 nM MIS for 24 hrs decreased only androstenedione and testosterone levels. After 48 hrs treatment, MIS significantly inhibited the production of androstenedione ($p<0.001$), androsterone ($p<0.05$), androstanediol ($p<0.001$), and testosterone ($p<0.001$). Thus, the effect of MIS was potentiated with a longer duration of treatment. In contrast, exposure of **immature** Leydig cells to MIS for up to 48 hrs did not inhibit production of any of the four androgens. These data indicate that MIS has a dual role in regulation of postnatal testicular maturation. MIS not only has an anti-proliferative action that helps maintain a normal complement of Leydig cells in the mature testis, but also modulates the production of androgens during Leydig cell differentiation. Supported in part by the Charles H. Hood Foundation and NICHD grant R29HD36768.

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CONNEXIN 43 (α1) AND CONNEXIN 32 (β1) ONTOGENY IN THE RAT PROSTATE AND EFFECTS OF NEONATAL ESTROGEN EXPOSURE

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Neonatal estrogenization interrupts rat prostatic development by reducing branching morphogenesis and by blocking epithelial cells from normal differentiation. Upon aging, ventral prostates exhibit extensive hyperplasia and dysplasia, suggesting that neonatal estrogens may predispose the prostate gland to precancerous lesions. Connexins (Cx) are gap junction (GJ) proteins, designated according to their molecular weight or into 2 subclasses, α and β. Six Cxs form a connexon, in the center of which a hemichannel is located, through which intercellular communication (GJIC) functions. GJIC is believed to be involved in the regulation of cell growth in developing tissues. In contrast, loss of GJIC is reported in cancer development. Developmental "switches" in the expression of GJ-protein-subclasses may have functional significance by differentially regulating GJIC. Steroid hormones can modulate GJIC and Cx expression in other cell systems. Since we have shown that neonatal estrogenization leads to alterations in androgen-receptor and estrogen-receptor (α and β)-expression in the rat prostate, the present study was undertaken to determine 1) the developmental pattern of Cx 43 and 32 expression in the rat prostate, and 2) whether they are altered in response to early estrogen exposure. Male rat pups were given 25μg estradiol or oil on days 1 - 5 of life. Prostates were removed on days 6, 10, 15, 30 and 90 of life and frozen sections were immunostained for Cx 43 and Cx32 using specific antibodies. Co-localization studies were performed with immunoflourescence. Gap junctions in undifferentiated epithelial cells at day 6 and 10 were composed of Cx 43 which always co-localized with basal cell cytokeratins (CK 5/15). Cx 32 expression was first observed between days 10-15 and co-localized to differentiated luminal cells (CK 8/18). Cx 43 and Cx32 never co-localized to the same cell indicating that GJIC differs between basal and luminal prostatic cells. While this pattern was not initially altered following estrogen exposure, adult estrogenized prostates exhibited an increased proportion of Cx 43 expressing cells which corresponds to the known accumulation of undifferentiated basal cells in response to neonatal estrogen exposure. These findings are highly significant in light of the human data where prostate carcinogenesis and progression are associated a switch from Cx 32 to Cx 43 expression in epithelium (Supported by NIH DK 40890.)

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INCREASED ANDROGEN RECEPTOR EXPRESSION CORRELATES WITH DEVELOPMENT OF AGE-DEPENDENT, LOBE-SPECIFIC SPONTANEOUS HYPERPLASIA OF THE BROWN NORWAY RAT PROSTATE.

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Androgens are essential for development and differentiated function, as well as cell proliferation and survival, in the prostate gland. Age-related changes in the hormonal milieu, marked by a decrease in the serum testosterone:estradiol (T:E) ratio, and perhaps the nuclear steroid receptor profile, contribute to the evolution of pathological changes such as benign prostatic hyperplasia and carcinoma of the prostate gland in older men. We have reported a similar phenomenon in Brown Norway (BN) rats in which the serum T:E ratio declined with age and despite the lower serum testosterone level, age-dependent prostatic hyperplasia developed in the dorsal (DP) and lateral (LP), but not in the ventral (VP) lobe. To evaluate the role of androgens, we compared the immunostaining of androgen receptor (AR) in young (4 mo) and old (24 mo) BN rat VP, DP and LP. AR immunostaining was present in the nuclei of all epithelial cells and some stromal cells throughout the prostatic ducts in both young and old rats. Whereas, AR immunostaining intensity decreased in the VP of old rats, it increased in the DP and LP of old rats. To validate immunocytochemical studies, Western blot analyses were performed. The AR level decreased by 30% in the VP of old rats, whereas AR levels increased 2.7-fold and 1.3-fold in the DP and LP, respectively, of old rats. Similarly, the percentage of cells staining positive for the proliferation marker, PCNA, was increased approximately 2-fold in the DP and LP as a function of age. The presence of higher levels of AR and increased number of PCNA positive cells in the DP and LP of old rats suggest that ARs may be involved in the lobe-specific cell proliferation that occurs with age. Additional evidence for lobe-specific regulation of AR expression was obtained following castration which down-regulated AR in the VP and DP but not LP of young and old rats. Nuclear immunostaining of AR returned by 7-10 days post-castration in the VP and DP even in the absence of androgen. Moreover, up-regulation of AR was more rapid in the VP and DP of old compared to young castrated rats. Taken together, these results suggest that lobe-specific differences in the regulation of AR expression and age-dependent increases in AR level might lead to development of lobe-specific hyperplasia in the BN rat prostate gland. (Supported by NIH grant P01-AG08321 and AFAR grant A98120)

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IDENTIFICATION OF FIBROBLAST GROWTH FACTOR RECEPTORS IN THE RAT EPIDIDYMIS: POSSIBLE INITIATORS OF SIGNALING PATHWAYS INVOLVED IN REGULATION OF GGT mRNA IV EXPRESSION.

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Secreted factors from the testis are involved in maintaining the structure and function of the epididymis. We previously showed that gamma-glutamyl transpeptidase (GGT) mRNA IV is highly expressed in the rat initial segment and is under the control of testicular factors. Efferent duct ligation (EDL), which prevents luminal fluid of testicular origin from reaching the epididymis, resulted in decreased GGT mRNA IV expression, protein level and activity. Basic fibroblast growth factor (bFGF) treatment returned GGT protein and activity levels in EDL initial segments to sham-operated control levels. We hypothesize that bFGF recognizes its receptor(s) on the apical surface of the epididymal epithelium and initiates signaling through the Ras-dependent ERK pathway, resulting in GGT mRNA IV expression. The aims of this study were 1) to determine the expression and localization of FGF receptors (FGFR) in the initial segment and 2) to determine if the Ras-dependent ERK pathway is involved in GGT mRNA IV expression. RT-PCR analysis revealed the presence of FGFRs 1-4, including the splice variants IIIb and IIIc of FGFR-1, -2, and -3. Western blot analysis confirmed the presence of FGFR protein in the initial segment. Preliminary data showed a substantial decrease in the phosphorylated forms of MEK and ERK following 12h EDL. Additionally, a decrease in ERK phosphorylation was seen following *in vivo* electroporation of a dominant negative Ras construct. These results are consistent with our hypothesis that testicular factors in the epididymal lumen are involved in signal transduction. Future experiments will examine receptor localization and signal transduction pathways involved in GGT mRNA IV expression. Our findings support the hypothesis that FGFRs are expressed in the initial segment and may contribute to signaling cascades that lead to GGT mRNA IV expression.
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ANALYSIS OF INFERTILITY IN MALE MICE DEFICIENT IN INOSITOL POLYPHOSPHATE 5-PHOSPHATASE (INPP5B): RESULTS FROM ASSAYS FOR SPERM FUNCTION.

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Inpp5b is a member of an enzyme family of inositol polyphosphate 5-phosphatases that hydrolyzes soluble and lipid inositol phosphate metabolites (e.g., IP_3, IP_4, PIP_2 and PIP_3) *in vitro*. *INPP5B* is 70% identical to *OCRL1*, a gene deficient in a human disorder, the oculocerebrorenal syndrome of Lowe, although no human disease has yet been linked to *INPP5B*. Knock-out mice deficient in inpp5b are viable, but males show reduced fertility and have abnormal testis histology characterized by vacuolization in Sertoli cells (Hellsten *et al.*, unpublished data). To analyze the basis of the reduced male fertility more thoroughly, several parameters of sperm function were examined. *Inpp5b*$^{-/-}$ males have significantly lower sperm counts in both standard (43% of counts of control males) and swim-up (16% of counts of control males) preparations of cauda epididymal sperm. Sperm motility is also dramatically reduced in sperm from *inpp5b*$^{-/-}$ males. Sperm from the mutant males are capable of undergoing capacitation (as assessed by observing normal levels of tyrosine phosphorylation), but have significantly reduced abilities to fertilize *zona pellucida* (ZP)-intact and ZP-free eggs *in vitro*. However, although the numbers are reduced, sperm from some *inpp5b*$^{-/-}$ males were observed to fertilize ZP-free eggs, indicating that there was not a complete failure of sperm maturation and that at least some sperm could undergo spontaneous acrosome reactions. In addition, sperm from one *inpp5b*$^{-/-}$ male were capable of fertilizing ZP-intact eggs (albeit with reduced efficiency as compared to controls), indicating that the sperm could undergo a ZP-induced acrosome reaction. Further analysis of the ability of the *inpp5b*$^{-/-}$ sperm to undergo ZP-induced acrosome reactions as well as the dependence of the subfertile phenotype on genetic background is ongoing. Taken together, these data suggest that the deficiency in the inpp5b enzyme could impair spermatogenesis and sperm maturation, and that phosphoinositols play a role in sperm maturation and function.

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MURINE GERM CELLS DO NOT REQUIRE FUNCTIONAL ANDROGEN RECEPTORS TO UNDERGO MEIOSIS FOLLOWING SPERMATOGONAL STEM CELL TRANSPLANTATION INTO NORMAL MOUSE TESTES. D.S. Johnston* and M.D. Griswold, Washington State University, School of Molecular Biosciences and Center for Reproductive Biology, Pullman, WA

Androgens and their effects are absolutely required for spermatogenesis in mammals. Although there is general agreement that androgen receptors are present in Leydig and Sertoli cells, the presence and potential function of the androgen receptor within germ cells remains controversial. To determine whether murine germ cells require androgen receptors to undergo meiosis, we used spermatogonial stem cell transplantation to introduce germ cells lacking functional androgen receptors into the seminiferous tubules of normal testes. Female testicular feminized (Tfm) mice hetero-zygous for the X-linked androgen receptor gene were mated with B6,129 Rosa 26 males, a transgenic line which is homozygous for the bacterial β-galactosidase gene and which expresses the marker in all germ cell types. The testes of the β-galactosidase expressing Tfm offspring were used as donors for spermatogonial stem cell transplantation. Busulfan treated B6,129 male mice were used as recipients according to published procedures. Seven weeks after the transplantation procedure the recipient testes were fixed and stained with X-Gal. Numerous colonies were seen within the testes of recipient animals. The colonization and differentiation of the Tfm germ cells within the recipient seminiferous tubules was consistent with the development of wild type transplanted germ cells in controls at this time point. Colonies of transplanted cells contained spermatids as the most advanced cell type. These results demonstrate that murine germ cells do not require functional androgen receptor to colonize, proliferate, differentiate and undergo meiosis following transplantation into the seminiferous tubules of normal recipient testes.

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IDENTIFICATION OF mRNA TRANSCRIPTS ASSOCIATED WITH HUMAN SPERMATOGENESIS: FEASIBILITY OF DIFFERENTIAL DISPLAY POLYMERASE CHAIN REACTION (ddPCR) TECHNIQUE.
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Limited data exist on the molecular basis of human spermatogenesis. Differential display polymerase chain reaction (ddPCR) allows partial amplification of cDNA sequences from subsets of mRNAs and subsequent display on a sequencing gel. Although this technique may be applied for a large-scale comparison of mRNAs expression between different well-characterized cell types, it is troublesome when applied to very complex tissues such as the testis that may display significant regional variability of spermatogenic function. We have recently developed a technique of testicular microdissection for identification of limited regions of spermatogenesis from subfertile men. This approach allows extensive microscopic examination of the entire testis and provides well characterized human testicular tissue for experimental use. In this study, we demonstrate the utility of ddPCR in identifying genes that are differentially expressed in the testes of men with normal spermatogenesis, compared to infertile men with pure, well-characterized Sertoli cell-only pattern. Six infertile azoospermic men with extensive histologic, cytologic and intra-operative microscopic confirmation of pure Sertoli-cell-only pattern were analyzed. The mRNAs isolated from biopsies of the twelve testes from this group were compared to those obtained from two men with normal spermatogenesis using ddPCR. We identified three distinct mRNA transcripts, referred to as dd4, dd5 and dd7, in both subjects with normal spermatogenesis that consistently failed to be expressed in all infertile men with Sertoli-cell-only pattern. Northern analysis confirmed the absence of expression of these three transcripts in testicular samples from the infertile group and high level expression in specimens from normal males. Transcript lengths of 1.9 kb for dd4, 1.4 kb for dd5 and 1.7 kb for dd7 were seen. While further characterization of these transcripts is required, their absence among infertile men with Sertoli-cell-only pattern and an absence of any spermatogenic activity, suggests a potential role for these gene products in normal spermatogenesis. The use of ddPCR, when applied to carefully characterized human testicular tissue, is an effective approach that can be applied for evaluation of gene expression associated with normal and abnormal spermatogenesis. These preliminary data support the use of ddPCR as a promising avenue to elucidate other novel genes associated with normal and pathologic human testicular function.

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EVIDENCE FOR THE PRESENCE OF L-TYPE Ca^{2+} CHANNELS IN HUMAN SPERMATOZOA
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Pharmacological studies suggest that voltage-operated Ca^{2+} channels (VOCCs) are expressed in human spermatozoa and are involved in induction of the acrosome reaction by zona pellucida and possibly progesterone. Molecular studies on rodent and human testis and spermatozoa have provided evidence, primarily for expression of α_{1C} (L-type) VOCC subunits in these cells (Bennoff, 1998). However, patch clamp studies on rodent spermatogenic cells have revealed the presence of T-type channels only (Darszon et al, 1999).
We have used single cell, confocal imaging to investigate the effects of the dihydropyridines, nifedipine and Bay K 8644, on $[Ca^{2+}]_i$ in Calcium Green-1-loaded, human spermatozoa. Though nifedipine is known to have antagonistic effects on non-L-type VOCCs (including those of rodent sermatogenic cells), Bay K 8644 has an agonistic effect on VOCCs which is believed to be specific to L-channels.
Upon exposure to nifedipine (5-10 μM), calcium green fluorescence increased by 5-10% in 51±8% of cells (mean±SEM; n=5). This effect began upon nifedipine application and fluorescence settled at a new steady-state level within 1.5-2 min. Upon exposure to Bay K 8644, 73±7% of cells (n=4) showed an *increase* in fluorescence. In most cases fluorescence settled at a new steady-state 20-100% above control levels. The response was not synchronised, latency of response in individual cells varying from seconds to minutes. Since (i) the action of Bay K 8644 is specific to L-type channels and (ii) the effects of the two dihydropyridines on Calcium Green-1 fluorescence were opposite but consistent with their characterised effects on L-type channels, we conclude that functional L-type channels are present in human spermatozoa. Furthermore, these data suggest that, in capacitated spermatozoa, a small proportion of the channels are in the open configuration at any one time (blocked by nifedipine) and that action of Bay K 8644 on these channels is sufficient to cause a slow depolarisation, leading to delayed activation of other channels.
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NITRIC OXIDE REGULATES THE cAMP PATHWAY OF HUMAN SPERMATOZOA DURING CAPACITATION.
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This study aimed to demonstrate a relationship between sperm nitric oxide production and capacitation and to characterise a possible interaction between nitric oxide and cAMP-related pathway in the control of human sperm capacitation and protein tyrosine phosphorylation. Spermatozoa were incubated for 30, 90, 240 minutes in Tyrode's medium with or without bovine serum albumin and nitric oxide was measured with the spin trap sodium N-methyl-D-glucamine dithiocarbamate. Under non-capacitating conditions, spermatozoa produced low levels of nitric oxide. However, under capacitating conditions, prominent nitric oxide adduct signals were obtained and a time-dependent increase of nitric oxide production was observed. These signals were totally abolished when spermatozoa were incubated with different nitric oxide synthase inhibitors. When spermatozoa were incubated for 30 minutes in Tyrode-bovine serum albumin medium with nitric oxide-releasing compounds (diethylamine- and spermine-NONOate), intracellular cAMP concentrations were higher than those of spermatozoa incubated in Tyrode-bovine serum albumin alone. In contrast, incubation with nitric oxide synthase inhibitors (L-NAME or L-NMMA) decreased intracellular sperm cAMP concentrations. Addition of L-NAME to spermatozoa decreased the level of tyrosine phosphorylation of two sperm proteins (105, 81 kDa) as well as the capacitation process. However, the presence of cAMP analogs (dibutyryl cAMP; Sp-cAMP) or of a phosphodiesterase inhibitor (3-isobutyl-1-methylxanthine) overcame this inhibitory effect on both capacitation and tyrosine phosphorylation. Taken together, these results indicate that nitric oxide is produced by capacitating human spermatozoa and that nitric oxide may act as a cellular messenger by modulating the cAMP pathway involved in capacitation and protein tyrosine phosphorylation.

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THE cGMP-SPECIFIC PHOSPHODIESTERASE INHIBITOR, SILDENAFIL, STIMULATES SPERM MOTILITY, HYPER-ACTIVATION AND CAPACITATION.

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Adenosine 3',5'-cyclic monophosphate (cAMP), an important second messenger, plays an important role in the metabolism and motility of human spermatozoa. Levels of cAMP are regulated by 2 processes: 1) synthesis from adenosine triphosphate (ATP) through the action of adenylyl cyclase, and 2) degradation of the cAMP by cyclic nucleotide phosphodiesterase (PDE). Because PDE inhibitors induce capacitation, we were interested in identifying the different types of PDE present in human spermatozoa and their role in capacitation. At least ten different gene families of PDE exist in mammalian tissues. Using specific inhibitors, we previously noted the presence of 3 types of PDE in human spermatozoa: a calcium-calmodulin-dependent (type-1), a cAMP-specific (type-4) and a cGMP-specific (type-5) PDE. The aim of this study was to investigate the effect of sildenafil, a type-5 PDE inhibitor, currently used in the treatment of male impotence, on human sperm functions. Percoll washed spermatozoa were incubated in BWW alone (negative control) supplemented with 3-isobutyl-1-methylxanthine (IBMX; positive control), a non-selective PDE inhibitor or with sildenafil. Sperm velocity, amplitude of the lateral head displacement and hyperactivation were significantly increased at different time intervals (30, 90 and 180 min). Moreover, sildenafil induced sperm capacitation as measured by the lysophosphatidylcholine-induced acrosome reaction. This 45% increase in sperm capacitation correlated with increased levels of tyrosine phosphorylation of two fibrous sheath proteins (p105/81). However, acrosome reaction was not induced by sildenafil. These results strongly suggest the involvement of a cGMP-specific PDE in the control of the events leading to human sperm capacitation.

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CD34 STEM CELL ANTIGEN TRANSCRIPTS PRESENT IN MATURE SPERM: CONTRIBUTION IN SPERM-EGG ADHESION AND FUSION.

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Objective: The cell surface expressed CD34 antigen is a highly glycosylated transmembrane (type I) protein, selectively expressed on stem/progenitor cells. It is thought to function in cell adhesion, facilitating/modulating interaction with local growth factors and to act directly as a signal transducer, through attachment to the cytoskeleton. CD34 expression has been implicated in regulating integrin levels, shown to be involved in murine sperm-oolemma binding. Changes in the levels of surface expression of this antigen are correlated with changes in adhesion. Expression of CD34 has been detected in mouse testis and brain, therefore, we sought to identify its presence in human germinal tissue and cells, such as sperm where it may contribute to binding and fusion with the egg.

Design: Human ovary, testis and sperm PCR products were cloned and analyzed by sequencing and alignment with published human CD34 sequence.

Materials and Methods: Both fresh and cryopreserved motile sperm were prepared and aliquoted. Smears were microscopically analyzed for detection of residual cytoplasm. 0.5gm of human testis and ovary tissue as well as 4 x 10^7 sperm/sample (n=6) was used for nucleic acid extraction, using SDS/ citric acid followed by alcohol precipitation. Total RNA served as template for RT-PCR using CD34 specific primers. Sequence alignment was analyzed using MacVector 5.0 program.

Results: Total RNA, isolated from mature fresh or cryopreserved motile sperm, testis and ovarian tissue was reverse transcribed into cDNA. 463 bp PCR products were generated from all cDNA templates. Sequencing data indicates there were no base changes among the testis, ovary and sperm PCR products.

Conclusions: We have demonstrated the presence of CD34 in mature, ejaculated, motile sperm, as well as ovary and testis, indicating this marker is present on germinal cells. Knowing the involvement of CD34 in adhesive events, decreased marker expression on sperm may be correlated with fertility reduction, due to a loss of sperm adhesive ability. (Supported by funds from the division of Molecular Genetics Dept. of Research NSUH and Dept OB/GYN The University of Arizona, Tucson, AZ).

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EFFECTS OF VASOACTIVE INTESTINAL PEPTIDE ON ACROSOME REACTION IN HUMAN SPERM.

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Purpose: Sperm acrosomal reaction (AR) is associated with the generation of cAMP by adenyl cyclase (AC). Vasoactive intestinal peptide (VIP), a neurotransmitter and a potent stimulator of AC activity, is widely distributed in the male urogenital tract. We investigated the presence of VIP in seminal plasma and its effects on AR *in vitro*.

Methods: Seminal plasma levels of VIP were determined in 57 men by a radioimmunoassay method. Sperm from 12 men were isolated by gradient centrifugation. Live sperm (20 x 10^6/ml) were incubated for 1 and 3 h under capacitating conditions (5% human serum albumin in BWW media, 37°C, 5% CO_2) in the presence (+) or absence (-) of VIP (1, 10 and 100ng/ml). Detection of AR status was by fluorescein-*Pisum sativum* agglutinin binding: 200 live (hypoosmotic swelling test) cells were counted and scored. To confirm the effects of VIP, sperm were incubated with anti-VIP antibody (Ab; n = 5). Statistical comparison was by Wilcoxon Signed test, and a p value of <0.05 was considered significant.

Results: The mean (± SD) concentration of VIP in seminal plasma was 15.9 ± 8.1 pg/ml (range 3.2 - 36.0).

	1 hour		3 hour	
	- VIP	+ VIP	- VIP	+ VIP
VIP alone	5.8 ± 2.1	6.5 ± 2.8	5.4 ± 1.7	12.2 ± 4.0 *
VIP+ Ab	4.7 ± 1.4	5.2 ± 1.0	6.7 ± 1.0	5.7 ± 2.2

Data are mean (% AR) ± SD for sperm incubated with VIP at 100ng/ml.
** $p < 0.002$ compared with VIP absent*

At concentrations of 1 and 10ng/ml, VIP has no effects on sperm AR.

Conclusions: Sperm acrosome reaction is induced by VIP. This effect is probably mediated by stimulation of AC activity. At even higher pharmacologic concentrations, VIP may exhibit greater potency to induce AR. One potential use is the enhancement of fertilization success in artificial insemination procedures in human and animal reproduction.

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HUMAN FOLLICULAR FLUID HAD A DETRIMENTAL EFFECT ON SPERM MOTILITY

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Introduction: Human follicular fluid (HFF) is released at time of ovulation and may be present at the time of in-vivo fertilization. A wide variability of effects of HFF on the motility in human spermatozoa has been reported, i.e. HFF enhance their hyper-activation-like motility, in addition, it has detriment effects on sperm motility by reducing the overall proportion of motile spermatozoa. Furthermore, HFF may be affect as inducer of the acrosome reaction (AR). Therefore, the purpose of this study was to investigates the influence of different concentrations of HFF on sperm motility and the use of HFF as alternative inducer of the AR.

Materials and Methods: Samples of HFF were collected from women undergoing trans-vaginal oocyte retrieval on the IVF programme. Human sperm samples were obtained from 6 fertile donors with normal sperm parameters. After liquefaction for 30 min motile spermatozoa were seperated by PureSperm® centrifugation (300 x g, 20 min). The washed pellet were overlaid with 0,5 ml Hams F-10 containing 10 mg/ml Human Serum Albumin (HSA) and spermatozoa were allowed to swim up for 60 min. This procedure yielded sperm preperations of > 90% motility. Under sterile double washed light paraffin oil 50.000 motile spermatozoa were incubated in 50 µl standard IVF medium (human tubal fluid medium (HTF)) containing 10 mg/ml HSA and different concen-trations of HFF (12.5, 25 and 50%) for 1, 4, and 12 h at 37°C in humidified 5% CO_2 in air. After the designated times, sperm motility was evaluated microscopically and the percentage motility of the sperm suspension in the control and each experimental group was estimated and then averaged. At least 300 spermatozoa of each group were analyzed. All results were assessed using Student's *t*-test. For the detection of the AR sperm were capacitated in Hams F-10 for 120 min and were then exposed to 50, and 100% HFF for another 60 min (short exposure). The AR was assessed by the flow cytometric analysis of CD46 antibody. Data were collected on a minimum of 10.000 cells.

Results: Treatment with HFF for 1 h resulted in a servere reduction in overall sperm motility (12.5% HFF, $P < 0.05$; 25 and 50% HFF, $P < 0.01$). After 4 h further decrease was seen with concentration of 50%. After 12 h of incubation time in the experimental group at 50% HFF a decrease was observed over 50% compared with the motility after 1 h (24.5 vs 45%, $P < 0.001$). The Spearman rank correlation between sperm motility and concentration of HFF was perfectly related ($r = -0.9069$). A short exposure of 50% HFF was sufficient significantly increase acrosomal loss (17.8 ± 1.7% vs 1.6±0.12%, $P < 0.05$).

Conclusions: HFF adversely affecting the movement characteristics of those spermatozoa that maintained their motility. Furthermore this effect was worsened by increasing concentration of HFF. These characteristics of movement may be correlated with capacitation and acrosome reaction, because a short exposure to HFF at concentrations of > 50% are capable of inducing the acrosomal loss in normal sperm populations.

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SHORT AND LONG TERM STORAGE OF GIANT PANDA SPERM

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Semen cryopreservation is an integral part of giant panda captive breeding programs. The development of short- and long-term storage techniques is needed to ensure a viable reserve of germplasm for future artificial reproduction. Semen collected by electroejaculation from 3 male pandas was extended in culture medium prior to recording initial motility (MOT), speed of progression (SOP) and percent live (% L, by eosin-nigrosin staining). Motility scores (MS) were calculated as MOT x SOP^2. To correct for differences in initial semen quality, all results were expressed as % of initial MS and % L (% IMS and % IL). In Experiment 1 the longevity of cooled sperm, both fresh and thawed, was examined for all 3 males. Extended fresh sperm was diluted 1:1 in TEST-Y buffer and examined for %IMS and %IL for 48 hr (male 1 and 2), and 214 (male 3) of incubation at 4°C. Additional ejaculates were frozen over liquid nitrogen vapor following dilution in TEST-Y buffer with 4% glycerol and cooling to 4°C. Thawed sperm from males 1 and 2 was evaluated for %IL at thaw (T0) and after 90 min (T90) at 4°C. Sperm from male 3 was incubated at 37° for 60 min prior to storage at 4°C, then evaluated until all sperm were dead. %IL of fresh sperm after 48 hr at 4°C was 96 and 100 for males 1 and 2 , respectively. In contrast, thawed sperm from these males exhibited just 77 and 68 %IL at T0. Although %IMS of fresh sperm declined precipitously over 48 hr at 4°C (5% for male 1), warming to 37°C increased %IMS to 50%. These data indicate that short-term storage at 4°C is superior to freezing when sperm is to be used within 48 hr of collection. In Experiment 2 sperm from male 3 was diluted in TEST-Y buffer and cooled by each of the following methods: 1) 30 min cool to 4°C; 2) 30 min cool to 4°C, then 30 min equilibration at 4°C; 3) 30 min cool to 4°C, then 2 hr equilibration at 4°C; 4) 2.5 hr. cool to 4°C. TEST-Y with glycerol was added to a final concentration of 4%. Vials were frozen over liquid nitrogen vapor for 15 min before 5 month storage in liquid nitrogen. Sperm was thawed for 1 min in a 37°C water bath and evaluated for %IMS and %IL at T0 before washing by centrifugation and resuspension in 0.5 ml culture medium. Sperm was reevaluated at 30 and 60 min at 37°C. There was no significant effect of pre-freeze cool method on %IMS or %IL.

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EFFECT OF FREEZE RATE ON CRYOSURVIVAL OF DOMESTIC DOG EPIDIDYMAL SPERM.

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Canine epididymal sperm, which is easily obtained during routine neutering of domestic dogs, is an excellent model for the development of semen cryopreservation methods for wild canids. In this experiment, four freeze rates were compared for their effect on post-thaw motility and plasma membrane integrity. The epididymides of three domestic dogs were minced into PBS and filtered to remove tissue. Sperm was evaluated for initial motility (MOT), speed of progression (SOP), and membrane integrity (%IL, using eosin/nigrosin stain). An initial motility score (IMS) was calculated as MOT x SOP^2. To correct for differences in initial sperm parameters, all results were expressed as a percent of the initial (%IMS and %IL). Sperm was extended with Test-Yolk buffer and glycerol for a final concentration of 3.2% glycerol before cooling at 30 min at 4°C. Four freeze rates were achieved with the following treatments: 1) LNV, 0.25 ml cooled sperm was aliquoted into 2 ml plastic cryovials which were frozen for 15 minutes over liquid nitrogen vapor on a 1-inch thick styrofoam platform; 2) COV. 0.25 ml cooled sperm in cryovials was placed horizontally onto the surface of solid CO_2 (dry ice) for 5 minutes; 3) COP, cooled sperm was pelleted in 0.05 ml drops on dry ice for 3 minutes; 4) LNP, cooled sperm was pelleted by immediate immersion in liquid nitrogen. Following freezing, sperm in all treatments was stored in liquid nitrogen for a minimum of 10 days before thawing in a 37°C water bath for 1 minute. %IL and %IMS was recorded immediately after thaw (T0). Sperm was washed by centrifugation and resuspended in BWW with BSA. The sperm was reevaluated for %IL and %IMS after 30 min (T1) and 60 min (T2) at 37° C. The effect of freeze treatment on %IL and %IMS was analyzed by ANOVA. Results were similar for each dog and so were combined for analysis. Freeze rate significantly affected the %IL at each time period ($p< .0001$). At T0, all treatment groups differed significantly from each other ($p< .05$) with the LNV group exhibiting the highest %IL followed by COV, then COP and finally LNP. The LNP was not of sufficient quality to warrant further evaluation. The ranking of the remaining groups at both T1 and T2 was the same as at T0 and all groups significantly differed from each other ($p< 0.05$). Freeze rate also significantly affected the %IMS, but only at T0 ($p= .0012$) and T1 ($p= .0048$). At T0, the LNV had a higher %IMS than all other treatments ($p< .05$) with COV higher than COP and LNP, which did not differ from each other. The ranking of the groups remained the same at T1, but LNV and COV did not differ significantly (the LNP group was not evaluated). By T2, all differences between groups were insignificant, but the COP group %IMS remained lowest. These data indicate that cryosurvival of canine epididymal sperm is reduced by pellet freezing. In addition, sperm frozen in vials over liquid nitrogen vapor exhibits greater post-thaw viability than sperm frozen at a faster rate on dry ice.

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PYRUVATE PROTECTS HUMAN SPERM FROM PEROXIDE-INDUCED DAMAGE.

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Sperm processing for assisted reproductive technologies (ART) frequently induces sublethal injury through peroxidative damage to sperm membrane, resulting in a significant loss of sperm motion and viability. Under these conditions, the natural antioxidant defenses are overwhelmed and the presence of synthetic oxyradical scavengers may offer protection to sperm. Sodium pyruvate, a non-enzymatic scavenger of H_2O_2, has been shown to protect mammalian kidney and neuronal cells from peroxide-induced injury. We evaluated the protective effects of sodium pyruvate against peroxide induced damage to human sperm motility and viability. Washed human sperm suspended in Ham's F10 (20-30 X 10^6/ml) were incubated (37°C) with varying concentrations of H_2O_2 (0-0.5 mM) in the presence and absence of sodium pyruvate (1mM) for up to 120 minutes. Sperm motility was evaluated using a Makler chamber and viability was checked by Eosin-Y dye exclusion. Incubation of normal human sperm with H_2O_2 resulted in a dose- and time-dependent loss of sperm motion and viability. H_2O_2 (0.5 mM) produced about 42% loss of motility accompanied by 25-30% loss of viability within 30 minutes. Presence of pyruvate in the incubation mixture offered a 50-60% protection against H_2O_2-induced sperm damage. Pyruvate had no intrinsic toxicity at the doses employed. Our results suggest that pyruvate is an effective protectant against peroxidative damage to sperm. Such scavengers may have potential clinical applications as adjuvants in sperm processing media employed in ART.

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BIOCHEMICAL MARKERS OF SPERM CELLULAR MATURITY AND THE INTEGRITY OF DNA DETERMINED BY THE CHROMATIN STRUCTURE ASSAY (SCSA) IN HUMAN SPERM.

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Introduction: Arrested sperm cellular maturation in men is evidenced by the retention of cytoplasm, diminished expression of the 64kDa chaperone protein (CP64, formerly CK-M), lack of plasma membrane remodeling, increased lipid peroxidation and consequential DNA fragmentation. We found that 40% of men with immature sperm also show a diminished expression of LDHC4 indicating defects of meiosis. Sperm DNA may also be assessed by SCSA, which is based on green and red fluorescence difference of double stranded and single stranded DNA. respectively. We studied the relationship between sperm immaturity and DNA structure.

Methods: In aliquots of sperm fractions, we determined maturity by CK activity, CP64 ratio and by CK-immunocytochemistry (% stained sperm with cytoplasmic retention, 2-3x100 sp. per sample). Other frozen aliquots were sent to South Dakota for SCSA. The sperm was exposed to pH 1.2 briefly, stained with acridine orange, and intensities of green and red fluorescence were measured for each of 5000 sperm per sample by flow cytometry.

Results: We found previously that the COMP value (proportion of sperm with abnormal DNA) of <30%> distinguishes fertile from diminished fertility men in an IVF study (HR,14,1039) Thus, we divided the 25 samples into two groups according to <30% (N=15) and >30% (N=10) COMP. The respective sperm maturity parameters in the two groups were as follows (mean±SEM): **CK activity**: 0.16±0.11 and 0.48±0.22 IU/10^8 (NS); **CP64-ratio:** 44.0±5.3% and 29±7.0% (NS); **stained sperm**: 21±2.7% and 36±5.3% (P=0.01). The correlation between COMP and stained sperm was r=0.56 (P=0.004). There was no significant correlation between COMP and the other sperm markers.

Conclusions: (1) There is a significant correlation between the incidence of sperm with cytoplasmic retention and abnormal DNA structure. Thus, we suggest that abnormal DNA may contribute to arrest of sperm maturation downstream. (2) There was no relationship between the DNA and the sperm markers of CK activity or CP64 ratio, perhaps because both % immature sperm and COMP are based on assessment of individual sperm, whereas the CK and CP64 ratio parameters reflect data of sperm populations (HD-32902, EPA-R827[illegible])

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SILDENAFIL INTERACTIONS WITH HUMAN SPERMATOZOA FROM NORMAL AND INFERTILE MEN

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Phosphodiesterases (PDE) are a class of intracellular enzymes that regulate the levels of cGMP and cAMP. Inhibition of cAMP-specific PDE stimulates sperm motility. Sildenafil, a widely prescribed oral medication for treating erectile dysfunction, is a selective inhibitor of cGMP-specific PDE-type 5 and may affect sperm motion and/or function. The present study investigates the effect of sildenafil on human sperm motility, viability, membrane integrity (HOST), and sperm penetration assay (SPA).

Spermatozoa obtained from normal donors and infertile men (n = 6 each) were washed using a mini-Percoll (80%) gradient, suspended in Ham's F-10 medium, and incubated with various doses of sildenafil (125, 250, and 750 ng/ml). Pentoxifylline (3 mM) was used as a positive control, and Ham's F-10 was used as a reagent control. Sperm motility/grade, viability, membrane integrity (by hypo-osmotic swelling test), and motion were evaluated at various time intervals. Hamster-ova sperm penetration assay (SPA) was used to evaluate overall sperm function.

Sildenafil did not affect normal or infertile sperm motility, viability, or membrane integrity at any dose under these conditions. Pentoxifylline significantly ($P<0.05$) enhanced motility of these sperm without affecting viability and membrane integrity ($P>0.05$). Sperm incubated with sildenafil and pentoxifylline from both normal donors and infertile patients demonstrated no significant change in SPA compared to the respective controls.

Sildenafil, at the doses evaluated, did not alter the motility, viability, membrane integrity, or ova-penetration characteristics of human spermatozoa from both normal donors and infertile patients.

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EFFECTS OF SERUM ALBUMIN AND METHYL-β-CYCLODEXTRIN ON HEAD-TO-HEAD AGGLUTINATION IN BOAR SPERMATOZOA

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When boar spermatozoa are incubated in a medium designed for IVF, they are agglutinated with others at the acrosomes (1). Our previous data (2,3) indicated that release of an epididymal protein "Anti-Agglutinin" (AA) from the sperm acrosomes is required for occurrence of the head-to-head agglutination. Moreover, extracellular calcium and bicarbonate play important roles in the control of this event. To reveal the mechanism of sperm agglutination, we examined the effects of serum albumin and methyl-β-cyclodextrin (MBC) on sperm agglutination and release of AA from the acrosomes. Spermatozoa were collected from two mature boars, washed and resuspended in a modified Krebs-Ringer bicarbonate containing 0.1% PVA (mKRB-P) that was supplemented with bovine serum albumin (BSA: 1-4 mg/ml), porcine serum albumin (PSA: 1-4 mg/ml), or MBC (1-10 mg/ml). The sperm suspension was incubated in a CO_2-incubator (38.5 °C) for 1 h, then used to determine percentages of head-to-head agglutinated spermatozoa and to detect sperm-bound AA by SDS-PAGE and Western blotting techniques. Both BSA and PSA raised the percentages of head-to-head agglutinated spermatozoa in a dose-dependent manner. The relative amount of sperm-bound AA was significantly less in the sperm samples incubated with either of these serum proteins as compared with amount in control samples. Moreover, MBC was as effective on enhancing sperm agglutination and decreasing sperm-bound AA as the albumin. Based on these data, the possible mechanism of sperm agglutination will be discussed.

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PREVENTION OF SPERM MEMBRANE DAMAGE IN TERATOSPERMIC FELIDS DURING REMOVAL OF PERMEATING CRYOPROTECTANTS.

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To develop an optimal sperm freezing protocol for cats, we assessed the addition and removal of glycerol (GLY) versus dimethylsulfoxide (DMSO) on sperm intact plasma membranes (IPM) from teratospermic (<40% normal sperm/ejaculate) domestic cats and clouded leopards (*Neofelis nebulosa*). Electroejaculates (3 males/cat group; 1 ejaculate/male) were diluted in Ham's F10+HEPES (HF10; 300 mOsm), washed and maintained at 25°C. Test solutions (300, 600, 1200 and 2400 mOsm) were prepared by dissolving GLY and DMSO in HF10. In Study 1, 10 µl of sperm suspension were added to 200-500 µl of each test solution, equilibrated (25°C) and/or returned to 300 mOsm by a single or 8 step (fixed volume) addition of deionized water (DW). In Study 2, 100 µl of 2 M GLY (2600 mOsm) solution were added either rapidly or drop-wise to 100 µl of domestic cat sperm suspension, equilibrated and returned to 300 mOsm in 8 fixed molar or volume steps with HF10. All samples were assessed by flow cytometry for IPM following equilibration or return to 300 mOsm using SYBR-14 (intact) and propidium iodide (non-intact) stain. Sperm from both groups maintained high proportions of IPM (range, 93-100%) after a single exposure to each test solution. When returned to 300 mOsm after exposure to 300, 600, 1200 and 2400 mOsm GLY or DMSO, sperm from domestic cats (mean, 100, 55, 10, 0.5%; respectively) and clouded leopards (mean, 100, 76, 38, 4.3%; respectively) exhibited a dramatic ($p<0.05$) decline in IPM following a single or 8 step fixed volume return with DW. Interestingly, return to 300 mOsm after exposure to 1 M GLY (1300 mOsm) in either 8 fixed molar or volume steps with HF10 resulted in no ($p>0.05$) membrane damage to domestic cat sperm (range, 98-100%). These results suggest that osmotic injury associated with removal of permeating cryoprotectants in sperm from teratospermic felids is prevented by step-wise dilution in an isotonic solution. (NIH KO1RR00135, Smithsonian Institution's Scholarly Studies Program and Smithsonian Women's Committee).

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CHYMOTRYPSIN-LIKE ACTIVITY IN EPIDIDYMAL AND EJACULATED SPERM FROM SEVERAL MAMMALIAN SPECIES.

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The presence of a chymotrypsin-like protease has been described in human (Mol Reprod Dev 38:222, 1994) and marine invertebrate sperm. This protease activity is involved in binding and penetration through the egg's coats and the acrosome reaction. However, this protease activity has not yet been demonstrated in other mammalian species. In this work we present evidence of a chymotrypsin-like activity in epididymal and ejaculated sperm from several mammalian species. Epididymal sperm were recovered from bull, rabbit, hamster, human, and mice. Ejaculated sperm were recovered from rabbit, humans, and Cebus monkey. The chymotrypsin-like activity was measured in the presence of 1 mM benzamidine, 50 mM Hepes, 10 µg/ml bestatin, 1 mM 1,10 phenanthroline, 1 mM EGTA, and 10% glycerol, pH 7.4. The chymotrypsin-like activity was assayed using the fluorogenic substrates Suc-Ala-Ala-Phe-AMC, Suc-Ala-Ala-Pro-Phe-AMC, Suc-Leu-Tyr-AMC, and Suc-Leu-Leu-Val-Tyr-AMC. The protein concentration ranged between 0.3 and 0.8 mg/ml. The assay was run at 25 °C and the fluorescence was monitored with excitation at 380 nm and emission at 460 in a Hitachi-Merck F-1050 spectrofluorometer. The results indicated that all the sperm evaluated exhibited chymotrypsin-like activity. This protease activity was higher in ejaculated than in epididymal sperm. Further studies are being carried out to determine the physiological role of this chymotrypsin-like protease. FONDECYT 197-1243.

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EFFECT OF GnRH ANTAGONISTS UPON THE *IN VIVO* FERTILIZATION IN THE RAT. P. Morales, C. Pastén*, E. Pizarro*. Facultad Ciencias de la Salud. Universidad de Antofagasta, P. O. Box 170. Antofagasta, Chile.

Previously we showed that in vitro sperm treatment with GnRH antagonists significantly inhibit zona binding in humans (Hum Reprod 14:2069, 1999). Here we show the effect of two GnRH antagonists, Ac-D-Nal[1]-Cl-D-Phe[2]-3-Pyr-D-Ala[3]-Arg[5]-D-Glu(AA)[6]-GnRH (Nal-Glu) and Ac[3,4]-dehydro-Pro[1],-p-fluoro-D-Phe[2], D-Trp[3,6]-GnRH (4pF), upon in vivo fertilization in the rat. Female Sprague-Dowley rats were treated in the afternoon of the proestrus with 2 µl of Nal-Glu or 4pF (1 and 10 mg/ml) delivered directly in one oviduct horn (experimental) and saline in the contralateral horn (control). The females were then caged with fertile males and the next day the mating was confirmed by the presence of sperm in the vagina. Twenty-four hours later, the females were sacrificed and the oviducts perfused with saline for oocyte/embryo recovery under a stereoscope. The results indicated that: a) with Nal-Glu 53±8% (mean±sem; n=10) and 0±0% (n=5) of the oocytes were fertilized in the experimental horn with 1 mg/ml and 10 mg/ml, respectively; in the control oviduct 93±3% and 100±0% of the oocytes were fertilized, respectively ($P<0.001$); and b) with 4pF 47±10% (n=5) and 15±8% (n=3) of the oocytes were fertilized in the experimental horn with 1 mg/ml and 10 mg/ml, respectively; in the control oviduct 83±3% and 92±3%, of the oocytes were fertilized, respectively ($P<0.001$). These results indicated that GnRH antagonists inhibit in vivo fertilization in the rat through a local mechanism. One possible explanation is that GnRH antagonists block putative binding sites for GnRH present in the sperm. FONDECYT 197-1243.

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INHIBITION OF SPERMATOZOAL CHROMATIN DECONDENSATION *IN VITRO* BY IRON

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Spermatozoa chromatin decondensation occurs following oocyte penetration. Nuclear protamines are removed from the sperm DNA, allowing chromatin remodeling. Defective chromatin decondensation can, thus, prevent development of the male pronucleus and fertilization. Decondensation can be produced *in vitro* by the combined actions of polyanionic compounds and reducing agents. This study investigated the impact of iron on *in vitro* human sperm decondensation, stimulated by heparin and glutathione. It was determined that iron produces a significant dose-dependent inhibition of decondensation at micromolar concentrations. The presence of an iron chelator, EDTA, in the incubate, resulted in almost complete abolition of the iron-induced inhibition. Addition of hydrogen peroxide to the incubate did not significantly attenuate the response observed using iron alone. This suggests that the iron-induced inhibition of decondensation was not related to Fenton reaction-mediated reactive oxygen species production and subsequent toxicity. The data indicates that iron can inhibit sperm decondensation *in vitro* by a process independent of reactive oxygen species production.

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FAILURE OF THE GNRH AGONIST, DESLORELIN, TO AFFECT SEMEN PRODUCTION OR AGGRESSIVE BEHAVIOR IN GERENUK (*LITOCRANIUS WALLERI WALLERI*)

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Maintaining surplus captive male gerenuk in bachelor groups can result in unacceptable aggression levels. Suppressing endogenous testosterone secretion has been suggested as a means of controlling aggressive behavior. The aim of this study was to investigate the effect of a slow release GnRH agonist (deslorelin) on LH and testosterone secretion, semen and sperm characteristics and behavior in male gerenuk. Deslorelin (6 mg) was implanted subcutaneously in three males. Immediately before, and two months after implantation body weights and testes volumes were recorded, ejaculates and blood samples were collected, and a GnRH challenge (with serial blood sampling) was conducted. Additional data was collected from a single male that received 4 supplemental implants. Quantitative behavioral data were collected 3/week for 30 min for the duration of the study, starting one month before deslorelin treatment. The mean incidence of behaviors associated with dominance and aggression in bachelor gerenuk groups (supplanting, foreleg kicking, sparring, head butt, marking and mounting) were compared before and after deslorelin treatment. A paired T-test showed no differences ($P>0.05$) in animal weight, testes volume, semen volume, sperm concentration, percent sperm motility, percent sperm plasma membrane integrity or percent normal sperm morphology before and after deslorelin treatment. Semen and sperm traits in the male that received 5 implants (30 mg total) were indistinguishable from pre-treatment ejaculates. The characteristic rise in LH, occurring ~10 min following administration of a GnRH challenge in untreated males, was not evident following treatment with deslorelin. No pre- and post-treatment differences ($P>0.05$) in the mean incidence of any behavioral traits were detected. The absence of a GnRH-induced increase in serum LH in treated males indicated that deslorelin suppressed pituitary responsiveness to endogenous GnRH, but without impacting testicular function. While future studies will investigate the potential of other GnRH agonists, more work is required to determine the impact of deslorelin on basal FSH secretion, as well as testicular gonadotropin receptor concentrations.

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EVIDENCE FOR THE EXPRESSION OF α_{1G} (T-TYPE) VOLTAGE OPERATED Ca^{2+} CHANNELS IN HUMAN MALE GERM CELLS.

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The results of pharmacological studies suggest that voltage-operated Ca^{2+} channels (VOCCs) are expressed in human spermatozoa and are involved in induction of the acrosome reaction by zona pellucida and possibly progesterone. Patch clamp studies on rodent spermatogenic cells have revealed the presence of T-type VOCC-like currents (Arnoult et al, 1996; Santi et al, 1996) but molecular studied have, to date, provided evidence primarily for α_{1C} (L-type) channels (Benoff, 1998).

We have used PCR and *in situ* hybridisation techniques to investigate the expression of the α_{1G} (T-type) VOCC α_1 subunit in human male germ cells. Using specific primers directed against a region close to the 3′ end of human brain-derived α_{1G}, we have obtained PCR products of the expected size from cDNA libraries derived from human testis and human spermatozoa. Sequencing of the product showed that it had >90% identity with human brain α_{1G}.

A digoxigenin-labelled DNA probe was generated from this PCR product and used to label sections of human testis. In some tubules a large number of germ cells were labelled. Very little labelling was seen in non-germ cells.

We conclude that human male germ cells express α_{1G} (T-type) VOCC subunits. It is therefore possible that α_{1G} is responsible for the T-like currents of rodent spermatogenic cells.

REFERENCES Arnoult, C. et al (1996) Proc Nat Aacad Sci USA 93:13004-1309; Benoff, S. (1998). Frontiers Biosci 3:d1220-1240; Santi, C. et al (1996) Am J Phys 271:C1583-C1593.

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METHOD OF ISOLATION AND PURIFICATION OF SPERM HEADS FROM HUMAN SPERMATOZOA: APPLICATIONS TO THE STUDY OF GLYCOLYTIC ACTIVITY IN THE SPERM HEAD. T.M. Rigau[2], R. Laporte[1], M. Ollero[1], J.E. Rodriguez-Gil[2], and J.G. Alvarez[1]. [1]Department of Ob/Gyn, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, and [2]Department of Animal Pathology and Production, School of Veterinary Medicine, Autonomous University of Barcelona, Spain.

Mammalian spermatozoa utilize the Embden-Meyerhof pathway for ATP production through anaerobic glycolysis. The presence of hexose transport systems (Angulo et al., J Cell Biochem 1998, 2:189) and hexokinase (Alexander et al. Mol Biol Cell 1998, 9:2 3) immunoreactivity in the head of mammalian sperm suggest that glycolytic activity may be present in the sperm head. In this st dy, human sperm heads were isolated by a combination of enzymatic hydrolysis, mild sonication, and low-speed centrifugation. The purity of the sperm head preparations was monitored by Western blot analysis using tail-α-tubulin-specific mAb TAP-952. Lactate dehydrogenase activity (LDH) was used as a marker for anaerobic glycolytic activity and it was measured in both head and tail preparations by spectrophotometric analysis using NADH and pyruvate as substrates. Semen samples were fractionated sing a discontinuous Percoll gradient, the 95% pellet washed twice by cent fugation at 800g in PBS, resuspended in 1ml of PBS containing 10mg/ml of α-chymotrypsin, and incubated at 37°C for 30min. Aliquots of 100μl were transferred to glass test tubes, immersed in a Branson sonication bath for 15 sec x 4, the aliquots combined, and centrifuged at 200g for 20min. The supernatant containing the sperm tails and the pellet containing the sperm heads were centrifuged again at 200g for 20min. The resulting tail and head fractions were combined and resuspended in PBS. The purity of the sperm head preparation was greater than 97% and that of the tails greater than 98%. No immunoreactive α-tub lin was detected in head preparations of human spermatozoa containing up to 20 million heads. LDH activity in the head was 0.12μmoles/min and 6.36μmoles x 10^8 cells in the tail preparation. A linear correlation was found between L H activity and α-tubulin concentration in the tail. The results of this st dy indicate that p rification of sperm heads using the method described herein can be used to assay glycolytic activity in the sperm head. In addition, human sperm express LDH activity in the head suggesting that it may have the capability of producing ATP from exogenous hexoses through anaerobic glycolysis. In situ production of ATP in the sperm head may be important during the early stages of fertilization.

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THE PREDICTIVE VALUE OF EPIDIDYMAL FULLNESS.

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Introduction: The purpose of this study was to evaluate the predictive value of subjective epididymal fullness (EF) in 1) predicting obstruction in azoospermic men *(group 1)* and 2) determining preoperatively the need for vasoepididymostomy (VE) in men presenting for vasectomy reversal *(group 2).*

Methods: Obstruction was confirmed at surgical exploration for group 1 following a biopsy that showed sufficient spermatogenesis. If insufficient spermatogenesis was seen then this side was classified as not obstructed. In group 2, the indications for VE were either no fluid seen from the testicular end of the vas or thick, pasty fluid devoid of sperm. Patients with sperm in the vasal fluid or clear or opalescent fluid without sperm underwent vasovasostomy (VV).

Results: The predictive value of EF was evaluated in 42 units (12 in group 1, 30 in group 2) and is summarized below.

	Group 1 (azoospermic)	Group 2 (vasectomy reversal)
Sensitivity	67%	33%
Specificity	100%	85%
+ predictive value	100%	20%
- predictive value	83%	92%

In group 1, 1 patient with bilateral EF had bilateral efferent duct obstruction and thus EF could not predict the site of obstruction.

Conclusions: 1) EF in azoospermic men is predictive of obstruction though the site cannot necessarily be localized. 2) The absence of EF in azoospermic men cannot rule out obstruction. 3) In vasectomized men, the absence of EF is predictive of vasal fluid that allows for VV. 4) EF may suggest but cannot predict unfavorable vasal fluid that requires VE.

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BACK TO THE BASICS - SPERM COUNTING CONVENTIONS

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Objectives: To determine the sperm counting conventions practiced by laboratories performing semen analysis.

Design: Different laboratories and individuals that perform semen analysis were sent the same diagram of sperm on a grid and asked to perform a count using their current laboratory protocol.

Material and Methods: A diagram representing a grid with sperm was sent to 150 laboratories throughout the United States that perform semen analysis. The instructions asked the participants to count the sperm on the grid as if they were performing an analysis in their own laboratory and to circle or highlight the sperm they were including. Labs that use a hemacytometer also were asked to determine the concentration of the original specimen assuming a 1:20 dilution. Results were analyzed to determine sperm counting conventions.

Results: 158 individuals from 84 labs returned results. 114 individuals counted the entire grid, 24 counted the center and four corner squares, 13 counted two rows, 4 counted three rows, 2 counted one row and 1 counted four rows. The mean and 95.5% confidence limits for all values when normalized to the entire grid count was 76.2 (58.8-93.6). 49% of participants *did not* count tail-less sperm heads; 1% *did* count head-less tails; 6% *did not* count sperm with tails that fell outside the grid; 1% *did* count sperm with heads that fell outside the grid; 1% *did not* count sperm that fell on the lines; 1% *did not* count sperm with abnormal morphology; and 7% of individuals reported a value that was an obvious miscount (sperm that were highlighted were not included in the given value or sperm were omitted from the count for no apparent reason). 12% of all laboratories had individuals using different counting conventions within the same lab. 108 individuals reported a concentration value for the hemacytometer dilution question. 10% calculated an incorrect answer based on their original count.

Conclusions: Many different counting conventions exist among and within laboratories that perform semen analysis. Counting conventions and concentration calculations should not be taken for granted and should be an integral part of any semen analysis training and quality assurance program.

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BACK TO THE BASICS - GRADING OF OVERALL SPERM MORPHOLOGY QUALITY IN A SEMEN SPECIMEN

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Objective: To determine whether laboratories performing sperm morphology analysis use % normal forms to classify the overall sperm morphology of a semen specimen as normal or abnormal.

Design: Retrospective analysis of results of two sperm morphology proficiency testing (PT) events in January and July of 1999.

Material and Methods: Labs were sent 2 unstained semen smears for sperm morphology analysis in each PT event (Lots P15, P16, P17, P18). They were instructed to report % normal sperm for each smear along with the classification system used, and to classify the specimen as normal or abnormal based on the criteria used in their lab.

Results: 209 individuals reported results for % normal morphology and 129 of these classified the specimens as normal (norm) vs. abnormal (abn).

		% METHOD USERS WITH CORRECT ANSWER*				
	% using method	P15	P16	P17	P18	Overall correct each system
Reference Lab (*based on these values)		abn	norm	norm	abn	
ASCP	34	40	87	100	92	80
MacLeod	1	0	100	--	--	50
Strict	13	56	78	75	100	77
WHO 2nd	6	50	86	100	100	84
WHO 3rd	30	16	100	80	95	73
Other	16	14	92	100	14	55
Overall correct each lot		32	90	88	84	

Conclusions: Variability exists in the determining whether overall sperm morphology is normal or abnormal. Since this is the real purpose of a lab test, the ambiguity found in this study has disturbing clinical implications. Based on these results it can be estimated that up to 30% of semen specimens are incorrectly categorized as normal or abnormal independent of the method used. Almost half of participants were unwilling or did not know how to use the % normal result to label a semen sample as abnormal or normal. The need is again confirmed for well-defined standards and training methods for sperm morphology analysis.

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STABILITY OF HUMAN SEMEN MEASURES AFTER SIMULATED OVERNIGHT SHIPMENT. SA Rothmann[1], J Quigley[1]*, S Selevan[2]*, W Robbins[3] and SD Perreault[4]. [1]Fertility Solutions Inc., Cleveland, OH, [2]US EPA, Wash., DC; [3]UCLA, Los Angeles, CA; [4]US EPA, RTP, NC.

Population-based studies of semen quality are hampered by logistics and expense of obtaining samples from men in diverse locations. The study evaluated stability of selected measures in unpreserved semen samples analyzed after simulated overnight shipment in the TRANSEM100™ shipping container (Fertility Solutions Inc.). Ejaculates were collected from 11 men and a complete semen analysis was performed. Overnight shipping was simulated by repackaging the sample in TRANSEM100™ and transporting to an off-site location until the next day when a complete semen analysis was performed. Changes in sperm concentration and morphology after shipping were not significantly different from zero (paired t test). Concentration (mean +/- SE) before and after was 54.8 +/- 6.2 vs. 58.1 +/- 7.0 million/ml. % Normal sperm using WHO criteria was 37.0 +/- 2.0% vs. 34.1 +/- 2.1% and using Strict criteria was 14.2 +/ 1.2% vs. 13.1 +/- 1.2%. After shipping viable sperm were found in all samples and motility was found in 10 of 11 samples. As expected, the % viable sperm declined significantly (68.6 +/- 4.4% vs. 45.7 +/- 6.2%) as did the % motile sperm (54.1% +/- 3.9 vs. 28.7 +/- 5.3%). The mean % change in viability (-34.4%, range -2.4 to -87.8%) was slightly less than that for motility (-48.0%, range -6.7 to -100%) and both measures were highly variable among donors. 9 samples were also analyzed for chromosome breakage using fluorescence *in situ* hybridization of tandem probes for chromosome 1 and no significant change in the % breaks was observed. These preliminary results suggest that it may be feasible to ship semen without preservatives by overnight carrier at ambient temperature in TRANSEM100™ and still obtain reliable information on sperm concentration and morphology as well sperm aneuploidy, desired measures of reproductive toxicity in epidemiological trials.

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EVALUATION OF HUMAN SEMEN SPECIMENS TRANSPORTED AT 5°C USING THE BIO-TRANZ™ SHIPPING SYSTEM

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Objective: Transport of unprocessed human semen specimens from the production site to distant laboratories for andrological evaluation and/or clinical use requires the development of proper protocols and devices for the shipment and maintenance of sperm viability during transport. Factors such as maintenance of proper temperature and the specific diluent used are considered to affect the viability and fecundity of semen specimens during transport. The Bio-Tranz™ shipper, which was designed to cool specimens (5°C) during transport, consists of a properly refrigerated Styrofoam unit with all its attachments for semen collection, dilution and transportation. The viability and longevity of semen specimens stored from the time of collection to the time at which the specimens were to be processed and used (24-h post-collection) was evaluated using the Bio-Tranz™ shipping technology.

Materials & Methods: Semen specimens (n=93) were assessed for percentage and grade of motility and for the sperm membrane functional integrity as measured by the hypo-osmotic swelling test (HOS) at collection time and 24-h after storage in the Bio-Tranz™ shipper (ZDL, Inc., Lexington, KY, USA). Furthermore, the recovered spermatozoa were washed and reconstituted in SpermPrep™ media and were assessed for longevity for additional 24 hours.

Results: The results obtained are shown in the table below

	Sperm Characteristics Assessed			
Treatments (n=93)	*Motility (%)*	*Grade (0-4)*	*HOS (%)*	*TMS ($X10^6$)*
TYB, 0-h	65.7±7.4	3.6±0.3	69.7±9.7	28.6±4.1
TYB, 24-h	57.8±8.7	3.2±0.2	61.3±10.2	29.7±5.2
SpermPrep™,24-h	39.5±9.6	2.9±0.2	58.1±9.3	17.2±4.1

Sperm characteristics between fresh specimens (0-h) and specimens prepared using TYB and stored via the Bio-Tranz™ shipper for 24-h were not different (P>0.05).

Conclusions: The results obtained show that collection and shipment of semen specimens via the Bio-Tranz™ shipper is possible. The Bio-Tranz™ shipper maintains adequate sperm viability and adequate longevity after 24-h of cryostorage. The use of the Bio-Tranz™ shipper is extremely convenient for patients that request semen processing services, such as, semen cryostorage, semen evaluation and semen preparation for IUI purposes or other assisted reproductive technologies. The technique could be of significant clinical and economic importance to the patient and to the treating physician at locations across the USA or elsewhere.

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PERCUTANEOUS EPIDIDYMAL SPERM ASPIRATION (PESA): REPRODUCIBILITY OF THE METHOD AND FEASIBILITY OF CRYOPRESERVATION.

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INTRODUCTION: ICSI has allowed men with obstructive azoospermia to father their own children. Sperm of those men can be retrieved through operative microscopical aspiration (MESA) or percutaneous aspiration (PESA). PESA is an outpatient procedure done under local anesthesia and it is cheaper than MESA. PESA has the theoretical disadvantage of ruining the epididymis anatomy (blind procedure), what could prevent a new aspiration. Additionally, it is stated that the ammount os sperm retrieved by this method would not be enough for freezing.

OBJECTIVE: Presentation of 52 PESA procedures, evaluating the reproducibility of the method in the same patient and the possibility of semen cryopreservation for further utilization.

MATERIAL AND METHODS: Between August 1996 and June 1999 we have performed 52 PESA procedures in 39 patients (mean age of 42 years and 34 years for the partners).The most frequent cause of sterility in these patients was vasectomy (n=30). Other etiologies included CAVD (n=4) and other causes (n=5). All the procedures have been performed in an office basis under local anesthesia (1% lidocaine for the spermatic cord blockade). Epididymal aspiration was made with a 27.5G needle adapted to a 1mL-tuberculin syringe filled with 0.05mL of Dulbecco's medium.

RESULTS: We have acquired motile sperm in 39/52 procedures (75%). PESA has been repeated twice in 9 patients and three times in 3 patients. We have been able to find motile sperm in all occasions in 7/9 (77.8%) patients in whom PESA has been performed twice and in 1/3 (33.3%) patients submitted to 3 procedures. In 12 (30.7%) patients we have cryopreserved semen either for a future ICSI procedure or simply to wait for oocyte procurement. Whenever the epididymal aspiration turned negative (13/52 procedures – 25%) the patients were submitted to TESA with a reported success rate of 76.9% (10/13 procedures). Only in 3/52 procedures it was not possible to retrieve sperm percutaneouslly. There were 9 clinical pregnancies (pregnancy/transfer rate of 27% and pregnacy/cycle of 17%) that resulted in 3 abortions, 3 ongoing pregnancies and 3 healthy babies. We have had no complications in any of the 52 PESA procedures.

CONCLUSION: PESA is a cost-effective, easy and safe method for sperm retrieval. It can be repeated more than one time in the same patient with high success rates. Additionally the sperm acquired by this technique can be cryopreserved.

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GENETIC EVALUATION OF AZOOSPERMIC AND OLIGOASTHENOZOOSPERMIC MEN.

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INTRODUCTION: In recent years ICSI has revolutionized the way male factor infertility has been looked upon. Several studies have shown that many infertile men, especially those with non-obstructive azoospermia , severe oligozoospermia or severe oligoasthenoteratozoospermia (OAT), have genetic defects as the main cause of their infertility. Genetic evaluation of those men is extremely important provided they are natural candidates for ICSI and that some of those genetic defects may be potentially transmitted to the embryos.

OBJECTIVE: Presentation of genetic testing (karyotipe, Y-chromossome microdeletion analysis, CF genetic testing) of 56 infertile men.

MATERIAL AND METHODS: We evaluated 56 patients with the diagnosis of non-obstructive azoospermia, severe OAT or severe oligozoospermia with karyotipe, Y -chromossome microdeletion analysis, CF genetic testing: 29 (51.8%) with azoospermia, 20 (35.7%) with severe OAT (< $5x10^6$ sperm/mL) and 7 (12.5%) with < 20 > $5xI0^6$ sperm/mL. Patients mean age was 31.8 years.

RESULTS: We have found alterations in 7/48 (14.6%) kariotypes and in 5/31 (16.1%) Y-chromossome analysis performed. Twenty three patients have been evaluated with both kariotype and Y –chromossome analysis. Overall, the incidence of genetic alterations detected within this group of patients was 21.42% (n=12). However, when we focused on patients with azoospermia, the incidence such alterations was significantly higher: 6/26 kariotypes (23%) and 3/14 microdeletion analysis (21.4%). Four patients (7.14%) were 47XXY-mosaic Klinefelter. Fifty-one patients have been studied for CF-related mutations with alterations being detected in 2 (4.1%). FSH levels were elevated in 13/45 (28.8%) patients (normal range: 2-10 UI/mL): 10/23 azoospermic (43.5%) and 3/22 (13.6%) OAT patients.

CONCLUSION: ICSI has allowed men hitherto considered infertile, to father children with their own genetic material, even though the cause of infertility in most cases remains undetermined. The identification of genetic alterations as the cause of male factor infertility with studies such as kariotype, Y-chromossome microdeletion analysis and CF genetic testing is mandatory in patients with either azoospermia sever oligozoospermia or severe OAT not only because of the high prevalence of genetic alterations in this patients but also because of the risk of transmisson of such genetic alterations to the offspring. Genetic counselling is highly recommended in these situations.

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RECOVERY OF FROZEN-THAWED BOVINE SPERMATOZOA VIA A CONVENTIONAL AND A STANDARDIZED SWIM-UP TECHNIQUE

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Objective: The objective of this study was to evaluate the recovery of frozen-thawed bovine spermatozoa via a conventional and a standardized swim-up technique

Materials & Methods: Frozen-thawed semen specimens (n=40) from 10 bulls were washed and reconstituted with 2.9% (w/v) sodium citrate extender containing 20% (v/v) chicken egg yolk (SC-EY). Reconstituted sperm specimens were used for selection via conventional swim-up and the standardized ZSC™ method. The swim-up method consisted of overlayering the sperm specimen with 1.0 mL of isolation media (Ham's F-10), and incubating at 37°C for 1hr. The sperm specimen (2.0mL) was placed into the ZSC™ conical cavity and the surrounding periconical and epiconical areas of the ZSC™ were filled with 1.0mL of isolation media, followed by 1hr of incubation (37°C). The isolation media was removed from swim-up (80% volume) and ZSC™ specimens (100% volume) at the end of incubation. Recovered isolation media specimens were assessed for volume (mL), sperm concentration ($x10^6$sperm /mL), the percentage and grade of motility (0 to 4), the occurrence of osmotic shock and the percentage of spermatozoa reactive to the hypoosmotic swelling (HOS) test.

Results: Swim-up and ZSC™ selected specimens were qualitatively similar to each other. However, higher numbers of spermatozoa were recovered when sperm specimens were processed via the ZSC™ method (1.5 fold increase) than with the conventional swim-up technique. Because the ZSC™ method enabled the recovery of up to 100% of the overlayered media, it also enabled the recovery of most of the spermatozoa that migrated from the sperm specimen into the isolation media without mixing the two, which was the case with the swim-up method and which could also contaminate the recovered specimen with dead and immotile spermatozoa.

Conclusions: The ZSC™ technique enabled the harvesting of the medium closest to the underlayered sperm specimen, which contributed to maximize the number of sperm recovered. When all assessed parameters were noted and all clinical improvements and efficiency of the two methods were compared, the ZSC™ method was superior to the swim-up technique.

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SPERM CHARACTERISTICS RECOVERED FROM TERATOZOOSPERMIC SPECIMENS USING A MULTI-LAYER SWIM-UP COLUMN

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Objectives: Selection of the highest quality sperm population is more critical as the Assisted Reproductive Technology (ART) method becomes more complex (i.e, IUI vs ICSI) and the need for using the "elite" spermatozoa from deficient specimens becomes a priority in improving the chances for fertilization and subsequent embryonic development. The sperm processing methods available today yield only one sperm sample of higher quality. A modified swim-up column (Multi-ZSC™) is currently available that enables the separation of four distinct subpopulations of spermatozoa from a processed semen specimen. The objective of this study was to assess the recovery and the qualitative distribution of selected sperm subpopulations from teratozoospermic (Terato) specimens via Multi-ZSC™.

Materials & Methods: Sperm specimens were obtained from 30 Terato (but otherwise normal) patients. The cutoff point for Terato status was ≤ 30 % normal forms by strict criteria. A 2.0mL semen aliquot was placed in the bottom cavity of the Multi-ZSC™ (ZDL, Inc., Lexington, KY). Each of the conical chambers, Chamber #1 (Ch-1) being the closest to the bottom cavity, and chamber # 4 (Ch-4) the furthest, were filled with modified Ham's F-10 medium and incubated for 90 min at 37°C.

Results: The results obtained are shown by in the table below:

Parameters	*Semen*	*Ch-1*	*Ch-2*	*Ch-3*	*Ch-4*
Count ($x10^6$)	179.0±14.7[a]	33.2±4.9	9.9±1.3	5.5±0.7	2.5±0.3
Motility %	55.1±1.8[a]	84.7±2.7	100.0±0.0	99.8±0.2	100.0±0.0
Grade (%)	27.1±2.5[a]	78.0±3.4	100.0±0.0	99.8±0.2	100.0±0.0
Morphology (%)	17.5±1.5[a]	41.3±2.2	49.6±2.4	54.1±2.5	58.0±2.5

[a]Significantly different (P<0.05) from populations of sperm harvested from the Multi-ZSC™

Conclusions: Sperm harvesting via the Multi-ZSC™ yielded spermatozoa of superior qualitative characteristics than raw specimens from Terato specimens. Furthermore, those improvements in qualitative measurements increased as the higher quality sperm migrated into the upper chambers of the Multi-ZSC™. A sperm subpopulation that best suits the ART procedure of choice may be selected from the appropriate chamber of the Multi-ZSC™ swim-up column, which may improve the fertilization outcome, subsequent embryonic development and onset of pregnancy. The process may be even most beneficial for the ICSI procedure where the sperm selection is done manually and the Multi-ZSC™ can offer more critical selection of spermatozoa.

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TESTICULAR SPERM RESULTS IN ELEVATED MISCARRIAGE RATES COMPARED TO EPIDYDIMAL SPERM IN AZOOSPERMIC PATIENTS J. Hallak*, F. F. Pasqualotto*, M. E. M Vieira*, A. R. C. Medeiros*, C. C. Rocha*, A. Iaconelli*, E. Borges*. Fertility Center for Assisted Reproduction, São Paulo, Brazil

For men with azoospermia, several sperm retrieval techniques are available. However, comparisons between spermatozoa retrieved from the testicles and epididymis regarding pregnancy and miscarriage rates are not well established. The objective of this study was to compare pregnancy and miscarriage rates with sperm retrieved from the testis and epididymis using intracytoplasmic sperm injection (ICSI). Hundred and eight consecutive patients who presented with azoospermia were included in our study, performing a total of 144 retrieval procedures. Of these procedures, 104 were testicular sperm aspirations (TESA) and 40 were epidydimal sperm aspirations (PESA) PESA was the first approach in obstructive patients (n = 68), whereas TESA was used when the former failed. For non-obstructive patients (n = 40), TESA was the method of retrieval. Eighty one and thirty cycles were performed with spermatozoa retrieved from the testicles and epididymis, respectively. There were no differences in pregnancy rates with testicular spermatozoa (n = 28) compared to epididymal spermatozoa (n = 13) (p = 0.1). However, the miscarriages rates were higher in spermatozoa retrieved from the testicles (n = 12) compared to the epididymis (n = 1) (p = 0.01). Although pregnancy rates were similar when ICSI was performed with spermatozoa retrieved from the testicles and epididymis, the use of testicular spermatozoa yields a significantly higher miscarriage rate. It is possible that the higher miscarriage rate seen in patients with spermatozoa retrieved from the testicles is linked to high genetic sperm abnormalities.

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IS TESE WORTHWHILE IN THE AZOOSPERMIC MALE WITH A HISTORY OF UNILATERAL OR BILATERAL CRYPTORCHIDISM? T. McCallum, D. Cunningham*, C. Burgess*, R.Oates, Boston Medical Center, Boston, Ma

Introduction/Objectives: Multiple studies have documented that approximately 45-55% of men with non-obstructive azoospermia (NOA) will have spermatozoa found within the testicular parenchyma. These sperm can be used in conjunction with ICSI to help achieve biological paternity. However, what are the rates of sperm retrieval for subgroups of men with NOA (Klinefelter's, post-chemotherapy, Y microdeleted, etc.)? The purpose of this study is to document the success of TESE for only those men with a history of unilateral or bilateral cryptorchidism.

Materials/Methods: 20 azoospermic men with a history of cryptorchidism and who underwent TESE constitute the study group. Their ages range from 23 to 46 years. Orchidopexies were performed anywhere from early childhood to adulthood. FSH ranged between 6 and 60. 8 (40%) patients had unilateral cryptorchidism, 4 of whom had an orchiectomy instead of an orchidopexy. 12 patients (60%) had bilateral cryptorchidism, 2 of whom had had a unilateral orchiectomy.

Results: Of the 8 men with unilateral cryptorchidism (5 right, 3 left), 6 had sperm harvested from their tissue. 3/4 in this group who had a solitary testis had sperm found. Of the 12 men with bilateral cryptorchidism, 6 had sperm recovered during TESE. Overall, 12/20 (60%) had spermatozoa in the testis parenchyma.

Conclusion: For the subgroup of men with NOA who have a history on unilateral or bilateral cryptorchidism, the rates of success for TESE appear quite good. Whatever the genetic/environmental mechanism(s) underlying the spermatogenic deficiency in patients with cryptorchidism, it does not preclude fatherhood in the era of ICSI. Unilateral patients fare better than bilateral patients. Various subgroups need to be identified and analyzed separately to provide each patient with as best prediction as possible.

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RELATIONSHIP BETWEEN PRONUCLEI MORPHOLOGY AND HUMAN PRE-EMBRYOS BY CYTOGENETIC ANALYSIS USING FLUORESCENCE IN SITU HYBRIDIZATION TECHNIQUE

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It is well defined that human oocytes obtained by *in vitro* fertilization techniques should be kept for 1 or more days in culture media in order to determine the cellular division rate and the morphological evaluation, parameters directly correlated with implantation rates. This approach involves ethical, medical, and laboratory aspects when issues like cryopreservation and disposal of unused embryos are taken in consideration. We sought to correlate PN morphology with cytogenetic analysis of human pre-embryos submitted to preimplantation genetic diagnosis (PGD). Ten intracytoplasmic sperm injection (ICSI) cycles were evaluated with a single PN analysis 16-18 hours after injection. A pre-embryo was considered normal (PN-nl), when the ponuclei were closed or aligned with alignment of nucleoli at the junction of the 2 PN. An embryo was classified as having good quality if 6 or more cells were present with < 20% fragmentation at least 68 hours after ICSI (day +3). Probes for chromosomes X, Y, 18,13 and 21 were analyzed through biopsy of one or more blastomeres (Vysis-Aneuvision) and transferred 96 hours after retrieval. The diagnosis was obtained in 47 of the 51 biopsied pre-embryos. Twenty-three pre-embryos were considered normal for the 5 chromosomes studied, being 43.5% with PN-nl compared to 25% with abnormal PN ($p<0.01$). Using morphology as the endpoint for pre-embryo evaluation at 68 hours after ICSI, 69,6% of them presented with PN-nl compared to 54.2% with abnormal PN ($p>0.05$). The most frequent cytogenetic alterations were euploidies (41%) and aneuploidies (33%), without correlation with PN morphology or pre-embryo at day +3. PN morphology based in the analysis of its alignment and nucleoli disposition within the embryo represents an efficient criteria for the selection of cytogenetically normal pre-embryos to be transferred in ICSI cycles.

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RECOVERY OF SPERMATOGENESIS AND SUCCESSFUL CONCEPTION AFTER BONE MARROW TRANSPLANT FOR ACUTE LEUKEMIA

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There have been four manuscripts to date reporting pregnancies despite bone marrow conditioning by either total body irradiation (Facon et al, 1993; Pakkala et al, 1994) or chemotherapeutically with busulfan and cyclophosphamide (Shepherd et al, 1996; Lipton et al, 1999). Though paternity was established in a couple of the cases, semen specimens revealed either zero sperm or extremely severe oligoasthenozoospermia. A case is described of a 25 year old male diagnosed with acute myeloid leukemia. Prior to chemotherapy he had a semen sample frozen. While in remission he had a bone marrow harvest performed. After relapse, he had bone marrow conditioning with busulfan and cyclophosphamide. Autologous bone marrow transplantation was performed on day 9. The couple came to our infertility center 5 years later. Interestingly a normal post-coital test was found on initial visit despite intercourse from 48 hours before. A semen analysis found the sperm concentration to be 30.8×10^6/mL with 54% motility, volume 1.8mL. Sperm viability was 94% and the HOS test was 82%. Sperm morphology using strict criteria found 5% normal and 1% slightly abnormal forms. The female partner was diagnosed with premature luteinization, luteal phase deficiency, and a large 54mm endometrioma. The endometrioma was laparoscopically removed and her premature luteinization was treated with leuprolide acetate 1mg days 1-3. She was also treated with progesterone vaginal suppositories in the luteal phase. She conceived and delivered a normal male at 42 weeks. Using similar treatment the couple conceived again 2 ½ years after delivery but she had a spontaneous abortion. A repeat semen analysis was performed 9 years after bone marrow conditioning and the sperm concentration was 12.5×10^6/mL with 62.0% motility. The normal morphology using strict criteria was 12% and the HOS test was 79%. This is the first case of the return of normal spermatogenesis and parentage following bone marrow conditioning. If the combination of busulfan and cyclophosphamide is found equally efficacious to total body irradiation for disease control, the former may prove to be advantageous to a younger couple who still may want to start a family later if the disease is put under control.

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ABSENCE OF SPERM WITH RAPID MOTILITY IS NOT DETRIMENTAL TO IN VITRO FERTILIZATION (IVF) OUTCOME MEASURES WHEN INTRACYTOPLASMIC SPERM INJECTION (ICSI) IS USED

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We have previously demonstrated that the absence of sperm with rapid motility after sperm separation was associated with a reduced pregnancy rate (PR) following intrauterine insemination and IVF using conventional insemination techniques. The objective of the current study was to investigate the association between rapid motility and IVF outcome when ICSI is used for insemination. All IVF cycles between 1/1/97 and 9/30/98 in which ICSI was used for insemination were reviewed. Only one cycle per patient was used to assure independence of the observations. Patients were excluded from this analysis if they were >43, they were using donor gametes, or they were using a gestational carrier. Patients were classified into groups by the presence/absence of sperm with rapid motility ("A" sperm) in the specimen before and after sperm preparation. Group 1 had 0% A sperm pre-wash and 0% A post-wash (n=45), group 2 had 0% A sperm pre-wash but some A sperm post-wash (n=47), and group 3 had A sperm both pre and post-wash (n=141). The outcome measures compared by group were fertilization rate, clinical PR and implantation rates. Statistical analysis demonstrated no difference in the fertilization, PRs, or implantation rates in the 3 groups. The average fertilization rates per patient in the three groups were 54.4%±26.5%, 59.4%±24.4%, and 61.6%±23.0%, respectively. The clinical PRs in the groups were 37.8%, 48.9%, and 40.4%, respectively. The implantation rates were 20.7%, 17.9%, and 23.5%, respectively. Absence of sperm with rapid motility pre-wash occurred in 92 (38.8%) of the ICSI cycles studied. Absence of sperm with rapid motility post-wash occurred in 49 (20.7%) of the ICSI cycles studied. These data suggest that the absence of sperm with rapid motility post-separation of sperm from seminal plasma is not detrimental to IVF outcome when ICSI is used for insemination.

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MICROSURGICAL TESE IN NON-OBSTRUCTIVE AZOOSPERMIA

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The aim of this study was to determine the need and efficacy of microTESE in azoospermic patients, prior to ICSI. During the procedure, seminiferous tubules were collected from different areas using a microsurgical microscope. Nine samples were obtained from each testis. Forty-two azoospermic patients underwent microTESE. The diagnosis of obstructive and non-obstructive azoospermia was done using a "testicular score" based on FSH levels, testicular volume, previous biopsy or surgery, volume and pH of ejaculate etc. Seminiferous tubules were evaluated by histology and after mincing under an inverted microscope. Spermatozoa were frozen and used for ICSI.

Results: Among 16 obstructive azoospermic patients no differences had been observed between the samples and 15 patients had motile spermatozoa. Among 26 non-obstructive azoospermic patients, 12 (46%) had spermatozoa and 7 (26%) revealed different seminiferous contents.

Azoospermic patients	No	Sperm+	%	DST*	%
Obstructive:	16	15	93	1	6
Non-Obstructive:	26	12	46	7	26

Sperm+: Sperm found, DST* different seminiferous tubules.

Conclusions: MicroTESE enables selection of seminiferous tubules which contain spermatozoa in a damaged testis. By using magnification an improved distinction between testicular segments and preservation of blood vessels, is achieved.

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Cryopreservation of Spermatozoa for Carcinoma or TESE

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Objective / Indications for IVF/ICSI with cryopreserved spermatozoa are: carcinoma patients before orchidectomy, chemotherapy or radiation (Cryo-Ca); cryptozoospermia (Cryo-Crypto); testicular sperm extraction (Cryo-TESE).
We asked for two questions: 1. Do pregnancy rates per embryo-transfer after sperm freezing differ between carcinoma patients and non carcinoma patients?
2. Do pregnancy rates per embryo-transfer differ between Cryo-TESE and fresh TESE?

Material and Methods / Spermatozoa were frozen following the Berlin protocol (Kaden 1985); after thawing IVF/ICSI and embryo-transfer were performed (Palermo 1992).

Results - Carcinoma vs. Cryptozoospermia

	Cryo-Carcinoma	Cryo-Cryptozoospermia
Cycles	23	31
Fertilization / oocyte	68%	59%
ET / cycle	100%	87%
Pregnancy / ET	**26%**	**26%**
Abortion / pregnancy	17%	14%

There was no difference in pregnancy rates per embryo transfer for carcinoma or non carcinoma patients.

Cryo- vs. fresh TESE

	Cryo-TESE	Fresh TESE
Cycles	87	30
Fertilization / oocyte	51%	60%
ET / cycle	93%	83%
Pregnancy / ET	**32%**	**30%**
Abortion / pregnancy	23%	22%

For TESE patients freezing did not decrease pregnancy chances per embryo-transfer.

Conclusion / Freezing of spermatozoa does not decrease pregnancy chances per embryo-transfer neither for carcinoma nor for TESE patients.

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HUMAN SPERM FUNCTION IN COCULTURE WITH AND WITHOUT DIRECT OVIDUCT CELL CONTACT

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Oviduct epithelial cells (OEC) of either human or bovine origin improve human sperm function in vitro, including increasing sperm survival time and motility retention. Studies were done to determine if this effect required direct cell contact between the sperm and OEC. Cryopreserved sperm from 6 normospermic donors were routinely washed and either: cultured in HTF with HSA (HTF+) medium alone (control); cocultured in HTF+ with bovine OEC (direct sperm-OEC contact); or cocultured in HTF+ with bovine OEC and a diffusible 0.4 μm membrane physically separating the sperm and OEC (no direct sperm-OEC contact). Measurements included sperm motility parameters via CASA and membrane integrity assessment (via hypo-osmotic swell test "HOS") over time. Sperm function was equivalently improved ($p<0.05$) for sperm in both coculture treatments as compared to the control. This included prolonged sperm motility and membrane integrity, and increased sperm velocity over time. This work suggests that at least some of the OEC factors which protect sperm function in vitro (and possibly in vivo) are diffusible and do not require direct physical sperm - OEC contact. Funded by NICHD #32851.

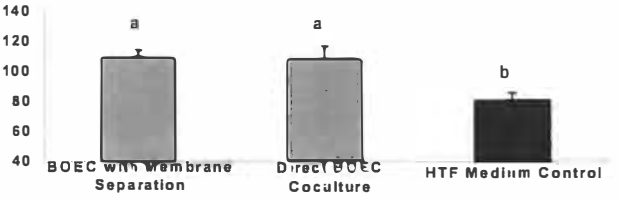

Figure. Time to loss of sperm motility (i.e.<5% progressively motile sperm) for cryopreserved human sperm in control media or coculture with and without direct sperm and OEC contact. [a,b]Differ at $p<0.01$.

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SPERM CHROMATIN DAMAGE REDUCES BLASTOCYST FORMATION

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A general paternal effect on reproductive outcomes has been previously shown in several studies. This study was done to evaluate the relationship between sperm chromatin (DNA) integrity and early embryonic development using a bovine IVF model. The flow cytometric Sperm Chromatin Structure Assay (SCSA) was used to identify bulls with high and average levels of sperm chromatin damage after summer heat stress. Different ($p<0.01$) mean percentages of sperm with abnormal chromatin were observed between the groups of bulls (high=27±3%, low=10±2%). Routine IVF with 300 in vitro matured oocytes/group was then done to assess fertilization and embryonic development rates over 6 days of culture resulting from sperm in either group. Equivalent fertilization rates were observed for sperm from both bulls with high and average levels of chromatin damage. However, subsequent development of embryos from sperm with more chromatin damage was reduced ($p<0.01$). These data suggest that a population of sperm with damaged chromatin may fertilize oocytes but not support further embryonic development to the blastocyst stage.

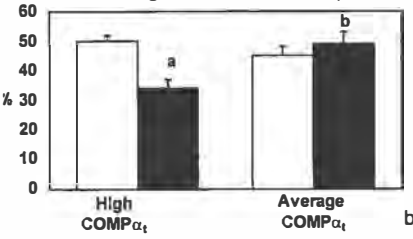

Figure. Percent oocytes cleaved □, and percent of cleaved oocytes going on to form blastocysts ■ using sperm from bulls with high or average percentages of sperm chromatin damage (COMPαt); n=300 oocytes and 3 bulls/group). [a,b]Superscripts means differ at $p<0.01$; $COMP\alpha_t$ = sperm cells outside of main population, showing those sperm with damaged chromatin. Supported by NICHD HD#32851, USDA #9803911 & EPA #G8C10458.

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Sperm counts amongst men with malignant disease: Implication for sperm storage counselling prior to chemotherapy

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Sperm counts are often perceived as general systemic 'health markers' in many disease states, and malignant disease is no exception. Our knowledge of the fertility status of cancer patients, both before and after chemotherapy is limited.. Chemotherapy and in particular alkylating agents produce significant damage to the testis, leading to sterility in a large number of cases.

We analysed data from 230 sperm storage patients. 211 were patients diagnosed with various forms of cancer and were storing sperm prior to chemotherapy. Sperm count and motility was compared with that of 19 patients storing sperm prior to vasectomy, to ascertain whether the diagnosis had a major bearing on traditional semen parameters. 4 major groups of disease were formed for analysis: Leukaemias (n=31); Lymphomas (Hodgkins and non-Hodgkins (n=87); Sarcomas (n=27) and Testicular (Seminoma and Teratoma).

Sperm counts from patients with testicular malignancies were significantly lower than all other groups (mean 20.7×10^6/mL s.e.m 2.26) which appears to be due entirely to orchidectomy prior to chemotherapy ($p<0.01$). Those of lymphoma, leukaemia and sarcoma patients did not differ significantly from each other, and were in the normal range (WHO, 1999). However when compared to a fertile control group of pre-vasectomy storage patients, all cancer groups showed significantly lower sperm count and motility. ($p<0.0001$). Concentration of motile sperm for Lymphoma, sarcoma and leukaemia were 25.2, 24.4 and 26.5×10^6/mL respectively, compared to the pre-vasectomy mean of 39.4×10^6/mL. The results clearly demonstrate that in many malignant disease states, fertility status is affected and should be borne in mind when counselling cancer patients prior to sperm storage. The mechanisms involved are unknown and may differ between cancer type. Disturbances in endocrine/paracrine function and associations with germ cell line apoptosis are suggested and investigations are underway to test this hypothesis.

World Health Organization (1999). Press syndicate of Cambridge.

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FERTILITY OF MOUSE SPERMATOZOA RETRIEVED FROM CADAVERS MAINTAINEDAT 4℃

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After the death of animals the spermatozoa within the testis and epididymis eventually disintegrate. We examined the motility, viability, and fertility of mouse spermatozoa after retrieval from the epididymis at various postmortem days. Cadavers were kept in a refrigerator (4℃). About 30% of the spermatozoa collected 10 days postmortem were viable, but they had a very limited ability to fertilize oocytes in vitro. When injected into the oocytes, however, the spermatozoa fertilized over 80% of the oocytes. Normal live fetuses were obtained even with immotile spermatozoa retrieved 20 days postmortem. If valuable male animals (of the mouse and other species) die unexpectedly and the facility for sperm cryopreservation is not immediately accessible, temporal storage of cadavers (or of epididymis/vas deferens) at 4℃ (in a regular refrigerator) followed by ICSI may rescue the genomes of individuals, and possibly their species, even if the spermatozoa are immotile.

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SPERM SELECTION BY A DEXTRAN/SWIM UP PROCEDURE INCREASES DE EMBRYO RATES FOLLOWING *IN VITRO* FERTILIZATION BY INTRAUTERINE INSEMINATION IN SUPEROVULATED EWES. PREDICTION OF FERTILITY BY CENTRIFUGAL COUNTERCURRENT DISTRIBUTION (CCCD) IN AN AQUEOUS TWO-PHASE SYSTEM

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The objective of this work was a comparative study of the fertilizing ability of three different ram sperm samples by means of *in vitro* parameters and analysis of centrifugal countercurrent distribution in an aqueous two-phase system.

A raw semen sample diluted with PBS (control sample), and two different samples obtained by a dextran/swim-up procedure with (swim-up+) or without NaCl and $NaHCO_3$ (swim-up-) in the selection medium, were used. Sperm motility, viability (assessed by CFDA/PI stain) and mitochondrial activity (by Rhodamine 123) were significantly higher in both selected samples than in the control one. Both swim-up obtained samples also achieved significantly higher fertilization rates with 64 h. interval between progestagen removal and AI. When the interval was 52 hours, the swim-up- sample fertilization rates were significantly higher than the swim-up+ and the control samples. Analysis by CCCD in an aqueous two-phase system revealed a very high correlation between the heterogeneity of the obtained profile together with the total recovered viability and the fertilization rate.

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SPERM CHROMATIN STRUCTURE IN GLOBOZOOSPERMIA: A CASE STUDY.

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Introduction: Nuclear abnormalities previously characterized in globozoospermic patients suggest problems in nuclear condensation during development. This study used the sperm chromatin structure assay (SCSA) and single cell gel electrophoresis assay (COMET) to assess if globozoospermia is associated with sperm chromatin structure abnormalities and/or DNA fragmentation. **Material & Methods**: The SCSA, a flow cytometric measure of susceptibility to acid-induced nuclear DNA denaturation *in situ*, quantifies abnormal chromatin structure by measuring red and green fluorescence of 5 x 10^3 sperm/sample with acridine orange staining. Fertile sperm have low SCSA values ($X\alpha_t$, $SD\alpha_t$ and $COMP\alpha_t$). COMET measures DNA fragmentation in individual sperm nuclei based on gel electrophoretic patterns. **Results**: Sperm concentration (113 mill/ ml) and motility (66%) were normal but there was complete acrosome deficiency. Light microscopic examination of this sample by Testsimplet® showed 68% round-headed, 30% cigar-shaped with an equatorial constriction, and 1% pinheaded sperm. The scatter plots of SCSA confirmed that sperm populations had a mixture of round and elongated sperm heads. Even though 100% of the sperm had abnormal head morphology, only 13% percent of the sperm demonstrated DNA denaturation ($COMP\alpha_t$) which is below our threshold of 15% $COMP\alpha_t$ for high fertility (HR 14;1039). Of interest, 13% of the sperm were also positive in the COMET assay supporting our previous observations (ECR 236;231) that SCSA positive cells are also positive for DNA fragmentation. **Conclusions**: It was unexpected but of great interest that a human sperm population with 100% sperm nuclear morphology abnormalities had a chromatin integrity at the molecular level that is equivalent to sperm populations in previous studies that were highly fertile. These data support the previous reports that ICSI of globozoospermia may result in fertility/pregnancy; the lower success rate may be due to other factors.

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ULTRASTRUCTURAL FINDINGS IN SEMINAL CYTOLOGY OF INFERTILE MEN WITH GENITAL INFECTIONS CAUSED BY *Chlamydia trachomatis* AND *Ureaplasma urealyticum*

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Among non gonococcal uretritis 60% of them are caused by *C. trachomatis* and *U. urealyticum*, organisms sexually transmited . These are frecuently presented like asymptomatic infections and involving a diminished sperm quality. The defects in motility and morphology of spermatozoa associated with these infections have not been explained clearly to date, although, in the case of *U. urealyticum* it has been shown that sperm motility is decreased by the physical adherence of the organism to the tail of the spermatozoon .

Even when some descriptions of the ultraestructure of semen in patients with *U. urealyticum and C. trachomatis* infections exist, there has not been a detailed description of alterations of the fine structure of the spermatozoa that can or can't be related to asthenozoospermia.

In this study we selected a group of one hundred infertile patients, who presented an alteration in sperm motility and morphology according with WHO. All patients were microbiologicaly tested for *U. urealyticum in a seminal sample* (Mycrotrim GU, Irving Sc.) and studied with a direct immunofluorescence test for *C. trachomatis*, using uretral smears (Syva Microtrac). An aditional sample of seminal liquid was fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide and embedded in epoxic resin by conventional metodology, sectioned at 90 nanometers and stained with uranile acetate and lead citrate for Transmission Electron Microscope (TEM) examination.

RESULTS. The microbiological studies showed 24% of patients infected with *U. urealyticum* and 20% infected with *C. trachomatis*, the TEM spermatic observation in these individuals showed similar findings: heavy intranuclear vacuolization, chromatine decondensation, nuclear and acrosomal deformities, alterations in the flagellar ultraestructure and binucleation. Beside these sperm alterations we also observed the constant presence of polymorphonuclear leucocytes, macrophages and epithelial cells, phagocytosis of spermatozoa and the presence of bacteria. In the cases of negative microbiological test, some of the sperm alterations observed in the positive group,there were also present but in a diminshed rate of incidence. Aditionally in 30% of the patients with negative microbiological test, the TEM seminal study showed chlamydial dense bodies and pleomorfic mycoplasmic elements; this would indicate the ineffectiveness of microbiological test to detect genital urinary infections caused by these infectious agents.

The alterations in the spermatic ultrastructure observed in association with infections by C trachomatis and U. urealyticum can be associatd although no specifically with spermatic motility problem and reproductive failure of the affected individuals.

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CHARACTERISTICS OF SEMINAL FLUID LEUKOCYTES IN MEN WITH AND WITHOUT CHRONIC PELVIC PAIN SYNDROME. C. H. Muller and R. E. Berger, Dept. of Urology, University of Washington, Seattle WA 98195

Seminal fluid may be of use in the diagnosis of inflammatory (category IIIa), non-inflammatory (category IIIb) chronic abacterial prostatitis/chronic pelvic pain syndrome (CPPS), and asymptomatic inflammatory prostatitis (category IV). We have previously compared seminal fluid from men in category IIIa vs. IIIb, and here report preliminary findings from a study of men with and without CPPS.

METHODS Semen and expressed prostatic secretion (EPS) were collected from men with CPPS category III, and from controls without pelvic pain. A complete analysis was performed on each sample, with special attention given to leukocytes (WBC). Concentrations of WBCs were measured by hemacytometer; the percentage of peroxidase-positive cells was determined; and when possible, reactive oxygen species (ROS) generated by washed WBCs were measured with and without stimulation by FMLP, using a luminol-based assay.

RESULTS In semen, equal numbers of round cells (WBC plus immature germ cells) were observed (2100 vs 1750/cu mm), but men with CPPS had a higher percentage of peroxidase-positive cells (27% vs. 5% of round cells, p=0.05) than controls. The concentration of peroxidase-positive cells/cu mm in both EPS and semen was higher for men with CPPS (2352 vs. 2 for EPS; 435 vs. 75 for semen, p=0.06). The men with CPPS tended to have more WBCs in EPS than the controls (median 1380 vs. 500 WBC/cu mm, NS). About half of all men had leukocytospermia but non-inflamed EPS; this was true for only a third of controls. Finally, ROS generation by unstimulated seminal cells was significantly higher in men with CPPS than in controls (4.2 vs. 0 rlu/sec per 1000 round cells, p<0.02). Stimulation by FMLP obscured this difference.

CONCLUSION Our preliminary results suggest that evaluation of seminal WBCs by peroxidase staining and ROS generation may assist in studying chronic abacterial and asymptomatic prostatitis. However, WBC in semen may have sources other than the prostate; this situation must be ruled out by examination of the EPS. Seminal WBCs in men with CPPS may have higher basal ROS then those of controls.

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EXPRESSION OF APOPTOSIS AND DNA REPAIR GENES IN INFERTILE MEN

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Objectives: The reason for nonobstructive azoospermia in many cases of male infertility is unclear. We examined the hypothesis that perhaps some of these men have alterations in DNA repair or apoptotic gene expression that result in decreased or aborted spermatogenesis.

Design: Case study of gene expression in infertile azoospermic men.

Methods: Individual testis cDNA libraries were generated from snap frozen testis biopsy tissue in men with both obstructive and nonobstructive azoospermia. Nonobstructive azoospermic histologies included germ cell aplasia and early maturation arrest. Testis cDNA libraries were radiolabelled and then hybridized to a cDNA expression array (Clontech, Inc) containing 98 known apoptosis and DNA repair genes. Differential testis gene expression patterns were compared between obstructed controls and men with nonobstructive azoospermia.

Results: Analysis of DNA repair genes revealed both qualitative and quantitative differences in expression patterns among men with nonobstructive azoospermia. This was especially obvious with the DNA repair genes ATM and PUR. Analysis of apoptosis-related genes revealed significant overexpression of MCH4, a cysteine protease, in a subset of nonobstructed men.

Conclusions: The expression of critical DNA repair genes within the testis varies widely among nonobstructed, azoospermic men. In addition, overexpression of the apoptosis gene MCH4 in some men provides strong corroborating evidence for the role of apoptosis in nonobstructive azoospermia.

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CARNITINE THERAPY OF OLIGOSPERMIC MEN

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Carnitine is cofactor of several enzymes involved in the transformation of fatty acids, but also required for sperm motility. We analysed 85 infertile men who received 3gr carnitine (Sigma Tau,Italy) during 3 months and 25 men without any therapy. Carnitine group was divided into A.severe oligospermic (sperm count < 5x10^6/ml,prog,motility < o.1o) and group B.oligospermic (s.c. < 2ox1o/ml,motility < o.2o). Results:Seminal carnitine raised in group A. from 565 to 951 umol/l, and in group B. from 656 to 1196 umol/l. The concentration in both groups was significantly higher after the therapy(r-o.911,p-o.ooo12).Sperm count improved in group A. from 1.36 to 3.9x1o/ml (r-o.715,p-o.oo14) and in group B from 14.11 to 23.o7x1o/ml (r-788,p-o.ooo37), Progressive motility is also better in group A.from o.o5 to o.14 (r-o.777,p-o.ooo1) and in group B. from o.14 to o.24 (r-787,p-o.ooo1). Swim up spermogram was improved in group B. from 1.1 to 9.2x1o/ml (r-755,p-o.ooo32). Non treated group showed similar results before and after this period. In conclusion we can say that treated group improved sperm count, progressive motility and group B.oligospermic improved also swim up spermogram.

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Y CHROMOSOME DAZ MICRODELETIONS BY STS-PCR IN MEXICAN OLIGO/AZOOSPERMIC MALES. Diaz M, Hernández-Zaragoza G*, Sandoval L*, Nuñez C*, Olivares N*, Rivas F*. Andrology Service, Gyneco-Obstetrics Hospital, and Genetics Division, CIBO, Instituto Mexicano del Seguro Social, Guadalajara, Mexico.

In 1976 it was suggested that terminal Yq euchromatin contains spermatogenesis-related genes (Tiepolo & Zuffardi, Hum Genet 34:119). Assignment has been reduced to the AZF (azoospermic factor) regions, and the critical point to DAZ (deleted in azoospermia) genes family at the subinterval 6D (Reijo et al. Nature Genet 10:383, 1995). Ten to 20 % of patients with spermatogenesis failure show microdeletions at the DAZ region, although this relationship is still controversial (Agulnik et al. Hum Mol Genet 7:1371,1998). By STS-PCR (Vollrath et al. Science 258:52,1992), 65 idiopatic oligo- or azoospermic males were typed with ten STSs (from cen to tel: sY132, sY152, sY154, sY147, sY149, sY254, sY255, sY202, sY158, sY277), all included within subintervals 6A to 6F. In five cases, All sites typed were deleted. The finding of approximately eight percent of DAZ microdeletions in the patients with abnormal spermatogenesis studied here is similar to the proportion observed in other studies (Krausz & Mc Elreavey, Front Bioscience 4:1, 1999), and stress that this technique is useful in male infertility diagnosis.

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ASTHENOZOOSPERMIA: ANALYSIS OF A LARGE POPULATION

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The spermatozoa require a long distance travel to meet and fertilize the oocyte, so sperm motility is a requisite for normal fertilization.

PATIENTS AND METHODS:

In this study, semen analysis data on 1165 men referred to the Fertility Laboratory; Department of Clinical Biochemistry; Hospital de Clínicas; University of Buenos Aires, were retrospectively examined (1992-1999). WHO guidelines were used. Samples were classified in three groups: (n=169) normozoospermic (N), (n=207) asthenozoospermic (A) and (n= 789) asthenozoospermic associated to oligo and/or teratozoospermia (C).

MAR-test was employed for immunologic studies. PAP stain was performed in order to determine the cytomorphology and evaluate the ratio germ cells/spermatozoa (C/E; reference value<2.5%).

RESULTS:

The frequency of leukemia (leukocyte concentration> 1.10^6 /ml ejaculate) was 5.8% in A, 7.7% in C and 5.3% in N (NS). Mar-test over to 40% was found in 6.0% of the A samples and 7.6% of the C (NS), while no positive values were observed in the N group ($p<0.001$). Statistically different was the ratio germ cells/spermatozoa between C and N ($p<0.001$) and C and A ($p<0.001$). No difference was found comparing N and A.

CONCLUSIONS:

a) Asthenozoospermia was observed in 19% of the infertile men.
b) Leucospermia was not related to asthenozoospermia.
c) The incidence of immunologic factor is higher in the groups under study.
d) Combined asthenozospermia is associated with a failure in germ cells/spermatozoa ratio.

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CAN VIAGRA® HELP? ITS ROLE IN THE TREATMENT OF INFERTILITY IN MALES WITH ERECTILE DYSFUNCTION

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Objectives: The role of Sildenafil Citrate (Viagra®) in the treatment of men with erectile dysfunction (ED) has been extensively described. Studies have shown that when Viagra® is administered to men with ED, it can bring about erection and subsequent ejaculation. The objective of this study was to evaluate the characteristics of ejaculates in men of reproductive age with ED, utilizing Viagra® while undergoing infertility evaluation and employment of intrauterine insemination (IUI) for their wives.
Design: A prospective study of three patients treated with Viagra® for ED at the Andrology Institute of America.
Materials & Methods: A total of 3 patients with ED received Viagra® and produced a total of 18 ejaculates for evaluation and sperm preparation for IUI. Each male was given the opportunity for up to 3 times to produce an ejaculate prior to being given Viagra®. Each ejaculate was produced at intercourse via the use of the Male Factor Pak. Seminal specimens were assessed and processed via the ZSC-II method for IUI. Each processed specimen was evaluated for its appropriateness for IUI.
Results: Sperm quantitative and qualitative measurements after production at intercourse were within normal range (WHO standards) with ejaculate volume: 2.6±0.4mL; concentration: $41.4\pm5.7 \times 10^6$ spermatozoa per mL; percent motility: 52.3±6.8; progressive motility: 3.1±0.4 (scale 0-4) and percent normal morphology: 46.2±6.6. When specimens were processed for IUI, the sperm quantity and quality was adequate to cause adequate conception. IUI specimens (0.7mL) contained $23.5\pm7.1 \times 10^6$ sperm/mL with 73.6±6.8% motility and 3.3±0.2 progressive motility (Scale of 0-4).
Conclusions: It appears that Viagra® can be an effective tool for the treatment of reproductive age ED males undergoing infertility workup or treatment in an IUI program. The ejaculates produced and the processed specimens for IUI were of adequate quality to establish a pregnancy in the couples undergoing infertility treatment.

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TESTICULAR HISTOLOGY OF MEN WITH NON-OBSTRUCTIVE AZOOSPERMIA OR SEVERE OLIGOSPERMIA CAUSED BY Y CHROMOSOMAL DELETIONS SJ Silber, Infertility Center of St. Louis, St. Luke's Hospital, St. Louis, MO, R Alagappan*, LG Brown,* DC Page,* Howard Hughes Medical Institute, Whitehead Institute and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA

Objective: It has been suggested that deletions in specific regions of the Y chromosome cause specific histologic testicular defects. We wished to determine whether the type of Y deletion had any impact on the specific histologic defect in azoospermic or severely oligospermic men.
Materials and Method: Twenty-one fertile men with Y chromosomal deletions were studied with testicular sperm extraction (TESE)-ICSI procedures and/or diagnostic testicular biopsy for quantitative analysis of spermatogenic defect. A total of sixteen azoospermic and five severely oligospermic patients were found to have Y chromosome deletions that were not present in their fertile fathers, brothers, or paternal uncles. The common deletion in all twenty-one men was AZFc. In two cases, AZFa was also deleted, and in three cases, AZFb was also involved. In sixteen cases, only AZFc was deleted. In four of the AZFc deleted cases (severe oligospermia), no histology was available. In seventeen cases histology was available.
Results: Of the twelve AZFc only deleted patients, five had Sertoli cell only, five had maturation arrest, and two had a mixed combination of Sertoli cell only and maturation arrest. In the three cases where AZFb was also deleted, two were Sertoli cell only and one was maturation arrest (in contrast to what has been proposed). In the two cases where AZFa was also deleted, both were Sertoli cell only. Sperm were found (often in minute quantities) in the sixteen cases where only AZFc was deleted. In the five cases involving AZFa and AZFb, no sperm were found.
Conclusions: We could not demonstrate for AZFb or AZFc a specific histologic defect in the testis other than a dramatic overall reduction in mature sperm. Deletions involving AZFa were unusual, and caused Sertoli cell only. The size and location of the Y deletion predicted the presence or absence of sperm but not the histology.

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Y CHROMOSOME DELETIONS AND THE FEASIBILITY OF ICSI **SJ Silber, Infertility Center of St. Louis, St. Luke's Hospital, St. Louis, MO, R Alagappan*, LG Brown*, DC Page*, Howard Hughes Medical Institute, Whitehead Institute and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA**

Objectives: Y chromosome deletions, not found in normal, fertile men, are found in 12% of azoospermic men and 7% of severely oligospermic men.
Design: In this updated study, we wished to determine the severity of testicular defects in azoospermic men with different Y chromosomal defects, and what impact different types of Y-deletions might have on the results with ICSI.
Materials and Methods: 205 infertile men with azoospermia or severe oligospermia underwent Y chromosomal mapping. 135 were azoospermic, with a histologic diagnosis of either maturation arrest or Sertoli cell only. 51 of these men (10 with Y deletions) chose to undergo testicular sperm extraction with ICSI. 70 men with severe oligospermia ($<2X10^6$/cc) also had Y DNA testing, and 31 (5 with Y deletions) of them underwent ICSI with ejaculated sperm.
Results: 16 of the 135 azoospermic men (12%) and 5 of the 70 severely oligospermic men (7%) were found to have deletions of the Y chromosome. None of the 100 controls had Y deletions, and none of the parents or male relatives of these men had Y deletions. 10 of the 16 azoospermic men who were Y-deleted underwent TESE-ICSI. Of those 10, 5 had sperm retrievable from the testis, and 2 of those 5 became pregnant in nine cycles and delivered. There was an additional pregnancy that miscarried. Five of the 6 oligospermic couples that were Y-deleted eventually became pregnant and delivered in nine cycles.
Conclusion: 1) Our studies continue to suggest that there are several genes on the non-recombining portion of the Y chromosome (NRY) in addition to DAZ that impinge on spermatogenesis. 2) In the azoospermic men who were Y-deleted, deletions limited to the (AZFc) region were associated with the presence of very small numbers of testicular sperm that were sufficient for ICSI. Larger Y deletions were associated with a total absence of testicular sperm. 3) This is consistent with the view that the Y chromosome has collected, during evolution of higher primates, many previous autosomal spermatogenesis genes, with results amplification and subsequent degeneration. This helps to explain why the human male has relatively poor spermatogenesis compared to other animals. 4) This molecular data may eventually be used to prognosticate the success or failure of TESE in azoospermic men who are not obstructed.

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TRANSMISSION OF Y DELETION TO MALE OFFSPRING BY ICSI SJ Silber, Infertility Center of St. Louis, St. Louis, MO, LG Brown*, DC Page*, Howard Hughes Medical Institute, White Institute and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA

Objectives: 13% of azoospermic and 7% of severely oligospermic infertile men have been found to have Y chromosomal deletions that are not found in their fathers and brothers. These deletions are found most commonly in the DAZ region of the Y (interval 6D-F), but often involve other areas of the Y as well.

Design: . Our purpose was to report on the children derived from ICSI or from TESE-ICSI of these sterile Y-deleted men.

Materials and Methods: A prospective review of eight pregnancies, and ten children arising from ICSI with sperm from azoospermic or severely oligospermic (<500,000 sperm) Y-deleted men was performed. Five Y-deleted azoospermic men with sperm recoverable from the testes, and six Y-deleted men who were severely oligospermic, underwent TESE-ICSI or ICSI procedures. A total of eighteen ICSI cycles were performed for these eleven couples, resulting in eight pregnancies. These pregnancies were followed to see if there would be any unusual outcome in the children of Y-deleted infertile men.

Results: Eight of the eleven Y-deleted couples eventually became pregnant. One miscarried. Of the remaining seven pregnancies, three were twins. Six of the ten babies were girls and four were boys. Of the ten delivered babies, the six girls had a normal XX karyotype, were healthy, and had no congenital abnormality. The four boys had exactly the same Y deletion as their father, but otherwise all had a normal XY karyotype. Three of the four boys appeared completely normal (aside from the Y deletion). One of the four boys had severe tricuspid and pulmonary atresia, and died in the first week after an attempt at surgical correction. All boys had normal-appearing genitalia.

Conclusions: 1) De novo Y-chromosomal deletions in azoospermic or severely oligospermic men do not prevent the delivery of normal offspring from ICSI using their sperm. 2) We have demonstrated that the Y deletion is transmitted to the male offspring of these azoospermic or oligospermic men via ICSI. We presume, therefore, that these offspring will also be infertile or sterile. 3) The heart malformation in one of these eight children is probably not related to the Y deletion, but does point to the need for careful counseling and caution.

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Seminologic alterations in infertile men, with infectious pathology of genitourinary tract

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Introduction: The infectious pathology of genitourinary tract has been associated to infertility; but frequently it is an asymptomatic process. Often, sexually transmitted diseases are related to chronical pelvic inflammatory disease, that affects salpinx and causes tubary obstruction in women; while in male they may cause orchitis, epididymitis, prostatic and seminal vesicles inflammation. However, still there isn't enough information about the seminal alterations, derived from these infectious processes.

Objectives: To describe the seminal anomalies that could be associated to genitourinary infection in infertile men.

Materials and method: 101 patients with primary or secondary infertility, who presented sugestive signs or symptoms of genitourinary infection, were selected.

A seminogram was practiced to all of them, according to WHO recomendations. This included: physicochemical evaluation; determination of sperm counting, motility and sperm vitality.

The cytomorphology was studied also in a papanicolau stained smear; as well as the presence of phagocytes by staining with neutral red and polymorphonuclear leucocytes by the myeloperoxidase reaction.The presence of genitourinary infection was investigated by aerobic and anaerobic sperm culture, specific culture for Mycoplasma ssp as well as fluorescence monoclonal antibodies test for Chlamydia trachomatis in urethral smears.

The seminologic alterations, and the presence of demonstrated infection were correlated.

Results: We found in this study; that from 54 patients with some positive microbiological test, 12.9% presented altered seminal pH, while in patients whit negative microbiological test, there were no pH alteration. It was an increase in the number of polymorphonuclear leucocytes in 24% of the positive cases and only in 5% of the negatives. The presence of phagocytes, was detected in 33% of the positive cases, and 16% of the negatives. 46% of positive cases, presented altered sperm count and 38% the negatives.

In the analysis of spermatic morphology we observed the presence of intra nuclear vacuoles, rolled, thicked, folded flagella more frequently in the patients with positive microbiological test, than in patients with negative test. The most frequently microorganisms founded were: *Streptococcus faecalis* (44.4%), *Staphylococcus aureus* (37.9%), *Staphylococcus epidermidis* (32.4%), Escherichia coli (18.5%) and Serratia ssp (10.2%). The anaerobical bacteria founded were: *Propionibacterium ssp*, *Bacteroides ssp*, and *Peptostreptococcus ssp. Chlamydia trachomatis* was present in 20% of the studied cases, and *Mycoplasma ssp* in 24% .

Conclusions: It was observed, that the seminal pH, the amount of polymorphonuclear leucocytes and phagocytes; and abnormal spermatozoa are useful parameters for diagnosing infectious pathology of the genitourinary tract in infertile men.

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NEURAL COMPUTATIONAL MODELING OF VARICOCELECTOMY OUTCOMES

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Objectives: Although varicocele is the most common cause of male subfertility, predicting individual patient responses to varicocelectomy remains an intractable problem. We modeled a pilot database of post-varicocelectomy semen analysis outcomes using neural computational techniques and traditional linear statistical methods.

Materials and Methods: Data from 29 patients who had undergone microsurgical varicocelectomy were collected. Improvement in the post-operative semen analysis was used as the outcome measure. 27 separate input features included such variables as patient age, prior exposure to toxins/radiation, and characteristics of the varicocele, etc. A neural network was constructed and compared to linear discrimmant function analysis (LDFA) and quadratic discriminant function analysis (QDFA). We wrote programs to implement LDFA and QDFA (C), and canonical back-propagation using the cross-entropy error function at the output node(C++), and 10 hidden nodes. We divided the data into a training set of 19 examples, and a test set of 10 examples. The N1/N1+N2 cross validation model was used. Programs were compiled and run on the UIC "Borg" parallel supercomputer, a Hewlett Packard HP 9000/800 V-server, containing fourteen 240 MHz 8200 CPUs (0.960 GLOPS peak per processor). The neural network trained to completion in 4140 iterations in 56 seconds.

Results: Modeling results are described in the following table. Classification accuracy is defined as the correct number of predictions divided by the total number of outcomes. ROC area (receiver operator characteristic curve area) combines sensitivity and specificity for all possible thresholds.

	Classification Accuracy	Sensitivity	Specificity	PVP	PVN	ROC Area
LDFA	40%	0%	100%	0%	40%	0.5
QDFA	40%	0%	100%	0%	40%	0.5
NNET	100%	100%	100%	100%	100%	1

Conclusions: The neural computation was superior to the linear analysis in every aspect. Both LDFA and QDFA completely failed to model the varicocelectomy dataset. The neural network provided an overall improved sensitivity and specificity for predicting improvement in post-varicocelectomy semen total motile count. We thus present a pilot neural computational model which, when scaled for larger datasets, may have predictive value for physicians performing varicocelectomy.

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A STUDY TO DETERMINE IF SPERM AUTOANTIBODIES CAUSE A REDUCTION IN THE HYPO-OSMOTIC SWELLING (HOS) TEST S. Jairaj*, J.H. Check and A. Bollendorf*, UMDNJ, Robert Wood Johnson Med. School at Camden, Div. Repro. Endo. & Infertility, Camden, NJ

Low HOS test scores were found to be associated with lower pregnancy rates (PRs). The mechanism seems to be related not so much to impaired fertilization but to inhibition of implantation. The defect may be present in males with normal or subnormal semen specimens. However, anecdotal experience suggested that the subset of males with antisperm antibodies (ASA) have a higher frequency of low HOS scores. The possibility exists that ASAs may impair the functional integrity of the sperm membrane. The study presented herein, artificially added ASAs to sperm to see if this could lower the HOS score. Furthermore, since the treatment of sperm laden with sperm autoantibodies with the protein digestive enzyme chymotrypsin prior to insemination has been found to improve PRs following intrauterine insemination, and to also improve low HOS scores, the study would determine if chymotrypsin could improve HOS scores if, in fact, they were lowered by the addition of ASAs. Donor sperm free from autoantibodies had an initial HOS measurement performed. The washed sperm specimen was then incubated with sperm autoantibodies and the HOS test was repeated. The mean HOS score on the initial sample was 87.7%. Following the addition of ASAs, there was not a significant drop in the HOS score (83.5%) (p=.079). The immunobead test, however, demonstrated that 100% of the sperm were bound with antibodies. The addition of chymotrypsin to the sperm now bound with ASA improved the HOS score to 89.7% and almost resulted in a significant improvement (p=.053). These data would suggest that ASAs do not impair the functional integrity of the sperm membrane as manifested by a low HOS test score. However, the possibility exists that the exposure time of sperm autoantibody was insufficient to impair the sperm membrane and maybe longer incubation could alter the findings.

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ERECTILE DYSFUNCTION (ED): PREVALENCE AND QUALITY OF LIFE. THE BOXMEER STUDY

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Introduction In 1994, the first epidemiological data on the prevalence of ED and a risk-factor analysis were published in the Massachusetts Male Aging Study (MASS). Recently, 3 population based studies on the prevalence of ED and its impact on quality of life were performed in the Netherlands. We report on the outcome of the Boxmeer study and compare the Dutch data with the MASS.

Methods Recruitment of subjects, employing a random selection mechanism that aims to provide representative samples of the population, was conducted in 1998 in the city of Boxmeer. Boxmeer has a population of 20,000. Data on ED were collected with a postal questionnaire. In case of non-response professional interviewers conducted a computer-aided telephone interview. The sexual function inventory (SFI) as developed by O'Leary and colleagues was used to assess ED (low scores indicating problems with achieving and maintaining erection). QoL was measured with a generic instrument, the SF-12. 1215 (69%) of 1771 invited men aged 40+ responded to the questionnaire. 1059 (72%) of the 1460 spouses responded to a female version of the questionnaire.

Results 13% of men reported ED. ED is age-related with 78% of men aged 70+ having an erection score of 0-5 compared to only 10% aged less than 55%. ED-related bother decreases with age. (Table 1)

Table 1

Age	% ED	% Bother
40 – 49	6	73
50 – 59	9	78
60 – 69	21	67
70 – 79	37	46

Conclusion In accordance with the MASS, the prevalence of ED increased with age in the Netherlands. However, the ED-related bother seems to decrease with age. This seems to be related to the fact that only 50% of men aged 70+ are still sexually active. It is remarkable that the prevalence of ED in the Netherlands is considerably lower than in Massachusetts. Possible explanations for this unexpected discrepancy are 1) the ED-data of the MASS are based on a post-hoc analysis in a surrogate population and 2) questions on ED-related bother were not asked in the MASS.

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SAFETY AND EFFECTIVENESS OF A NEW SALINE-FILLED TESTICULAR PROSTHESIS

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Objectives: Testis prostheses have been used for more than 50 years to replace a missing or removed testes. Earlier this decade, the manufacture of silicone gel-filled testis prostheses was discontinued because of concern generated by the safety profiles of other kinds of implants. We assessed the safety and effectiveness of a new, saline-filled implant as a testicular replacement.

Design: Multicenter, open label, prospective clinical trial with a single treatment group.

Methods: Testis prostheses were placed in adult and adolescent patients in 18 centers in the U.S. over a one year period. All patients underwent formal rheumatologic as well as urologic evaluation prior to and after prosthesis placement. In addition, self-assessment and quality of life instruments were applied to determine prosthesis effectiveness.

Results: A total of 145 patients underwent prosthesis placement. Seventy seven (53%) of patients were >17 years old, and 47% were <17 years old. Among adult patients, 76% were single or divorced. Post-surgical occurrences were observed in 17% of patients, and included discomfort or pain (5%); scrotal edema (1.4%); infection (0.7%); extrusion (0.7%); deflation (0.7%) and pulmonary emboli (0.7%). No patient reported rheumatologic symptoms at 6 months follow-up. Mean Rosenberg self-esteem scores were stable or improved in all categories.

Conclusions: In short term follow-up, a new saline-filled testis prosthesis appears safe and well tolerated in patients undergoing testicular replacement. Further rheumatologic and urologic follow-up is planned to monitor long-term safety of the device.

Funding: Clinical trial funded by Mentor Corporation.

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THE VASO-CONSTRICTOR EFFECT OF ENDOTHELIN-1 IN THE PENIS IS OVERRIDDEN DURING ERECTION . Mills, T., Lewis, R., Pollock, D., Stopper, V., Wingard, C. Department of Physiology and Endocrinology, Department of Surgery, Urology Section, Vascular Biology Center. Medical College of Georgia, Augusta, Georgia, USA.

Studies from this laboratory have shown that when endothelin-1 (ET-1) is injected into the cavernous sinuses, it exerts a strong vaso-constrictor action resulting in a reduced erectile response. However, our studies also showed that treatment with specific antagonists to ET-1 receptors (ET_A and ET_B) failed to alter erection. These studies demonstrated that the cavernosal circulation is sensitive to the vaso-constrictor effects of ET-1 but did not establish a role for ET-1 in the normal rat erection. To test the hypothesis that a mechanism is activated during erection which prevents ET-1 from causing vaso-constriction, ET-1 (50 pmoles/rat) was injected into the cavernous sinuses *before* or *during* erection of 80 - 120 day old Holtzman rats. When injected 3 minutes *before* erection, the subsequent erectile response was reduced 44 ± 9% indicating a vaso-constrictive action. However, when ET-1 (50 pmole/rat) was injected *during* erection, the subsequent erectile response was unaffected (+6 ± 8%) showing that vaso-constrictor action of ET-1 was prevented. To test the potential role of nitric oxide in masking the vaso-constrictor effect of ET-1, we injected ET-1 during erection induced by intracavernosal injection of the nitric oxide donor drug, NOR-1 (20 µg/Kg). When injected during the NO induced erection, the vaso-constrictor effect of ET-1 was blocked. Based on these findings, we suggest that ET-1 helps maintain the penis in a flaccid state while during the erectile response, NO overrides the vaso-constrictor effect of ET-1.

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CAVERNOSAL CELL CYTOTOXICITY UNDER ENVIRONMENTAL STRESS.

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There is evidence to support the negative impact of certain drugs and environmental toxins on male erectile function. An in vitro scientific model would be a valuable tool to facilitate the evaluation of direct toxicity of these agents to cavernosal smooth muscle cells. We evaluated the cytotoxicity of environmental toxins such as hydrogen peroxide (H_2O_2), nicotine, ethanol and peroxynitrites (OONO-) using a New Zealand white rabbit penile cavernosal smooth muscle cell culture model. Rabbit penis was removed en bloc and the corpora cavernosa were dissected into 3-5 mm segments. Primary culture was initiated with these explants in DMEM-10% FBS media, and smooth muscle cells were characterized by morphological criteria. Monolayer cavernosal cells were grown to confluency in 48-well tissue culture dishes and cells were incubated with varying concentrations of H_2O_2 (0-3.0 mM) , OONO- (0-1.0 mM) nicotine (0-100 µM) and ethanol (0-100%) for short (3 hr) or prolonged periods (7 days). Cytotoxicity was assessed by either chromium or LDH assay (*Cytotox 96, Promega*, Madison, WI). Our results showed that incubation of cavernosal cells with H_2O_2, OONO- and ethanol induced a dose-dependent increase in cytotoxicity. Pretreatment with catalase (500 U) offered a significant (>90%) protection from peroxide-induced cytotoxicity. Presence of nitric oxide potentiated peroxide-induced cell damage. Nicotine had no significant toxicity at the physiologically relevant concentrations. Our results suggest that rabbit cavernosal smooth muscle cell culture is a viable model for the quantitative evaluation of in vitro cytotoxicity and the cavernosal cells are susceptible to environmental toxins such as peroxide, ethanol and peroxynitrites. Additional mechanisms/metabolites may be involved in the nicotine-induced erectile dysfunction.

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METABOLIC AND ULTRASTRUCTURAL ABNORMALITIES IN SEMEN FROM SPINAL CORD INJURED MEN.

M. Monga, K. Dunn* and M. Rajasekaran. UCSD Medical Center, San Diego, CA 92103.

Spinal cord injury (SCI) in men impairs fertility due to a combination of ejaculatory dysfunction and poor semen quality. The present study was designed to characterize the abnormalities in seminal constituents and sperm ultrastructure in ejaculates obtained from SCI men. Following a written consent for study participation, SCI men (n=7) had undergone electrovibratory stimulation (EVS) using Ferticare. Bladder was emptied by clean catheterization and sperm wash media (Ham's F10) was instilled into the bladder prior to stimulation. Comparative samples were obtained by masturbation from normal fertile donors (n=4). Both antegrade and retrograde samples were collected and semen evaluation was conducted according to WHO guidelines. Sperm samples were fixed in 3.0% glutaraldehyde followed by 1.5% osmium tetroxide for electron microscopy. Seminal plasma was incubated with normal human sperm and motility was evaluated using Makler chamber and intracellular ATP content was measured using a firefly luciferase bioluminescent assay kit (Sigma Chemicals, St. Louis, MO). Transforming growth factor (TGF)-β1 levels in seminal plasma were measured by an ELISA kit. Our results showed the presence of live or dead sperm in a majority of ejaculates (5/7). Two samples had severe asthenospermia (motility 0-5%) accompanied by a significant decrease in sperm viability. Incubation of normal sperm with SCI seminal plasma induced a concentration and time-dependent decrease in sperm motility accompanied by a significant drop (45-65%) in intracellular ATP content. TGF-β1 levels (ng/mL) in SCI men were significantly elevated (341.68 when compared to 100.15 in normal controls). Ultrastructural evaluation revealed degenerative changes and significant axonemal defects in a majority of sperm. Our studies suggest that seminal constituents of SCI men are detrimental to sperm movement and the ultrastructural degenerative changes may contribute to the impaired sperm motility and viability in these SCI patients. (Supported in part by a grant from American Paraplegic Society.)

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ROLE OF GROWTH FACTORS IN AGE-INDUCED MOLECULAR CHANGES IN MALE ERECTILE TISSUE.

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Age-related alterations in the penile expression of growth factors such as transforming growth factor (TGF)-β1 and vascular endothelial growth factor (VEGF) may contribute to the penile cavernosal tissue morphological changes associated with aging. The present study evaluated age-related changes in the gene expression of growth factors (TGF β1, VEGF) in the penile tissue of Brown-Norway rats. Young (4 months) and old (19 months) Brown-Norway male rats (NIA, Bethesda, MD) were employed for these studies. Animals were euthanized, the penile tissue rapidly removed and total RNA extracted using TRIZOL® reagent (GIBCO-BRL). Specific primers (TGF-β1, VEGF) were custom synthesized and Access RT-PCR System (Promega) was used for mRNA expression analysis. β-actin was used as an internal control for all RT-PCR reactions and the products were analyzed on 1.2% agarose mini-gel system. A computerized image analysis system was employed to quantify the band intensity. Penile tissues of both young and old animals showed the gene expression for both TGF-β1 and VEGF. No age-related difference in TGF-ß1 mRNA levels in the penile tissue was observed. RT-PCR of VEGF mRNA produced four distinct products, corresponding to four different splice variants of VEGF mRNA. Semi-quantitative analysis revealed decreased levels of VEGF mRNA expression for all splice variants in the old population of rats. The most significant decrease was detected between bands corresponding to splice forms 164 (21%) and 120 (18%). Our results suggest that the observed alterations in the gene expression of growth factors such as VEGF may contribute to the abnormal age-related morphological changes in the erectile tissue. (Supported in part by a *American Foundation for Urological Diseases* (AFUD) Summer Scholarship.)

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ENDOTHELIN-1 SUPPRESSES NITRIC OXIDE SYNTHASE EXPRESSION IN HUMAN CAVERNOSAL CELLS.

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Penile erection is known to be modulated by endothelium-derived nitric oxide (NO) and endothelins (ET-I). These endothelium-derived products frequently exhibit opposing effects on physiological and inflammatory processes. The interactions between pro-inflammatory agents and the endothelium-derived products in the erectile tissue are not known. In the present study, we sought to determine the induction of nitric oxide synthase (NOS) mRNA expression by pro-inflammatory agents (lipopolysaccharide [LPS] and γ-interferon [INF]) and its possible modulation by ET-1 in isolated human cavernosal smooth muscle cells (HCSMC). Monolayer cells (80-90% confluence) at passages (1 to 3) were incubated with an induction mixture comprised of LPS (10μg/mL) and recombinant human γ-IFN (250 U/ml) in the absence/presence of ET-1 (10^{-9} mol/L). The incubation was continued for 72 hours and the cells were harvested at 0, 12, 24, 48, and 72 hours for RNA extraction. The polymerase chain reaction (PCR) was carried out in an automatic DNA thermal cycler using NOS-specific primers. Electrophoresis of the PCR products was performed and the molecular weight marker was used for the identification and quantification of PCR products using densitometric image analysis. Stimulation of cells with LPS/γ-IFN caused a time-dependent increase in iNOS-specific amplification product of 462 bp. Maximum iNOS expression (three- to five-fold) was observed at 48 hours. Presence of ET-1 in the incubation mixture resulted in a 30% reduction in induced iNOS mRNA levels in HCSMC. These findings suggest that pro-inflammatory cytokines have a significant inducible effect on nitric oxide synthesis which is partially blocked by ET-1. The isolated cavernosal cell culture model is a viable system for studying such interactions. Further studies are needed to elaborate the role of such interactions. Pharmacological development of ET-1 inhibitors could potentiate erectile function by both a direct inhibition of vasoconstrictive effects of ET-1 and an indirect upregulation of NO synthesis.

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DIAGNOSIS AND MANAGEMENT OF HIGH-FLOW PRIAPISM M. A. Arap*, A. A. Figueiredo*, P. M. Góes, F. F. Pasqualotto*, P.H. Egydio*, C. Gromatzky*, A. M. Lucon*, S. Arap* Section of Andrology, Department of Urology, Clinicas Hospital, Medical School of the University of São Paulo, Brazil

High-flow priapism is a rare but well recognized entity mainly caused by traumatic perineal lesions. Diagnosis and treatment are usually performed by superseletive arterial embolization of the traumatic fistula. We sought to evaluate our experience in the diagnosis and management of high-flow priapism secondary to a perineal blunt trauma. Three patients presented with high flow priapism to our emergency service. Two of them were adult (18 and 23 years old) and one was a child (8 years old). All these patients had a history of perineal trauma and presented with painless erection that started 3 to 5 hours after the trauma. The two adult patients were treated with a superselective arterial embolization, resulting in immediately detumescence. In the young boy, the arterial embolization was not possible due to the very small caliber of the artery. However, during the arteriography, the detumescence occurred due to arterial thrombosis, closing the fistula. All cases had a satisfactory outcome and, after three years of follow-up, the two adults maintain regular sexual activities, with no erectile dysfunction. The young boy is in the third month after resolution and his mother refers matinal erection. Patients with high flow priapism usually delay medical assistance because erection is painless. Diagnosis and treatment are made by arterial exploration. Radioscopic visualization and embolization of the fistula results in immediate detumescence in most of the cases, making arteriography a safe and efficient procedure.

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COMPARISON OF TWO INJECTION COLOR DUPLEX DOPPLER ULTRASOUND OF THE PENIS WITH CAVERNOSOMETRY AND CAVERNOSOGRAPHY IN THE DIAGNOSIS OF VENO-OCCLUSIVE ERECTILE DYSFUNCTION R. Wang, L.V. Ho*, Sathyanarayana*, and R.W. Lewis, Medical College of Georgia, Augusta, GA

INTRODUCTION AND OBJECTIVES: Color duplex doppler ultrasound (CDDU) has been used as a screening tool for vasculogenic erectile dysfunction (ED). Our previous study demonstrated that two injection CDDU could significantly affect the diagnosis of this test. This study seeks to compare the two injection CDDU with the pharmacocavernosometry and pharmacocavernosography (CC) in diagnosis of veno-occlusive ED.

MATERIALS AND METHODS: CDDU was obtained before and after trimix injection (0.5 mcg PGE_1, 5 mg phentolamine and 150 mg papaverine). The second injection was given if the patient did not achieve a full erection. The criteria for veno-occlusive ED includes peak systolic velocity $\geq$ 30 cm/sec, end diastolic velocity > 3 cm/sec and post injection arterial diameter > 0.7 mm. The same group of patients then underwent CC. The criteria for veno-occlusive ED by CC includes maintenance flow rate > 20 and < 50 ml/min in moderate cases and $\geq$ 50 ml/min in severe cases or a plateau pressure on gravity cavernosometry < 60 mmHg. The cavernosography was also performed to locate the site(s) of venous leakage.

RESULTS: 24.5% (49/200 patients) had CDDU studies that were diagnostic of veno-occlusive ED, including 6 patients whose diagnosis was changed from arteriogenic to veno-occlusive ED. Of 30 patients with veno-occlusive ED by CDDU, 29 had their diagnosis confirmed by CC ($p > 0.05$). Seven other patients with a normal CDDU who underwent CC were found to have no veno-occlusive ED except one ($p > 0.05$). Cavernosography demonstrated that veno-occlusive ED was seen in the intermediate and deep venous system; superficial and deep venous system; superficial, intermediate and deep venous system; and superficial and intermediate venous system in 30%, 25%, 11% and 7% respectively.

CONCLUSION: Our study suggests that the two injection CDDU is comparably effective to CC in diagnosing veno-occlusive ED without the morbidity. However, a cavernosography may be required to localize venous leak if surgical intervention is warranted.

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SURGICAL TREATMENT OF PEYRONIE'S DISEASE WITH BOVINUM PERICARDIUM GRAFT P. H. Egydio, A. M. Lucon, F. F. Pasqualotto, J. Hallak, C. Gromatzki, P. M. Goes, and S. Arap. Section of Andrology, Department of Urology, Clínicas Hospital, Medical School of the University of Sao Paulo

An incision or excision of the plaque with graft have been used in the treatment of severe penile curvature avoiding shortening of the penis. The literature is rich in reports regarding the different types of albuginea substitutes such as dermal grafts, safein vein patch, temporalis fascia, deep dorsal vein patch and others. However, the ideal substitute remains to be found. The purpose of this study was to assess the use of bovine pericardium as an albuginea substitute. Seven patients with a stabilized curvature with 60° bending or more were treated with an incision of the plaque and with a bovine pericardium patch. The inclusion criteria for our study included patients with rigid erections and normal duplex scan. The bovine pericardium graft was used to cover the albuginea defect after the plaque incision. The bovine pericardium was treated after conservation procedures as used in heart valves and vascular grafts. The mean age was 56.4 years old (range 40 to 62), and the mean follow up was 3.2 months (range 1 to 6 months). The mean surgical time was 120 minutes (range 100 to 150). Graft rejection was not noted, and all patients maintained the erection status after the procedure with preservation of penile sensibility. Also, for the five patients who had their penile length measured intraoperatively before and after the pericardium graft replacement, there were a medium gain in the penile length of 2 cm (range 1 to 3 cm). The bovine pericardium has been demonstrated to be a good albuginea substitute with low cost and high affectivity. Due to the fact that the patch is stored, the time spent during the surgery is lower compared to other types of grafts that need another surgery for obtaining the grafts such as temporalis fascia, dermal grafts and other autologous grafts.

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PLACEBO CONTROLLED, RANDOMIZED, DOUBLE-BLIND, PHASE II INTRACAVERNOSAL INJECTION COMPARISON STUDY OF THE PHARMACODYNAMICS OF SAFETY OF TWO FORMULATIONS OF PAPAVERINE HCL, PHENTOLAMINE MESYLATE AND ALPROSTADIL TO CAVERJECT™ IN PATIENTS WITH MALE ERECTILE DYSFUNCTION. R. Tapia; L. Mora*; M. Solomayor*; J. Galán*; J. Castañeda*; R. Castell*; M. Lipesker* and F. Lowrey*. In Mexico City: Clínica Londres, Instituto Nacional de la Nutrición Salvador Zubirán and Centro Médico Nacional Siglo XXI; Centro Medico Nacional de Occidente, Guadalajara, Jal; Hospital Muguerza, Monterrey, NL, Mexico, ECA, México and Zonagen, Inc, Houston, Texas

Objectives: The objectives of the study were to determine whether pharmacodynamic and safety of two different combinations of papaverine HCL, phentolamine mesylate and alprostadil, are similar to that of an alprostadil-only formulation in male patients with moderate to severe erectile dysfunction, who previously experienced lack of efficacy on oral treatment.

Material and Methods: A total of 72 patients were enrolled in the study at 6 sites. All except one patient completed the study (all four treatments).

Treatment	Papaverine Dose (mg)	Phentolamine Dose (mg)	Alprostadil Dose (mcg)
1: Placebo per 0.5 mL	0	0	0
2: Caverject per 1 mL	0	0	20
3: Trimix # 1 per 0.5 mL	15	0.5	5
4: Trimix # 2 # per 0.5 mL	3.75	2.5	2.5

This was a randomized to treatment sequence, placebo controlled, phase II crossover study design, using a 4 x 4 Latin Square. There were four study visits. During each visit the patients self assessed their erectile response (pre-dose and after 5, 10, 20, 30, 45, 60, 75, 90 and 120 minutes) and a close monitoring of adverse events (pre and after 5, 15, 30 and 60 minutes) and vital signs were also performed. There was a one week (not less than 5 days) period between visits.

Criteria for Evaluation: The primary efficacy variable was the patient's self-assessment of erection score. The score has a range of 1 to 4 where: 1 = no evidence of any tumescence or erection 2 = partial tumescence (not likely to be sufficient for penetration) 3=greater tumescence sufficient for vaginal penetration but not fully rigid and 4 = full rigidity. Based on the patient's self-assessment of erection score, four response variables were analyzed: R_{max} = maximum response (1 to 4) recorded. $T_{first \geq 3}$ = time to the first response ≥ 3. $D_{\geq 3}$ = period of time response is ≥ 3

% Success = percent of responses ≥ 3 (both defined as only one $R_{\geq 3}$ and as 2+ consecutive $R_{\geq 3}$). Safety was assessed based on monitoring of treatment-emergent adverse events (frequency, intensity, duration and relation to treatment).

Results: Mean values for the 72 patients were: 54.4 years old (range: 24, 74), 28.0 BMI (range: 19, 39) and 78.9% did smoke tobacco.

Summary of Efficacy Results (PP population N=70) [a]

Treatment Group	Response Variable (Mean Value)				
	R_{max}	$T_{first \geq 3}$ (min)	$D_{\geq 3}$ (min)	Success One $R_{\geq 3}$ (%)	Success 2+ Consecutive $R_{\geq 3}$ (%)
Placebo	1.1	–	–	0	0
Caverject	2.9	15.8	45.7	45 (64)	39(56)
Trimix # 1 (15,0.5,5)	2.9	18.8	60.5	46 (66)	46 (66)
Trimix # 2 (3.75, 2.5, 2.5)	3.1	17.5	59.3	50 (71)	47 (67)
P-values					
Trimix #1 – Caverject	0.5030	0.7671	0.0496	0.796	0.071
Trimix # 2 – Caverject	0.0349	0.5907	0.0065	0.297	0.074
Trimix # 1 – Trimix # 2	0.1480	0.4046	0.4332		

[a] Two patients were excluded from the efficacy Per Protocol population because of a protocol violation and one patient withdrew his consent. There were 8 serious adverse events (7 patients), 4 when patients received Trimix # 1 and 4 when received Trimix # 2. All these serious adverse events were priapisms. All 8 priapisms were resolved with treatment (mainly oral pseudoephedrine, aspiration of cavernous bodies, ejaculation, exercise and local ice) and were judged by the investigator to be treatment related. Conclusion: In this placebo controlled, randomized, intracavernosal injection comparison study in 72 patients with moderate to severe erectile dysfunction, efficacy and safety data are summarized as follows. When patients received Trimix formulations as compared to Caverject, they had a greater mean maximum response (R_{max} 3.1 to 2.9) nd stayed longer (about 15 minutes more) with an erection response sufficient for vaginal penetration ($R_{\geq 3}$).

The percentage of successful patients (defined as 2+ consecutive $R_{\geq 3}$, instead of only one $R_{\geq 3}$) was significantly higher among the Trimix formulations (56-67%) compared to Caverject (56%).

With Trimix formulations, as compared to Caverject, the incidence of adverse events was lower. In particular, penile pain was less frequently reported with Trimixes (30% to 50% less). Also, among Trimix patients, penile pain tended to be less severe and had a shorter duration after injection.

There were 8 priapisms in the base trial, all among the Trimix formulations. The reported incidence of priapism is not considered indicative of stand ard clinical use of the two mixtures since no up-titration we s allowed in this fixed-dose Phase II A clinical trial..

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NEUROENDOCRINE MECHANISMS UNDERLYING AGING OF THE HUMAN MALE REPRODUCTIVE AXIS: NOVEL HYPOTHESIS FORMULATION AND TESTING VIA A PHYSIOLOGICALLY INTERLINKED FEEDBACK AND FEEDFORWARD BIOMATHEMATICAL CONSTRUCT

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Neuroendocrine axes in the intact organism act as ensemble dynamic feedback and feedforward-coupled homeostatic systems (*Endo Rev* 9:3, 1998). In aging, disruption of network linkages provides a potential general hypothesis for disorderly uni- and pluri- hormonal output without overt failure of any single endocrine gland (*PNAS* 93:14100, 1996). Since the behavior of an interactive, nonlinear, multinodal, time-delayed and dose-responsive connectionistic axis is difficult to envision intuitively, here we test aging hypotheses via an explicit biomathematical (stochastic differential equation) construct of the GnRH, LH, testosterone (Te) axis (*AJP* 275:E157, 1998). We specifically test six relevant hypotheses of disrupted feedback connectivity or altered nodal (GnRH, LH, or Te) function. Five of six models are refuted as improbable based on published clinical characteristics of aging-related changes in the older male (*J Androl* 20:1, 1999). Only a parsimonious aging hypothesis of impaired LH feedforward on Te combined with ensuant restraint of Te feedback on GnRH/LH emulates the expected uni- and bivariate entropy alterations in LH and Te release, singly and jointly, with diminished Te and elevated LH release. Accordingly, further studies will be relevant to assess the utility and limitations of this investigative strategy in adumbrating existing or novel hypotheses of altered network behavior in the reproductive axes in health and disease.

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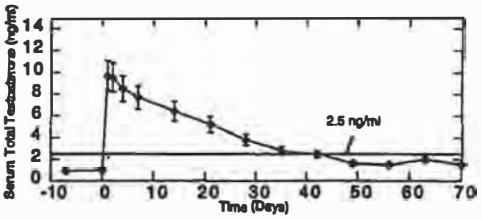
SAFETY AND PHARMACOKINETICS OF TESTOSTERONE RELEASE FROM A BIODEGRADABLE POLYMER IN HYPOGONADAL MEN. John K. Amory[1], Brad D. Anawalt*[1], Phillip D. Blaskovich*[2], Janet Gilchriest*[1], E. S. Nuwayser*[2], and Alvin M. Matsumoto[1]. [1]VA-Puget Sound, University of Washington, Seattle, WA and [2]BIOTEK, Inc. Woburn, MA

Introduction: Men with hypogonadism require testosterone (T) replacement for healthy bone, muscle and sexual function. T is administered by daily transdermal patches or intramuscular injections every 1-3 weeks. Biodegradable polylactide-co-glycolide polymers are used currently for long-term drug delivery in humans. Such polymers containing T could provide a better means of long-term T therapy. We therefore studied T release from these polymers in hypogonadal men.
Methods: We enrolled 7 hypogonadal men, regularly treated with T, in an open-label, prospective study of T-microcapsule administration. Subjects were enrolled if serum total T levels were less then 2.5 ng/ml after 4 weeks with no T therapy. Subjects were injected with a single dose of 267 mg of T-microcapsule, and serum total T was assayed at 1,2,7 days and weekly for 10 weeks.
Results: Serum Total Testosterone (±SEM) in Human Subjects (N=7)



No significant adverse reactions were seen. Two subjects complained of transient tenderness and fullness at their injection sites.
Conclusions: A single injections of 267 mg of T-microcapsules results in normal serum T levels for up to 7 weeks. T-microcapsules may be a safe and convenient method for the long-term treatment of male hypogonadism. It is possible that increased doses of T-microcapsule will result therapeutic T levels for longer periods of time. This is being tested with 534 mg injections of T-microcapsule.

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LACK OF AGE EFFECT ON DIHYDROTESTOSTERONE SUPPRESSION BY GI198745, A NOVEL, DUAL 5 ALPHA REDUCTASE INHIBITOR. RV Clark, BB Morrill*, LF Puertolas*, LJ Haberer*, Glaxo Wellcome R & D, Research Triangle Park, NC

Dihydrotestosterone (DHT) is formed from testosterone (T) by 5 alpha reductase (5AR). DHT is a potent androgen, and DHT plays a key role in the development of benign prostatic hyperplasia (BPH) and androgenetic alopecia (AGA). Suppression of DHT can alter the progression of both conditions. GI198745 is a potent inhibitor of both type 1 and 2 5AR isozymes, and is being evaluated for the treatment of BPH and AGA. As the age range of individuals with these conditions is broad, the effect of age on DHT suppression by GI198745 was studied.

This study was a parallel design in which 36 healthy male volunteers received a single dose of 5.0 mg of GI198745, and DHT levels were measured pre-dose, 24h, 3, 7, 14, 21, 28 days, and 4 months post-dose. Age groups were Young: 20-49y, Middle: 50-69y, and Mature: >70y. DHT suppression was >80% of baseline at 24h, >60% at 28d, and was resolved by 4 months. No statistically significant differences between age groups were observed in % DHT suppression at 24h, % maximal DHT suppression which occurred at 3 days, or % DHT suppression (Supp) at other time points.

Table: Mean ± SD % DHT compared to baseline

Age	N	% DHT at 24h	Max % DHT Supp	% DHT at 4m
Young	12	- 82.3 ± 9.4	- 87.6 ± 6.3	+ 26.0 ± 43.9
Middle	12	- 83.5 ± 4.0	- 88.6 ± 2.2	+ 13.6 ± 16.7
Mature	12	- 86.4 ± 3.1	- 89.9 ± 2.4	+ 14.3 ± 26.8

In conclusion, GI198745 induces marked suppression of DHT within 24h following a single dose, and this effect persists for several days. There is no age effect on the magnitude of DHT suppression, and clinical use of this compound does not require individualization of dose for age.

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ETHNIC DIFFERENCES IN LEYDIG CELL ORGANELLE CONTENT MAY PREDISPOSE TESTES OF ASIAN MEN TO A HEIGHTENED SENSITIVITY TO STEROIDAL CONTRACEPTIVES. L. Johnson, J.W. Polasek*, W.B. Neaves, C. Wang, R.S. Swerdloff, and X.H. Wang; Texas A&M University, College Station, TX; Southwestern Medical School, Dallas, Texas; Harbor-UCLA Medical Center, CA; Jiangou Family Planning Research Institute, Nanjing, China

Asians appear to be more susceptible to androgen or androgen-progestogen suppression of spermatogenesis than other ethnic groups. The objective was to determine if ethnic differences in sensitivity correspond to differences in Leydig cell organelle content. Testes from 12 Chinese men were compared to those from 8 Hispanic men and 12 Caucasian men of ages 29 ± 3, 30 ± 2, and 29 ± 3 yrs., respectively. Testes were fixed by vascular perfusion with glutaraldehyde, further fixed in osmium, embedded in Epon, and evaluated by stereology using 0.5 μm sections stained with toluidine blue to determine the volume of Leydig cell cytoplasm. From thin sections, electron micrographs were evaluated by stereology for volume density of various organelles including smooth endoplasmic reticulum (SER). Paired parenchymal weight was less ($p<0.05$) in Chinese men than Hispanic or Caucasian men (23 ± 2, 45 ± 3, and 36 ± 2 g, respectively). Given differences in parenchymal weight, the volume density of Leydig cell cytoplasm (6.02 ± 0.43, 4.35 ± 0.36, and 4.59 ± 0.51%) was greater ($p<0.05$), and volume density of SER in Leydig cell cytoplasm (53.0 ± 4.7, 62.2 ± 3.9, and 44.7 ± 1.8%) had a trend ($p>0.05$) to be greater in Chinese men than Caucasian, the volume density of SER in testicular parenchyma (3.19 ± 0.23, 2.71 ± 0.23, and 2.05 ± 0.23%) was greater ($p<0.05$) in Chinese than Caucasian men. Hispanic men had intermediate values of SER in parenchyma not different from the other groups. The total SER volume per men (0.66 ± 0.05, 1.18 ± 0.16, and 0.69 ± 0.08 ml) was greater ($p<0.05$) in Hispanic than the other two groups which were similar to each other. However, the volume of SER/g (30.4 ± 2.2, 25.8 ± 2.2, and 19.5 ± 2.2 μl) was greater ($p<0.05$) for Chinese than Caucasian men, and Hispanic men were intermediate. Asian men may have evolved to require a greater volume of Leydig cell SER for normal testicular function; however, this potential need for a greater volume of steroidogenic machinery may predispose them to a heightened negative spermatogenic response to steroidal contraceptives (NIH N01 HD-8-3281).

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ANDROGENS MODULATE Po GENE EXPRESSION IN PERIPHERAL NERVES.
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Recently, we have demonstrated that progesterone derivatives are able to stimulate the gene expression of the most abundant myelin protein of the sciatic nerve of male rats, the glycoprotein Po (Melcangi et al., *Neuroscience*, **85**, 569-578, 1998; *J. Neurosci. Res.*, **56**, 349-357, 1999). On the basis of these observations, it was felt important to ascertain whether others classes of sex steroids (e.g., androgens) might exert a similar effect. The present results show that androgens may play a role in the modulation of Po gene expression. We have shown that: 1) the messenger for the androgen receptor (AR) is present in the rat sciatic nerve but not in cultured Schwann cells; 2) castration performed in adult male rats at the age of 3 month, induces, 3 month later, a decrease of Po mRNA levels in the sciatic nerve; this effect is counteract by the subsequent treatment with dihydrotestosterone (DHT), the 5α–reduced metabolite of testosterone; 3) castration is also able to significantly decrease in the sciatic nerve the activity of the enzyme 5α–reductase (which converts testosterone into DHT); 4) DHT is able to stimulate Po gene expression in cultured Schwann cells. These observations indicate that androgens may exert their effect on Po gene expression via indirect mechanisms, i.e., by modulating neuronal influences reaching the Schwann cells. However, alternative mechanisms may also be taken in consideration (action via the progesterone receptor?).
Altogether these observations indicate that also androgen, like progesterone derivatives, are able to stimulate Po gene expression, indicating an interesting role of steroid hormones in the modulation of peripheral myelinization (The financial support of Telethon Italy, grant n° E.765, is gratefully acknowledged).

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ANDROGENIC EFFECTS OF TIBOLONE REPLACEMENT THERAPY IN WOMAN DURING MENOPAUSE

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Androgenic symptoms in the women during menopause are a common hobby-horse in unities of menopause. In this way, the steroid gonado-mimetic tibolone, with estrogen, progestin and small androgenic properties, seems to be an effective therapy for this problem.
The authors contribute results obtained with the study of 58 menopausical women before, during end after of treatment with this steroid showing data obtained in clinic exploration plasmatic levels of LH, FSH, Stradiol, Progesterone, Delta-4-Androstendione, Testosterone, DHEA, DHEA-S, PRL, T-3, T-4, TSH, and 17-cetosteroids, and answer to a life quality questionnar offered to women before, during and after of treatment with oral tibolone, 2,5 mg/day, and continuous line with very positive results.

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HIPERANDROGENISME AND HIPERINSULINISME IN WOMEN WITH POLICYSTIC OVARIAN DISEASE

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The blood levels of IGF-1 are not in general significantly in more elevated in woman with policystic ovarian. The biologic activity of IGF-1 can be elevated by a decrease in levels of IGFBP-1, that serves as regulator of the action of IGF-1.
Different groups of research fellows have propossed that is a synegism betwen free IGF-1 and LH, capable of increase the production of androgens by hiperactivity of cytochromo P450-17-alfa. More over, the hiperandrogeny has probed to be a consequence of hiperinsulinemy and a decrease of levels of insuline by use of diazixide or the diminiution of weight decrease androgen secretion.
The authors contribute the results of 128 women with hyperandrogenisme assiciated to policystic ovarian disease and results of tratment with cyproterone, Gn-RH, spirolactone and hipocaloric diet.

BURROUGHS WELLCOME FUND

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SUPPORT FOR REPRODUCTIVE SCIENTISTS

CAREER AWARDS IN THE BIOMEDICAL SCIENCES INCLUDING AWARDS IN REPRODUCTIVE SCIENCE

BWF's career awards provide U.S. and Canadian biomedical scientists with up to $574,000 to support their advanced postdoctoral training and initial faculty appointment.

Through its Career Awards Program, the Burroughs Wellcome Fund provides support for reproductive scientists who are early in their careers by helping make the critical transition to becoming independent investigators. Career awards provide support ranging from $445,000 for four years to $574,000 for six years to bridge the advanced postdoctoral years and the first three years of faculty service. Each year, BWF makes at least 20 awards, including awards targeted at the reproductive sciences.

Candidates must have completed at least 12 months but not more than 48 months of postdoctoral research training by the application deadline. Approximately half of the awards will go to researchers with a Ph.D. degree and half to those with an M.D. or M.D.-Ph.D. degree. For candidates with M.D. degrees, postdoctoral training excludes clinically oriented residencies that do not contain a major research component. Recipients may spend part of the award period at an institution in the United Kingdom or the Republic of Ireland.

Recent BWF career awardees in reproductive science: Bruce T. Lahn, Ph.D., University of Chicago; William L. Kraus, Ph.D., Cornell University; Kelle H. Moley, M.D., Washington University School of Medicine.

The application deadline for career awards that begin in the year 2001 is October 2, 2000.

OTHER REPRODUCTIVE SCIENCE AWARDS:

- BWF supports one research fellowship annually in obstetrics and gynecology, which provides $144,000 over three years. The award, open to U.S. and Canadian postdoctoral obstetrician-gynecologists, is administered in partnership with the American Association of Obstetricians and Gynecologists Foundation (AAOGF). Contact AAOGF for information (804/924-9921).
- BWF supports one award annually to help U.S. obstetrician-gynecologists working in the basic reproductive sciences bridge the postdoctoral and initial faculty years. The award is made through the Reproductive Scientist Development Program (RSDP), a consortium supported by the National Institute of Child Health and Human Development, professional societies, and foundations. BWF provides $240,000 for the first three faculty years, and RSDP supports the postdoctoral years. Contact RSDP for information (415/476-9047).

ADDITIONAL BWF FUNDING OPPORTUNITIES

Several other programs may be of interest to U.S. and Canadian researchers in reproductive science. Contact BWF for more information and application deadlines.

- Clinical Scientist Awards in Translational Research, which provide $750,000 over five years, support established independent physician-scientists whose work bridges basic research and patient care and who will serve as mentors to the next generation of physician-scientists.
- New Investigator Awards in the Pharmacological or Toxicological Sciences, which provide $210,000 over three years, support scientists early in their careers who will bring new approaches and novel thinking to their fields.
- BWF Research Travel Grants provide support to work for brief periods (from two weeks to six months) in the United Kingdom or Ireland.

The Burroughs Wellcome Fund is an independent private foundation dedicated to advancing the medical sciences by supporting research and other scientific and educational activities.

Complete information and application forms for all BWF award programs are available on the Fund's website at www.bwfund.org