MARCH/APRIL 2005
Supplement

Journal of ANDROLOGY

American Society of Andrology

30th Annual Meeting

March 30-April 5, 2005
Seattle, Washington

Program and Abstracts

Published by THE AMERICAN SOCIETY OF ANDROLOGY

schedule at a glance

XVIII North American Testis Workshop:
"Testicular Cell Dynamics and Endocrine Signaling"
March 30 – April 2, 2005

Andrology Lab Workshop
"Sperm Morphology Workshop"
April 2, 2005

ASA 30th Annual Meeting

"Androgens and Their Target Organs"
Saturday, April 2 – Tuesday, April 5, 2005
Location: Princessa (unless otherwise noted)

FRIDAY, APRIL 1, 2005

12:00 p.m.	**Executive Council Meeting** *Location: Douglas Boardroom*
2:00 p.m. – 6:00 p.m.	**Registration** *Location: Lobby Alcove*

SATURDAY, APRIL 2, 2005

7:00 a.m. – 7:00 p.m.	**Registration** *Location: Lobby Alcove*
4:00 p.m. – 9:30 p.m.	**Exhibit Hall Open** *Location: Leonesa Foyer*
6:00 p.m. – 6:10 p.m.	**Welcome and Opening Remarks** *President: William J. Bremner, MD, PhD* *University of Washington* *Local Arrangements Chair:* *Charles H. Muller, PhD* *University of Washington,* *Department of Urology* *Program Chair:* *Christina Wang, MD* *Harbor-UCLA Medical Center &* *Los Angeles Biomedical Research Institute*
6:10 p.m. – 6:30 p.m.	**Distinguished Andrologist Award**
6:30 p.m. – 7:30 p.m.	**ASA KEYNOTE LECTURE** **The Y Chromosome** *David Page, PhD* *Whitehead Institute* *(Introduced by William J. Bremner, MD, PhD)*
7:30 p.m. – 9:30 p.m.	**Welcome Reception** *Location: Leonesa Foyer*

SUNDAY, APRIL 3, 2005

7:00 a.m. – 6:00 p.m.	**Registration** *Location: Lobby Alcove*
7:00 a.m. – 8:00 a.m.	**Past President's Breakfast** *Location: Douglas Boardroom*
7:00 a.m. – 4:00 p.m.	**Exhibit Hall Open** *Location: Leonesa Foyer*
8:00 a.m. – 9:00 a.m.	**AUA LECTURE** **Androgens and the Prostate Cancer Prevention Trial** *Ian M. Thompson Jr., MD* *University of Texas Health Sciences Center* *(Introduced by Jon Pryor, MD)*
9:00 a.m. – 9:15 a.m.	**Distinguished Service Award**
9:15 a.m. – 10:45 a.m.	**SYMPOSIUM I – REGULATORS OF PROSTATE CANCER GROWTH: POTENTIAL NOVEL THERAPIES** *Co-chairs: Gail Prins, PhD, and Ian Thompson Jr., MD* **Androgen Receptors as a Therapeutic Target in Androgen Independent Prostate Cancer** *Steven Balk, MD, PhD* *Beth Israel Deaconess Medical Center* **Stress Induced Antiapoptotic Genes and Clusterins as Targets for Prostate Cancer Therapy** *Martin Gleave, MD* *University of British Columbia* **Prostate Cancer Genomics: Identification of Potential Therapeutic Targets** *Peter Nelson, MD* *University of Washington, Seattle*
10:45 a.m. – 12:30 p.m.	**Poster Session I (with refreshments)** *Location: Leonesa Ballroom*
12:30 p.m. – 2:00 p.m.	**Lunch (on your own)**
12:30 p.m. – 2:00 p.m.	**Women in Andrology Luncheon and Roundtable Discussion** (not included in registration fee; tickets required)
2:00 p.m. – 3:30 p.m.	**Concurrent Oral Sessions**
3:30 p.m. – 4:00 p.m.	**Refreshment Break**

schedule at a glance

4:00 p.m. – 5:00 p.m. **WOMEN IN ANDROLOGY LECTURE**
Novel Glycolytic Enzymes and Sperm Motility
Deborah A. O'Brien, PhD
University of North Carolina at Chapel Hill
(Introduced by Janice Bailey, PhD)

5:00 p.m. – 6:00 p.m. **DEBATE: THERE ARE SIGNIFICANT INCREASED RISKS IN THE OFFSPRING AFTER ART**
Moderator: Paul Turek, MD
UCSF
Yes *- Dolores Lamb, PhD*
Baylor College of Medicine/Urology

No *- Peter N. Schlegel, MD*
The New York Weill/Cornell Medical Center

6:30 p.m. – 8:30 p.m. **Trainee Forum and Mixer**
Location: Leonesa Foyer

MONDAY, APRIL 4, 2005

7:00 a.m. – 6:00 p.m. **Registration**
Location: Lobby Alcove

7:00 a.m. – 4:30 p.m. **Exhibit Hall Open**
Location: Leonesa Foyer

7:00 a.m. – 8:00 a.m. **MINORITY AFFAIRS BREAKFAST AND MEETING**
Missing Persons: Minorities in the Health Professions, and Implications for the Nation's Health
Keith C. Norris, MD
Location: Blewett Suite, 7th Floor
Sign up at the registration desk by 2:00 p.m. on Sunday, April 3.

8:00 a.m. – 9:00 a.m. **LECTURE**
Gene Therapy for Erectile Dysfunction
Wayne J. G. Hellstrom, MD
Tulane University School of Medicine
(Introduced by Catherine Naughton, MD)

9:00 a.m. – 9:15 a.m. **Young Andrologist Award**

9:15 a.m. – 10:45 a.m. **SYMPOSIUM II – ANDROGEN REGULATION OF REPRODUCTIVE TISSUES**
(honoring C. Alvin Paulsen, MD)
Co-chairs: David DeKretser, MD, and Richard Sherins, MD

Nongenomic Steroid Receptors on Sperm – Signaling and Function
Michaela Luconi, PhD
University of Florence

Androgens and Epididymal Genomics
Shayesta Seenundun, BSc
McGill University

Androgen Regulation of Vas Deferens Proteins
Laurent Morel, PhD
University Blaise Pascal

10:45 a.m. – 12:00 p.m. **Poster Session II (with refreshments)**
Location: Leonesa Ballroom

12:00 p.m. – 2:00 p.m. **Lunch (on your own)**

12:00 p.m. – 1:30 p.m. **LAB SCIENCE FORUM AND LUNCHEON**
Vasectomy and Reversal: A View from the Lab Bench
Sheldon Marks, MD
International Center for Vasectomy Reversal
Location: Menzies Suite
(not included in registration; tickets required)

12:00 p.m. – 1:30 p.m. **Editorial Board Luncheon**
Location: Stevens

2:00 p.m. – 3:00 p.m. **Concurrent Oral Sessions**

3:00 p.m. – 4:00 p.m. **LECTURE**
Toward a Comprehensive Genetic Analysis of Male Fertility Using Drosophila
Barbara Wakimoto, PhD
University of Washington
(Introduced by Kate Loveland, PhD)

4:00 p.m. – 4:30 p.m. **Refreshment Break**

4:30 p.m. – 6:00 p.m. **SYMPOSIUM III – CELL BIOLOGY OF FERTILIZATION**
Co-chairs: Monica Vazquez-Levin, PhD, and Pablo Visconti, PhD

Sperm Acrosome Membranes, Matrix and Exocytosis
John C. Herr, PhD
Center for Research in Contraceptive and Reproduction

schedule at a glance

Regulation of Sperm-egg Interactions and Egg Membrane Dynamics
Janice P. Evans, PhD
Johns Hopkins University Bloomberg School of Public Health

Sperm Binding and Humanized Zona Pellucida
Tanya Hoodbhoy, PhD
NIDDK

7:00 p.m. – 11:00 p.m. **Annual Banquet at the Columbia Tower Club**
(not included in registration fee; tickets required)

TUESDAY, APRIL 5, 2005

7:00 a.m. – 8:00 a.m. **2006 Program Committee Meeting**
Location: Douglas Boardroom

7:30 a.m. – 11:00 a.m. **Registration**
Location: Lobby Alcove

8:00 a.m. – 9:30 a.m. **SYMPOSIUM IV – MALE CONTRACEPTION**
Co-chairs: Ronald D. Swerdloff, MD, and Geoffrey M.H. Waites, PhD

Agents Acting Directly on the Testis: Will They be Reversible?
C. Yan Cheng, PhD
Population Council Center for Biomedical Research

Will There Ever be an Epididymal Male Contraceptive?
Barry T. Hinton, PhD
University of Virginia

Combination of Hormonal and Physical Agents – Are They More Efficacious and Why?
Amiya P. Sinha-Hikim, PhD
Harbor-UCLA Medical Center & Los Angeles BioMedical Research Institute

9:30 a.m. – 10:30 a.m. **INTERNATIONAL LECTURE**
Mutations in Male Infertility: Of Mice and Men
Yoshitake Nishimune, MD
Osaka University, Institute for Microbial Diseases
(Introduced by Patricia S. Cuasnicu, MD)

10:30 a.m. – 10:45 a.m. **Refreshment Break**

10:45 a.m. – 12:15 p.m. **SYMPOSIUM V – HOT TOPICS IN ANDROLOGY**
Co-chairs: Patricia Morris, PhD, and Nina Davis, MD

Prostatis – What is New?
Richard E. Berger, MD
University of Washington, Seattle

Cell-Specific Knockout of the Androgen Receptor in Testis – What are the Physiological Implications?
Chawnshang Chang, PhD
University of Rochester

Germ Cell Transplantation – Where Will This Technology Go?
Ina Dobrinski, DVM, PhD
University of Pennsylvania

12:15 p.m. **ASA Business Meeting, Trainee Awards**

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president's welcome

William J. Bremner, MD, PhD

It is my great pleasure to welcome you to the 30th Annual Conference of the American Society of Andrology in the beautiful city of Seattle. Our annual meeting is one of the key functions of our society, and I am certain that this will be a productive and enjoyable one for ASA members and guests. Its success is due in large part to its limited size and intimate setting, which foster collegiality at both formal and informal events. There will be many opportunities in the coming days to interact in ways that stimulate scientific exchange and plans for collaboration.

I have had the good luck of working during the past year with a terrific cast of people who were responsible for planning and coordinating this event. On behalf of all of us, I wish to thank them for their great work. Dr. Christina Wang, the Annual Meeting Program Chair, has been a fountain of energy and has assembled an outstanding program blending basic science and clinical applications of andrology. The meeting has a theme of "Androgens and Their Target Organs," with many outstanding presentations related to this area. In addition, the Program Committee has done an excellent job of incorporating the breadth of interests of society members; for example, with lectures and symposia on the Y chromosome, prostate cancer, assisted reproductive technology, erectile dysfunction, epididymal function, vasectomy, male infertility, fertilization, male contraception, and germ cell transplantation. A record number of abstracts will be presented, representing many disciplines of male reproductive research from colleagues around the world.

Dr. David Page will open the meeting with the ASA Keynote Lecture "The Y Chromosome." There are a number of presentations around the meeting's theme, including the symposia "Androgen Regulation of Reproductive Tissues," in honor of C. Alvin Paulsen, and "Regulators of Prostate Cancer Growth: Potential Novel Therapies," as well as the AUA Lecture, "Androgens and the Prostate Cancer Prevention Trial," by Ian Thompson. Deborah O'Brien will present this year's Women in Andrology Lecture, "Novel Glycolytic Enzymes and Sperm Motility," and the International Lecture is "Mutations in Male Infertility" by Yoshitake Nishimune. The Laboratory Science Forum and Luncheon will feature Dr. Sheldon Marks speaking on vasectomy reversal. We are fortunate that immediately before the meeting, the 18th North American Testis Workshop will take place March 30-April 2, 2005, and will address "Testicular Cell Dynamics and Endocrine Signaling." This provides the opportunity to come a few days early to the ASA meeting and participate in an outstanding workshop with a basic science emphasis. The Andrology Lab Workshop, chaired by Dr. Michael Palladino, will take place Saturday, April 2, 2005, and will offer instruction in all aspects of human sperm morphology classification. Under Dr. Wang's dedicated leadership, the 2005 Annual Meeting provides a rich and wide-ranging program that offers something of interest for everyone in our field.

There are plenty of professional and social opportunities for trainees this year. Be sure to attend the Trainee Forum and Mixer on Sunday evening – a great way to meet new friends and learn more about andrology careers in a relaxed setting. Everyone is invited to the Welcome Reception Saturday evening. There will also be a Minority Affairs Breakfast and a Women in Andrology Luncheon and Roundtable Discussion. A highlight of this year's meeting is the annual banquet on Monday night. This year we will dine and dance at the Columbia Tower Club, an elegant room atop the 76-story Bank of America Tower in downtown Seattle. The Club offers the finest of local seafood and other Seattle specialties, along with a spectacular view of the city and surrounding mountain ranges. It promises to be an unforgettable evening, and I hope you all will join me there.

I want to thank our corporate sponsors and the NIH for the generous donations that have made the educational and social events of this meeting possible. I extend my appreciation on behalf of all of us to the Development Committee, Gail Prins, Chair, and Dana Ohl and Bill Roudebush for a very successful year. Charles Muller, chair of the Local Arrangements Committee, with the able assistance of Janice Evans, has done an outstanding job of organizing activities in Seattle. Finally, we all thank Sue O'Sullivan, our Society and Industry Relations Coordinator, for her masterful handling of everything.

The success of our annual meeting depends on the tireless behind-the-scenes efforts of the people in our executive office, WJ Weiser & Associates. Debbie Roller, who runs the day-to-day ASA operations, coordinated this meeting throughout the year with endless energy and enthusiasm. Ann Marie DuPlessis, our events coordinator, provided all the hotel and social arrangements and much more. Alison Heimburger coordinated all publications for the meeting. Wendy Weiser, our executive director, runs a fantastic operation, and her direction has enabled our society to grow significantly over the past year. My very warm thanks also to the officers and council members of ASA for their tireless efforts and for a successful year.

I hope you will take time following the meeting to explore the attractions of Seattle and the Pacific Northwest. I look forward to seeing you all in the coming week. Enjoy the 2005 meeting!

With best regards,
William J. Bremner, MD, PhD
President, American Society of Andrology
Professor and Chairman, Department of Medicine
The Robert G. Petersdorf Endowed Chair in Medicine
University of Washington School of Medicine

Past Presidents of the American Society of Andrology

1975-1977	Emil Steinberger	1990-1991	Howard Nankin
1977-1978	Don W. Fawcett	1991-1992	David W. Hamilton
1978-1979	C. Alvin Paulsen	1992-1993	Ronald S. Swerdloff
1979-1980	Nancy J. Alexander	1993-1994	Bernard Robaire
1980-1981	Philip Troen	1994-1995	Glenn R. Cunningham
1981-1982	Richard M. Harrison	1995-1996	Marie-Claire Orgebin-Crist
1982-1983	Richard J. Sherins	1996-1997	Arnold M. Belker
1983-1984	Andrzej Bartke	1997-1998	Terry T. Turner
1984-1985	Rudi Ansbacher	1998-1999	Richard V. Clark
1985-1986	Anna Steinberger	1999-2000	Barry T. Hinton
1986-1987	William D. Odell	2000-2001	J. Lisa Tenover
1987-1988	Larry L. Ewing*	2001-2002	Barry R. Zirkin
1988-1989	C. Wayne Bardin	2002-2003	Jon L. Pryor
1989-1990	Rupert Amann	2003-2004	Gail S. Prins

*Deceased

American Society of Andrology

OFFICERS

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Treasurer	Gail A. Cornwall, PhD
Past President	Gail S. Prins, PhD

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Future Meetings Committee	Erwin Goldberg, PhD; Evanston, IL
International Liaison Committee	Patricia S. Cuasnicu; Buenos Aires, Argentina
Journal of Andrology	Matthew P. Hardy, PhD; New York, NY (Co-Editor)
	Peter N. Schlegel, MD; New York City, NY (Co-Editor)
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Local Arrangements Committee	Charles H. Muller, PhD; Seattle, WA
Membership Committee	Janice L. Bailey, PhD; Sainte-Foy, Quebec, Canada
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Public Affairs and Policy Committee	Rebecca Z. Sokol, MD, MPH; Los Angeles, CA
Publications Committee	Bernard Robaire, PhD; Montreal, Quebec, Canada
Testis Workshop	Matthew P. Hardy, PhD; New York, NY
Trainee Affairs	Rex A. Hess, PhD; Urbana, IL

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NOTICE TO READERS

Every effort has been made to ensure that the information printed here is correct; however, details are subject to change.

general information

about seattle washington

Seattle is home to Pikes Place Market, a jubilant, open-air celebration of fresh regional fruits and vegetables, seasonal flowers, herbs, seafood, spices, cheeses, hand-crafted work by artisans, eclectic shops, and fine restaurants and eateries – many with views of ferry and freighter traffic on Elliott Bay. Here in this revered nine-acre community with its cracked walkways and uneven cobblestone streets, the scent of sweet peas mingles with Dungeness crabs and spicy teas, street musicians compete with "free sample!" vendors, and weird things make their appearance: bottom-dwelling monkfish and rubber-necked geoducks.

The regional bounty is rich: salmon, shellfish, sweet onions, mushrooms, stone fruits, berries and asparagus. The culture overflows with liquid delights – coffee, microbrews and wine. Given its easy proximity to the Pacific Rim, Seattle's collaborative cuisine also takes full advantage of Asian ingredients and embraces the city's many cultural influences to create cutting-edge fusions and ambrosial works of art.

Seattle's natural beauty inspires even the most committed couch potatoes. Add the inherent heartiness of the natives, and it's obvious why REI and Eddie Bauer found their niche here. After all, this is home to the likes of twin brothers Jim and Lou Whittaker, among the most famous American mountain climbers of the 20th century, who stroll up and down Mount Rainier about as often as most of us go out for a latte. The best in urban recreation is at your toes and at your fingertips around Seattle—spectacularly scenic golf, kayaking and canoeing, fishing and clamming, hiking, urban parks including Discovery Park (Magnolia neighborhood), Seward Park (Lake Washington) and Woodland Park Zoo and Rose Gardens. A short drive out of the city leads to skiing, snowboarding, river rafting, hiking, and some of the most scenic parks around: Mt. Rainier National Park, Mount St. Helens National Volcanic Monument, North Cascades National Park, Olympic National Park and Rainforest, and Columbia River Gorge National Scenic Area.

A trip to Seattle offers more opportunities to experience arts and culture than you can probably fit in, but you'll have fun trying. Seattle's cultural scene continues to flourish with nationally respected opera, ballet, art galleries, museums, festivals, and theater. In fact, Seattle boasts more theaters than any comparably sized U.S. city, and the number of total performances ranks second only to New York. Seattle is one of only six American cities with resident major symphony, opera and ballet companies.

Built in 1962 for the Seattle World's Fair, the rotating 607-ft. Space Needle was hailed as futuristic and daring. Now this internationally recognized symbol of Seattle (which, by the way, held its own during the 7.6 earthquake in February 2001) is a quirky reminder of the city's creative spirit.

Seattle has plenty to offer you during your stay, and the ASA looks forward to experiencing it with you!

hotel information

Grand Hyatt Seattle
721 Pine Street
Seattle, Washington 98101
Phone: (206) 774-1234
Fax: (206) 774-6120
Website: www.grandhyattseattle.com

Room Rate:
$173.00 single/double, 15.6% Washington State sales & room tax, subject to change.

Reservations can be made through the Hotel's Reservation Department by calling **(206) 774-1234** or **(800) 233-1234**. Please make sure to mention the special, group code, "**G-AMSA**," in order to receive the special rate. The cut-off date for these special room rates is **March 8, 2005**. There is no deposit required, but a credit card is required to guarantee the reservation, and there is a 24-hour cancellation policy (guest must cancel by 3:00 p.m. the day prior to arrival). If a guest cancels within 24 hours, there will be a $75.00 charge. A guest who does not cancel and does not show up will be charged one night's room and tax. Check-in time is 3:00 p.m. and check-out time is 12:00 p.m.

airline information

United Airlines is the official airline for the ASA 30th Annual Meeting. If you or your travel agent call the United Airlines toll-free number, **(800) 521-4041**, to book your reservations, you will receive a 5% discount off the lowest applicable discounted fare, including First Class, or a 10% discount off full fare unrestricted coach fares, purchased 7 days in advance. An additional 5% discount will apply when tickets are purchased at least 30 days in advance of your travel date. Discounts also apply on Shuttle by United and United Express. Call United's Specialized Meeting Reservations Center at **(800) 521-4041** to obtain the best fares and schedule information. Make sure you refer to Meeting ID Number "**531CB.**" Dedicated reservation agents are on duty 7 days a week from 8:00 a.m. – 10:00 p.m. EST.

ground transportation

Transportation to/from Airport:
Airport Express Service
(206) 626-6088
Recorded Schedule Information
(206) 624-5077 / (800) 426-7532
Gray Line of Seattle Assistance

Airport Express is the least expensive way to go between downtown Seattle hotels and Seattle-Tacoma (SeaTac) International Airport.

Airport Express Service operates between SeaTac Airport and the Crowne Plaza, Fairmont Olympic, Hilton Hotel, Grand Hyatt, Madison Renaissance, Seattle Sheraton, Warwick Hotel and Westin Hotel. Ask your hotel front desk staff for details or call Gray Line of Seattle.

- Adults:
  One Way $8.50
  Roundtrip $14.00
- Children (2-12 years):
  One-way $6.00
  Roundtrip $10.00
  Children less than 2 years old travel for free.

Car Rental:
Avis Rent A Car is the official rental car company for the ASA Meeting. You are not required to use them but we encourage you to take advantage of their special offer.

You must return the car at same renting location or additional surcharges may apply. All rates include unlimited free mileage. Rates do not include any state or local surcharges, tax, optional coverage or gas refueling charges. Weekend daily rates are available from 12:00 p.m. Thursday through Monday at 11:59 p.m. When making your reservations, dial **(800) 331-1600** and mention the code "**J996845**" to receive discounted rates.

events & activities

Welcome Reception
Saturday, April 2, 2005
7:30 p.m. – 9:30 p.m.
Location: Leonesa Foyer
Join us on Saturday, April 2, 2005, at 7:30 p.m. for a Welcome Reception, to connect with friends and colleagues. Admission to the reception is included in your ASA registration fee (not included if you only register for the Testis Workshop and/or Andrology Lab Workshop).
Cost: included in your ASA registration fee; additional tickets can be purchased for $30.00 each.
Business casual or casual attire is appropriate.

Trainee Forum and Mixer
Sunday, April 3, 2005
6:30 p.m. – 8:30 p.m.
Location: Leonesa Foyer
The ASA **T**rainee **F**orum and Mixer provides the opportunity for Trainee members to meet other trainees as well as meet with more established members of the Society. This is a relaxed, informal event with appetizers and drinks provided. Senior members of the Society will be present for an informal "forum and discussion group" setting to answer your questions about relevant topics such as grant-writing, searching for a post-doc or job, alternative PhD career paths, succeeding in the clinic or lab, etc. All members of the Society are welcome. Please check the appropriate box on the registration form if you will be attending.
Business casual or casual attire is appropriate.

2005 Women in Andrology Luncheon and Roundtable Discussion
Sponsored by the American Society of Andrology
Sunday, April 3, 2005
12:30 p.m. – 2:00 p.m.
Location: Stevens
The Women in Andrology group, which exists to promote the visibility and contributions of women within the Society and the field of Andrology, meets for lunch each year at ASA's Annual Meeting. At this year's luncheon, to be held Sunday, April 3, at 12:30 p.m., we are planning Roundtable Discussions led by a discussion leader on a number of topics. (Note: The Women in Andrology lecture is also on Sunday, April 3, from 4:00 p.m. – 5:00 p.m. The lecture is included in your ASA meeting registration fee.)
Cost: $35.00 per person. Please pre-register for this event.
Business casual or casual attire is appropriate.

Minority Affairs Breakfast and Meeting
Missing Persons: Minorities in the Health Professions & Implications for the Nation's Health
Monday, April 4, 2005
7:00 a.m. – 8:00 a.m.
Location: Blewett Suite, 7th Floor
At this breakfast meeting, Dr. Keith Norris will discuss the paucity of minorities in the health professions, the need to achieve academic success, and the moral imperative for mentoring and role modeling at the early stages (k-12). Highlight disparities in health care and the unique opportunities minority researchers and clinicians have to impact on these differences and how these findings can be transformed into fundamental advances for all persons.
Sign up at the registration desk by 2:00 p.m. on Sunday, April 3.

Lab Science Forum and Luncheon
Monday, April 4, 2005
12:00 p.m. – 1:30 p.m.
Location: Menzies Suite
Join us for the 2005 Lab Science Forum and Luncheon entitled "Vasectomy and Reversal: A View from the Lab Bench," with Sheldon Marks, MD, a microsurgical specialist, founder of the International Center for Vasectomy Reversal, member of the Society of Reproductive Surgeons, and Diplomat of the American Board of Urology.
Cost: $35.00 per person. Please pre-register for this event.

Annual Banquet
Monday, April 4, 2005
7:00 p.m. – 11:00 p.m.
Location: The Columbia Tower Club

Join us for the ASA 2005 Annual Banquet! On Floors 75 and 76 of the Bank of America Tower in downtown Seattle, the location alone makes every visit to the Club a special event. The Club's beautifully decorated rooms offer panoramic, breathtaking views of both the downtown skyline and Capitol areas, as well as the historic countryside.
Cost: $65.00 per person. Please pre-register for this event.
Business casual attire is appropriate.

message from the program chair


Christina Wang, MD

Welcome to Seattle and the 30th Annual Meeting of the American Society of Andrology, for another three days of lively scientific exchange and interactions. I would like first and foremost to thank the 2005 Program Committee (Janice Bailey PhD; Gail Cornwall, PhD; Mitch E. Eddy, PhD; Janice P. Evans, PhD; Michael D. Griswold, PhD; Matt Hardy, PhD; John C. Herr, PhD; Jonathan P. Jarow, MD; Kate Loveland, PhD; Alvin Matsumoto, MD; Deborah A. O'Brien, PhD; Steven M. Schrader, PhD; Jacquetta Trasler, MD, PhD; Paul J. Turek, MD; Monica Vasquez-Levin, PhD; Pablo E. Visconti, PhD) for their commitment to the ASA. Their rapid and helpful responses to my emails led to rapid interchanges leading to the early development of the 2005 Program. This year's Annual Meeting adopted a theme "Androgens And Their Target Organs." The theme sessions included a symposium dedicated to Dr. C. Alvin Paulsen, one of our founding members, past president of the ASA and mentor of numerous andrologists throughout the world, on "Androgen Regulation of Reproductive Tissues," where Drs. Luconi, Ms. Seenumdun and Dr. Morel will discuss the action of androgens and other steroids on sperm, epididymis and vas deferens. The other sessions within the theme include discussion by Dr. Ian Thompson on his reflections on "Androgens and the Prostate Cancer Prevention Trial" two years after the completion of this important study. Scattered in different symposia throughout the meeting will be other androgen related topics, including "Androgen Receptors as Therapeutic Target in Androgen Independent Prostate Cancer," by Dr. Balk; "Cell-Specific Knockout of Androgen Receptor in Testis – What are the Physiological Implications?" by Dr. Chang; and "Combination of Hormonal and Physical Agents – Are They More Efficacious and Why?" by Dr. Sinha-Hikim.

The meeting will open with the ASA Lecture by Dr. David Page of the Whitehead Institute, MIT, on "The Y Chromosome," describing the work his laboratory has performed across two decades. Other invited lectures on genes and male infertility include "Toward a Comprehensive Genetic Analysis of Male Fertility Using Drosophila" by Dr. Wakimoto, and "Mutations in Male Infertility: Of Mice and Men" by Dr. Nishimuni, our International and ParentPlus lecturer. We re-introduce the debate for 2005 with Drs. Lamb and Schlegel, discussing the important topic of "Risks in the Offspring After ART." Other lectures by ASA members include "Gene Therapy of Erectile Dysfunction," by Dr. Hellstrom, and "Novel Glycolyte Enzymes and Sperm Motility" by our Women in Andrology lecturer, Dr. O'Brien. Symposia topics include Prostate Cancer Growth (Antiapoptotic Genes and Clusterins by Dr. Gleave; Prostatic Cancer Genomics by Dr. Nelson), Cell Biology of Fertilization (Sperm Acrosome Membranes by Dr. Herr; Sperm-egg Interaction by Dr. Evans; and Sperm Binding by Dr. Hoodboy); Male Contraception (Agents Acting on Testis by Dr. Chang and on the Epididymis by Dr. Hinton); and a "hot" topic symposium ("Prostatites" by Dr. Berger, and "Germ Cell Transplantation" by Dr. Dobrinski).

The Council decided that this year there would not be a post-graduate session, because our annual meeting follows the Testis Workshop. The Andrology Laboratory Workshop organized by Michael Palladino, PhD, will focus on Sperm Morphology, following the extremely well received workshop last year. We will again have our Trainee Forum and Mixer, which provides the opportunity for trainee members to meet other trainees, as well as more established members of the society. This year's Minority Affairs Breakfast and Meeting will include a presentation by Dr. Keith Norris entitled, "Missing Persons: Minorities in the Health Professions, and Implications for the Nation's Health." Dr. Sheldon Marks will speak about "Vasectomy and Reversal: A View from the Lab Bench" at the Lab Science Forum.

This year there were 136 abstracts submitted, which will be presented in two simultaneous oral sessions on both April 3 and 4, 2005, and two poster sessions on the same dates.

This exciting program is made possible by the support from the National Institutes of Health, our members, and our industry sponsors. The support that the program committee received from the ASA presidents, Gail Prins and Bill Bremner, resulted in the program being finalized in the fall of 2003, allowing the ASA to have positive responses and commitment from all the speakers selected by the program committee. Again, I would like to thank the 2005 program committee, the guidance given by Sally Perreault and Debbie O'Brien (2003 and 2004 program chairs), Wendy Weiser, Debbie Roller, and the ASA executive office, the local arrangement committee, and the many sponsors of our program.

Enjoy the Seattle 2005 meeting.

Program and Abstract Review Committee

Christina Wang, M.D.; Torrance, CA (Chair)
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ASA lectureship award

David Page, PhD

David C. Page has conducted fundamental studies of mammalian sex chromosomes and their roles in germ cell development, with special attention to the function, structure and evolution of the Y chromosome. His laboratory recently completed the sequencing of the human Y chromosome in conjunction with the Washington University Genome Sequencing Center. The foundations of this sequencing project were built across two decades. Dr. Page's laboratory first reported DNA-based deletion maps of the Y chromosome in 1986 (updated 1992), comprehensive clone-based physical maps of the chromosome in 1992 (updated 2001), and systematic catalogs of Y-linked genes in 1997 (updated 2003).

These genomic studies have led to unanticipated biological insights. Dr. Page's laboratory reconstructed the evolution of today's X and Y chromosomes from an ancestral pair of autosomes that existed 300 million years ago. His laboratory discovered molecular evolutionary mechanisms by which the Y chromosome became functionally specialized, in male germ cell development and spermatogenesis. Indeed, as demonstrated by Dr. Page's laboratory, most of the chromosome's genes are members of Y-specific families that are expressed exclusively or predominantly in testicular germ cells. Dr. Page's laboratory discovered and characterized the most common genetic cause of spermatogenic failure in humans, namely, deletion of the *AZFc* region of the Y chromosome.

In conjunction with colleagues at Washington University, Dr. Page's laboratory discovered that most of the Y chromosome's testis genes exist as mirror-image pairs on massive palindromes. They determined that these palindromes are sites of frequent gene conversion and, thus, that the male-specific chromosome is intensely recombinogenic despite the absence of conventional crossing over.

Having explored the chromosomal basis of human sex reversal (XX maleness) in the 1980's, Dr. Page is now turning his attention to the question of germ cell sex determination in mammals, and to the development of the embryonic ovary.

In 2004, Dr. Page became Interim Director of the Whitehead Institute. He is also Professor of Biology at the Massachusetts Institute of Technology and Investigator at the Howard Hughes Medical Institute. In 1992, he founded the Whitehead Task Force on Genetics and Public Policy. He is Editor (with Matthew Scott) of *Current Opinion in Genetics and Development* and was a founding Editor (with Eric Lander and Richard Lifton) of the *Annual Review of Human Genetics and Genomics*. His honors include the Francis Amory Prize (with Robin Lovell-Badge and Peter Goodfellow) from the American Academy of Arts and Sciences, *Science* magazine's Top 10 Scientific Advances of the Year (twice: 1992 and 2003), a MacArthur Prize Fellowship, and the Curt Stern Award from the American Society of Human Genetics (2003).

Dr. Page trained in the laboratory of David Botstein, at MIT, while earning an MD magna cum laude from Harvard Medical School and the Harvard-MIT Health Sciences and Technology Program. Dr. Page also holds a BA with Highest Honors in Chemistry from Swarthmore College, which awarded him an honorary doctorate in 1989.

Serono Lectureship Recipients

1980	C. Alvin Paulsen	1993	Leroy Hood
1981	Pierre Soupart	1994	Michael D. Griswold
1982	Kevin J. Catt & Maria L. Dufau	1995	Marie-Claire Orgebin-Crist
1983	J. Michael Bedford	1996	Norman B. Hecht
1984	C. Wayne Bardin	1997	Patrick C. Walsh
1985	David M. De Kretser	1998	Jurrien Dean
1986	Ronald S. Swerdloff	1999	Neal First
1987	Roger V. Short	2000	Bert O'Malley
1988	Roger Guillemin	2001	John D. Gearhart
1989	Frank S. French	2002	David Botstein
1990	David C. Page	2003	Victor D. Vacquier
1991	Tony M. Plant	2004	Judith Kimble, PhD
1992	Yves Clermont		

distinguished andrologist award


Mitch Eddy, PhD

The American Society of Andrology is pleased to recognize Edward Mitchell (Mitch) Eddy, PhD, as the 2005 Distinguished Andrologist for his outstanding contributions to advancing our knowledge of the molecular basis of male reproductive function, as well as his exceptional service as a leader and mentor in the reproductive biology community.

A native of Kansas, Mitch Eddy went to Kansas State University and obtained his BS in Zoology in 1962 and MS in 1964. He then received his PhD in anatomy at the University of Texas Medical Branch in 1967, followed by post-doctoral work at Harvard Medical School 1967-1970. His career as an independent researcher began in the Department of Biological Structure at the University of Washington in 1970, when he started as an assistant professor and then rose through the ranks to Professor in 1982. He also served as Vice Chair and Acting Chair of his department. In 1983, he left Washington for North Carolina, where he now serves as Head of the Gamete Biology Group and Deputy Chief of the Laboratory of Reproductive and Developmental Toxicology at the National Institute of Environmental Health Sciences in the National Institutes of Health.

Dr. Eddy's research has covered a broad range of topics related to the biology of male germ cells, from the origin of the germ line to spermatogenesis, spermiogenesis, epididymal maturation and sperm function. He has maintained a theme of evolving as a scientist and adapting new experimental approaches or state-of-the-art technologies to questions related to male germ cells throughout his career. Emerging from his background as a morphologist, his lab performed analyses with monoclonal antibodies of the surfaces of sperm, primordial germ cells and Leydig cells (well before the term "proteome" was coined). He has also pursued projects related to intracellular regions of the sperm, with studies of the fibrous sheath of the sperm flagellum and acrosomal components. A driving force for much of Dr. Eddy's scientific investigations has been his desire to determine which genes encode proteins with key roles in mitotic, meiotic and post-meiotic phases of male germ cell development. Dr. Eddy's group was among the first to apply molecular biology approaches in andrological research. He became interested in the analysis of the expression, function and regulation of male germ cell-specific genes, with one example being his work on spermatocyte-specific heat shock protein (HSP) isoforms. HSP70-2 was found to be a component of the synaptonemal complex in spermatocytes and as a chaperone for the cell cycle regulatory kinase CDK1, promoting formation of the CDK1-cyclin B1 complex. Dr. Eddy was among the first to apply gene knockout technology to studies of male reproduction. His lab demonstrated that HSP70-2 is essential for male reproductive function, as *Hsp70-2*-null male mice were infertile, lacking spermatids and sperm due to spermatogenic arrest at prophase of meiosis I in the G2-M transition. A colleague remarks that this "characterization of the role of [this HSP protein] in the folding other proteins and his demonstration of the essential nature of this gene in knockout studies were among the most elegant and convincing studies ever done on germ cell gene expression." Dr. Eddy continues to pursue ways to bring current methodologies to andrology research, with one of his more recent projects being his involvement in an effort to generate ESTs from mouse germ cell libraries and to develop spermatogenesis-specific microarrays.

His colleagues continually note his generosity with his time and skills for collaborations. Dr. Eddy's utilization of knockout methodologies have led to many fruitful interactions with other reproductive biologists in studies of the *in vivo* functions of a number of gene products, including estrogen receptor-a, fertilin b, AKAP4, protamine 2, and, most recently, glyceraldehyde 3-phosphate dehydrogenase (GAPD-S). GAPD-S was another project that came from Dr. Eddy's lab's interest in male germ cell-specific genes. *Gapd-s* was isolated from a mouse spermatogenic cell expression library by Dr. Eddy, and found to be specific to spermatogenic cells and expressed specifically in post-meiotic germ cells. In sperm, GAPD-S is tightly associated with the fibrous sheath along the length of the flagellum. Dr. Eddy's collaboration in a study headed by Debbie O'Brien, with further help from Sally Perreault, has demonstrated a critical role for GAPD-S in sperm motility and male fertility.

Dr. Eddy's research accomplishments and leadership in the field of reproductive biology have been widely acknowledged by the community with an NIH Merit award in 1997, and serving as the program chair for two of the most significant meetings in our field, the Gordon Research Conference on Mammalian Gametogenesis and Embryogenesis in 1994 and the annual meeting of the Society for the Study of Reproduction in 2001. He has been an editorial board member of several journals in a range of disciplines, including the *American Journal of Andrology*, *Endocrinology*, and *Environmental Health Perspectives*. He is currently an Associate Editor of *Biology of Reproduction*. He has also been a speaker at numerous prestigious conferences, including Testis Workshops, Gordon Research Conferences, International Symposia on Spermatology, AUA Summer Research Conference, and at the annual meetings of the Society for the Study or Reproduction, the Endocrine Society, the European Society of Human Reproduction and Embryology, and, of course, the ASA. He has been consistently involved in the Triangle Consortium for Reproductive Biology, a day-long gathering for reproductive biologists from the NIEHS, EPA, University of North Carolina, Duke University, and North Carolina State University. Furthermore, through his involvement with and presentations to other groups of scientists, he has introduced non-andrologists to the challenges and rewards of studying male reproduction.

Dr. Eddy is also noted as a gifted educator and mentor, as well as for his breadth and depth of his knowledge of current and past research in reproductive biology. One colleague describes his reviews as "encyclopedic and challenging, as they typically present volumes of information and then ask us to re-frame our thoughts about the topic." He is also noted as being "extremely approachable and willing to engage in scholarly discussions with scientists of all ages," from peers to first-year students. Colleagues warmly acknowledge his generous and unselfish nature as a scientist and as a mentor of young reproductive biologists.

Dr. Eddy has served on three committees of the ASA, and the editorial board of the *Journal of Andrology*, and is currently a member of the ASA Executive Council, as well as a member of the Board of Directors of the Society for the Study of Reproduction.

Mitch Eddy can be considered a "triple threat" – an outstanding and constantly forward-thinking researcher, a dedicated teacher and mentor, and a conscientious citizen of the scientific community. For all of these wonderful traits and with gratitude that we can count him as a colleague in andrology and the Society, the ASA is proud to honor Dr. Eddy as its Distinguished Andrologist for 2005.

Distinguished Andrologists

1976	Roy O. Greep & M.C. Chang	1990	Marie-Claire Orgebin-Crist
1977	Robert E. Mancini	1991	Philip Troen
1978	Robert S. Hotchkiss	1992	C. Wayne Bardin
1979	Thaddeus Mann	1993	Anna Steinberger
1980	John MacLeod	1994	Richard J. Sherins
1981	Alexander Albert	1995	Rupert P. Amann
1982	Eugenia Rosemberg	1996	J. Michael Bedford
1983	Kristen B.D. Eik-Nes	1997	Brian P. Setchell
1984	Mortimer B. Lipsett	1998	Ryuzo Yanagimachi
1985	Robert H. Foote	1999	Richard D. Amelar
1986	Alfred D. Jost	2000	Bayard T. Storey
1987	Emil Steinberger	2001	Frank French
1988	Yves W. Clermont	2002	Geoffrey Malcolm Hasting Waites
1989	C. Alvin Paulsen	2003	David M. de Kretser
		2004	Ronald Swerdloff

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

distinguished service award

Barry T. Hinton, PhD

With immense gratitude, the ASA names Barry T. Hinton, PhD, its recipient of the 2005 Distinguished Service Award.

Dr. Hinton received his BSc in Pharmacology and Biochemstry with honors from the University of East London in 1974. He then earned his PhD in Reproductive Physiology with Dr. Brian Setchell of the Babraham Institute in 1979 with his dissertation research entitled, "The concentration and movement of luminal fluid in the testis and epididymis." Following that, Dr. Hinton came to the United States for post-doctoral training as a Rockefeller Foundation Fellow in the laboratory of Dr. Stuart Howards at the University of Virginia School of Medicine. Dr. Hinton remained at the University of Virginia to take an assistant professorship in Cell Biology in 1983 and rose through the ranks to become a Professor of Cell Biology in 1995. His research interests continue to focus on the function of the male reproductive tract, particularly the epididymis. His scientific expertise in reproductive biology has been recognized not only by his service as program chair of the 1998 ASA meeting, but also by his service on several study sections of the NIH, and his being co-organizer of the 1999 NIH Workshop on Male Contraception, and co-organizer of the Third International Epididymal Meeting in 2002. Dr. Hinton was also the recipient of the ASA's Young Andrologist Award in 1989.

Dr. Hinton has served the ASA in virtually every possible capacity. He has served on six different committees, and has been chair of three of these: the Student (now Trainee) Affairs Committee, the International Liaison Committee and, most notably, the Program Committee for the 1998 meeting in Long Beach, Calif. He has been a member of the editorial board of the *Journal of Andrology.* He was elected to serve on the Executive Council for 1987-1990, as Secretary for 1992-1995, and as Vice President / President / Past President for 1998-2000. His presidency is noted by those who served with him on the Executive Board and Executive Council as a time of Dr. Hinton bringing forward numerous innovative ideas, fueled by his strong desire to ensure that the ASA maintains its leadership in the male reproductive sciences.

Dr. Hinton's dedication to trainees has also contributed in tremendous ways to the Society. In addition to chairing the ASA committee, which oversees trainee activities such as the soirée and travel awards, he also has faithfully brought members of his own lab to the ASA meetings. Several of his trainees have won the ASA's New Investigator or Trainee Merit awards, a testament to Dr. Hinton's qualities as a mentor. He has also won numerous teaching awards in eight separate years at the University of Virginia; obviously, he is consistently a favorite educator of the students he teaches. As one colleague notes, "Barry has not only been a major influence on the ASA, he has helped build its next generation."

Dr. Hinton exemplifies the heart and soul of the ASA with his collegiality and enthusiasm. Finally, more than a few of his colleagues noted Dr. Hinton makes the annual meetings worth attending, most especially for the spirit he shows on the dance floor and his knack for getting others to join him. The ASA is indebted to Barry Hinton for his innovative leadership, his dedicated service, and his warmth and caring for the science and the people of the ASA.

Distinguished Service Award Recipients

1994	C. Alvin Paulsen	2000	Bernard Robaire
1995	Andrzej Bartke	2001	Gail S. Prins
1996	Philip Troen	2002	Terry T. Turner
1997	Marie-Claire Orgebin-Crist	2003	Arnold M. Belker
1998	Rupert P. Amann	2004	J. Lisa Tenover
1999	David W. Hamilton		

young andrologist award

Janice L. Bailey, PhD

The American Society of Andrology is delighted to recognize Janice L. Bailey, PhD, as the 2005 Young Andrologist for her notable contributions in sperm biology. Dr. Bailey grew up in Manitoba, Canada, and attended the University of Manitoba, where she earned her BS in Agriculture and Animal Science in 1987 and then her MSc in 1988, with her thesis research on the fluidity and function in porcine sperm membranes. Her interest in sperm biology then extended to cellular signaling. She performed her doctoral research with Dr. Mary Buhr at the University of Guelph on calcium regulation of bovine and porcine sperm, earning her PhD in 1992. She then pursued post-doctoral research with Dr. Bayard Storey at the University of Pennsylvania, where she developed her interest in changes in calcium levels during capacitation and with acrosome exocytosis. She joined the faculty of Laval University in Québec as an assistant professor in 1994, in the Department of Animal Sciences. She was promoted to associate professor and granted tenure in 1999, and was then promoted to full professor in 2003.

Her current research interests represent a marriage of all her previous experiences as well as some newer interests in other areas of andrology. She performs research in a variety of systems, including mouse, cattle, and pig, with an interest in both basic science and applied research questions, including reproductive toxicology and cryopreservation of sperm. Her studies of bull sperm cryopreservation led to her discovery of the phenomenon of "cryocapacitation," in which the freezing process triggers a subset of capacitation-like signaling pathways. Most recently, she has utilized phosphoproteomics to study substrates of tyrosine kinase activated during capacitation in boar sperm, and is currently pursuing a proacrosin binding protein that is phosphorylated in a calcium-regulated manner during capacitation. Her interest in reproductive toxicology has led her to study the effects of organochlorines (present in high amounts in the foods of Inuit populations in northern Canada) on reproductive function, including on fertilization processes and on offspring through in utero and lactational exposure. Dr. Bailey is also well respected for her collaborations with many different investigators, particularly within the *Centre de recherche en biologie de la reproduction* at Laval University. Her research accomplishments have been acknowledged with speaking invitations at a number of meetings and conferences including several noteworthy international meetings such as the International Spermatology meeting in South Africa and the International Congress of Andrology in Montreal. Her excellence as a scientist has also been recognized by invitations for editorial service. She has served on the editorial board of the *Journal of Andrology* since 1997, and presently is an Associate Editor. She also recently joined the editorial board of *Biology of Reproduction*.

She has been participating in didactic graduate and undergraduate teaching at Laval University since her arrival there in 1994. Part of this challenge was taking a semester-long immersion course in French so she could teach to the Francophone students in Québec. As a research mentor, she has supervised or co-supervised 21 master's or doctoral students and five post-doctoral fellows, as well as 15 undergraduate students.

Her service to the American Society of Andrology and reproductive biology as a whole is exemplary. She has been a member of the ASA since 1992, and has served on five different committees. She has also served as co-chair and then chair of the Membership Committee since 2002 and, under her leadership, the membership of the ASA has increased from 631 to 814. She also was elected to the Executive Council and served in this capacity from 1999 to 2002, and was elected the president of the Women in Andrology for 2004-2005. Her service and leadership extend well beyond the ASA, as she served on the Executive Council of the Canadian Society for Animal Science, on the Membership and Local Arrangements Committees, as chair of the Endowment Subcommittee for the Society for the Study of Reproduction, and on the Program Committee for the International Embryo Transfer Society.

The ASA is extremely fortunate to count Janice Bailey as one of its up-and-coming leaders in the field of andrology research and in the Society, and for this the ASA honors Dr. Bailey with the 2005 Young Andrologist Award.

Young Andrologist Award Recipients

1982	L.J.D. Zaneveld	1990	Luis Rodriguez/Rigau	1998	William R. Kelce
1983	William B. Neaves	1991	Patricia M. Saling	1999	Stuart E. Ravnik
1984	Lonnie D. Russell	1992	Gary R. Klinefelter	2000	Matthew P. Hardy
1985	Bruce D. Schanbacher	1993	Robert Chapin	2001	Jacquetta Trasler
1986	Stephen J. Winters	1994	Wayne J.G. Hellstrom	2002	Christopher L.R. Barratt
1987	Ilpo T. Huhtaniemi	1995	Christopher DeJonge	2003	Joanna E. Ellington
1988	Larry Johnson	1996	Paul S. Cooke	2004	Kate Loveland
1989	Barry T. Hinton	1997	Gail A. Cornwall		

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 2005 New Investigator Award will be announced during the Annual Business Meeting on Tuesday, April 5, at 12:15 p.m.

New Investigator Award Recipients

1983	Thomas T. Tarter	1991	Robert Viger	1998	Dolores D. Mruk
1984	Peter S. Albertson	1992	John Kirby	1999	Jacques J. Tremblay
1985	Randall S. Zane	1993	Michael A. Palladino	2000	Jeffrey J. Lysiak
1986	Mark A. Hadley	1994	Linda R. Johnson	2001	Alexander T.H. Wu
1987	Peter Grosser	1995	Mehdi A. Akhondi	2002	Ebtesam Attaya
1988	Stuart E. Ravnik	1996	Wei Gu, Daniel B. Rudolph	2003	Mustafa Faruk Usta
1989	Tracy L. Rankin	1997	Loren D. Walensky	2004	Darius Paduch
1990	Donna O. Bunch				

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course objectives & CME credit information

XVIII north american testis workshop

TESTICULAR CELL DYNAMICS AND ENDOCRINE SIGNALING

The theme for the 2005 Testis Workshop is "Testicular Cell Dynamics and Endocrine Signaling." Recent years have brought an influx of new information into the field of male reproduction. Several laboratories have been able to apply the genomics approach to gene expression in the male, revealing previously unknown patterns of gene expression and genes products that were localized in male reproductive tract tissues and cells for the first time. These discoveries paved the way for the next wave, an opportunity to analyze male reproductive biology and the processes by which sperm are formed in the seminiferous tubule and androgen is synthesized in the interstitium of the testis. The different levels of organization in the testis, including the stages of spermatogenesis, enzymatic steps of steroidogenesis and the intracellular signaling pathways of hormones, are now more amenable for study and selection of potential targets for drug development. The 2005 Workshop has been organized into six sessions: Genetics; Development; Endocrine Axis; Steroidogenesis; Spermatogenesis; and Clinical Correlates. Each of the two full days of the meeting will also have two short, 10-minute, oral talks that will be selected from among the submitted abstracts. This component infuses late-breaking developments into the program. Two poster sessions are also scheduled.

Participants will achieve the following learning goals and objectives:

- Describe the genetic basis of sex determination in mouse models with X and Y chromosome translocations.
- Recognize the identity of sperm nuclear characteristics that are critical for fertilization and successful intracytoplasmic sperm injection (ICSI).
- Identify the first descriptions of testicular steroid hormone measurement in humans and the relation between androgen concentration and spermatogenesis; pathways of androgen synthesis in human testes, and the relationship between mutations in genes encoding steroidogenic enzymes and hypogonadism.
- Identify the use of gene profiling as a tool in defining developmental changes during puberty.
- Describe the transitions in RNA processing in sperm and the importance of intercellular signaling between sperm and Sertoli cells.

andrology lab workshop

SPERM MORPHOLOGY WORKSHOP

The 2005 Andrology Laboratory Workshop, taking place on April 2, 2005, will be a laboratory-based training class with "hands-on" exploration of microscopic sperm morphology assessments. The workshop will teach the two most popular morphology schemes used by fertility specialists today: the WHO Third Edition (WHO 3), based on a traditional normal sperm morphology classification, and the WHO Fourth Edition (WHO 4), also known as "Strict Criteria," where normal is defined according to very stringent assessment.

The workshop will begin with an overview of sperm morphology classification including history, relationship to fecundity, the downward trend of percent normal, and the rationale for different morphology schemes. WHO 3 classification will be taught by experts who use this system. Stained slides will be projected through a microscope onto a classroom screen. Two to three hundred sperm will be classified as the slide is moved. Discussions of classification rationale will be encouraged. Students will then be given stained smears and will conduct a morphology assessment at the microscope, with the faculty present to help.

Breakout modules will be used for special aspects of morphology analysis to allow participants to explore their own specific areas of interest. Modules will include: 1. Semen Smear Preparation – this will include an instructional video, instructor, and samples available for practice. 2. Staining Procedures – Vendors of sperm stains will have modules to demonstrate their stains or instructional video will be used if more appropriate (e.g. modified Pap stain). Examples of each type of stained smear will be available for microscopic viewing. 3. CASA techniques – Vendors of CASA systems can demonstrate their systems with the same slide that was used in the training exercise. This portion of the workshop will extend over the lunch hour so participants can have lunch and move between modules to ensure small group participation within a module.

The format of the afternoon session will be very similar to the morning training session. WHO 4 will be taught with projected images described by experts using this classification system. Each participant will then analyze a stained smear at the microscope. Participants will re-analyze the slide using WHO 3 to demonstrate the effect that learning other schemes has on an individual's assessment.

The need for standardization in semen analysis will be presented including a discussion on standardization practices in the European Union. The workshop will then move into a presentation on quality control. Internal and external quality control methods including proficiency testing and statistics will be presented. The workshop will conclude with a summary and moderated discussion.

Participants will achieve the following learning goals and objectives:

- Describe the history of sperm morphology and its clinical significance.
- Name and perform sperm morphology assessments by WHO 3rd and Strict (WHO 4th) Criteria.
- Distinguish the differences between the two morphology classification systems.
- Observe semen smear preparation and practice staining techniques.
- Demonstrate CASA morphology/morphometry methods.
- Explain the importance of standardization in semen analysis.
- Review quality control (QC) measures.
- Utilize QC methods to evaluate and interpret sperm morphology results. End with recap and moderated discussion.

annual meeting

ANDROGENS AND THEIR TARGET ORGANS

The ASA 30th Annual Meeting will be held from April 2 - 5, 2005. This year, we have a theme, "Androgens and their Target Organs," but the program covers advances in all aspects of the field of andrology. The program consists of plenary lectures on broad topics, focused lectures on specific topics, and symposia consisting

of shorter talks on selected subjects. A diverse group of invited speakers, MDs and PhDs were selected to include distinguished senior scientists, as well as promising younger investigators. Platform talks and posters, based on abstracts submitted by our members and colleagues, round out the program and provide a forum for active discussion.

The ASA 2005 program objectives include:

- Recognize the influence of the Y chromosome in men
- Identify mutations that may lead to male infertility
- Describe the benefits and problems of assisted reproductive technology for male infertility
- Identify the role of androgens in prostate cancer
- Recognize novel approaches to the treatment of prostate cancer
- Describe the role of androgens in reproductive tissues
- Relate basic discoveries to male contraceptive development
- Evaluate sperm function and motility
- Describe recent knowledge in the cell biology of fertilization
- Describe the recent advances for erectile dysfunction

CME ACCREDITATION

This activity has been planned and implemented in accordance with the Essentials Areas and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of the University of Oklahoma College of Medicine, the American Society of Andrology Annual Meeting, Testis Workshop, and Andrology Lab Workshop. The University of Oklahoma College of Medicine is accredited by the ACCME to provide continuing medical education for physicians.

The University of Oklahoma College of Medicine designates this educational activity for a maximum of **40.25** category 1 credits toward the AMA Physician's Recognition Award. (**Testis Workshop** for a maximum of **16.75** category 1 credits; **Andrology Lab Workshop** for a maximum of **8.25** category 1 credits, and the **ASA Annual Meeting** for a maximum of **18.25** category 1 credits) Each physician should claim only those credits that he/she actually spent in the educational activity.

mark your calendars!

ASA 31ST ANNUAL CONFERENCE

April 8 – 11, 2006

Hyatt Regency Chicago on the Riverwalk

Chicago, Illinois

Andrology Lab Workshop: April 8, 2006

Postgraduate Course: April 8, 2006

ASA Annual Meeting: April 8 – 11, 2006

schedule of events

XVIII North American Testis Workshop

Testicular Cell Dynamics and Endocrine Signaling
March 30 – April 2, 2005
Location: Princessa (unless otherwise noted)
Registration fee includes entry into the workshop, a syllabus, continental breakfasts and refreshment breaks.

WEDNESDAY, MARCH 30, 2005

6:00 p.m. – 8:30 p.m. **Registration**
Location: Lobby Alcove

7:00 p.m. **Welcome Address**
Matthew Hardy, PhD

7:15 p.m. – 8:30 p.m. **Keynote Address:**
The Genetics of Sex Determination
Eve Eicher, PhD
The Jackson Lab
(Introduced by MaryAnn Handel, PhD)

THURSDAY, MARCH 31, 2005

7:00 a.m. – 6:00 p.m. **Registration**
Location: Lobby Alcove

7:30 a.m. – 8:30 a.m. **Continental Breakfast**
Location: Leonesa Ballroom

8:30 a.m. – 9:30 a.m. **Benchmark Lecture: Hormonal Regulation of Male Reproductive Tract Development**
Luis Parada, PhD
UT Southwestern

PLATFORM SESSION I: DEVELOPMENT
Chair: Janice Bailey, PhD

9:30 a.m. – 10:15 a.m. **Anti-Mullerian Hormone**
Richard Behringer, PhD
The University of Texas MD Anderson Cancer Center

10:15 a.m. – 10:45 a.m. **Break**

10:45 a.m. – 11:30 a.m. **Seminiferous Cord Formation and Germ Cell Survival**
Michael Skinner, PhD
Washington State University

11:30 a.m. – 12:15 p.m. **Male Gametes Derived From Embryonic Stem Cells**
Niels Geijsen, PhD
Center for Regenerative Medicine and Technology, Massachusetts General Hospital

12:15 p.m. – 1:30 p.m. **Break**

PLATFORM SESSION II: THE ENDOCRINE AXIS
Chair: Deborah O'Brien, PhD

1:30 p.m. – 2:15 p.m. **Structure of the LH Receptor**
J. David Puett, PhD
University of Georgia

2:15 p.m. – 3:00 p.m. **SF-1 Rescue Mice**
Leslie Heckert, PhD
University of Kansas

3:00 p.m. – 3:30 p.m. **Break**

3:30 p.m. – 4:15 p.m. **Estrogens in Testis Biology**
Philippa Saunders, PhD
MRC Human Reproductive Sciences Unit, Centre for Reproductive Biology

4:15 p.m. – 5:00 p.m. **Brief presentations from submitted abstracts**

5:00 p.m. **Poster Session I**
Location: Leonesa Ballroom

FRIDAY, APRIL 1, 2005

7:00 a.m. – 6:00 p.m. **Registration**
Location: Lobby Alcove

7:30 a.m. – 8:30 a.m. **Continental Breakfast**
Location: Leonesa Ballroom

8:30 a.m. – 9:30 a.m. **Benchmark Lecture: Steroidogenesis**
L. Walter Miller, MD
University of California – San Francisco

PLATFORM SESSION III: STEROIDOGENESIS
Chair: Vassilios Papadopoulos, PhD

9:30 a.m. – 10:15 a.m. **Neuroendocrine Regulation of the Leydig Cell Development**
Peter O'Shaughnessy, PhD
University of Glasgow Veterinary School

10:15 a.m. – 10:45 a.m. **Break**

10:45 a.m. – 11:30 a.m. **Mitochondrial Function in Steroidogenesis**
Dale "Buck" Hales, PhD
University of Illinois – Chicago

11:30 a.m. – 12:15 p.m. **Steroid Dehydrogenases**
William L. Duax, PhD
Hauptman-Woodward Medical Research Institute

12:15 p.m. – 1:30 p.m. **Break**

PLATFORM SESSION IV: SPERMATOGENESIS
Chair: Patricia DeLeon, PhD

1:30 p.m. – 2:15 p.m. **Pubertal Onset of Spermatogenesis**
Tony M. Plant, PhD
University of Pittsburgh

schedule of events

2:15 p.m. – 3:00 p.m.	**Messenger RNA Polyadenylation in Germ Cells** *Clint MacDonald, PhD* *Texas Tech University*
3:00 p.m. – 3:30 p.m.	**Break**
3:30 p.m. – 4:15 p.m.	**Drivers of Germ Cell Differentiation** *Kate Loveland, PhD* *Monash Institute of Reproduction and Development*
4:15 p.m. – 5:00 p.m.	**Brief presentations from submitted abstracts**
5:00 p.m.	**Poster Session II** *Location: Leonesa Ballroom*
6:00 p.m.	**Reception for Testis Workshop registrants** *Location: Leonesa Ballroom*

SATURDAY, APRIL 2, 2005

7:00 a.m. – 7:00 p.m.	**Registration** *Location: Lobby Alcove*
7:30 a.m. – 8:30 a.m.	**Continental Breakfast** *Location: Leonesa Ballroom*

PLATFORM SESSION V: CLINICAL CORRELATES
Chair: Michael Griswold, PhD

8:30 a.m. – 9:15 a.m.	**Benchmark Lecture: Sperm Contributions to the Embryo** *Ryuzo Yanagimachi, PhD* *University of Hawai'i Medical School*
9:15 a.m. – 10:30 a.m.	**Androgen Regulation of Human Spermatogenesis** *Jon Jarow, MD* *Johns Hopkins University*
10:30 a.m. – 11:30 a.m.	**Cyclic GMP-signaling in Sperm Chemotaxis** U. Benjamin Kaupp, PhD *Helmholtz Research Center – Juelich*

Andrology Lab Workshop

Sperm Morphology Workshop
April 2, 2005
Location: Eliza Anderson (unless otherwise noted)
Supported by contributions in kind from
Conception Technologies, Fertility Solutions, Inc.,
Fisher Scientific Company, LLC,
Hamilton Thorne Biosciences, Inc., MidAtlantic Diagnostic, Inc.,
Olympus America, Inc., and Penetrating Innovations, LLC.
Registration is limited to 50 people.
Registration fee includes a syllabus and refreshment break.
Peer approved by ABB for 0.8 CEU.

SATURDAY, APRIL 2, 2005

7:00 a.m. – 7:00 p.m.	**Registration** *Location: Lobby Alcove*
8:00 a.m. – 8:30 a.m.	**Overview of Sperm Morphology** **Classification Systems** *Susan Rothmann, PhD, HCLD* *Fertility Solutions, Inc.*
8:30 a.m. – 10:30 a.m.	**WHO 3rd Morphology Classification Method – Interactive Instruction** *Aniela Bollendorf, MT* *Cooper Center for IVF*
10:30 a.m. – 12:30 p.m.	**Breakout Modules (slide preparation, staining and CASA)** **Lunch (on your own)** *Michael Palladino, PhD* *Monmouth University* *Fertility Solutions, Inc.*
12:30 p.m. – 2:15 p.m.	**Strict Criteria Classification Method – Interactive Instruction** *David Karabinus, PhD, HCLD* *Genetics and IVF Institute* *Susan Rothmann, PhD, HCLD* *Fertility Solutions, Inc.*
2:15 p.m. – 3:45 p.m.	**Standardization in Semen Analysis** *Lars Björndahl, PhD* *Birmingham Women's Hospital, UK*
3:45 p.m. – 4:00 p.m.	**Refreshment Break**
4:00 p.m. – 4:45 p.m.	**Importance of Quality Control and Accurate Statistics** *Stephen Simon, PhD* *Children's Mercy Hospital* *Steven Schrader, PhD* *NIOSH, CDC*
3:45 p.m. – 4:00 p.m.	**Q & A**

schedule of events

ASA 30th Annual Meeting

Androgens and Their Target Organs
Saturday, April 2 – Tuesday, April 5, 2005
Location: Princessa (unless otherwise noted)

FRIDAY, APRIL 1, 2005

12:00 p.m. — **Executive Council Meeting**
Location: Douglas Boardroom

2:00 p.m. – 6:00 p.m. — **Registration**
Location: Lobby Alcove

SATURDAY, APRIL 2, 2005

7:00 a.m. – 7:00 p.m. — **Registration**
Location: Lobby Alcove

4:00 p.m. – 9:30 p.m. — **Exhibit Hall Open**
Location: Leonesa Foyer

6:00 p.m. – 6:10 p.m. — **Welcome and Opening Remarks**
President: William J. Bremner, MD, PhD
University of Washington

Local Arrangements Chair:
Charles H. Muller, PhD
University of Washington, Department of Urology

Program Chair: Christina Wang, MD
Harbor-UCLA Medical Center & Los Angeles Biomedical Research Institute

6:10 p.m. – 6:30 p.m. — **Distinguished Andrologist Award**

6:30 p.m. – 7:30 p.m. — **ASA KEYNOTE LECTURE**
The Y Chromosome
David Page, PhD
Whitehead Institute
(Introduced by William J. Bremner, MD, PhD)

7:30 p.m. – 9:30 p.m. — **Welcome Reception**
Location: Leonesa Foyer

SUNDAY, APRIL 3, 2005

7:00 a.m. – 6:00 p.m. — **Registration**
Location: Lobby Alcove

7:00 a.m. – 8:00 a.m. — **Past President's Breakfast**
Location: Douglas Boardroom

7:00 a.m. – 4:00 p.m. — **Exhibit Hall Open**
Location: Leonesa Foyer

8:00 a.m. – 9:00 a.m. — **AUA LECTURE**
Androgens and the Prostate Cancer Prevention Trial
Ian M. Thompson Jr., MD
University of Texas Health Sciences Center
(Introduced by Jon Pryor, MD)
Supported in part by an unrestricted educational grant from the American Urological Association, Inc.

9:00 a.m. – 9:15 a.m. — **Distinguished Service Award**

9:15 a.m. – 10:45 a.m. — **SYMPOSIUM I – REGULATORS OF PROSTATE CANCER GROWTH: POTENTIAL NOVEL THERAPIES**
Co-chairs: Gail Prins, PhD, and Ian Thompson Jr., MD

Androgen Receptors as a Therapeutic Target in Androgen Independent Prostate Cancer
Steven Balk, MD, PhD
Beth Israel Deaconess Medical Center

Stress Induced Antiapoptotic Genes and Clusterins as Targets for Prostate Cancer Therapy
Martin Gleave, MD
University of British Columbia

Prostate Cancer Genomics: Identification of Potential Therapeutic Targets
Peter Nelson, MD
University of Washington, Seattle

10:45 a.m. – 12:30 p.m. — **Poster Session I (with refreshments)**
Location: Leonesa Ballroom
Supported in part by unrestricted educational grants from GlaxoSmithKline, Lilly ICOS LLC, Ortho-McNeil Pharmaceutical, Inc., and Pfizer, Inc.

12:30 p.m. – 2:00 p.m. — **Lunch (on your own)**

12:30 p.m. – 2:00 p.m. — **Women in Andrology Luncheon and Roundtable Discussion**
(not included in registration fee; tickets required)

2:00 p.m. – 3:30 p.m. — **Concurrent Oral Sessions**
Location: Menies Suite

Oral Session I: Clinical Andrology
Co-chairs: Alvin M. Matsumoto, MD, Seattle, WA; Daniela Bomgardner, MD, Charlottesville, VA

02:00 PM #1 — **CORRELATING ANDROGEN AND ESTROGEN STEROID RECEPTOR EXPRESSION WITH CORONARY CALCIFICATION AND ATHEROSCLEROSIS IN MEN WITHOUT KNOWN CORONARY ARTERY DISEASE**
Peter Liu, MBBS PhD, Rose Christian, MD, Ming Ruan, BSc, Virginia Miller, PhD and Lorraine Fitzpatrick, MD (Presented By: Peter Liu, MBBS PhD)

02:15 PM #2 — **ARE TOTAL TESTOSTERONE LEVELS AFFECTED BY CHANGES IN OBESITY? MASSACHUSETTS MALE AGING STUDY RESULTS**
Beth Mohr, MS, Amy O'Donnell, MPH and John McKinlay, PhD (Presented By: Beth Mohr, MS)

02:30 PM #3 **ABSORBTION OF ORAL TESTOSTERONE IN OIL IS AUGMENTED BY 5-ALPHA REDUCTASE INHIBITION IN MAN**
John Amory, MD, Stephanie Page, MD, PhD and William Bremner, MD, PhD (Presented By: John Amory, MD)

02:45 PM #4 **ARE MEN WITH ERECTILE DYSFUNCTION MORE LIKELY TO HAVE DIABETES MELLITUS THAN MEN WITHOUT ERECTILE DYSFUNCTION—EVIDENCE FROM A LARGE MANAGED CARE CLAIMS DATABASE?**
Peter Sun, MD, PhD, Ann Cameron, PhD, Allen Seftel, MD, Ridwin Shabsigh, MD, Craig Niederberger, MD and Andy Guay, MD (Presented By: Peter Sun, MD, PhD)

03:00 PM #5 **DYSREGULATION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE PHOSPHORYLATION MECHANISMS IN DIABETES-ASSOCIATED ERECTILE DYSFUNCTION**
Arthur Burnett, M.D., Melissa Kramer, B.A., Robyn Becker, B.A. and Biljana Musicki, Ph.D. (Presented By: Arthur Burnett, M.D.)

03:15 PM #6 **INCREASED SPERM OOCYTE PENETRATION CAPACITY FOLLOWING MAGNETIC CELL SEPARATION**
Tamer Said, M.D., Sonja Grunewald, M.D., Uwe Paasch, M.D., Ph.D., Christian Kriegel, Liang Li, Ph.D., Hans-Juergen Glander, M.D., Ph.D. and Ashok Agarwal, Ph.D. (Presented By: Tamer Said, M.D.)

Oral Session II: Spermatozoa/Male Infertility
Co-chairs: Steven M. Schrader, PhD, Cincinnati, OH; Carl Lessard, PhD, Bar Harbor, ME
Location: Eliza Anderson

02:00 PM #7 **EFFECT OF HSP60 AND GRP78 ON HUMAN SPERM FUNCTIONS**
Catherine Lachance, MSc, Mathieu Boilard, PhD and Pierre Leclerc, PhD (Presented By: Catherine Lachance, MSc)

02:15 PM #8 **DEFECTIVE SPERM TAIL FORMATION IS PHENOTYPE OF REPRO2 SPERM, AN ENU-INDUCED MOUSE MUTATION**
Carl Lessard, PhD, Suzane A Hartford, John C Schimenti, PhD and Mary Ann Handel, PhD (Presented By: Carl Lessard, PhD)

02:30 PM #9 **IN VITRO STIMULATION OF HUMAN SPERM MOTILITY**
Michaela Luconi, PhD, Gianni Forti, MD and Elisabetta Baldi, PhD (Presented By: Michaela Luconi, PhD)

02:45 PM #10 **A MUTATION IN THE PROTAMINE 1 GENE OF TWO INFERTILE MEN CREATES A PUTATIVE NEW SITE FOR PHOSPHORYLATION**
Naoko Iguchi, PhD, Sicheng Yang, MD, PhD, Dolores Lamb, PhD and Norman Hecht, PhD (Presented By: Naoko Iguchi, PhD)

03:00 PM #11 **IDENTIFICATION OF PP60C-SRC AS THE ENZYME RESPONSIBLE FOR TYROSINE PHOSPHORYLATION AND CONSEQUENT HYPERACTIVATION DURING MOUSE SPERM CAPACITATION**
Mark Baker, PhD and Robert Aitken, PhD, ScD (Presented By: Mark Baker, PhD)

03:15 PM #12 **TWO FIBRINOGEN-LIKE PROTEINS, FGL1 AND FGL2 ARE DISULFIDE-LINKED SUBUNITS OF AN OLIGOMER SECRETED BY THE HAMSTER CAUDA EPIDIDYMIDIS THAT BINDS DEFECTIVE SPERMATOZOA**
Subir Nagdas, Ph.D., Virginia Winfrey, B.S., Michael Melner, Ph.D. and Gary Olson, Ph.D. (Presented By: Subir Nagdas, Ph.D.)

3:30 p.m. – 4:00 p.m. **Refreshment Break**

4:00 p.m. – 5:00 p.m. **WOMEN IN ANDROLOGY LECTURE**
Novel Glycolytic Enzymes and Sperm Motility
Deborah A. O'Brien, PhD
University of North Carolina at Chapel Hill
(Introduced by Janice Bailey, PhD)

5:00 p.m. – 6:00 p.m. **DEBATE: THERE ARE SIGNIFICANT INCREASED RISKS IN THE OFFSPRING AFTER ART**
Moderator: Paul Turek, MD
UCSF
Yes *- Dolores Lamb, PhD*
Baylor College of Medicine/ Urology

No *- Peter N. Schlegel, MD*
The New York Weill/Cornell Medical Center

6:30 p.m. – 8:30 p.m. **Trainee Forum and Mixer**
Location: Leonesa Foyer

MONDAY, APRIL 4, 2005

7:00 a.m. – 6:00 p.m. **Registration**
Location: Lobby Alcove

7:00 a.m. – 4:30 p.m. **Exhibit Hall Open**
Location: Leonesa Ballroom

7:00 a.m. – 8:00 a.m. **MINORITY AFFAIRS BREAKFAST AND MEETING**
Missing Persons: Minorities in the Health Professions, and Implications for the Nation's Health
Keith C. Norris, MD
Location: Blewett Suite, 7th Floor
Sign up at the registration desk by 2:00 p.m. on Sunday, April 3.

schedule of events

8:00 a.m. – 9:00 a.m. **LECTURE**

Gene Therapy for Erectile Dysfunction
Wayne J. G. Hellstrom, MD
Tulane University School of Medicine
(Introduced by Catherine Naughton, MD)
Supported in part by unrestricted educational grants from GlaxoSmithKline, Lilly ICOS LLC, Ortho-McNeil Pharmaceutical, Inc., and Pfizer, Inc.

9:00 a.m. – 9:15 a.m. **Young Andrologist Award**

9:15 a.m. – 10:45 a.m. **SYMPOSIUM II – ANDROGEN REGULATION OF REPRODUCTIVE TISSUES**
(honoring C. Alvin Paulsen, MD)
Co-chairs: David DeKretser, MD, and Richard Sherins, MD
Supported in part by an unrestricted educational grant from Solvay Pharmaceuticals.

Nongenomic Steroid Receptors on Sperm – Signaling and Function
Michaela Luconi, PhD
University of Florence

Androgens and Epididymal Genomics
Shayesta Seenundun, BSc
McGill University

Androgen Regulation of Vas Deferens Proteins
Laurent Morel, PhD
University Blaise Pascal

10:45 a.m. – 12:00 p.m. **Poster Session II (withrefreshments)**
Location: Leonesa Ballroom
Supported in part by unrestricted educational grants from GlaxoSmithKline, Lilly ICOS LLC, Ortho-McNeil Pharmaceutical, Inc., and Pfizer, Inc.

12:00 p.m. – 2:00 p.m. **Lunch (on your own)**

12:00 p.m. – 1:30 p.m. **LAB SCIENCE FORUM AND LUNCHEON**
Vasectomy and Reversal: A View from the Lab Bench
Sheldon Marks, MD
International Center for Vasectomy Reversal
Location: Menzies Suite
(not included in registration; tickets required)

12:00 p.m. – 1:30 p.m. **Editorial Board Luncheon**
Location: Stevens

2:00 p.m. – 3:00 p.m. **Concurrent Oral Sessions**

Oral Session III: Testis
Co-chairs: Jacquetta M. Trasler, MD, PhD, Montreal, Quebec; Sarah Netzel-Arnett, PhD, Rockville, MD
Location: Eliza Anderson

02:00 PM #13 **DISSECTING THE AXONEME INTERACTOME: THE MAMMALIAN ORTHOLOGUE OF CHLAMYDOMONAS PF6 INTERACTS WITH SPAG6, THE MAMMALIAN ORTHOLOGUE OF CHLAMYDOMONAS PF16**
Zhibing Zhang, MD, Ph.D, Brian Jones, Waixing Tang, Stuart Moss, Clement Ho, Jean Bennett, Michael Baker and Jerome Strauss Iii, MD, Ph.D
(Presented By: Zhibing Zhang, MD, Ph.D)

02:15 PM #14 **TESTICULAR FAILURE AND LEARNING DEFICITS IN ADULT XXY MICE**
Yanhe Lue, M.D., David Jentsch, Ph.D., Christina Wang, M.D., Amiya Sinhahikim, Ph.D., Wael Salameh, M.D. and Ronald Swerdloff, M.D. (Presented By: Yanhe Lue, M.D.)

02:30 PM #15 **ROLE OF TESTISIN (PRSS21) IN MALE FERTILITY**
Sarah Netzel-Arnett, Ph.D., Thomas Bugge, Ph.D. and Toni Antalis, Ph.D.
(Presented By: Sarah Netzel-Arnett, Ph.D.)

02:45 PM #16 **EFFECTS OF THE ANTIESTROGEN ICI 182,780 ON MALE REPRODUCTIVE TRACTS IN THE MATURE DOG AND MARMOSET MONKEY**
Kay Carnes, MS, Carla Morrow, MS, DVM, Paul Klopfenstein, DVM, Sheila McCullough, MS, DVM, Debra Sauberli, DVM and Rex Hess, PhD (Presented By: Kay Carnes, MS)

Oral Session IV: Toxicology/Immunology
Co-chairs: Mitch Eddy, PhD, Research Triangle Park, NC; Ebtesam Attaya, Lubbock, TX
Location: Blewitt Suite

02:00 PM #17 **RELATIVE ROLES OF TESTOSTERONE AND FSH IN THE INHIBITION OF SPERMATOGONIAL DIFFERENTIATION IN IRRADIATED RATS**
Gunapala Shetty, Ph.D., Connie Weng, M.D., Ph.D., Zhen Zhang, M.D., Ph.D., Sarah Meachem, Ph.D. and Marvin Meistrich, Ph.D.
(Presented By: Marvin Meistrich, Ph.D.)

02:15 PM #18 **15-DEOXY-$\Delta^{12,14}$-PROSTAGLANDIN J2 INDUCES REACTIVE OXYGEN SPECIES-MEDIATED AND CASPASE-DEPENDENT MECHANISMS IN LEYDIG PROGENITORS**
Anke Diemert, M.D., Emanuela Clavarino, Ph.D., Keumsil Hwang, M.S., Laurence Walch, Ph.D. and Patricia Morris, Ph.D.
(Presented By: Anke Diemert, M.D.)

02:30 PM #19 **GALECTIN-3 AND CANDIDATE BINDING LIGANDS IN HUMAN PROSTASOMES**
Sarika Saraswati, M.S., Jennifer Jones, M.S., Ashley Block, B.S., Cheryl Lichti, Ph.D. and Alan Diekman, Ph.D.
(Presented By: Sarika Saraswati, M.S.)

schedule of events

02:45 PM #20 **HISTONE H4 HYPERACETYLATION IN RAT ZYGOTIC PRONUCLEI FOLLOWING CHRONIC PATERNAL CYCLOPHOSPHAMIDE EXPOSURE**
Tara S. Barton, BSc, Bernard Robaire, PhD and Barbara F. Hales, PhD
(Presented By: Tara S. Barton, BSc)

3:00 p.m. – 4:00 p.m. **LECTURE**
Toward a Comprehensive Genetic Analysis of Male Fertility Using Drosophila
Barbara Wakimoto, PhD
University of Washington
(Introduced by Kate Loveland, PhD)
Supported in part by an unrestricted educational grant from Promega Corp.

4:00 p.m. – 4:30 p.m. **Refreshment Break**

4:30 p.m. – 6:00 p.m. **SYMPOSIUM III – CELL BIOLOGY OF FERTILIZATION**
Co-chairs: Monica Vazquez-Levin, PhD, and Pablo Visconti, PhD

Sperm Acrosome Membranes, Matrix and Exocytosis
John C. Herr, PhD
Center for Research in Contraceptive and Reproduction

Regulation of Sperm-egg Interactions and Egg Membrane Dynamics
Janice P. Evans, PhD
Johns Hopkins University Bloomberg School of Public Health

Sperm Binding and Humanized Zona Pellucida
Tanya Hoodbhoy, PhD
NIDDK

7:00 p.m. – 11:00 p.m. **Annual Banquet at the Columbia Tower Club**
(not included in registration fee; tickets required)

TUESDAY, APRIL 5, 2005

7:00 a.m. – 8:00 a.m. **2006 Program Committee Meeting**
Location: Douglas Boardroom

7:30 a.m. – 11:00 a.m. **Registration**
Location: Lobby Alcove

8:00 a.m. – 9:30 a.m. **SYMPOSIUM IV – MALE CONTRACEPTION**
Co-Chair: Ronald D. Swerdloff, MD, and Geoffrey M.H. Waites, PhD
Supported in part by an unrestricted educational grant from Auxilium Pharmaceuticals, Inc.

Agents Acting Directly on the Testis: Will They be Reversible?
C. Yan Cheng, PhD
Population Council Center for Biomedical Research

Will There Ever be an Epididymal Male Contraceptive?
Barry T. Hinton, PhD
University of Virginia

Combination of Hormonal and Physical Agents – Are They More Efficacious and Why?
Amiya P. Sinha-Hikim, PhD
Harbor-UCLA Medical Center & Los Angeles BioMedical Research Institute

9:30 a.m. – 10:30 a.m. **INTERNATIONAL LECTURE**
Mutations in Male Infertility: Of Mice and Men
Yoshitake Nishimune, MD
Osaka University, Institute for Microbial Diseases
(Introduced by Patricia S. Cuasnicu, MD)
Supported in part by an unrestricted educational grant from ParentPlus, LLC.

10:30 a.m. – 10:45 a.m. **Refreshment Break**

10:45 a.m. – 12:15 p.m. **SYMPOSIUM V – HOT TOPICS IN ANDROLOGY**
Co-chairs: Patricia Morris, PhD, and Nina Davis, MD
Supported in part by unrestricted educational grants from American Medical Systems, Inc., and Mentor Corp.

Prostatis – What is New?
Richard E. Berger, MD
University of Washington, Seattle

Cell-Specific Knockout of the Androgen Receptor in Testis – What are the Physiological Implications?
Chawnshang Chang, PhD
University of Rochester

Germ Cell Transplantation – Where Will This Technology Go?
Ina Dobrinski, DVM, PhD
University of Pennsylvania

12:15 p.m. **ASA Business Meeting, Trainee Awards**

poster session 1

SUNDAY, APRIL 3, 2005
10:45 a.m. – 12:30 p.m.
Location: Leonesa Ballroom

ASSISTED REPRODUCTION & INFERTILITY

Poster #21 **INCREASED SPERM OOCYTE PENETRATION CAPACITY FOLLOWING MAGNETIC CELL SEPARATION**
Tamer Said, M.D., Sonja Grunewald, M.D., Uwe Paasch, M.D., Ph.D., Christian Kriegel, Liang Li, Ph.D., Hans-Juergen Glander, M.D., Ph.D. and Ashok Agarwal, Ph.D. (Presented By: Tamer Said, M.D.)

Poster #22 **INFLUENCE OF SPERM DEOXYRIBONUCLEIC ACID DAMAGE ON REPRODUCTIVE OUTCOMES WITH ICSI**
Armand Zini, MD, James Meriano, B. Sc., Karim Kader, MD, Keith Jarvi, MD, Carl Laskin, MD and Kenneth Cadesky, MD (Presented By: Armand Zini, MD)

Poster #23 **IN VITRO STIMULATION OF HUMAN SPERM MOTILITY**
Michaela Luconi, PhD, Gianni Forti, MD and Elisabetta Baldi, PhD (Presented By: Michaela Luconi, PhD)

Poster #24 **THE EFFECACY OF CHOOSING SPERM BY NUCLEAR CHARACTERISTICS PRIOR TO INTRACYTOPLASMIC SPERM INJECTION FOR REFRACTORY IN VITRO FERTILIZATION CASES WITH SPERM WITH ABNORMAL SPERM CHROMATIN STRUCTURE ASSAY TESTS**
Jerome Check, M.D., Ph.D., Donna Summers-Chase, M.S., Danya Horwath, B.S., Wei Yuan, Ph.D. and Theresa Weisak, Ph.D. (Presented By: Jerome Check, M.D., Ph.D.)

Poster #25 **PSYCHOLOGICAL STRESS AND SEMEN QUALITY AMONG MEN UNDERGOING INTRAUTERINE INSEMINATION**
Fabio Pasqualotto, MD, PhD, Eleonora Pasqualotto, MD, PhD, Ks Ogliari, MD, PhD, Shyam Allamaneni, MD and Ashok Agarwal, PhD (Presented By: Shyam Allamaneni, MD)

Poster #26 **ANALYSIS OF PARTIAL AZFc DELETION ON THE Y CHROMOSOME IN JAPANESE POPULATION**
Futoshi Matsui, MD, Eitetsu Koh, MD, PhD, Jin Choi, BS, Mikio Namiki, MD, PhD and Atsumi Yoshida, MD, PhD (Presented By: Futoshi Matsui, MD)

Poster #27 **THE INTUSSUSCEPTED VASOVASOSTOMY: A SIMPLE, NOVEL TECHNIQUE**
Aaron Milbank, MD, Wayne Kuang, MD and Anthony Thomas, MD (Presented By: Aaron Milbank, MD)

Poster #28 **WITHDRAWN**

Poster #29 **ROLE OF INHIBIN B IN THE EVALUATION OF MALE INFERTILITY**
Shyam Allamaneni, M.D., Philip Kumanov, M.D., A Tomova, M.D., Rupesh Raina, M.D. and Ashok Agarwal, Ph.D. (Presented By: Shyam Allamaneni, M.D.)

Poster #30 **A MUTATION IN THE PROTAMINE 1 GENE OF TWO INFERTILE MEN CREATES A PUTATIVE NEW SITE FOR PHOSPHORYLATION**
Naoko Iguchi, PhD, Sicheng Yang, MD, PhD, Dolores Lamb, PhD and Norman Hecht, PhD (Presented By: Naoko Iguchi, PhD)

Poster #31 **DETERMINATION OF COENZYME Q10 IN HUMAN SEMINAL PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION**
Ke Li, Xuejun Shang and Suang Chen (Presented By: Ke Li)

Poster #32 **SPERM COATED WITH ANTISPERM ANTIBODIES DO NOT RESULT IN A HIGHER MISCARRIAGE RATE IN PREGNANCIES ACHIEVED BY IN VITRO FERTILIZATION AND INTRACYTOPLASMIC SPERM INJECTION**
Lynn Sansoucie, M.T., B.S., Brittney Katsoff, B.A. and Jerome Check, M.D., Ph.D. (Presented By: Lynn Sansoucie, M.T.,B.S.)

MALE SEXUAL DYSFUNCTION

Poster #33 **MULTIPLE OBSERVATIONS IN MEN WITH ED IN NATIONAL TADALAFIL STUDY IN THE US (MOMENTUS)**
Abraham Morgentaler, MD, FACS, James Barada, MD, Craig Niederberger, MD, Carmen S Garcia, PhD, Fanni Natanegara, PhD, Sanjeev Ahuja, MD and David G Wong, MD (Presented By: Abraham Morgentaler, MD, FACS)

Poster #34 **SELF-ESTEEM AND ERECTILE FUNCTION AFTER 9 MONTHS OF SILDENAFIL CITRATE TREATMENT**
Stanley E. Althof, PhD, Michael O'Leary, MD, MPH, Joseph C. Cappelleri, PhD, Richard L. Siegel, MD, Arthur R. Crowley, MD MPH, James Harnett, PharmD, Li-Jung Tseng and Suzanne Collins
(Presented By: Stanley E. Althof, PhD)

Poster #35 **PULMONARY HYPERTENSION IMPAIRS NEUROGENIC AND ENDOTHELIUM-DEPENDENT RELAXATION OF PENILE ERECTILE TISSUE IN RATS**
Serap Gur, PhD, Supat Thammasitboon, MD, Joseph A. Lasky, MD, Muammer Kendirci, MD, Philip J Kadowitz, PhD and Wayne JG Hellstrom, MD (Presented By: Serap Gur, PhD)

Poster #36 **THE PARP-1 INHIBITOR INO-1001 PRESERVES ERECTILE FUNCTION IN RATS AFTER CAVERNOSAL NERVE INJURY**
Muammer Kendirci, MD, Serap Gur, PhD, Csaba Szabo, MD, PhD, Trinity Bivalacqua, MD, PhD and Wayne JG Hellstrom, MD (Presented By: Muammer Kendirci, MD)

Poster #37 **VENOUS SURGERY HAS A ROLE IN TREATING PATIENTS WITH ERECTILE DYSFUNCTION: CLINICAL EVIDENCE OF SYNERGIC EFFECT WITH SILDENAFIL**
Cheng-Hsing Hsieh, MD, Geng-Long Hsu, MD, pei-Ying Ling, MD, Hsien-Sheng Wen, MD and Long-Jin Chi, MD
(Presented By: Cheng-Hsing Hsieh, MD)

Poster #38 **LONG-TERM POTENCY FOLLOWING RADICAL PROSTATECTOMY**
Craig Zippe, MD, Rupesh Raina, MD, Kalyana Nandipati, MD and Ashok Agarwal, PhD
(Presented By: Craig Zippe, MD)

Poster #39 **THE ROLE OF NON–ORAL TREATMENTS FOR EARLY PENILE REHABILITATION AFTER RP**
Rupesh Raina, MD, Kalyana Nandipati, MD, Ashok Agarwal, PhD and Criag Zippe, MD
(Presented By: Rupesh Raina, MD)

Poster #40 **CAN PURE LOCAL ANESTHESIA FOR OUTPATIENT PENILE SURGERIES BE STRICTLY FOLLOWED?**
Geng-Long Hsu, MD, Cheng-Hsing Hsieh, MD, Pei-Ying Ling, MD, Hsien-Sheng Wen, MD and Long-Jin Chi, MD
(Presented By: Geng-Long Hsu, MD)

Poster #41 **IS HIGH CHOLESTEROL A RISK FOR ERECTILE DYSFUNCTION? RESULTS BASED ON THE PENILE BLOOD FLOW INDEX (PBFI)**
Fabio Pasqualotto, MD, PhD, C Gromatzky, MD, PhD, M Cocuzza, MD, PhD, Rupesh Raina, MD, Jorge Hallak, MD, Am Lucon, MD, PhD, Shyam Allamaneni, MD and Ashok Agarwal, PhD (Presented By: Shyam Allamaneni, MD)

Poster #42 **IS BALLOONING FORMATION A NECESSARY COMPLICATION OF TUNICAL REPAIR?**
Cheng-Hsing Hsieh, MD, Geng-Long Hsu, MD, Pei-Ying Ling, MD, Hsien-Sheng Wen, MD and Long-Jin Chi, MD
(Presented By: Cheng-Hsing Hsieh, MD)

Poster #43 **THE ASSOCIATION BETWEEN MINIMUM TIME TO ONSET OF ACTION OF SILDENAFIL AND MINIMUM EFFECTIVE PLASMA SILDENAFIL LEVELS**
Harin Padma-Nathan, MD, Richard L. Siegel, MD, Vera J. Stecher, PhD and Herb Deriesthal
(Presented By: Harin Padma-Nathan, MD)

SPERMATOGENESIS/TESTIS

Poster #44 **INTERLEUKIN- 1α; AND INTERLEUKIN-1 RECEPTOR ANTAGONIST IN NORMAL OR IMPAIRED HUMAN SPERMATOGENESIS**
Natalia Rozwadowska, M.Sc, Dorota Fiszer, PhD, Piotr Jedrzejczak, PhD, Wlodzimierz Kosicki, PhD and Maciej Kurpisz, Prof PhD MD (Presented By: Natalia Rozwadowska, M.Sc)

Poster #45 **CKS2 REPRESSES CDK2/CCNA1-ASSOCIATED KINASE ACTIVITY**
Ebtesam Attaya, Ph.D., Stuart Ravnik, Ph.D. and Clinton Macdonald, Ph.D. (Presented By: Ebtesam Attaya, Ph.D.)

Poster #46 **DISSECTING THE AXONEME INTERACTOME: THE MAMMALIAN ORTHOLOGUE OF CHLAMYDOMONAS PF6 INTERACTS WITH SPAG6, THE MAMMALIAN ORTHOLOGUE OF CHLAMYDOMONAS PF16**
Zhibing Zhang, MD, Ph.D, Brian Jones, Waixing Tang, Stuart Moss, Clement Ho, Jean Bennett, Michael Baker and Jerome Strauss Iii, MD, Ph.D (Presented By: Zhibing Zhang, MD, Ph.D)

Poster #47 **EFFECT OF FINASTERIDE AND TESTOSTERONE UNDECANOATE ON REPRODUCTIVE FUNCTION IN MALE RATS**
Yue Jia, PHD, Xiaodong Wang, MD, Yugui Cui, MD, Xinghai Wang, MD, Jiansun Tong, MD, Fusong Di, MD and Jiahao Sha, PHD (Presented By: Yue Jia, PHD)

Poster #48 **CASPASES ARE EXECUTIONERS OF APOPTOSIS DURING THE FIRST WAVE OF SPERMIOGENESIS IN THE RAT**
Ricardo Moreno, PhD, Carlos Lizama, BSc and Natalia Urzúa, BSc (Presented By: Ricardo Moreno, PhD)

Poster #49 **METALLOTHIONEIN EXPRESSION IN MOUSE TESTIS AFTER BUSULFAN TREATMENT**
Sang Chul Han, MS, Sun-Hee Lee, MS, Seung Han Oh, MS, Jin Hyun Jun, Ph.D. and Yong-Seog Park, Ph.D. (Presented By: Sang Chul Han, MS)

Poster #50 **INVOLVEMENT OF P38 MAPK AND NITRIC OXIDE IN INDUCING MALE GERM CELL APOPTOSIS VIA INTRINSIC PATHWAY SIGNALING AFTER HOMONE DEPRIVATION**
Yanira Vera, MS, Mark Castanares, Concepcion Nunez, Yanhue Lue, MD, Christina Wang, MD, Ronald Swerdloff, MD and Amiya Sinha Hikim, PhD (Presented By: Yanira Vera, MS)

Poster #51 **THE USE OF AN IMMORTALIZED SERTOLI CELL LINE IN SERTOLI-GERM CELL COCULTURE**
Katja Wolski, B.S., Norbert Walther, PhD, Don Cameron, PhD and Christiane Kirchhoff, PhD (Presented By: Katja Wolski, B.S.)

Poster #52 **NUCLEAR RECEPTOR COFACTORS IN EFFERENT DUCTULES OF MOUSE, RAT, HAMSTER, AND MONKEY**
Carla Morrow, DVM, MS, Kay Carnes, MS, Cleida Oliveira, PhD, Jim Ford, Jr, PhD, Tameka Phillips, MS, Dusty Sachen and Rex Hess, PhD (Presented By: Carla Morrow, DVM, MS)

Poster #53 **NITRIC OXIDE FROM ACTIVATED TESTICULAR MACROPHAGES: A NEGATIVE PARACRINE MODULATOR OF TESTOSTERONE SYNTHESIS IN RAT LEYDIG CELLS**
Ben A. Weissman, Enmei Niu, Chantal M. Sottas, James C. Hutson and Matthew P. Hardy (Presented By: Chantal M. Sottas)

SEMEN ANALYSIS/SPERM

Poster #54 **IDENTIFICATION OF PP60C-SRC AS THE ENZYME RESPONSIBLE FOR TYROSINE PHOSPHORYLATION AND CONSEQUENT HYPERACTIVATION DURING MOUSE SPERM CAPACITATION**
Mark Baker, PhD and Robert Aitken, PhD, ScD (Presented By: Mark Baker, PhD)

Poster #55 **ADVANTAGE OF COMBINING MAGNETIC CELL SEPARATION WITH SPERM PREPARATION TECHNIQUES**
Tamer Said, M.D., Uwe Paasch, M.D., Ph.D., Sonja Grunewald, M.D., Thomas Baumann, Liang Li, Ph.D., Hans-Juergen Glander, M.D., Ph.D. and Ashok Agarwal, Ph.D. (Presented By: Tamer Said, M.D.)

Poster #56 **THE RELATIONSHIP BETWEEN HUMEN SEMEN CHARACTERISTICS AND SPERM APOPTOSIS: A PILOT STUDY**
Zuying Chen, MD, Russ Hauser, MD, ScD, Alexander Trbovich, MD, PhD, Jan Shifren, MD, David Dorer, PhD, Linda Godfrey-Bailey,, MSN, APRN, BC, and Narendra Singh, MBBS, MS (Presented By: Zuying Chen, MD)

Poster #57 **NEW LUBRICATING GEL FOR SEMEN COLLECTION DOES NOT DAMAGE MOUSE EMGRYOS, MUCOSAL MEMBRANES OR MALE FACTOR CONDOMS**
Joanna Ellington, DVM, PhD, G. Dennis Clifton, PharmD and Julie A. Schimmels, MS (Presented By: Joanna Ellington, DVM, PhD)

Poster #58 **ESTIMATION OF NUCLEAR PROTAMINE TO HISTONE RATIO IN THE SPERMATOZOA OF FERTILE AND INFERTILE MEN**
Xiaoyang Zhang, MD, Maria San Gabriel, Ph. D. and Armand Zini, MD (Presented By: Xiaoyang Zhang, MD)

Poster #59 **THE CONCENTRATION OF SPERMS ASSESSES BY 20MICRON CAPLIAARY FILLEDSLIDESIS UNDERESTIMATED. A METHOD FOR CORRECTION**
Jan Vermeiden, PhD, Joseph McDonnell, MSc and Diarmaid Douglas-Hamilton, MSc (Presented By: Jan Vermeiden, PhD)

Poster #60 **LECTIN CHARACTERIZATION OF MEMBRANE SURFACE CARBOHYDRATES IN POULTRY SPERMATOZOA**
Jesús Peláez, DVM, PhD and Julie Long, PhD (Presented By: Jesús Peláez, DVM, PhD)

Poster #61 **IDENTIFICATION OF A DECAPACITATION FACTOR: A CASE FOR PLATELET-ACTIVATING FACTOR-ACETYLHYDROLASE**
William Roudebush, PhD, Jim Zhu, MS, Dorothy Mitchell-Leef, MD, Carlene Elsner, MD, Daniel Shapiro, MD, Hilton Kort, MD and Joe Massey, MD (Presented By: William Roudebush, PhD)

Poster #62 **ESTIMATION OF SPERM DEOXYRIBONUCLEIC ACID DAMAGE BY GENE-SPECIFIC POLYMERASE CHAIN REACTION (PCR) ANALYSIS**
Maria San Gabriel, Ph. D., Xiaoyang Zhang, MD and Armand Zini, MD (Presented By: Maria San Gabriel, Ph. D.)

Poster #63 **BETA-CYCLODEXTRIN PLUS CHOLESTEROL PROTECTS PORCINE SPERM FROM THE EFFECTS OF COLD SHOCK**
Hannah Galantino-Homer, VMD, PhD, DACT, Wenxian Zeng, PhD, Susan Megee, BS, Modesty Dallmeyer, BS, Dawna Voelkl, DVM and Ina Dobrinski, Dr.vet.med., PhD, DACT (Presented By: Hannah Galantino-Homer, VMD, PhD, DACT)

ANDROGENS/HORMONES

Poster #64 **THE ACCURATE DEFINITION OF REFERENCE INTERVALS FOR THE DIAGNOSIS OF ANDROGEN AND REPRODUCTIVE HORMONE STATUS IN MEN**
David De Kretser, MBBS, MD, FRACP, FAA, FTSE, David Handelsman, MBBS, FRACP, PhD, Ken Sikaris, MD, Carol Holden, PhD, Nick Balasz and Rob McLachlan, MBBS, FRACP, PhD (Presented By: David De Kretser, MBBS, MD, FRACP, FAA, FTSE)

Poster #65 **HOW DO AGE-RELATED DECLINES IN TESTOSTERONE AFFECT HIGH DENSITY LIPOPROTEIN CHOLESTEROL? LONGITUDINAL RESULTS FROM THE MASSACHUSETTS MALE AGING STUDY**
Beth Mohr, MS, Stephanie Page, MD PhD, Andre Araujo, MA, William Bremner, MD PhD, Amy O'Donnell, MPH, Brian Walsh, MD and John McKinlay, PhD (Presented By: Beth Mohr, MS)

Poster #66 **CUBITAL LH AND TESTOSTERONE CONCENTRATIONS ARE NOT CORRELATED TO SCROTAL TESTOSTERONE VALUES IN YOUNG MEN WITH UNILATERAL VARICOCELE**
Thorsten Bach, M.D., Dietrich Pfeiffer, M.D. and Roland Tauber, M.D. (Presented By: Thorsten Bach, M.D.)

Poster #67 **EXPECTATIONS TOWARDS A NOVEL MALE FERTILITY CONTROL METHOD AND POTENTIAL USER TYPES**
Klaas Heinemann, MD, MBA and Farid Saad, DVM (Presented By: Farid Saad, DVM)

Poster #68 **DOES DECLINING CORTISOL CAUSE INCREASED ADIPOSITY? RESULTS FROM THE MASSACHUSETTS MALE AGING STUDY**
Amy O'Donnell, MPH, Beth Mohr, MS, Sophia Zilber, MS, Andre Araujo, MA and John McKinlay, PhD (Presented By: Amy O'Donnell, MPH)

Poster #69 **CORRELATING ANDROGEN AND ESTROGEN STEROID RECEPTOR EXPRESSION WITH CORONARY CALCIFICATION AND ATHEROSCLEROSIS IN MEN WITHOUT KNOWN CORONARY ARTERY DISEASE**
Peter Liu, MBBS PhD, Rose Christian, MD, Ming Ruan, BSc, Virginia Miller, PhD and Lorraine Fitzpatrick, MD (Presented By: Peter Liu, MBBS PhD)

FERTILIZATION

Poster #70 **REACTIVE OXYGEN SPECIES PROMOTE THE PHOSPHORYLATION OF MEK-LIKE PROTEINS DURING HUMAN SPERM CAPACITATION**
Cristian O'Flaherty, PhD, Eve De Lamirande, PhD and Claude Gagnon, PhD (Presented By: Cristian O'Flaherty, PhD)

Poster #71 **IMMUNOLOCALIZATION OF N-CADHERIN IN HUMAN SPERM AND EVALUATION OF ITS INVOLVEMENT IN FERTILIZATION**
Clara Marín-Briggiler, PhD, Ezequiel Lentz, (student), Florencia Veiga, MSc, Fernanda Gonzalez-Echeverría, MSc and Mónica Vazquez-Levin, PhD (Presented By: Clara Marín-Briggiler, PhD)

Poster #72 **IDENTIFICATION OF EPITHELIAL CADHERIN PROTEIN FORMS IN HUMAN SPERM AND EVALUATION OF THEIR LOCALIZATION IN WHOLE CELLS**
Ezequiel Lentz, (Studeent), Clara Marin-Briggiler, PhD, Florencia Veiga, MSc and Mónica Vazquez-Levin, PhD (Presented By: Mónica Vazquez-Levin, PhD)

Poster #73 **SUBCELLULAR DISTRIBUTION OF FUCOSE BINDING SITES ON HUMAN SPERM**
Kate Donigan, BS anticipated 05/2005, Jennifer Venditti, BS and Barry Bean, PhD (Presented By: Kate Donigan, BS anticipated 05/2005)

Poster #74 **SUBCELLULAR IMMUNOLOCALIZATION OF ALPHA-L-FUCOSIDASE IN HUMAN SPERM CELLS**
Jennifer Venditti, B.S., Kate Donigan, B.S., anticipated 2005 and Barry Bean, Ph.D. (Presented By: Jennifer Venditti, B.S.)

Poster #75 **INVESTIGATION ON THE CROSS-REACTIVE ANTIGENS BETWEEN UREAPLASMA UREALYTICUM (UU) AND HUMAN SPERM MEMBRANE PROTEINS**
Chen Xu, PhD, MD, Jianli Shi, PhD and Yuancong Zhou, PhD (Presented By: Chen Xu, PhD, MD)

ENVIRONMENT/TOXICOLOGY

Poster #76 **HISTONE H4 HYPERACETYLATION IN RAT ZYGOTIC PRONUCLEI FOLLOWING CHRONIC PATERNAL CYCLOPHOSPHAMIDE EXPOSURE**
Tara S. Barton, BSc, Bernard Robaire, PhD and Barbara F. Hales, PhD (Presented By: Tara S. Barton, BSc)

Poster #77 **SERUM TESTOSTERONE LEVELS AND PUBERTY IN RATS TREATED WITH DI(2-THYLHEXYL)PHTHALATE (DEHP)**
Qiang Dong, MD & PhD, Chantal Sottas, BA, Renshan Ge, PhD, Benson Akingbemi, PhD & DVM and Matthew Hardy, PhD (Presented By: Qiang Dong, MD & PhD)

Poster #78 **EFFECTS OF CADMIUM ON UBIQUITINATION PATHWAYS AND STRESS SIGNALING IN A PRIMARY SERTOLI CELL-GONOCYTE CO-CULTURE SYSTEM**
Xiaozhong Yu, MD, Ph.D, Jaspreet Sidhu, Ph.D, Sungwoo Hong, Bs and Elaine Faustman, Ph.D (Presented By: Xiaozhong Yu, MD, Ph.D)

Poster #79 **ESTROGEN-INDUCED ACCUMULATION OF FAT CELLS IN CORPORA CAVERNOSA PENIS DEPENDS UPON ESTROGEN EXPOSURE DURING CRITICAL PERIOD OF PENILE DEVELOPMENT**
Hari Goyal, DVM, PhD, Tim Braden, PhD, Carol Williams, MSc, Prasad Dalvi, MD, Mahmoud Mansour, DVM, PhD and John Williams, PhD (Presented By: Hari Goyal, DVM, PhD)

Poster #80 **GENE EXPRESSION PROFILING TO IDENTIFY MECHANISMS REGULATING TESTICULAR RESISTANCE OR SENSITIVITY TO INORGANIC LEAD (PB)**
Colleen Millan, MA, Rebecca Sokol, MD, MPH, Ian Hurley, PhD, Joel Marmar, MD and Susan Benoff, PhD (Presented By: Colleen Millan, MA)

Poster #81 **EFFECTS OF CHEMOTHERAPY FOR TESTICULAR CANCER ON REPRODUCTIVE ORGANS, SPERM COUNT, AND SPERM MOTILITY IN THE MALE RAT**
Adrienne Bieber, BSc, Ludovic Marcon, MSc, Barbara Hales, PhD and Bernard Robaire, PhD (Presented By: Adrienne Bieber, BSc)

Poster #82 **NEXT DAY DETERMINATION OF SPERM MOTILITY AFTER OVERNIGHT SHIPMENT OF SEMEN**
Robert Bennett, B.S., Gabor Huszar, M.D. and Mike Janes, Ph.D. (Presented By: Robert Bennett, B.S.)

poster session 2

MONDAY, APRIL 4, 2005
10:45 a.m. – 12:00 p.m.
Location: Leonesa Ballroom

ASSISTED REPRODUCTION & INFERTILITY

Poster #83 **PRESENCE OF SPERMATOZOA IN THE RETE TESTIS AND EPIDIDYMIS IN NON-OBSTRUCTED AZOOSPERMIC MEN**
Dimitrios Tsalikis, MD, Kimon Tsoukanelis, MD, Elias Pappas, MD, Anastasios Sylakos, MD, Nikolaos Kanakas, MD, PhD, Dimitrios Baltogiannis, MD. PhD and Nikolaos Sofikitis, MD, PhD (Presented By: Nikolaos Sofikitis, MD, PhD)

Poster #84 **COMPARING THE OUTCOMES OF IN VITRO FERTILIZATION BY TWO DIFFERENT DENSITY GRADIENT SEPARATION METHODS**
Sun-Hee Lee, M.Sc., Sang Chul Han, M.Sc., Hye Kyung Byun, M.Sc., Mi Kyoung Koong, MD, Ph. D. and Yong-Seog Park, Ph. D. (Presented By: Sun-Hee Lee, M.Sc.)

Poster #85 **COULD BIOTECHNOLOGY IMPROVE OUTCOME AFTER IUI?**
R.P. Amann, PhD, J.M. Dejarnette, PhD and C.M. Marshall, MS (Presented By: R.P. Amann, PhD)

Poster #86 **IS REACTIVE OXYGEN SPECIES (ROS) AN INDEPENDENT MARKER OF MALE FACTOR INFERTILITY?**
Kiran Nallella, M.D, Ravindrakumar Guruswamy, M.D and Ashok Agarwal, Ph.D, HCLD (Presented By: Ravindrakumar Guruswamy, M.D)

Poster #87 **INTRAINDIVIDUAL VARIATIONS AND THE EFFECT OF ANTIBIOTIC THERAPY ON HUMAN SEMINAL PLASMA IGA ANTIBODIES AGAINST CHLAMYDIA TRACHOMATIS**
Weidinger Stephan, M.D., Sbornik Martin, M.D., Ring Johannes, M.D., Ph.D. and Kohn Frank M., M.D., Ph.D. (Presented By: Weidinger Stephan, M.D.)

Poster #88 **ETIOLOGY OF MALE INFERTILITY AS DIAGNOSED WITHIN A COMPREHENSIVE INFERTILITY SERVICE PROGRAM**
Kiran Nallella, M.D., Nabil Aziz, M.D., Ravindrakumar Guruswamy, M.D., Sushil Prabakaran, M.D. and Ashok Agarwal, Ph.D. (Presented By: Kiran Nallella, M.D.)

Poster #89 **SELECTIVE SEROTONIN REUPTAKE INHIBITOR (SSRI) CAUSES SPERM CHROMATIN DAMAGE IN SMOKING MEN**
Clarke St. Dennis, PhD, Julie Schimmels, MS, Robert Short, PhD, G. Dennis Clifton, PharmD, David Grubb, MD, Don Evenson, PhD and Joanna Ellington, PhD (Presented By: Joanna Ellington, PhD)

Poster #90 **CYTOGENETIC DAMAGE IN MALE INFERTILITY**
Fotini Papachristou, MD, Theodoros Lialiaris, MD, Christos Kalaitzis, MD, PhD, Dimitrios Baltogiannis, MD, PhD, Nikolaos Sofikitis, MD, PhD and Stavros Touloupidis, MD, PhD (Presented By: Fotini Papachristou, MD)

Poster #91 **THE EFFECT OF TREATING SPERM WITH AUTOANTIBODIES WITH A PROTEIN DIGESTIVE ENZYME PRIOR TO INTRAUTERINE INSEMINATION ON PREGNANCY OUTCOME**
Jerome Check, M.D., Ph.D, Robert Hamburger, B.A., Wendy Hourani, B.A., Brittney Katsoff, B.A. and Kimberly McMonagle, M.T. (Presented By: Jerome Check, M.D., Ph.D)

Poster #92 **REPRODUCTIVE POTENTIAL OF SPERM WITH CHROMATIN DEFECTS DECLINES DURING EPIDIDYMAL PASSAGE AS REVEALED BY INTRACYTOPLASMIC SPERM INJECTION**
Ryota Suganuma, Marvin L. Meistrich, and Ryuzo Yanagimachi

MALE SEXUAL DYSFUNCTION

Poster #93 **INSUFFICIENT RESPONSE TO VENOUS SURGERY: IS PENILE VEIN RECURRENT OR RESIDUAL?**
Geng-Long Hsu, MD, Cheng-Hsing Hsieh, MD, Pei-Ying Ling, MD, Hsien-Sheng Wen, MD and Long-Jin Chi, MD (Presented By: Geng-Long Hsu, MD)

Poster #94 **REDOX SIGNALING MECHANISMS AND APOPTOTIC RESPONSE IN HUMAN CAVERNOSA UNDER OXIDATIVE STRESS**
Suresh Sikka, PhD, Xiangbin Zeng, PhD and Wayne JG Hellstrom, MD (Presented By: Suresh Sikka, PhD)

poster session 2

Poster #95 **TIMING OF SEXUAL INTERCOURSE POST TADALAFIL IN VARIOUS POPULATIONS OF MEN IN MOMENTUS (MULTIPLE OBSERVATIONS IN MEN WITH ED IN NATIONAL TADALAFIL STUDY IN THE US)**
Laurence Levine, MD, FACS, Albert Levy, MD, James McMurray, MD, Carmen S Garcia, PhD, Fanni Natanegara, PhD, David G Wong, MD and Sanjeev Ahuja, MD (Presented By: Laurence Levine, MD, FACS)

Poster #96 **CHRONIC INHIBITION OF PHOSPHODIESTERASE-5 INDUCES LONG-TERM POTENTIATION OF ERECTILE FUNCTION IN AGED BUT NOT YOUNG RATS**
Arthur Burnett, M.D., Robyn Becker, B.A., Hunter Champion, M.D., Ph.D., Tongyun Liu, M.S., Melissa Kramer, B.A. and Biljana Musicki, Ph.D. (Presented By: Arthur Burnett, M.D.)

Poster #97 **SILDENAFIL CITRATE TREAT ERECTILE DYSFUNCTION AFTER KIDNEY TRANSPLANTATION**
Yong Zhang, MD, Delin Guan, MD, Tongwen Ou, MD, Yong Wang, MD, Xiao Chen, MD and Nianzeng Xing, MD (Presented By: Yong Zhang, MD)

Poster #98 **RESPONSE TO SILDENAFIL CITRATE THERAPY FOLLOWING NERVE SPARING RADICAL PROSTATECTOMY: IMPACT OF RISK FACTORS**
Ashok Agarwal, PhD, Rupesh Raina, MD, Kalyana Nandipati, MD, Vivek Subbiah, MD and Craig Zippe, MD (Presented By: Rupesh Raina, MD)

Poster #99 **EFFICACY AND TREATMENT SATISFACTION OF PDE-5 INHIBITORS IN MANAGEMENT OF ERECTILE DYSFUNCTION FOLLWING RADICAL PROSTATECTOMY: SHIM ANALYSIS**
Kalyana Nandipati, MD, Rupesh Raina, MD, Ashok Agarwal, PhD and Craig Zippe, MD (Presented By: Kalyana Nandipati, MD)

Poster #100 **EFFECTIVE TREATMENT WITH VIAGRA® (SILDENAFIL CITRATE) IS ASSOCIATED WITH REDUCED BOTHER IN MEN WITH ERECTILE DYSFUNCTION**
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(Presented By: Mónica Vazquez-Levin, PhD)

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Gilles Frenette, BSc, Christine Légaré, MSc, Fabrice Saez, PhD and Robert Sullivan, PhD
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Suresh Yenugu, Ph.D, Katherine Hamil, M.S., Frank French, M.D. and Susan Hall, Ph.D
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poster session 2

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Stephanie T. Page, MD, PhD, Daniel W. Lin, MD, Peter S. Nelson, MD, John K. Amory, MD, Alvin M. Matsumoto, MD and William J. Bremner, MD, PhD (Presented By: Stephanie T. Page, MD, PhD)

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(Presented By: Tetsuo Hayashi, BS, MD, PhD)

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Thorsten Bach, M.D., Dietrich Pfeiffer, M.D. and Roland Tauber, M.D. (Presented By: Thorsten Bach, M.D.)

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SUNDAY, APRIL 3, 2005
2:00 P.M. – 3:30 P.M.

Concurrent Oral Session I: Clinical Andrology
Co-chairs: Alvin M. Matsumoto, MD, Seattle, WA;
Daniela Bomgardner, MD, Charlottesville, VA

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02:00 PM

CORRELATING ANDROGEN AND ESTROGEN STEROID RECEPTOR EXPRESSION WITH CORONARY CALCIFICATION AND ATHEROSCLEROSIS IN MEN WITHOUT KNOWN CORONARY ARTERY DISEASE

Peter Y. Liu*, Rose C. Christian, Ming Ruan, Virginia M. Miller and Lorraine A. Fitzpatrick, Mayo Clinic, Rochester, MN and *Harbor UCLA, Torrance, CA.

Steroid receptors including the androgen receptor (AR), estrogen receptor alpha (ERa) and beta (ERb) are expressed in key vascular tissues including endothelial cells and vascular smooth muscle cells (VSMCs). However, the relative abundance and importance of these receptors in the coronary artery is not well defined, particularly in men. We therefore examined AR, ERa and ERb expression as a function of key components of atherosclerosis, namely plaque and calcium area, in male human coronary arteries. Coronary arteries were obtained at autopsy from 24 men without known coronary artery disease. Coronary calcification was measured by contact microradiography and atherosclerotic plaque area was quantified histologically. Coronary artery cross-sections were immunostained for AR, ERa and ERb, and then measured semi-quantitatively in each arterial wall layer (intima, adventitia and media). AR, ERb and ERa were expressed in all artery wall layers, but most avidly in the media ($P<0.001$). ERb exceeded ERa expression ($P<0.0005$). AR expression in the media correlated negatively with plaque area ($P=0.006$, $R=-0.55$) whereas intimal ERb expression correlated positively with plaque area ($P=0.012$, $R=0.50$). We conclude that both AR and ERb are important in relatively early coronary atherosclerosis, but inversely so, since decreasing AR and increasing ERb expression correlate with more extensive atherosclerosis. ERb is the predominate estrogen receptor in coronary arteries harvested from men without known coronary artery disease. Interventional studies are required to assess the functional significance of these observations.

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ARE TOTAL TESTOSERONE LEVELS AFFECTED BY CHANGES IN OBESITY?

Beth A. Mohr, Amy O'Donnell, John McKinlay, Watertown, MA

Introduction: Obesity has reached epidemic proportions in the United States, dramatically contributing to disease states. Obesity has been inversely correlated with total testosterone (TT) levels. We investigated whether TT levels are affected by changes in obesity.
Methods: Data were obtained from the Massachusetts Male Aging Study, a population-based random cohort of men aged 40-70 at baseline (T_1) and observed 3 times (T_1: 1987-89; T_2: 1995-97; T_3: 2002-04). Serum TT was measured via radio-immunoassay on morning blood samples. Using data from T_1 and T_3, four obesity (defined as body mass index ^{3} 30 kg/m^2) change categories were constructed: obese at neither time, T_1 only, T_3 only, and T_1 & T_3. Of the 1709 recruited, 672 men who participated at both times and who had complete data were included. Analysis of variance was used to model T_3 TT as a function of obesity change with adjustment for T_1 TT, age, chronic illness, medication, alcohol, smoking, and physical activity. Pairwise comparisons between the neither group and the other obesity change categories were adjusted for multiple comparisons using the Bonferroni procedure.
Results: The number of men in the neither, T_1 only, T_3 only, and T_1 & T_3 group was 467 (69%), 20 (3%), 90 (13%), and 95 (14%), respectively. Change in obesity was significantly associated with T_3 TT ($p<0.0001$). In adjusted models, mean T_3 TT for the T_3 only group ($p<0.0001$) and for the obese at T_1 & T_3 group ($p<0.002$) were significantly lower than the mean for the neither group (mean in ng/dL, 95% confidence interval (CI)): T_3 only (354, CI: 323-384), T_1 & T_3 (369, CI: 340-399), vs. neither (431, CI: 418-444).
Conclusion: Men who became obese or stayed obese had significantly lower TT levels than men who were not obese at T_1 or T_3. Reducing obesity may be an effective and safe method for improving TT levels of aging men.

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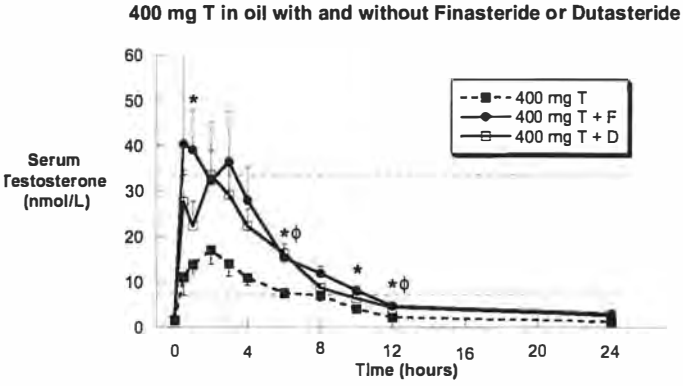
ABSORPTION OF ORAL TESTOSTERONE IN OIL IS AUGMENTED BY 5-ALPHA REDUCTASE INHIBITION IN MAN

John K. Amory, MD, Stephanie T. Page, MD, PhD, William J. Bremner MD, PhD, Dept. Medicine, University of Washington

Introduction: Testosterone (T), administered orally in oil, can result in therapeutic elevations in serum T in men. To determine if the 5-alpha reductase inhibitors dutasteride (D) and finasteride (F) would augment serum T levels after dosing, we conducted a study of oral T, alone or combined with D or F.
Methods: Seven men were administered a GnRH antagonist to suppress T production. Subjects were then administered 400 mg of T in oil. Serum T and DHT was measured prior to T and 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 hours after T administration. Subjects then underwent T administration and blood sampling after premedication first with oral F and then with oral D.
Results: D or F were equally effective at increasing serum T levels and normalizing serum DHT



levels after oral administration compared with the T alone (Fig).
Conclusions: The administration of oral T in oil combined with Dutasteride or Finasteride results in therapeutic increases in serum T and DHT. Additional study of these combinations as novel forms of androgen therapy are warranted.

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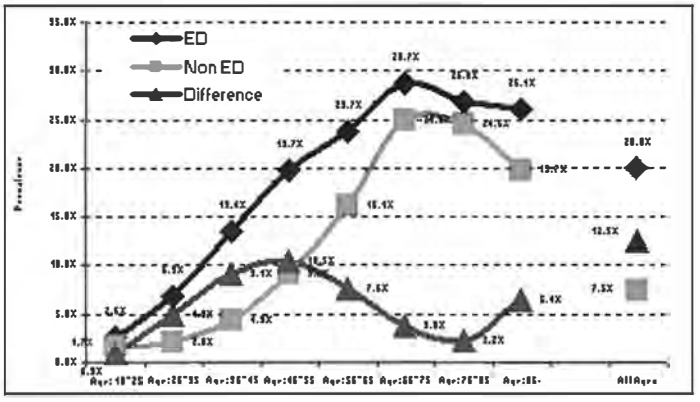
ARE MEN WITH ERECTILE DYSFUNCTION MORE LIKELY TO HAVE DIABETES MELLITUS THAN MEN WITHOUT ERECTILE DYSFUNCTION – EVIDENCE FROM A LARGE MANAGED CARE CLAIMS DATABASE?

P Sun, A Cameron, A Seftel, R Shabsigh, C Niederberger, A Guay; Eli Lilly & Company, Indianapolis IN

Men with diabetes mellitus (DM) are more likely to develop ED than men without DM, but there is less evidence to show that men with ED are more likely to have DM than men without ED. We used a retrospective cohort study design to compare DM prevalence rates in an ED cohort (n=285,436) and a Non-ED cohort (n=1,584,230) from a large national managed care claims database (28 million lives, 51 plans). DM prevalence rates were estimated and compared, and odds ratios were derived from a logistic model that controlled for age, region and 20 concurrent conditions (e.g., hyptertension, hyperlipidemia). 20.0% of men with ED had DM compared to 7.5% of men without ED. Figure 1 presents age-specific prevalence comparisons. Controlling for confounding factors, the odds for men with ED to have DM was 59% higher than the odds for men without ED (odds ratio: 1.59, *P*=0.0001). This supports the hypothesis that ED and DM share a common risk factor, endothelial dysfunction, and suggests clinicians should evaluate men with ED for DM.



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DYSREGULATION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE PHOSPHORYLATION MECHANISMS IN DIABETES-ASSOCIATED ERECTILE DYSFUNCTION

A.L. Burnett, M.F. Kramer, R.E. Becker, B. Musicki. Johns Hopkins Hospital, Baltimore, MD.

Impaired endothelial nitric oxide synthase (eNOS) function is associated with erectile dysfunction in diabetes mellitus, but the exact molecular basis for the eNOS defect in the diabetic penis remains unclear. We investigated whether hyperglycemia increases O-linked N-acetylglucosamine (O-GlcNAc) modification of eNOS at Ser-1177 in the penis, preventing phosphorylation at this primary positive regulatory site on the enzyme and thereby hampering mechanisms of the erectile response. Diabetes was induced in male rats by alloxan (140 mg/kg, ip). Five weeks after induction of diabetes, erectile response was evaluated and penes were excised at baseline and after electrical stimulation of the cavernous nerve (CNS) in the presence or absence of recombinant human vascular endothelial growth factor (rhVEGF)-165 injected intracavernosally. Erectile response to CNS was significantly decreased in diabetic compared to control rats. The diabetic rat penis exhibited significantly increased O-GlcNAc modification of eNOS and significantly decreased phosphorylation of eNOS (Ser-1177) and Akt (Ser-473) both at baseline and after CNS compared to the control rat penis. Phosphorylation of eNOS on other sites (Ser-633, Ser-615, and Thr-495) was not affected by diabetes. While in control rats rhVEGF-165 increased erectile response in parallel with an increase in phosphorylation of eNOS (Ser-1177) in the penis, rhVEGF did not improve erectile response in diabetic rats and correspondingly did not increase phosphorylation of eNOS (Ser-1177) in the diabetic rat penis. This study demonstrates that eNOS function in the penis is impaired in diabetes by a specific glycosylation mechanism, by which the enzyme is rendered incapable of activation by normal fluid shear stress stimuli and growth factor signaling.

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INCREASED SPERM OOCYTE PENETRATION CAPACITY FOLLOWING MAGNETIC CELL SEPARATION

Tamer Said[1], Sonja Grunewald[2], Uwe Paasch[2], Christian Kriegel[2], Liang Li[1], Hans-Juergen Glander[2], Ashok Agarwal[1]. [1]Cleveland Clinic Foundation, USA, [2]University of Leipzig, Germany.

The inclusion of apoptotic sperm during *in vitro* fertilization may be one of the reasons for suboptimal success rates. The binding with annexin-V microbeads during magnetic cell separation (MACS) can effectively eliminate apoptotic spermatozoa. Our aim was to evaluate the oocyte penetration capacity of the annexin-V negative (non-apoptotic) fraction using the zona free hamster oocyte penetration assay (SPA). Semen specimens collected from 16 donors were subjected to double density gradient centrifugation (DGC) followed by MACS. A non-separated aliquot of each sample served as control. All aliquots were subjected to capacitation followed by the SPA. Results were evaluated as the percentage of oocytes penetrated by sperm (SPA) and the average number of sperm penetrated per oocytes (sperm capacitation index, SCI). The sperm quality was monitored in terms of motility and the presence of apoptotic markers: caspase 3 activation (CP3), disruption of transmembrane mitochondrial potential (TMP) and externalized phosphatidylserine (EPS). Annexin-negative sperm were characterized by highest motility, intact TMP, low CP3 and EPS levels compared to controls and annexin positive sperm. Similarly, annexin negative sperm had the highest SPA and SCI values (table). In conclusion, annexin negative sperm display superior quality and oocyte penetration capacity compared to sperm prepared by routine DGC. The selection of non-apoptotic spermatozoa by MACS may help improve IVF outcome.

Parameter	Controls	Annexin-negative	Annexin-positive
Motility (%)	76.2 ±8.6	83.2 ±8.1**	19.2 ±9.7**
CP3 (% active)	8.9 ±8.1	3.7 ±1.2*	57.6 ±16.1**
TMP (% intact)	87.9 ±11.1	92.2±8.4**	39.1±15.7**
EPS (% positive)	5.8±3.2	3.4 ±1.7	54.9 ±18.1**
SPA (% penetrated)	33.8 ±6.9	44.5 ±12.6**	20.8 ±5.3**
SCI	1.5 ±0.6	1.8 ±0.3*	1.2 ±0.4*

Values are expressed as mean ± standard deviation. **p<0.01; * p<0.05 in comparison to control

SUNDAY, APRIL 3, 2005
2:00 P.M. – 3:30 P.M.

Concurrent Oral Session II: Spermatozoa/Male Infertility
Co-chairs: Steven M. Schrader, PhD, Cincinnati, OH;
Carl Lessard, PhD, Bar Harbor, ME

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EFFECT OF HSP60 AND GRP78 ON HUMAN SPERM FUNCTIONS
Catherine Lachance, Mathieu Boilard, Pierre Leclerc. Centre de Recherche en Biologie de la Reproduction, Université Laval, Québec, Canada.

Ejaculated spermatozoa must undergo a series of biochemical transformations to fertilise the egg. These changes collectively called capacitation include increase in intracellular pH, calcium, cAMP and tyrosine phosphorylation. It occurs naturally during the sperm transit within the female genital tract. We identified two oviductal proteins, heat shock protein 60 (HSP60) and glucose regulated protein 78 (GRP78), that bind to bull spermatozoa and might affect their activities. In order to determine whether these two chaperone proteins affect specific functions of human spermatozoa, they were incubated in capacitating media supplemented with recombinant HSP60 or GRP78. Following a 4 hour incubation, protein tyrosine phosphorylation was evaluated by western blot using a monoclonal anti-phosphotyrosine antibody. The acrosomal integrity was evaluated using the binding pattern of Pisum sativum agglutinin coupled to FITC. The presence of intrinsic HSP60 and GRP78 in fractionated human spermatozoa was detected by western blot using monoclonal antibodies against HSP60 and GRP78 respectively. The localisation of endogenous HSP60 was demonstrated by indirect immunofluorescence. None of the two proteins affected the protein tyrosine phosphorylation pattern when spermatozoa were incubated under control conditions. Sperm treatment with IBMX provoked a 10 fold increase in protein phosphotyrosine content which was decreased when HSP60 or GRP78 were included in the incubation medium. Similarly, these two chaperone proteins diminished the percentage of spontaneous acrosome reaction of human spermatozoa. The endogenous expression of HSP60 and GRP78 were detected in the demembranated head and cytosolic fractions, respectively. Immunofluorescence studies revealed a mid-piece localisation of endogenous HSP60. Our study suggests that these two proteins might affect human sperm capacitation.

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02:15 PM

DEFECTIVE SPERM TAIL FORMATION IS PHENOTYPE OF *repro2* SPERM, AN ENU-INDUCED MOUSE MUTATION
Carl Lessard, Suzane A Hartford, John C Schimenti, and Mary Ann Handel. The Jackson Laboratory, Bar Harbor, ME

Human asthenozoospermia is often associated with flagellar defects of the sperm, but the etiology of these defects is unclear. Family studies suggest a genetic role in some cases of sperm tail abnormalities. An ENU-mutagenesis strategy has been developed to generate mouse models for human infertility syndromes. ENU-mutagenized males were bred in a three generation breeding scheme to produce potential homozygous recessive males who were screened by a fertility test. Infertile mutant males were analyzed in an "infertility clinic" to determine andrological parameters of their infertility. Mutant males from the *repro2* family had normal body and paired seminal vesicle weights, suggesting normal hormonal levels; but their testes weigh less than testes from control males (184±17 mg for mutant vs 247±13 mg for control, paired testis weight). Epididymal sperm were reduced in number (2.2×10^6 spz/ml for mutant vs 25×10^6 spz/ml for control) and all sperm were morphologically abnormal, exhibiting defective heads and tails. Mutant epididymal sperm were not motile and exhibited poor IVF success. Abnormal spermatid head shape was apparent in step 9 spermatids and absence of normal spermatid tail was obvious by step 15. Scanning and transmission EM analyses revealed that *repro2* sperm have short, stubby tails. The mitochondrial sheath, axonemal complex and outer dense fibers were present in the most anterior regions. However, principal piece structures, such as fibrous sheath, were rare and not well organized. Immunostaining with antibodies against tail structures (ODF2, KLC3 and tubulin) confirmed that these proteins were present in the spermatid cytoplasm, but they were not spatially organized into tail structures. To fine map and ultimately clone the mutated gene, polymorphic markers were used to map the ENU-mutation to a specific chromosomes. The mutation mapped to a 5 cM region on Chr 5. Although this region includes 133 genes, it is expected that further recombinations will narrow the candidate interval. These observations on the *repro2* mutation provide evidence for genetic control of sperm tail formation in the mouse. This might ultimately be applied to further our understanding of some cases of sperm tail dysplasia in human males. This study was supported by N1H PO1 HD43127 to JJE, MAH & JCS.

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02:30 PM

IN VITRO STIMULATION OF HUMAN SPERM MOTILITY
Michaela Luconi, Gianni Forti, Elisabetta Baldi. Dept. Clinical Physiopathology, Andrology Unit, University of Florence, Florence, Italy

Human testicular sperm are immotile and to fertilize they need to acquire the ability to actively swim under the control of different factors acting during transit from the epididymis to the site of fertilization. Defects in sperm motility underlay asthenozoospermia, one of the main seminal pathology associated to male infertility. Thus, in vitro sperm treatments able to improve the number of motile sperm collected for assisted reproductive techniques (ART) can help infertile couples to conceive. Among the sperm kinases regulating motility, protein kinase A (PKA) plays a major role. We demonstrate that pharmacological inhibition of phosphatidylinositol 3-kinase (PI3K) by LY294002 stimulates a significant increase in motility in both unselected and swim up-selected sperm, leading to an increase in sperm recovery for ART. Similarly, in vitro addition of physiological concentrations of bicarbonate (HCO_3^-) results in a dose- and time-dependent increase in sperm motility as well as in hyperactivation parameters. The stimulatory effect of HCO_3^- and LY294002 is mediated by an increase in cAMP production and in tyrosine phosphorylation of one of the main component of the fibrous sheath in sperm tails, the A kinase anchoring protein AKAP3. We show that due to tyrosine phosphorylation, AKAP3 is able to recruit and activate PKA in sperm tails through an increased binding to its regulatory/catalytic subunits as shown by the increased amount of PKA co-immunoprecipitated with AKAP3 in HCO_3^- and LY294002-stimulated sperm samples. Our findings contribute to elucidate the molecular mechanisms underlying the physiological and pharmacological stimulation of motility in human sperm, demonstrating that HCO_3^- and LY294002 converge on the same intracellular signaling pathway involving stimulation of adenylate cyclase and tyrosine phosphorylation of AKAP3, finally leading to PKA recruitment and activation in sperm tails.

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02:45 PM

A MUTATION IN THE PROTAMINE 1 GENE OF TWO INFERTILE MEN CREATES A PUTATIVE NEW SITE FOR PHOSPHORYLATION

Naoko Iguchi[1], Shicheng Yang[1], Dolores J. Lamb[2], Norman B Hecht[1], [1]University of Pennsylvania, Philadelphia, PA, [2]Baylor College of Medicine, Houston, TX.

Heterozygous mutations in genes post-meiotically expressed can affect all spermatids because the germ cells mature in a clonal syncytium. In the mouse the disruption of one copy of the gene encoding protamine (Prm) 1 or 2 reduces the amount of the respective Prm and leads to reduced chromatin compaction, damage to the sperm DNA, and infertility (Cho et al., 2001, 2003). Using the phenotype generated by Prm gene targeting as a model for human male infertility, we screened DNA from 34 infertile men whose sperm exhibit one or more defects consistent with the Prm deficiency. Prm 1 and 2 alleles were analyzed using a PCR-direct sequencing method. In a survey of the promoters, exons, and introns of these two genes, we found several novel mutations that were specific to these infertile men compared to fertile controls. One heterozygous mutation found in two infertile men changed an arginine to serine in an arginine cluster of Prm 1. Such a mutation could create a new phosphorylation site for the SR protein-specific kinase 1, an enzyme known to phosphorylate human Prm 1 (Papoutsopoulou et al., 1999). Phosphorylation at this new site in an arginine cluster of Prm 1 could impact Prm 1 binding to DNA, ultimately affecting chromatin compaction and leading to infertility by changing the conformation and DNA binding properties of the mutant Prm 1 allele. Other mutations in the promoters of protamines are under investigation. Based on mouse models, precise temporal and quantitative regulation of transcription, translation and post-translational modifications are imperative for normal chromatin compaction and transcriptional silencing during spermiogenesis. Our results suggest that mutations that affect such processes may be major contributing factors for certain idiopathic infertilities.

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03:00 PM

IDENTIFICATION OF PP60C-SRC AS THE ENZYME RESPONSIBLE FOR TYROSINE PHOSPHORYLATION AND CONSEQUENT HYPERACTIVATION DURING MOUSE SPERM CAPACITATION

Mark A. Baker and R. John Aitken. University of Newcastle, NSW, Australia

Upon ejaculation, spermatozoa undergo a series of post-translational modifications in order to acquire the competence to fertilize the oocyte. Collectively, this complex process has been termed "capacitation". A fundamental attribute of capacitation is a redox-regulated, cAMP-dependent increase in the overall level of tyrosine phosphorylation. For over three decades, the importance of PKA in the regulation of this pathway has been recognized however, to date, the molecular identification of the intermediate tyrosine kinase has remained elusive. Using difference in 2D gel electrophoresis (DIGE), we have established and identified the major proteins that become tyrosine phosphorylated during capacitation including enolase, tubulin, hsp70. Based on these results, we have determined that pp60c-src, a non-receptor protein tyrosine kinase, is a key mediator of sperm capacitation, and in particular hyperactivation. Consistent with this, pp60c-src was shown to co-immunoprecipitate with PKA, the major inducer of tyrosine phosphorylation in spermatozoa. pp60c-src also co-precipitated with AKAP4 and ODF-2, two known substrates for tyrosine phosphorylation in capacitating sperm. Upon addition of the src-kinase inhibitors su6656 and PP1 a decrease in specific tyrosine phosphorylated proteins occurred. The major consequence of this event was a loss of the ability of the spermatozoa to undergo hyperactivated motility, a critical component of capacitation.

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03:15 PM

TWO FIBRINOGEN-LIKE PROTEINS, FGL1 AND FGL2 ARE DISULFIDE-LINKED SUBUNITS OF AN OLIGOMER SECRETED BY THE HAMSTER CAUDA EPIDIDYMIDIS THAT BINDS DEFECTIVE SPERMATOZOA

S.K. NagDas, V.P. Winfrey, M.H. Melner, G.E.Olson. Vanderbilt University School of Medicine, Department of Cell and Developmental Biology, Nashville, TN 37232

Previously, we identified a 64kDa glycoprotein (termed HEP64), secreted by principal cells of the hamster proximal cauda epididymidis that specifically binds defective spermatozoa. Both luminal fluid and sperm-associated HEP64 are assembled into disulfide-linked oligomers of ~260kDa and ~280kDa. Recently, we cloned HEP64 and identified it as the fibrinogen-like protein fgl2. The objective of the present study was to identify the subunit composition of the fgl2-containing oligomers. Both co-immunoprecipitation analysis, using anti-fgl2, and sequential non-reduced followed by disulfide-reduced, SDS-PAGE identified both fgl2 and a 31 kDa polypeptide as subunits of each oligomer. Likewise SDS-PAGE analysis of an epididymal fraction enriched for polymerized fgl2 also identified a major polypeptide of 31kDa. Using proteomic analyses and a PCR-based cloning strategy, the 31kDa polypeptide has been fully sequenced and identified as fibrinogen-like protein-1 (fgl1). Northern blotting and *in situ* hybridization demonstrated low fgl1 expression in the caput and corpus regions, but high expression in principal cells of the proximal cauda epididiymidis, as previously found for fgl2. Immunostaining using a fgl1-specific polyclonal antibody revealed that fgl1 also binds the defective sperm population in the cauda epididymidis. This study is the first to show an association of fgl1 and fgl2 and demonstrates that they are disulfide-linked subunits of the 260 and 280 kDa oligomers secreted by the epididymidis. The specific binding and polymerization of the fgl1/fgl2 oligomers onto defective spermatozoa appears to represent a mechanism to shield the viable sperm population and/or the epididymal epithelium from degenerating spermatozoa contained within the tubule lumen.

Supported by NIH HD20419.

MONDAY, APRIL 4, 2005
2:00 P.M. – 3:00 P.M.

Concurrent Oral Session III: Testis
Co-chairs: Jacquetta M. Trasler, MD, PhD, Montreal, Quebec; Sarah Netzel-Arnett, PhD, Rockville, MD

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02:00 PM

DISSECTING THE AXONEME INTERACTOME: THE MAMMALIAN ORTHOLOGUE OF CHLAMYDOMONAS PF6 INTERACTS WITH SPAG6, THE MAMMALIAN ORTHOLOGUE OF CHLAMYDOMONAS PF16

Zhibing Zhang[1], Brian H. Jones[1], Waixing Tang[2], Stuart B. Moss[1], Clement Ho[1], Jean Bennett[2], Michael E. Baker[3] and Jerome F. Strauss III[1]
[1]Center for Research on Reproduction and Women's Health, [2]Department of Ophthalmology, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, 19104, and [3]Department of Medicine, University of California, San Diego, CA 92093

Cell motility and the movement of surface fluids are dependent upon flagella and cilia. The core structure of these organelles, the axoneme, is remarkably conserved across species, having in common nine doublets of microtubules with the associated force generating dynein arms and radial spokes surrounding two central singlet microtubules (the central apparatus), which contain associated proteins. The central apparatus is thought to play a key role in translating the microtubule sliding caused by dynein into the flagellar waveform and maintaining structural integrity of the axoneme. However, the central apparatus proteins involved in these processes have not been fully elucidated, and the network of interactions among them which allow these events to take place in a compact space have not been defined. PF6, a component of the *Chlamydomonas* central apparatus, is localized to the 1a projection of the C1 microtubule. The *Chlamydomonas* pf6 mutant has paralyzed flagella and lacks the 1a projection, indicating that PF6 has a critical role in axonemal structure and function. To investigate the function of PF6 in mammals, human and mouse PF6 were cloned. A 7.5 kb transcript is highly expressed in both human and mouse testis. PF6 message appears to be expressed at more modest levels in organs that contain cilia-bearing cells. The mouse PF6 message is detected on day 16 after birth, when pachytene spermatocytes are present, suggesting that mouse *Pf6* gene is expressed during meiosis. A rabbit anti-mouse PF6 polyclonal antibody was generated against a recombinant fragment of PF6 representing amino acid residues 548 to 875. In Western blot analyses on testes extracts two bands were detected, a 250 kDa protein consistent with the size of the translated protein encoded by the 7.5 kb cDNA sequence and a more abundant 97 kDa protein, consistent with the predicted molecular mass of a second PF6 cDNA clone generated by PCR from mouse testis RNA which appears to be an alternative splice variant. In epidimymal sperm, the most prominent immunoreactive band was 37 kDa, suggesting that there is proteolytic processing of the larger PF6 protein isoforms. Immunohistochemical studies on testis sections demonstrated that PF6 is present in the cytoplasm of round spermatids, and more signal was found in the condensing spermatids. The antibody detected PF6 antigen along the tail of permeabilized epididymal sperm. Electron microscope immunocytochemistry revealed that PF6 was localized to the central pair of the axoneme, a location that is consistent with the *Chlamydomonas* PF6 protein. To investigate whether PF6 can interact with SPAG6, another axoneme protein localized to the C1 microtubules in the central pair, CHO cells were cotransfected with PF6/pEGFP-N_2 and SPAG6/Dsred-N_1 plasmids. PF6 protein, which when expressed alone is cytoplasmic, decorated the microtubules of PF6/SPAG6 co-transfectants, and co-localized with SPAG6. PF6 protein could also be pulled down by anti-SPAG6 antibody in the cotransfected CHO cells. Finally, the 37 kDa PF6 band was virtually absent from the epididymal sperm of SPAG6-deficient mice. These findings reveal a previously unknown feature of the axoneme interactome, creating a new framework for understanding how the central apparatus regulates mammalian axoneme function.

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TESTICULAR FAILURE AND LEARNING DEFICITS IN ADULT XXY MICE

YH Lue, JD Jentsch*, C Wang, AP SinhaHikim, WA Salameh, RS Swerdloff, Dept. of Med, LAbiomed at Harbor-UCLA, Torrance, CA.
* Dept. of Psychology, UCLA, LA, CA.

Klinefelter syndrome (XXY males) occurs in about 1.2 per 1000 liveborn male births. XXY mice were generated by utilizing a four-generation breeding scheme that involves the use of a structurally rearranged Y chromosome, Y*. Approximately 50% of the live born male offspring in the fourth generation were XXY mice identified by karyotyping. To characterize testicular phenotype, groups of 7 adult (2 or 3-month-old) XXY and their littermate XY mice were studied. There were no significant differences in body weight, but testis weights of adult XXY mice (22.50±1.03 mg) were markedly decreased compared with those of the controls (81.98±4.56 mg). Serum testosterone levels were significantly ($P<0.05$) decreased in XXY mice (0.26±0.08 ng/ml) as compared with their XY littermates (0.57±0.08 ng/ml). The FSH levels in XXY mice (48.63±1.37 ng/ml) were significantly increased compared with those of XY (41.10±1.54 ng/ml) controls. However, LH levels were not significantly different between XXY (0.30±0.13 ng/ml) and XY (0.13±0.07 ng/ml) mice. Testicular histology of adult XXY mice showed small seminiferous tubules with varying degree of intraepithelial vacuolization and completely devoid of germ cells. Hypertrophy and hyperplasia of Leydig cells were observed in the interstitium. Interestingly, in one XXY mouse, we found a few seminiferous tubules containing zygotene and pachytene spermatocytes, but no round spermatids. Additional behavioral studies were conducted to determine whether XXY mice exhibited learning deficits. The acquisition of a Pavlovian tone-food association was assessed in groups of 7 adult (6 or 12-month-old) XXY and XY littermates. The rate of learning of the association was significantly slower in XXY mice, providing evidence for impaired medial temporal lobe function in XXY mice. We conclude that adult XXY mice have testicular failure and learning deficiencies, similar to its human counterpart, Klinefelter syndrome.

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ROLE OF TESTISIN (PRSS21) IN MALE FERTILITY

Sarah Netzel-Arnett[1], Thomas H. Bugge[2], Toni M. Antalis[1], [1]Department of Physiology and Surgery, University of Maryland School of Medicine, Rockville, MD; [2]National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD.

Proteolytic modification of testicular proteins is required for germ cell differentiation, sperm maturation and sperm-egg interactions. Protease activity is tightly regulated and inappropriate cellular proteolysis can affect fertility. Testisin is a glycosyl-phosphatidylinositol (GPI)-anchored serine protease that is abundantly expressed in male germ cells. In epididymal sperm, Testisin is found predominantly in the midpiece and cytoplasmic droplet. We are utilizing recombinant proteins and have generated monoclonal antibodies in order to delineate Testisin-specific activities. To assist in understanding the physiological role of Testisin in male reproduction, we recently generated a mouse model of complete Testisin deficiency (Testisin -/-), through disruption of the Testisin coding sequence by homologous recombination. Wild type (+/+), heterozygous (+/-) and null (-/-) progeny were born at the expected Mendalian ratio of 1:2:1, respectively, indicating that Testisin is dispensable for development. In short term fertility studies, Testisin -/- males showed a reduced number of pregnancies relative to their wild type littermates. However, Testisin deficiency did not ablate or severely impair spermatogenesis as elongated spermatids and spermatozoa were still present in testis and epididymis, respectively. Detailed histopathological analyses of testes epididymides and sperm of Testisin null mice are in progress. The Testisin null mouse promises to be an interesting and unique model for studying mechanisms of male fertility.

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02:45 PM

EFFECTS OF THE ANTIESTROGEN ICI 182,780 ON MALE REPRODUCTIVE TRACTS IN THE MATURE DOG AND MARMOSET MONKEY

Kay Carnes[1], Carla Morrow[1], Paul Klopfenstein[1], Sheila McCullough[2], Debra Sauberli[2], and Rex A. Hess[1] Departments of [1]Veterinary Biosciences and [2]Clinical Medicine, University of Illinois at Urbana-Champaign, Urbana IL

The antiestrogen ICI 182,780 inhibits efferent ductule function resulting in fluid accumulation and luminal dilation, which produces adverse effects on sperm maturation and fertility in several rodent species. Because these ductules have different patterns of organization in rodent and large mammalian species, we hypothesized that species differences in physiological responses to the antiestrogen could result from these morphological divergent pathways. Beagle dogs were treated either with 0 or 40 mg/kg of ICI for 120 days and marmoset monkeys were treated with 0, 10, 50, or 200 mg/kg of ICI for 30 or 60 days. Semen was evaluated by CASA and serum testosterone (T) levels were analyzed by radioimmunoassay. Estrogen receptor alpha (ERa) and sodium/hydrogen exchanger-3 (NHE3) were evaluated by immunohistochemistry. In both dogs and marmosets, ERa expression was decreased by ICI, but NHE3 showed species differences in response, with the marmoset showing dose-dependent increase but a decrease in the dog. Both species had increases in serum T, but the dog showed greater changes than the monkey. Epithelial height was decreased and luminal diameter was increased at the higher dosages in the monkey, but the dog epithelium showed no effect. Sperm concentration was reduced in the dog, but not monkey. Oocyte penetration was reduced in the monkey. The increase in T appears to counter decreases in estrogen activity, as NHE3 appears to be regulated by both estrogen and androgen. In conclusion, ICI induces species-specific effects, but also some common responses, the differences possibly dependent upon T concentrations. Support: CONRAD Program and NIH T32 ES07326. ICI was provided by AstraZeneca.

MONDAY, APRIL 4, 2005
2:00 P.M. – 3:00 P.M.

Concurrent Oral Session IV: Toxicology/Immunology
Co-chairs: Mitch Eddy, PhD, Research Triangle Park, NC; Ebtesam Attaya, Lubbock, TX

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02:00 PM

RELATIVE ROLES OF TESTOSTERONE AND FSH IN THE INHIBITION OF SPERMATOGONIAL DIFFERENTIATION IN IRRADIATED RATS

Gunapala Shetty, Connie C. Weng, Zhen Zhang, Sarah J. Meachem, Marvin L. Meistrich.
UTMD Anderson Cancer Center, Houston Texas 77030.

Simultaneous suppression of both testosterone (T) and FSH reverse the radiation-induced block in spermatogonial differentiation in $LBNF_1$ rats. Treatment with an androgen receptor antagonist indicated that T is an inhibitory factor, but the relative role of FSH was not clear. Previously we showed that rec human **F**SH slightly but significantly inhibits spermatogenic recovery in GnRH-suppressed irradiated rats. In the present study hypophysectomy or treatment with a GnRH antagonist within a week after irradiation restored the percent of differentiated tubules (TDI) to 100% at 11 weeks after irradiation compared to a TDI of 1% in irradiated rats. Treatment with T in 6-cm Silastic capsules during weeks 1-11 after irradiation inhibited the TDI to 56%, and 47% in hypophysectomized and GnRH antagonist-treated rats respectively. However, due to weaker suppression of ITT levels by hypophysectomy than by GnRH antagonist treatment, addition of T increased the ITT concentration to 20 ng/g-testis in hypophysectomized irradiated rats compared to only 11 ng/g-testis in GnRH antagonist-treated irradiated rats. These results indicate that T can inhibit spermatogenic recovery in irradiated rats independent of pituitary hormones, especially FSH. However, the greater spermatogenic recovery in the T-treated hypophysectomized rats than the T plus GnRH-treated rats despite higher ITT concentrations suggests that a pituitary factor, most likely **F**SH, is partly involved in this inhibition of spermatogonial differentiation. In a second approach, daily injections of anti-rat ovine FSH antibody given after irradiation did not increase the TDI in irradiated-only, GnRH antagonist+flutamide- or GnRH-antagonist+T-treated irradiated rats suggesting that FSH does not have a major role in the inhibition of spermatogonial differentiation after irradiation.

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15-DEOXY-$\Delta^{12,14}$-PROSTAGLANDIN J_2 INDUCES REACTIVE OXYGEN SPECIES-MEDIATED AND CASPASE-DEPENDENT MECHANSIMS IN LEYDIG PROGENITORS

[1]Anke Diemert, [1]Emanuela Clavarino, [1]KeumSil Hwang, [1]Laurence Walch, and [1,2]Patricia L. Morris. [1]Population Council and [2]The Rockefeller University, New York, NY 10021

Prostaglandin (PG) receptors are developmentally expressed in the testis and believed to play a substantial role in this immunoprivileged environment. This study characterizes the effects and pro-apoptotic mechanisms of the prostaglandin (PG) metabolite 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) in progenitor Leydig cells (PLC) isolated and purified from 18-day-old rat testes. The data demonstrate that 15d-PGJ_2 can induce apoptosis in PLC through a PPAR-γ-independent but caspase-dependent mechanism. Apoptosis could partially be prevented by the pan caspase-inhibitor ZVAD-fmk; neither a PPARγ- nor a PPARα-antagonist was able to inhibit the 15d-PGJ_2-induced processes. Following treatment of primary progenitor cells, a time-dependent activation of the mitogen activated protein kinase cJun-N-terminal kinase (JNK), and heat shock protein 70 (HSP 70), and the tumor suppressor p53 were demonstrated. Furthermore, 15d-PGJ_2-treated Leydig cell progenitors displayed inhibition of the steroidogenic acute regulatory (StAR) protein, and cleavage of the androgen and glucocorticoid receptors. We identified generation of Reactive Oxygen Species (ROS) as an early-onset mediator of this process. This was shown by a rapid increase of intracellular ROS-levels following 15d-PGJ_2 treatment of PLC, as demonstrated by flow cytometry. Furthermore, pre-incubation with the N-Acetyl-L-cysteine (NAC), an antioxidant that inhibits ROS generation, was able to prevent the activation of JNK and p53 and the induction of the 15d-PGJ_2-induced apoptosis. Studies supported by R01 HD39024 (PLM).

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GALECTIN-3 AND CANDIDATE BINDING LIGANDS IN HUMAN PROSTASOMES

S. Saraswati, J. Jones, C. F. Lichti, A. S. Block, A. B. Diekman, Department of Biochemistry and Molecular Biology, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas

Prostasomes are membranous vesicles that are secreted by the human prostate and incorporated into seminal plasma. These extracellular organelles promote sperm motility and exhibit immunosuppressive properties that ostensibly protect spermatozoa in the female reproductive tract. Studies from our laboratory identified galectin-3 in prostasomes. Galectin-3 is a 30 kDa, β-galactoside binding protein that has multiple immunomodulatory functions. To examine the surface localization of galectin-3 on prostasomes, proteins on the prostasome surface were labeled with membrane impermeable biotin and β-galactoside binding lectins were affinity purified on immobilized lactose. The identification of biotinylated galectin-3 indicated its localization on the prostasome surface. To validate the immunochemical identification of galectin-3 in prostasomes, β-galactoside binding proteins were affinity purified from prostasome extracts and subjected to microsequence analysis. An approximately 30 kDa protein band was identified as galectin-3, confirming the presence of this lectin in prostasomes. An approximately 16 kDa protein band was identified as a functional, truncated galectin-3 variant. This finding implicates a potential regulatory mechanism of galectin-3 function in prostasomes. Additional purified proteins were identified as lactoferrin, olfactomedin, prolactin-inducible protein, and Mac-2 binding protein. Although direct association with lactosyl affinity matrix cannot be discounted, the previous characterization of Mac-2 binding protein as a galectin-3 ligand suggested that at least a subset of the identified proteins represent candidate galectin-3 binding ligands. Additional studies will further investigate galectin-3 binding ligands in prostasomes and will examine the contribution of galectin-3 to the immunosuppressive properties of prostasomes.

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HISTONE H4 HYPERACETYLATION IN RAT ZYGOTIC PRONUCLEI FOLLOWING CHRONIC PATERNAL CYCLOPHOSPHAMIDE EXPOSURE

Tara S. Barton [1], Bernard Robaire[1, 2] and Barbara F. Hales[1]. Departments of [1]Pharmacology and Therapeutics and [2]Ob\Gyn, McGill University, Montreal, QC, Canada.

Preconceptional paternal cyclophosphamide (CPA) exposure leads to advanced male pronuclear formation, dysregulated zygotic gene activation as well as increases in pre- and post-implantation loss. Histone acetylation is intimately associated with transcriptionally competent parental genomes and chromosomal integrity in the zygote. The goal of our study was to determine the effect of paternal CPA exposure on histone H4 acetylation in early preimplantation embryos. Sprague-Dawley rats were given CPA by gavage for 4-5 weeks. Males were mated with control females; 1-cell and 2-cell stage embryos were collected and histone acetylation was assessed using anti-histone H4 (acetyl K5) immunofluorescence. Optical sections were recorded using confocal laser scanning microscopy and images were analyzed quantitatively. Histone H4 acetylation fluorescence intensity of both parental pronuclei in zygotes fertilized by spermatozoa chronically exposed to CPA was significantly disrupted, compared to corresponding controls. Male and female pronuclei were hyperacetylated ($p<0.003$) at pronuclear stage (PN) 2; maintained a hyperacetylated state through PN3 ($p<0.001$), but were not different from controls at PN4-5. Both male and female pronuclear areas, identified by DAPI immunofluorescence, in embryos sired by CPA exposed males were larger ($P\leq0.001$) at PN3 and PN4. Pronuclear stage distribution of embryos sired by CPA-exposed males was also disrupted; fewer ($p<0.001$) zygotes were observed at PN1&2 while a greater number ($p=0.008$) had progressed to PN4, compared to controls. These results suggest that preconceptual paternal CPA treatment produces chromatin modifications very early post-fertilization potentially leading to heritable epigenetic instabilities in later development. Supported by CIHR.

POSTER SESSION I
SUNDAY, APRIL 3, 2005
10:45 A.M. – 12:30 P.M.
Location: Leonesa Ballroom

ASSISTED REPRODUCTION & INFERTILITY

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INCREASED SPERM OOCYTE PENETRATION CAPACITY FOLLOWING MAGNETIC CELL SEPARATION

Tamer Said[1], Sonja Grunewald[2], Uwe Paasch[2], Christian Kriegel[2], Liang Li[1], Hans-Juergen Glander[2], Ashok Agarwal[1]. [1]Cleveland Clinic Foundation, USA, [2]University of Leipzig, Germany.

The inclusion of apoptotic sperm during *in vitro* fertilization may be one of the reasons for suboptimal success rates. The binding with annexin-V microbeads during magnetic cell separation (MACS) can effectively eliminate apoptotic spermatozoa. Our aim was to evaluate the oocyte penetration capacity of the annexin-V negative (non-apoptotic) fraction using the zona free hamster oocyte penetration assay (SPA). Semen specimens collected from 16 donors were subjected to double density gradient centrifugation (DGC) followed by MACS. A non-separated aliquot of each sample served as control. All aliquots were subjected to capacitation followed by the SPA. Results were evaluated as the percentage of oocytes penetrated by sperm (SPA) and the average number of sperm penetrated per oocytes (sperm capacitation index, SCI). The sperm quality was monitored in terms of motility and the presence of apoptotic markers: caspase 3 activation (CP3), disruption of transmembrane mitochondrial potential (TMP) and externalized phosphatidylserine (EPS). Annexin-negative sperm were characterized by highest motility, intact TMP, low CP3 and EPS levels compared to controls and annexin positive sperm. Similarly, annexin negative sperm had the highest SPA and SCI values (table). In conclusion, annexin negative sperm display superior quality and oocyte penetration capacity compared to sperm prepared by routine DGC. The selection of non-apoptotic spermatozoa by MACS may help improve IVF outcome.

Parameter	Controls	Annexin-negative	Annexin-positive
Motility (%)	76.2 ±8.6	83.2 ±8.1**	19.2 ±9.7**
CP3 (% active)	8.9 ±8.1	3.7 ±1.2*	57.6 ±16.1**
TMP (% intact)	87.9 ±11.1	92.2 ±8.4**	39.1±15.7**
EPS (% positive)	5.8±3.2	3.4 ±1.7	54.9 ±18.1**
SPA (% penetrated)	33.8 ±6.9	44.5 ±12.6**	20.8 ±5.3**
SCI	1.5 ±0.6	1.8 ±0.3*	1.2 ±0.4*

Values are expressed as mean ± standard deviation. **p<0.01; * p<0.05 in comparison to control

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INFLUENCE OF SPERM DEOXYRIBONUCLEIC ACID DAMAGE ON REPRODUCTIVE OUTCOMES WITH ICSI

Armand Zini, MD, James Meriano, B. Sc., Karim Kader, MD, Keith Jarvi, MD, Carl Laskin, MD and Kenneth Cadesky, MD
(Presented By: Armand Zini, MD)

There is some preliminary evidence to show that human sperm DNA damage may have an adverse effect on reproductive outcomes. The objective of this study was to further examine the influence of sperm DNA damage on reproductive outcomes (fertilization, embryo quality and pregnancy) after intra-cytoplasmic sperm injection (ICSI). We conducted a prospective study of consecutive infertile couples (n=60) undergoing ICSI at a single IVF center between September 2003 and March 2004. Sperm DNA damage was monitored by flow cytometry analysis of acridine orange-treated spermatozoa and expressed as the percentage of spermatozoa with DNA denaturation (DD). Couples were sub-grouped according to sperm DD results: Group 1: 0-15% (n=23); Group 2: >15-30% (n=26); Group 3: >30% (n=11). We observed no differences among the 3 groups with regard to maternal age, E2 serum levels, day of hCG injection, sperm concentration, motility and morphology, oocyte maturation, fertilization, pregnancy or blastocyst rates. Group 3 (> 30% DD) had a significantly higher rate of multinucleation among the embryo cohorts compared to groups 1 or 2 (20.0% vs. 10% and 8.8% respectively, $P = 0.04$). There was a trend toward an increased spontaneous pregnancy loss rate in Group 3 ($P > 0.05$). In summary, we observed a significant relationship between sperm DNA damage and the prevalence of multinucleated blastomeres in ICSI embryos. However, we did not observe any significant relationships between sperm DNA damage and fertilization or pregnancy rates with ICSI. Our data suggest that sperm DNA damage may adversely affect embryo quality. The selection of only high quality embryos for transfer may account for the lack of association between sperm DNA damage and pregnancy outcome.

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IN VITRO STIMULATION OF HUMAN SPERM MOTILITY

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Human testicular sperm are immotile and to fertilize they need to acquire the ability to actively swim under the control of different factors acting during transit from the epididymis to the site of fertilization. Defects in sperm motility underlay asthenozoospermia, one of the main seminal pathology associated to male infertility. Thus, in vitro sperm treatments able to improve the number of motile sperm collected for assisted reproductive techniques (ART) can help infertile couples to conceive. Among the sperm kinases regulating motility, protein kinase A (PKA) plays a major role. We demonstrate that pharmacological inhibition of phosphatidylinositol 3-kinase (PI3K) by LY294002 stimulates a significant increase in motility in both unselected and swim up-selected sperm, leading to an increase in sperm recovery for ART. Similarly, in vitro addition of physiological concentrations of bicarbonate (HCO_3^-) results in a dose- and time-dependent increase in sperm motility as well as in hyperactivation parameters. The stimulatory effect of HCO_3^- and LY294002 is mediated by an increase in cAMP production and in tyrosine phosphorylation of one of the main component of the fibrous sheath in sperm tails, the A kinase anchoring protein AKAP3. We show that due to tyrosine phosphorylation, AKAP3 is able to recruit and activate PKA in sperm tails through an increased binding to its regulatory/catalytic subunits as shown by the increased amount of PKA co-immunoprecipitated with AKAP3 in HCO_3^- and LY294002-stimulated sperm samples. Our findings contribute to elucidate the molecular mechanisms underlying the physiological and pharmacological stimulation of motility in human sperm, demonstrating that HCO_3^- and LY294002 converge on the same intracellular signaling pathway involving stimulation of adenylate cyclase and tyrosine phosphorylation of AKAP3, finally leading to PKA recruitment and activation in sperm tails.

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THE EFFICACY OF CHOOSING SPERM BY NUCLEAR CHARACTERISTICS PRIOR TO INTRACYTOPLASMIC SPERM INJECTION FOR REFRACTORY IN VITRO FERTILIZATION CASES WITH SPERM WITH ABNORMAL SPERM CHROMATIN STRUCTURE ASSAY TESTS

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The sperm chromatin structural assay has been associated with poor pregnancy outcome. There are varying opinions as to the effectiveness of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) with sperm from males with DNA fragmentation indices (DFI) >30%. We previously evaluated the effect of ICSI with sperm with DFI >30% in patients failing to conceive after several IVF cycles and we found a 10% live delivery rate which was half the rate of males with DFI scores ≤30%. There have been some data suggesting that isolating sperm for ICSI by magnifying the sperm ~6000 x and chosing sperm based on nuclear characteristics can improve pregnancy outcome in refractory IVF cases. The present study evaluated the efficacy of attempting ICSI with sperm obtained by nuclear characteristics in refractory IVF cases with males with DFI scores >30%. Thirteen cases were evaluated. Only one pregnancy (7.7%) was achieved and that was an ongoing pregnancy. The percent of sperm with normal nuclei for this couple was 28%. Though this was a small study, these data are consistent with the previous outcome for DFI abnormalities in refractory IVF cases. Isolation of sperm by nuclear characteristics does not seem to improve ongoing pregnancy rates for refractory IVF cases where the male partner has a DFI score >30%.

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PSYCHOLOGICAL STRESS AND SEMEN QUALITY AMONG MEN UNDERGOING INTRAUTERINE INSEMINATION

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To test the hypothesis that psychological stress can influence the semen quality in men undergoing intrauterine insemination. Forty-two women who underwent an intrauterine insemination from September 2002 to April 2004 were included in this prospective study. A regular semen analysis was performed in all cases as part of our routine male infertility evaluation (T1). The result of this analysis was compared with the semen sample collected on the day of the intrauterine insemination procedure (T2). The mean difference between semen analysis at T1 and T2 was 3.1± 0.8 months. The mean T1 sperm concentration ± standard deviation was 52.45 ± 32.65 and 57.34 ± 39.54 (r = 0.45; p = 0.79) for T2. Of the 42 patients, sperm concentration increased in 8, no change in 12 and 22 showed decreased sperm count. The mean T1 sperm motility ± standard deviation was 62.42 ± 17.23 and 57.32 ± 21.52 (r = 0.87; p = 0.2) for T2. Of the 42 patients, sperm motility increased in 19, no change in 12 and 11 had decreased motility. The mean T1 normal sperm morphology according to World Health Organization ± standard deviation was 22.4 ± 8.35 and 21.1 ± 7.45 (r = 0.91; p = 0.88) for T2. Of the 42 patients, normal sperm morphology increased in 3, no change in 34 and 5 had decreased sperm morphology. This study provides no evidence of a significant decline in semen quality or an inverse correlation between semen quality and psychological stress of patients undergoing intrauterine insemination.

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ANALYSIS OF PARTIAL AZFc DELETION ON THE Y CHROMOSOME IN JAPANESE POPULATION

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Y chromosome microdeletion is involved in spermatogenesis impairment. Recently a 1.6-Mb deletion polymorphism of the Y chromosome has been described. These partial AZFc deletions are known as gr/gr, b1/b3 and b2/b3 deletions and derive from homologous recombination between palindromic sequences in the Y chromosome. A specific primer (sY1201, sY1206, sY1291, sY1191, sY1161, sY1197, sY1258), has been used for PCR based screening of a large group of Japanese normospermia (n = 54). sY1291, sY1258 and sY 1201 deletion have been found in of 29.6% (16/56), 11.1% and 7.4% respectively. The other primers are specific.

On the other hand, we evaluated oligo/azoospermic patients The gr/gr deletions found in six patients with oligo/azoospermia (3.2 %, 6/182). The b1/b3 or b2/b3 deletion detected respectively in three or two men with oligo/azoospermia (b1/b3; 1.1%, 2/182, b2/b3; 1.6 %, 3/182). In addition, other novel partial AZFc deletion patters were found in four oligo/azoospermia. The deletion involved sY1206, sY1291. The single deletion of sY 1291 is present 36.8 % patients with oligo/azoospermia (67/182). This primer is considered to have potential for polymorphism. Thus, sY1291 is not reliable primer for detection of deletion for Japanese population. On the other hand, we found a total of 6 gr/gr deletions. Four of gr/gr deletions were from the azoospermia, two were from the severe oligo spermia. No gr/gr deletions were found in the control group. There was significant statistical difference in the frequency of gr/gr deletions between infertile and control populations in Japan.

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THE INTUSSUSCEPTED VASOVASOSTOMY: A SIMPLE, NOVEL TECHNIQUE

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Objective: Common contemporaneous methods for performing vasovasostomy include the two layer vasovasostomy and the modified one layer vasovasostomy, both of which require at least 12 sutures. The intussuscepted vasoepididymostomy is now an accepted and commonly used method for anastomosing the epididymis to the vas deferens. We postulated that the principle of intussusception could be adapted to vasovasostomy to create a widely patent, water-tight and simply crafted anastomosis.

Methods: We compared the two layer VV (2LVV) technique to the Intussusception VV (IVV) with respect to patency, leak and ease of performance as assessed by anastomotic time and number of haptic events. We performed 5 IVV's and 5 2LVV's on human vasa deferentia. The IVV is performed using 9-0 Nylon double armed suture (2.5 cm). The technique incorporates the principles of a horizontal mattress closure. The 1st suture is placed with both needles passed full thickness from outside to inside the lumen on the left hand sided vas, one at 5:30 and one at 6:30 on a clock face. The other 2 sutures are placed in a similar fashion but at the 1:30/ 2:30 and 9:30/10:30 locations. All needles are passed from inside the lumen to outside the lumen on the right hand side of the vas deferens in matching positions. Three 9-0 nylon sutures (remnants) are then placed through the muscularis to further bolster the anastomosis. The 2LVV was performed as previously described. We recorded the anastomotic time, # sutures used, suture breakage, needle damage, patency as assessed by irrigation and suture passage, and presence or absence of leak. Following completion of the patency/leak tests, all specimens were opened to subjectively assess patency at the anastomosis and to determine if one technique produced wider anastomoses.

Results: Objectively, the average anastomotic time for the 2LVV and the IVV were 36 minutes and 20 minutes, respectively ($p=0.0001$). A total of 3 sutures were used for each of the IVV's. All 10 anastomoses were patent and no leaks were dependent. There were two haptic events (bent needles) both in the 2LVV group. The lumens of the IVV anastomoses appeared larger than the lumen of the same vas distant from the anastomosis. This was not observed in the 2LVV specimens where the lumen at the anastamosis was similar to the lumen in the remainder of the vas segment.

Discussion: This pilot study demonstrates that the intussuscepted vasovasostomy is a technically simple method for vasal approximation associated with a high ex-vivo patency rate. It appears that this technique, by incorporating the principle of the horizontal mattress, provides a lumen at the anastomosis that is larger than the lumen elsewhere in the vas. Definitive statements regarding the clinical superiority of this technique must await in vivo studies.

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WITHDRAWN

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ROLE OF INHIBIN B IN THE EVALUATION OF MALE INFERTILITY

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Inhibin B is an important modulator of spermatogenesis through the regulation of the FSH biosynthesis and acts as an intragonadal regulatory factor on the para- and autocrine level. The objective of this study was to assess if inhibin B provides additional information about spermatogenesis in males. Thirty patients with fertility problems participated in this prospective study. Volume of the testes was assessed with Prader orchidometer. Serum levels of inhibin B (nanograms/L), luteinizing hormone (LH)(IU/L), and follicle-stimulating hormone (FSH)(IU/L), were assessed. Inhibin B serum levels were measured by ELISA. The mean age of the patients was 30.4 ± 8.07 years. The median (25, 75 percentiles) levels of different hormones were: inhibin B: 88 (32.25, 184.25), FSH: 10.06 (3.26, 16.44), and LH: 5.83 (3.25, 8.18). A good negative correlation was observed between inhibin B and FSH levels ($r = -0.794$, $P = 0.000$), indicating their primary interaction. Inhibin B showed a strong correlation with testicular volume and semen parameters compared to FSH (Table). Our results indicate that estimation of inhibin B levels in patients with infertility may provide useful information on spermatogenesis, as it is a direct marker of the spermatogenesis than FSH as seen in our study. Inhibin B is more precise in reflecting the testicular volume as it is secreted from testicular seminiferous tubules, which make up the testicular volume.

Variable	FSH	Inhibin B
Right volume (mL)	r = -0.396, P = 0.30	r = 0.618, P = 0.000
Left volume (mL)	r = -0.443, P = 0.01	r = 0.619, P = 0.000
Concentr.(10^6/mL)	r = -0.384, P = 0.043	r = 0.548, P = 0.003
Motility (%)	r = -0.249, P = NS	r = 0.440, P = 0.019
WHO morph. (%)	r = -0.29, P = NS	r = 0.369, P = NS

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A MUTATION IN THE PROTAMINE 1 GENE OF TWO INFERTILE MEN CREATES A PUTATIVE NEW SITE FOR PHOSPHORYLATION

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Heterozygous mutations in genes post-meiotically expressed can affect all spermatids because the germ cells mature in a clonal syncytium. In the mouse the disruption of one copy of the gene encoding protamine (Prm) 1 or 2 reduces the amount of the respective Prm and leads to reduced chromatin compaction, damage to the sperm DNA, and infertility (Cho et al., 2001, 2003). Using the phenotype generated by Prm gene targeting as a model for human male infertility, we screened DNA from 34 infertile men whose sperm exhibit one or more defects consistent with the Prm deficiency. Prm 1 and 2 alleles were analyzed using a PCR-direct sequencing method. In a survey of the promoters, exons, and introns of these two genes, we found several novel mutations that were specific to these infertile men compared to fertile controls. One heterozygous mutation found in two infertile men changed an arginine to serine in an arginine cluster of Prm 1. Such a mutation could create a new phosphorylation site for the SR protein-specific kinase 1, an enzyme known to phosphorylate human Prm 1 (Papoutsopoulou et al., 1999). Phosphorylation at this new site in an arginine cluster of Prm 1 could impact Prm 1 binding to DNA, ultimately affecting chromatin compaction and leading to infertility by changing the conformation and DNA binding properties of the mutant Prm 1 allele. Other mutations in the promoters of protamines are under investigation. Based on mouse models, precise temporal and quantitative regulation of transcription, translation and post-translational modifications are imperative for normal chromatin compaction and transcriptional silencing during spermiogenesis. Our results suggest that mutations that affect such processes may be major contributing factors for certain idiopathic infertilities.

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DETERMINATION OF COENZYME Q_{10} IN HUMAN SEMINAL PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

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A simple and reliable high performance liquid chromatographic (HPLC) method has been developed and validated for the analysis of coenzyme Q_{10} in human seminal plasma. After protein being deposited with methanol, coenzyme Q_{10} and coenzyme Q_9 (internal standard) were extracted with hexane and then the organic layer was collected and evaporated to until dry with nitrogen at 45 °C. HPLC separation of the sample was performed on a Lichrospher C_{18} column and detected by absorbance at 275 nm. A mobile phase composed of isopropanol: methanol: tetrahydrofuran in the ratio of 55: 39: 6 (v/v) was found to be the most suitable for this separation at a flow rate of 1.0 mL/min and enabled the baseline separation of CoQ_{10} and CoQ_9 from interferences with isocratic elution. Under the chromatographic conditions described, CoQ_{10} and CoQ_9 had retention times of approximately 5.83 min and 4.97 min, respectively, and good separation and detectability of CoQ_{10} in human seminal plasma sample were obtained. The method proved to be linear in the range of CoQ_{10} from 0.01 mmol/mL to 10.00 mmol/L. The relative standard deviations of within- and between-assay for CoQ_{10} analysis were 0.81 and 1.83 %, respectively. The extraction recoveries of CoQ_{10} from spiked human seminal plasma samples at three concentrations were 94.1 ~ 99.0 %. The method has been successfully applied to the study of the level discrepancy of CoQ_{10} between the fertile and infertile men.

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SPERM COATED WITH ANTISPERM ANTIBODIES DO NOT RESULT IN A HIGHER MISCARRIAGE RATE IN PREGNANCIES ACHIEVED BY IN VITRO FERTILIZATION AND INTRACYTOPLASMIC SPERM INJECTION

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There has been controversy about whether males with antisperm antibodies (ASA) produce fetuses with a greater risk for miscarriage. The present study evaluated whether the presence of ASA >50% by immunobead testing was associated with a greater risk of miscarriage than women with levels <50%. There were 360 cycles of in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) evaluated where clinical pregnancies were achieved. Only cycles where there were at least 5 eggs retrieved were included. The miscarriage rate was 22.5% (81/360). The miscarriage rate for 63 couples where ASA levels were >50% was 23.8% (n=15) vs. 22.2% (66/297) where ASA levels were ≤50% (p=NS). There have been some previous studies suggesting that pregnancies following intrauterine insemination (IUI) of sperm coated with ASA are at risk for higher miscarriage rates. The data presented here do not seem to corroborate these previous conclusions. However, the possibility exists that the process of ICSI obviates the mechanism by which ASA cause a higher miscarriage rate when IUI is used.

MALE SEXUAL DYSFUNCTION

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MULTIPLE OBSERVATIONS IN MEN WITH ED IN NATIONAL TADALAFIL STUDY IN THE US (MOMENTUS)

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This open-label study was designed to demonstrate the efficacy and safety of tadalafil 20 mg, dosed as needed (up to once a day), for 12 weeks in various populations of men with erectile dysfunction (ED). 1911 men were enrolled into 8 predefined groups: (1-3) Caucasian (n=608), Black American (n=293), or Hispanic (n=242) men, £ 65 yrs with no diabetes or depression, (4-5) men £ 65 yrs with clinical depression, no diabetes (n=178), or with diabetes, no depression (n=198), 6) men > 65 yrs with no diabetes or depression (n=188), 7) men who met enrollment criteria but were not included in any other group (n=155), and 8) patients with ED due to spinal cord injury (n=49). Patients in all sub-groups had a significant change from baseline (P<0.001) in the International Index of Erectile Function (IIEF) EF domain score and positive responses to Sexual Encounter Profile (SEP) Q3. (Table). Statistical analysis showed that tadalafil was as efficacious in the Black American and Hispanic men as in the Caucasians. 9% of patients reported headache and 3% each, nasal congestion, dyspepsia, flushing or back pain. Tadalafil 20 mg was efficacious and well tolerated in all sub-groups studied.

Sub-Group	*ΔIIEF EF*	*ΔSEP3* (%)*	*GAQ1** (%)*
Caucasian	10.5	49.0	92.6
Black American	9.5	50.5	89.6
Hispanic	9.5	56.4	97.2
Clinical Depression	11.7	55.5	89.4
Diabetes	9.4	44.8	84.8
Over 65 years old	8.8	39.6	81.0
Not included in any other group	7.6	32.5	77.4
Spinal cord injury	11.9	51.7	87.8

* SEP3 (Did your erection last long enough for you to have successful intercourse? Yes/No)

**Global assessment question (Has the treatment you have been taking during this study improved your erections? Yes/No).

Reported % of Yes responses.

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SELF-ESTEEM AND ERECTILE FUNCTION AFTER 9 MONTHS OF SILDENAFIL CITRATE TREATMENT

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Short-term (up to 12 wk) studies have indicated that treatment of erectile dysfunction (ED) with sildenafil can improve self-esteem (SE). We evaluated the longer-term effect (up to 36 wk) of sildenafil on SE in men with ED and correlated changes in SE with changes in erectile function (EF). This was a 36-week open-label (OL) extension of a 12-week US-based double-blind placebo-controlled (DBPC) trial of sildenafil in men aged ³18 y diagnosed with ED. All subjects in the OL extension received sildenafil, but remained blind to their DBPC treatment. Changes in SE and EF from OL baseline (end of DBPC study) to week 36 were assessed using the Self-Esteem And Relationship (SEAR) questionnaire (0=worst, 100=best) and the **EF** domain of the International Index of Erectile Function (1=worst, 30=best), respectively. Changes in SE and EF were correlated using Pearson coefficients. Mean changes in SE score from OL baseline to week 36 were 27.5 (P<0.0001) for the DBPC placebo group (n=108) and 5.8 (P=0.0044) for the DBPC sildenafil group (n=96). Mean changes in **EF** scores were 8.6 (P<0.0001) and 1.4 (P=0.06) for the DBPC placebo and sildenafil groups, respectively. Week 36 SE and **EF** scores were similar between DBPC treatment groups. Correlations between changes in SE scores and changes in EF scores were 0.68 (P<0.0001) for the DBPC placebo group and 0.33 (P=0.0016) for the DBPC sildenafil group. These data suggest that restoring erectile function provides long-term improvements in self-esteem. DBPC placebo subjects who later took sildenafil reached favorable SE and EF scores similar to those of DBPC sildenafil subjects, and significant correlations were observed between changes in SE and changes in **EF**.

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PULMONARY HYPERTENSION IMPAIRS NEUROGENIC AND ENDOTHELIUM-DEPENDENT RELAXATION OF PENILE ERECTILE TISSUE IN RATS

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Erectile dysfunction has an increased prevalence in hypertensive patients. In rats, injection of monocrotaline (MCT) causes pulmonary hypertension (PH) that leads to right ventricular heart failure. The aim of this study was to assess whether nitric oxide (NO)-dependent autonomic nerve- and endothelium-dependent relaxations of corpus cavernosum (CC) were altered in a MCT rat model. Male rats were injected with 60 mg/kg of MCT (i.v.) to induce PH. After 3 wks, mean pulmonary arterial pressure (mPAP), medial hypertrophy index (% wall thickness of pulmonary artery: % WT), right ventricular hypertrophy (right ventricle/left ventricular + septum weight ratio: RV/LV+S) were evaluated. CC was isolated and mounted in organ baths and electrical stimulation (EFS)-evoked nerve or agonist-mediated tension responses were examined. MCT produced fatal PH at 3 wks. Maximum NO-dependent nerve-mediated relaxation was reduced approximately 80% by PH. Maximum NO-mediated relaxation of phenylephrine-precontracted CC to acetylcholine was reduced >45% by PH. There were no significant differences between PH and control rats for relaxant response of CC to sildenafil, vardenafil, isoprenaline and sodium nitroprusside. Our data suggest that PH acutely attenuates NO-dependent autonomic nerve and endothelial function and decreases the erectile tissue response. Future studies are needed to elucidate the underlying mechanisms of these alterations.

	Control	MCT	*p value*
mPAP(mgHg)	16.00 ± 1.18	36.60 ± 6.11	< 0.05
%WT	8.42 ± 0.67	18.17 ± 1.78	< 0.001
RV/LV+S (%)	23.95 ± 1.13	44.09 ± 2.48	< 0.001
EFS	63.80 ± 7.95	13.25 ± 7.70	< 0.001
ACh	56.50 ± 2.10	30.79 ± 8.46	< 0.001

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THE PARP-1 INHIBITOR INO-1001 PRESERVES ERECTILE FUNCTION IN RATS AFTER CAVERNOSAL NERVE INJURY

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Objectives: PARP activation during neuronal injury plays a role in neurotoxicity. The aim of this study was to investigate the role of PARP activation and the effects of the potent competitive PARP-1 inhibitor INO-1001 on erectile function in a cavernous-nerve-injury model.
Materials-Methods: SD rats (n=24) were divided into: group I, sham; group II, bilateral cavernous-nerve-crush injury with vehicle treatment; and group III bilateral cavernous-nerve-crush injury treated with INO-1001. One week after surgical intervention, all animals underwent in vivo erectile response studies. PARP activation, nitrotyrosine, and iNOS were assayed using IHC. Serum levels of INO-1001 were measured by HPLC. Penile tissues were analyzed for malondialdehyde and myeloperoxidase. The results were statistically compared.
Results: The mean ICP/MAP and total ICP values to be significantly reduced in group II compared to group I. In the group III, these values remained unchanged compared to group I but significantly increased compared to group II. There was a marked reduction in PARP staining in group III, indicating that the compound inhibits activity of its target enzyme. There was no change in malondialdehyde and myeloperoxidase levels, but there was a marked increase in tyrosine nitration in group III. Reduced expression of iNOS was noted in the penile tissues in group III.
Conclusion: These data document increased activation of PARP with bilateral cavernous-nerve-crush injury in rats, which results in severe impairment of erectile function. Treatment with the PARP inhibitor INO-1001 reduced the degree of nitrosative stress and exhibited significant cavernous neuroprotection and preservation of erectile function after cavernous nerve injury.

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VENOUS SURGERY HAS A ROLE IN TREATING PATIENTS WITH ERECTILE DYSFUNCTION: CLINICAL EVIDENCE OF SYNERGIC EFFECT WITH SILDENAFIL

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To elucidate the benefit of venous surgery in treating impotence, we report on the synergic effect of it and sildenafil in patients who failed to response to this drug preoperatively. From July 1998 to July 2003, a total of 128 these patients were diagnosed as veno-occlusive dysfunction. Subsequently 65 men underwent venous surgery and were assigned as the surgical treatment group. The remaining 63 men were just followed and were allocated as control group. All were evaluated with the abridged five-item version of international index of erectile function (IIEF-5) scoring every 3 months and cavernosography required in surgery group. Sildenafil (12.5-100 mg) was prescribed postoperatively since they were not satisfied with venous surgery alone. The IIEF-5 scoring in the control group changed from a preoperative mean IIEF-5 score of 9.9±3.9 to 9.7±4.5 postoperatively. In the surgical treatment group, however, the mean preoperative IIEF-5 score of 9.8±4.3 increased to 15.1±5.0 ($p<0.001$) after surgery, further increased to 20.9±4.1 ($p<0.0001$) after the addition of sildenafil. Although there was no significant difference between the two groups' preoperative IIEF-5 score, there was a statistically significant difference between treatments ($p<0.001$). The follow-up period was 37.0±11.5 months. Overall 61 men (93.8%) reported a positive response to sildenafil after venous stripping surgery. In contrast only 8 patients (12.7%) felt a beneficial response in the control group ($p<0.001$). It appears that sildenafil and venous surgery may provide an encouraging solution to impotent patients who are non-responders to sildenafil. This suggests that the pathophysiology of erectile dysfunction may not just be due to intracavernous fibrosis resulting from the abnormality of transforming growth factor-ß1, as sildenafil is only a type of phosphodiesterase-5 inhibitor.

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LONG-TERM POTENCY FOLLOWING RADICAL PROSTATECTOMY

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The reported potency rates after radical prostatectomy (RP) vary from 11-86% and are often reported 12-24 months after surgery. 5-year potency status after RP has not been reported in the literature. We obtained 1 and 5 year potency data on a prospective RP population of 141 sexually active patients between 1997-1999. Mean age was 65.08 ± 6.68 and mean follow-up; 6.4 ± 1.5 years. The following data was obtained: sexually active or not, natural erections, nerve sparing status (NS), erectaids used, reasons for sexual inactivity (loss of interest, cardiovascular factors, urinary incontinence, loss of spouse, hormonal treatment). At **One-Year Analysis**: 113/141(80%) patients were sexually active (including drug therapy and erectaids); 28 (20%) were sexually inactive. The reasons for sexual inactivity included incontinence (15/28, 53%), loss of interest in sex (10, 36%); loss of libido (3/28, 11%; hormonal therapy). Of the 113 patients; 4 (3.5%) had natural erections sufficient for intercourse, 55 (48.7%) were using sildenafil, 26 (23%) intracavernosal injections (ICI), 19 (16.8%) vacuum constriction device (VCD), 9 intraurethral alprostadil (MUSE). At **Five-Year Analysis**: 70/113 (62%) remained sexually active. Of the 70 patients, 16 (22.9%) had natural erections sufficient for intercourse (15/16 NS), 21/70 (30%) were still using sildenafil, 10 (14.3%) IC injections, 5 (7%) VCD, 11 (15.7%) were using combination therapy, sildenafil with VCD, ICI or MUSE. Additional 7 (10%) patients switched to tadalafil alone. At 5 years 38% (43/113) were sexually inactive. The reasons included loss of interest -17 (39.5%), cardiovascular/ neurologic diseases- 18 (42%), hormonal tx-3 (7%), loss of partner -3 (7%) and 2, other surgeries. At 5 years, sexual activity following RP decreases 50%, most of them due to loss of interest and associated medical co-morbidities. The vast majority (77.8%) of RP patients are sildenafil/erectaid dependent, with only 22.2 % having natural erections sufficient for intercourse.

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THE ROLE OF NON–ORAL TREATMENTS FOR EARLY PENILE REHABILITATION AFTER RP

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To assess whether vacuum constriction devise (VCD), intraurethral alprostadil (MUSE) and intracavernous (IC) injections can facilitate earlier sexual activity, and potentially an earlier return of natural erection following nerve sparing (NS) RP. Out of 171 pt. undergoing NS–RP for localized prostate cancer (T1-T2, GS <6 and PSA ≤ 4) were enrolled in a) early treatment arm; MUSE (125 micro gram or 250 micro gram) [N=56]; VCD (manual or battery operated) [N=74] and IC injections (10 microgram PGE1) [N=6] and b) no treatment (control group). All patients advised to use erectaids 2-3 times/ wk. Average time after RP to the initiation of early treatment was 3.9 wks. At mean f/u of 6 mo. return of natural erections sufficient for vaginal intercourse was observed in 15/38 (39.5%) in MUSE group and 10/60 (17%) in VCD group compared to 4/35 (12%) in the control group. Return of natural erection sufficient for vaginal penetration were only significantly higher in early MUSE group vs. control ($P< 0.05$). At a mean f/u of 6 mo. 32% (18/56) in MUSE group, 25% (14/74) in VCD group and 67% (4/6) in ICI injections group discontinued the treatment. The most common causes of discontinuation in early rehabilitation program were; (MUSE [urethral burning], IC injections [pain and fear of injections] and VCD [lack of spontaneous and rigid erections]. Early pharmacological intervention following RP facilitates early sexual intercourse, early patient/spousal sexual satisfaction, and potentially an earlier return of natural erections.

Treatment Used	Disc. N (%)	NE Sufficient for Intercourse N (%)	NE Not Sufficient for Intercourse N (%)
MUSE (n=56)	18 (32.2)	15 /38 (39.5)	13/38 (34.2)
VCD (n=74)	14 (19)	10/60 (16.7)	9/60 (15)
ICI (n=6)	4(67)	1/2	1/2
Control (n=35)		4/35 (11.5)	9/35(25.7)
NE- natural erections; Disc. -Discontinued			

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CAN PURE LOCAL ANESTHESIA FOR OUTPATIENT PENILE SURGERIES BE STRICTLY FOLLOWED?

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Although topical blockage for penile surgeries has been substantially reported, its methodology, simplicity, reliability and reproducibility were not beyond controversy. We report on several cases of topical blocks as local anesthesia in patients who were indicated for penile surgeries. From August 1992 to May 2004, a total of 1121 men, aged from 19 to 87 years, underwent penile surgeries in which 45, 203, 708 and 165 patients received venous patches, modified Nesbit procedure, venous surgery and penile implantation respectively on an outpatient procedure with the assistance of the application of acupuncture. They were categorized into the patch, implant, Nesbit's and venous group correspondingly. A method of proximal dorsal nerve block, peripenile infiltration and ventral injection is sufficient for patients in the first three groups, a novel method of crural block, however, is mandatory in order to anesthetize the cavernous nerve for implantation purpose. The anesthetic effects and postoperative results were satisfactory. Common immediate side effects included puncture of the vessels, subcutaneous ecchymosis, transient palpitations and acceptable pain, but there were no significant late complications. In the implant group, however, 6.1% patients (10/165) experienced aching over the perineum for 1 to 2 weeks postoperatively. There was no statistical difference in scoring using a visual analog scale between the four groups. This topical blockage proved to be reliable, simple, and safe with fewer complications. They offer the advantages of less morbidity, protection of privacy, reduced effects of anesthesia, and a more-rapid return to activity with which the modern society trends demands.

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IS HIGH CHOLESTEROL A RISK FOR ERECTILE DYSFUNCTION? RESULTS BASED ON THE PENILE BLOOD FLOW INDEX (PBFI)

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Color duplex Doppler ultrasound (Doppler), is the most reliable and least invasive means of screening for vasculogenic erectile dysfunction (ED). The purpose of this study was to evaluate whether high cholesterol level is a co-morbid factor in vasculogenic ED using Doppler parameters. One hundred thirty-five patients with ED, mean ± SD IIEF score of 8 ± 2.5 had their cavernous arteries assessed by Doppler. Patients were divided into Group 1 (G1, n = 61) patients with normal cholesterol levels (143.45 ± 21.8) and Group 2 (G2, n = 74) patients with high cholesterol levels (247.11 ± 33.73). The PBFI was estimated by the changes in the diameter of the cavernous arteries and the respective rate of systolic peak following the injection of 70 mg of papaverine. Doppler parameters were analyzed using Student's t test. Mean ages in G1 and G2 were 53.18 ± 13.61 and 53.95 ± 10.41 years, respectively. ED was diagnosed in 86.89% of patients (53/61) in G1 and 82.44% (61/74) of patients in G2. No significant differences were seen in the parameters evaluated with the Doppler in patients with or without high levels of cholesterol (table). Our preliminary data shows that high cholesterol levels are not directly associated with ED. Further studies are required to prove this hypothesis.

Doppler parameters	G1	G2	P
Mean peak systolic velocity	31 ± 16.5	29.6 ± 17.5	0.08
End diastolic arterial velocity	4.99 ± 3.4	5.36 ± 1.61	0.06
Resistive index	0.82 ± 0.12	0.79 ± 0.15	0.09
Mean arterial diameter	0.85 ± 0.21	0.8 ± 0.22	0.09
Mean PBFI	204 ± 99.05	200 ± 90.15	0.08

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IS BALLOONING FORMATION A NECESSARY COMPLICATION OF TUNICAL REPAIR?

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To study retrospectively the outcomes of the tunical repair on the complicated ballooning region by using finer suture material in recent decade, 35 patients were seen from February 1993 to May 2000 for recurrence of penile curvature and gradual loss of erectile function resulting from previous tunical surgeries in spite of the fact that 2/3-0 nylon were used. All of them subsequently underwent tunical revision and penile venous stripping surgery by using finer suture materials. Before July 1998, a total of 15 men received 4-0 polyglactin for tunical repair and assigned as polyglactin group. Later a total 20 patients underwent similar surgery by using 6-0 nylon suture and designated as nylon group. A total number of 29 patients (11 of the 15 and 18 out of the 20) were available for follow-up by using the abridged five-item version of the International Index of Erectile Function (IIEF-5) scoring system and cavernosograms. The IIEF-5 scoring changed from a preoperative mean IIEF-5 score of 10.3±4.2 and 9.9±4.1 to a postoperative values of 21.6±2.3 and 21.9±2.1 corresponded to polyglactin and nylon group respectively. The morphologic results were considered excellent except for one patient in each group. Although there was no significant difference between the two groups, there remained significance in that the finer suture materials can consistently solve the unnecessary complication of tunical ballooning. This finding suggested that a proper repair of the tunica in which a bilayered architecture is well recognized and is a determinant of surgical success; and in contrast to what is believed that a stronger and coarser suture is essential in preventing from a resumption of penile curvature. We therefore believe that the tunical function can only be preserved using exact repair of the collagen bundles of the outer longitudinal layer which is assured in our series, although larger studies will be required.

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THE ASSOCIATION BETWEEN MINIMUM TIME TO ONSET OF ACTION OF SILDENAFIL AND MINIMUM EFFECTIVE PLASMA SILDENAFIL LEVELS

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Introduction: Although a relation exists between plasma drug levels necessary to inhibit phosphodiesterase 5 and efficacy, there has been no comparison of plasma levels to the minimum time of onset of action in an at-home clinical setting. A double-blind study using Rigiscan measurements and visual sexual stimulation showed that erections can occur as early as 12 min in men with erectile dysfunction (ED) taking a 50-mg oral dose of sildenafil (*BJCP*, 53:61S-65S, 2002). This study examined the association between the minimum time to onset of an erection after taking sildenafil with known plasma levels of sildenafil at comparable times. Methods: Men with ED who were successfully treated with sildenafil (100 mg) for >2 months were randomized to sildenafil (n=115) or placebo (n=113) for 4 weeks of double-blind treatment. After taking study drug in a fasted state (>2 h), patients recorded the time (stopwatch) needed to obtain an erection hard enough that led to successful sexual intercourse. The onset data were compared with total plasma levels of sildenafil determined from pharmacokinetic studies performed in healthy volunteers (n=16) of similar demographics (*BJCP*, 53:53S-60S, 2002). Results: Onset of action was seen within 14 and 20 min of sildenafil dosing with 35% and 51% of patients, respectively, having >1 erection leading to successful intercourse ($P<0.05$ compared with placebo). In healthy subjects, the mean total plasma levels after a 100-mg dose of sildenafil at 15 min and 30 min postdose were 20 ng/mL and 130 ng/mL. In these subjects, the maximum plasma concentration (C_{max}) occurred at 1 h (T_{max}) and was 286 ng/mL.
Conclusions: Clinical onset of action of sildenafil was observed as early as 14 min. The plasma levels associated with this time point were approximately 7% of the C_{max}. This comparison suggests that the erectile response to sildenafil can begin well before the median T_{max} of 1 h is reached.

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INTERLEUKIN-1α AND INTERLEUKIN-1 RECEPTOR ANTAGONIST IN NORMAL OR IMPAIRED HUMAN SPERMATOGENESIS

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There is a growing body of evidence that cytokines may exhibit modulatory activity on development of reproductive cells. It is well documented that IL-1 gene system is expressed in rodents and human male gonad and IL-1α is produced mainly by Sertoli cells while IL-1β mainly secreted in interstitium by testicular macrophages. In adult rat testis expression of IL-1α seems to be stage and developmentally regulated. Analysis of the mRNA expression of the two main genes of IL-1 system: IL-1α and IL-1RA, in different compartments of human normal testis and in biopsies from testis with impaired spermatogenesis was performed using the real-time PCR technique. mRNA samples were obtained from different cell subfractions and/or homogenates from testicular oligobiopsies. We have found distinct pattern of IL-1α and IL-1RA expression (mRNA) in two functional compartments of human testis (interstitial versus intratubular one). The mRNA level for IL-1α gene was high intratubularly, especially in fraction with round spermatids and low in interstitial cell fraction. The IL-1 RA gene was intensively transcribed in the round spermatid fraction. The analysis of mRNA expression in tissue homogenates from testis with maturation arrest, Sertoli cell only syndrome (SCOS) and obstructive azoospermia showed the inversely related balance between these two factors. The biopsies from SCOS patients showed exceptionally high expression for IL-1α in proportion to IL-1RA mRNA. The maturation block of spermatogenesis (azoospermia) was connected with almost equal expression of both these genes. When a spermatogenesis was close to normal (obstructive azoospermia) the expression pattern resembled the physiological status of testis (prevalence of IL-1 mRNA over IL-1RA). In conclusion, we may underline that IL-1α mRNA is inversely related to expression of IL-1RA according to the patophysiological status of the testis with the exceptional dynamics of tuning between these two analyzed factors.

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CKS2 REPRESSES CDK2/CCNA1-ASSOCIATED KINASE ACTIVITY

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In order to study the CDKs and interacting proteins that control meiosis, we examined CDK2β, the alternatively spliced isoform of cyclin dependent kinase 2 (CDK2). We used the yeast two-hybrid system to identify binding proteins of CDK2β, and identified the mouse homolog of CDC28 Kinase Subunit 2 (CKS2). Previously, we showed that CDK2β binds to cyclin A1 and to CKS2 in mouse testis. This suggested a role for CKS2 in regulating the prophase to metaphase I transition controlled by the cyclin A1/CDK2β complex. In agreement with this, *Cks2*$^{-/-}$ mice are sterile due to a block in spermatogenesis at metaphase I of meiosis (Spruck et al., Science 300: 647-650, 2003). I show here that the major biochemical effect of the absence of *Cks2* was an increase of CCNA1/CDK2-associated kinase activity in the testes of *Cks2*$^{-/-}$ mice. This suggests that the role of CKS2 is to modulate the activity of CCNA1/CDK2β kinase, and that its absence results in unregulated activity and a halt in spermatogenesis. Surprisingly, we also found that RT-PCR analysis showed that an alternate form of *Cks2* was being transcribed in testes of *Cks2*$^{-/-}$ mice, and that western blotting showed *Cks2*$^{-/-}$ mice produced a protein that was immunoreactive with the CKS antibody. This suggests that a translatable *Cks2* mRNA is present in testes of *Cks2*$^{-/-}$ mice, and that caution must be taken in interpretation of results from the *Cks2*$^{-/-}$ mice.

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DISSECTING THE AXONEME INTERACTOME: THE MAMMALIAN ORTHOLOGUE OF CHLAMYDOMONAS PF6 INTERACTS WITH SPAG6, THE MAMMALIAN ORTHOLOGUE OF CHLAMYDOMONAS PF16

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Cell motility and the movement of surface fluids are dependent upon flagella and cilia. The core structure of these organelles, the axoneme, is remarkably conserved across species, having in common nine doublets of microtubules with the associated force generating dynein arms and radial spokes surrounding two central singlet microtubules (the central apparatus), which contain associated proteins. The central apparatus is thought to play a key role in translating the microtubule sliding caused by dynein into the flagellar waveform and maintaining structural integrity of the axoneme. However, the central apparatus proteins involved in these processes have not been fully elucidated, and the network of interactions among them which allow these events to take place in a compact space have not been defined. PF6, a component of the *Chlamydomonas* central apparatus, is localized to the 1a projection of the C1 microtubule. The *Chlamydomonas* pf6 mutant has paralyzed flagella and lacks the 1a projection, indicating that PF6 has a critical role in axonemal structure and function. To investigate the function of PF6 in mammals, human and mouse PF6 were cloned. A 7.5 kb transcript is highly expressed in both human and mouse testis. PF6 message appears to be expressed at more modest levels in organs that contain cilia-bearing cells. The mouse PF6 message is detected on day 16 after birth, when pachytene spermatocytes are present, suggesting that mouse *Pf6* gene is expressed during meiosis. A rabbit anti-mouse PF6 polyclonal antibody was generated against a recombinant fragment of PF6 representing amino acid residues 548 to 875. In Western blot analyses on testes extracts two bands were detected, a 250 kDa protein consistent with the size of the translated protein encoded by the 7.5 kb cDNA sequence and a more abundant 97 kDa protein, consistent with the predicted molecular mass of a second PF6 cDNA clone generated by PCR from mouse testis RNA which appears to be an alternative splice variant. In epidimymal sperm, the most prominent immunoreactive band was 37 kDa, suggesting that there is proteolytic processing of the larger PF6 protein isoforms. Immunohistochemical studies on testis sections demonstrated that PF6 is present in the cytoplasm of round spermatids, and more signal was found in the condensing spermatids. The antibody detected PF6 antigen along the tail of permeabilized epididymal sperm. Electron microscope immunocytochemistry revealed that PF6 was localized to the central pair of the axoneme, a location that is consistent with the *Chlamydomonas* PF6 protein. To investigate whether PF6 can interact with SPAG6, another axoneme protein localized to the C1 microtubules in the central pair, CHO cells were cotransfected with PF6/pEGFP-N_2 and SPAG6/Dsred-N_1 plasmids. PF6 protein, which when expressed alone is cytoplasmic, decorated the microtubules of PF6/SPAG6 co-transfectants, and co-localized with SPAG6. PF6 protein could also be pulled down by anti-SPAG6 antibody in the cotransfected CHO cells. Finally, the 37 kDa PF6 band was virtually absent from the epididymal sperm of SPAG6-deficient mice. These findings reveal a previously unknown feature of the axoneme interactome, creating a new framework for understanding how the central apparatus regulates mammalian axoneme function.

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EFFECT OF FINASTERIDE AND TESTOSTERONE UNDECANOATE ON REPRODUCTIVE FUNCTION IN MALE RATS

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To investigate whether 5á-reductase and dihydrotestosterone (DHT) play a role in spermatogenesis and reproductive function we administered a 5α-reductase-2 inhibitor, Finasteride (Fi), with or without TU to adult male SD rats for 12 weeks. Animals were divided into four groups with 5 rats per group: C; control, F; Fi alone (5mg/Kg, oral), T; TU alone (20mg/Kg, SC) and FT; Fi+TU, respectively. We showed that: in comparison with control, 1).the weight of testis was reduced by TU or Fi+TU but not by Fi ($P>0.05$). TU increased while Fi decreased the weight of ventral prostate. The effect of Fi on prostate weight was reversed by TU in the FT group. 2). Fi suppressed the serum DHT but increased the testosterone (T) levels, while TU increased both; group FT showed no suppression of the Serum DHT and increased T level. 3). Fi or TU and Fi+TU induced increased apoptosis of germ cells. 4). As anticipated Fi markably inhibited the activity of 5α-reductase in the testis. The activity of 5α-reductase in testis of group Fi+TU was decreased compared with TU group. 5). F, TU and Fi+TU all showed decreased epididymal sperm count. We conclude from this study, high doses of 5α-reductase-2 inhibitor (Fi) suppressed male reproductive function by inhibiting activity of 5α-reductase of gonad and inducing apoptosis of testis cells, but the combination of TU+Fi does not enhance suppression of either T or Fi alone.

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CASPASES ARE EXECUTIONERS OF APOPTOSIS DURING THE FIRST WAVE OF SPERMIOGENESIS IN THE RAT

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Mammalian spermatogenesis is a highly regulated process which starts during puberty and it kept a continuous production of spermatozoa throughout the male reproductive life. Spermatogenesis process begins when germ stem cells (spermatogonia) proliferate and undergo between 9-10 rounds of mitosis. After that, they enter into meiosis and then the haploid cell (spermatid) differentiates into spermatozoa. It has been documented that during puberty there is a dramatic increased in the death of germ cells, however there is no information about its regulation or control.

Caspases are a family of cisteine-proteases activated during apoptosis. Procaspase-2 and -8 are activated by oligomerization of death receptors like FAS or necrosis tumoral factor receptor. On the other hand, stress stimulis induce the activation of procaspase-9 which in turn activates caspasa-3. caspase-8 could also activate caspasa-9 and caspasa-3 may increased the apoptosis rate by processing procaspase-9. In this work we have study the activation of caspases during the first wave of spermatogenesis, in order to determine the role of these proteases in the execution of germ cell apoptosis. TUNEL (+) germ cell we observed during all the studied period between 5 and 40 days old rats. Numbers of TUNEL (+) germ cells reached a maximum in 25 days old rat and they were significantively lower during other ages. It is interesting that TUNEL (+) cells appeared frequently in groups of 3 or more cells and rarely were observed alone. We did not detect any Sertoli cell labeled with TUNEL. Immunohistochemistry using a specific antibody against active casapse-3 (PROMEGA) indicated that number of caspasa-3 active cells peaked at 25 days old rat and decreased dramatically at 35 days. A similar result was observed by immuhistochemistry with an antibody specific to active caspasa-9 (Cell Signaling). Active caspase-3 and-9 (+) cells were rarely observed in adult testis. Confocal scanning laser microscopy indicated that there is a co-localization of TUNEL (+) and active caspasa-3 (+) and active caspasa-9 (+). We isolated germ cells and assayed the enzyme activity of casapase-3, -8 and -9 using a colorimetric assay with specific peptides as substrates, and we observed the same pattern of activation as described with immunohistochemistry. We did not detect FAS between 5-15 days old rats. Germ cell with a clear positive signal for FAS appeared at 20 days and peaked in 25 days old rats. Confocal scanning laser microscopy indicated that most of the TUNEL (+) cell co-localize with FAS (+) cells. Isolated FAS (+) germ cells had a significatively higher activity of caspase-3, -8 and -9 than FAS (+) cells.

Our Results strongly suggest the participation of caspase-3, -8 and -9 in the apoptosis during puberty of the rat. We proposed that activation of FAS or another related death receptor could trigger activation of procasapase-8 and/or probably proaspase-2.

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METALLOTHIONEIN EXPRESSION IN MOUSE TESTIS AFTER BUSULFAN TREATMENT

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Metallothionein (MT) is a group of low molecular weight proteins, and has a unique amino acid composition such as a large content (approximately 30%) of cysteine residues. The function of MT is known to be related to the proliferation of cells and the detoxification of heavy metals. Spermatogonia is always proliferating, but were stopped by busulfan treatment for cancer therapy. We evaluated histological change and expression pattern of metallothionein during spermatogenesis in mouse testis after busulfan treatment. Experimental group of male ICR mice was treated with a single I.P. injection of 40 mg busulfan/kg of body weight. Control group received a single injection of 0.2 ml of a mixture of dimethyl sulphoxide (DMSO) and saline. Mouse testis were collected on day 1 to day 70 after busulfan treatment in experimental groups and on day 43 in control group. The tissues were prepared for light microscopy and immunohistochemistry for metallothionein (MT). By busulfan treatment, the ratio of testis to body weight was decreased gradually to 6 weeks, after that the ratio increased gradually. In histological observation of experimental group, testicular germ cells gradually disappeared until 3 weeks. Spermatogonia were firstly disappeared and spermatozoa were maintained upto 3 weeks. In immunohistochemical study, MT were expressed in proliferating spermatogonia of control and experimental group. MT expression was gradually decreased in experimental group until 3 weeks after treatment. Interestingly, we observed that recurrence of spermatogonia and expression of MT in experimental group after 4 weeks of the busulfan injection at the same time. Base on our results, busulfan seems to be affect on proliferation of spermatogonia, but not on differentiation process of spermatogenesis. The busulfan treated mouse may be showed failure of spermatogenesis, however, they have a potency of spermatogenesis after recovery time. We suggests that MT have relationships with the proliferation of male germ cells and recurrence of spermatogenesis.

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INVOLVEMENT OF p38 MAPK AND NITRIC OXIDE IN INDUCING MALE GERM CELL APOPTOSIS VIA INTRINSIC PATHWAY SIGNALING AFTER HOMONE DEPRIVATION

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In earlier studies, we have shown that the mitochondria-dependent intrinsic pathway signaling is the key pathway for testicular germ cell apoptosis. To gain further insight into the molecular mechanisms that regulate germ cell apoptosis, we characterized the up-stream modulators of the intrinsic pathway signaling after suppression of intratesticular testosterone (T). Groups of adult male rats were given a daily injection of vehicle for 14 days or GnRH antagonist (GnRH-A) for 2, 5, and 14 days. Within 2 days of GnRH-A treatment testicular T concentrations declined markedly to 14.0% of control values and plasma T levels fell below detectable limits. The mean incidence of apoptotic germ cells (expressed as numbers per 100 Sertoli cells) exclusively at stages VII-VIII increased significantly (108.1 ± 7.4; $P < 0.001$) by Day 5, and increased another 1.9-fold (over the 5-day treatment value) on Day 14 after GnRH-A treatment when compared to controls, where no apoptosis was detected. Activation of p38 mitogen-activated protein kinase (p38 MAPK), critical mediator of a variety of environmental stresses, and induction of inducible nitric oxide synthase (iNOS), which is critical for NO-mediated cell death, were clearly evident in the testicular lysates, coincident with the fall in testicular T concentrations as early as 2 days after GnRH-A treatment. These events were followed by a marked alteration in the mitochondrial Bax and Bcl-2 ratio, cytosolic translocation of mitochondrial cytochrome c, caspase activation, and PARP cleavage. Taken together, these results indicate that withdrawal of gonadotropins and consequently intratesticular T induces germ cell apoptosis in the testis by stimulating p38 MAPK and NO-mediated intrinsic pathway signaling. Supported by NIH grants HD 39293 and GM 08683.

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THE USE OF AN IMMORTALIZED SERTOLI CELL LINE IN SERTOLI-GERM CELL COCULTURE

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The current method for studying the interactions between Sertoli cells and germ cells *in vitro* consists of using primary Sertoli cell isolates, making these kinds of studies difficult and expensive. Using an established cell would be advantageous, but very few Sertoli cell lines exist. Most available Sertoli cell lines do not possess FSH receptor and are thus insufficient for studying how FSH is involved in the binding dynamics of Sertoli and germ cells. Hence, the development of a Sertoli cell line that supports germ cell binding and expresses FSH receptor is of special interest. The sk9 Sertoli cell line, established from H-2K^b-tsA58 transgenic mice, expresses mRNA for FSH receptor and, as such, is a good candidate for use in Sertoli-germ cell binding studies. This study utilized these cells in cocultures with mouse spermatocytes and round spermatids. Some cocultures contained FSH (recombinant hFSH AFP8468A, 10ng/ml) and/or testosterone (Sigma, 100 nM) and were incubated 44 hrs at 32°C. The number of germ cells bound to Sertoli cells was determined using ImageJ. Round spermatids appeared to exhibit a tighter binding to the Sertoli cells than did spermatocytes, since more spermatids remained attached following gentle washing. Hormones increased the number of bound germ cells and appeared to stimulate tubule formation. These results suggest hormones enhance binding *in vitro* between sk9 Sertoli cells and isolated germ cells in a manner similar to that reported when using primary isolated Sertoli cells. The apparent response of the sk9 cells to hormones indicates that this cell line is potentially useful in the study of cell-cell interactions between Sertoli and germ cells *in vitro*.

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NUCLEAR RECEPTOR COFACTORS IN EFFERENT DUCTULES OF MOUSE, RAT, HAMSTER, AND MONKEY

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In male C57BL/6J mice, the antiestrogen ICI 182,780 alters efferent ductule structure and function leading to infertility. However, other species show response differences, such as increases in lysosomes in rats and increase in NHE3 in marmoset monkeys, yet all these species show luminal dilation. Because steroid ligands show nuclear receptor cofactor-specific recruitment in mediating tissue and cell-specific effects in the female, we are testing the hypothesis that responses to ICI in males may also depend upon the cofactor environment. To evaluate potential species differences, immunohistochemistry was used to identify the following cofactors: Steroid Receptor Coactivator (SRC)-1,2,3; Repressor of Estrogen Activity (REA), Silencing Mediator for Retinoid and Thyroid Receptor (SMRT); and Nuclear CoRepressor (NCoR). SRC1 displayed strong nuclear, minimal cytoplasmic, and no microvillus staining among rodents; in marmosets, microvilli stained but not nuclei or cytoplasm. SRC2 staining was perinuclear in mice, nuclear and perinuclear in rats, and microvilli in hamsters. SRC3 was cytoplasmic and nuclear in rodents, with nuclear staining highest in mice and least in rats. In mice, rats, hamsters and marmosets, 90, 75, 0, and 0% of nuclei stained for REA, respectively. For SMRT, the percentages were 90, 75, 30 and 20%, respectively. REA and SMRT were also always found in cytoplasm. NCoR stained microvilli intensely in mice but nuclei showed speckled staining. In rats, NCoR was perinuclear, but in hamsters, 1/3 of the nuclei showed speckled staining. When compared to females, males differed considerably for select cofactors. Thus, differences in cofactor expression may contribute to species-specific male responses to estrogens and antiestrogens. Support: CONRAD Program; NIH T32 HD07028 and ES07326.

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NITRIC OXIDE FROM ACTIVATED TESTICULAR MACROPHAGES: A NEGATIVE PARACRINE MODULATOR OF TESTOSTERONE SYNTHESIS IN RAT LEYDIG CELLS

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Leydig cells reside in the testicular interstitium along with blood vessels, lymphatics and macrophages. Previous reports have shown that testicular macrophages (TMs) express the inducible isoform of nitric oxide (NO) synthase (iNOS), although they failed to generate NO during incubations in vitro. Leydig cells have also been reported to express NOS and generate NO. Since NO is known to have a suppressive effect on testosterone production, the goal of the present study was to determine whether NO is a paracrine signal of TM origin or, rather, an autocrine effector synthesized by Leydig cells. To this end, we measured NO production and iNOS expression levels in TMs and Leydig cells. Purified Leydig cells and TMs were cultured for 24 h in the presence or absence of the proinflammatory factor lipopolysaccharide (LPS). INOS was undetectable in Leydig cells by DNA gene array and RT-PCR. NO production by Leydig cells as measured by the concentration of nitrite accumulated in spent culture media (Griess method), was unaffected by LPS (10 mg/ml). In contrast, a high level of iNOS mRNA expression was recorded in LPS-activated TMs. This observation was associated with a 10-fold increase in NO formation following LPS activation. Our results show that TMs are a possible source of NO that is locally produced in the testis under pathological conditions. We conclude that NO is a modulator of Leydig cell testosterone production through paracrine signaling.
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SEMEN ANALYSIS/SPERM

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IDENTIFICATION OF PP60C-SRC AS THE ENZYME RESPONSIBLE FOR TYROSINE PHOSPHORYLATION AND CONSEQUENT HYPERACTIVATION DURING MOUSE SPERM CAPACITATION

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Upon ejaculation, spermatozoa undergo a series of post-translational modifications in order to acquire the competence to fertilize the oocyte. Collectively, this complex process has been termed "capacitation". A fundamental attribute of capacitation is a redox-regulated, cAMP-dependent increase in the overall level of tyrosine phosphorylation. For over three decades, the importance of PKA in the regulation of this pathway has been recognized however, to date, the molecular identification of the intermediate tyrosine kinase has remained elusive. Using difference in 2D gel electrophoresis (DIGE), we have established and identified the major proteins that become tyrosine phosphorylated during capacitation including enolase, tubulin, hsp70. Based on these results, we have determined that pp60c-src, a non-receptor protein tyrosine kinase, is a key mediator of sperm capacitation, and in particular hyperactivation. Consistent with this, pp60c-src was shown to co-immunoprecipitate with PKA, the major inducer of tyrosine phosphorylation in spermatozoa. pp60c-src also co-precipitated with AKAP4 and ODF-2, two known substrates for tyrosine phosphorylation in capacitating sperm. Upon addition of the src-kinase inhibitors su6656 and PP1 a decrease in specific tyrosine phosphorylated proteins occurred. The major consequence of this event was a loss of the ability of the spermatozoa to undergo hyperactivated motility, a critical component of capacitation.

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ADVANTAGE OF COMBINING MAGNETIC CELL SEPARATION WITH SPERM PREPARATION TECHNIQUES

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The selection of vital, non-apoptotic spermatozoa is a prerequisite for achieving optimal conception rates in assisted reproductive techniques (ART). Magnetic cell sorting (MACS) using annexin-V microbeads has been shown to effectively separate apoptotic and non-apoptotic spermatozoa. The objective of our study was to optimize the integration of MACS in standard sperm preparation methods. Semen specimens collected from 14 healthy donors were separated into 3 aliquots, which were prepared by: 1) density gradient centrifugation (DGC); 2) DGC followed by MACS; and 3) simple HTF wash followed by MACS. Different aliquots were evaluated by estimation of semen parameters (motility, viability, morphology), as well as, markers of apoptosis [levels of active caspase 3 (CP3) and integrity of transmembrane mitochondrial potential (TMP)]. CP3 and TMP were measured using fluorochrome dyes coupled with flow cytometry.
The combination of DGC and annexin-V MACS procedure was superior to all other sperm preparation methods in terms of providing motile, viable and non-apoptotic spermatozoa. No significant differences were seen in terms of morphology (table).
The results of our study clearly indicate the advantage of integrating MACS as a part of sperm preparation. The combination of DGC and MACS represents the optimum approach for sperm preparation, which in turn may positively affect ART success rates.

Sperm preparation	Motile (%)	Viable (%)	Morphology (%)	CP3 (%)	Intact TMP (%)
DGC	74.4±12.3*	75.5±10.8**	21.3±10.0	17.8±12.2**	73.6±16.3*
HTF+MACS	49.9±19.6**	71.6±11.8**	21.9±8.7	14.6±16.4*	75.2±7.8
DGC+MACS	82.5±9.2	82.9±6.7	21.4±8.9	5.8±5.1	83.5±11.5

Values are expressed as mean ± standard deviation. **p<0.01; * p<0.05 in comparison to the protocol using MACS after DGC.

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THE RELATIONSHIP BETWEEN HUMEN SEMEN CHARACTERISTICS AND SPERM APOPTOSIS: A PILOT STUDY

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Introduction: Conventional semen analysis provides information on the clinical status of male fertility. However, the results of the semen analysis are only moderately predictive of an individual's fertility. It is important to develop new methodology and assays to improve the clinical diagnosis of male infertility. In the present study, we explored the associations between human semen characteristics and the percent of apoptosis in ejaculated sperm. Methods: We collected semen samples from 24 consecutive male patients who presented to the Vincent Memorial Andrology Laboratory at Massachusetts General Hospital (MGH) for routine semen analysis. Sperm concentration and motility were measured using computer assisted sperm analysis (CASA). Morphology was assessed using Tygerberg Strict criteria. The DNA diffusion assay was used to assess the percentage of apoptosis in ejaculated sperm. In this assay, cells were mixed with agarose and placed into a microgel on a microscopic slide. The cells were stained with YOYO-1 dye, and apoptotic cells were viewed under a fluorescent microscope.
Results: Among the 24 men, the mean (SD) sperm concentration, percent motility, percent progressive motility, and normal morphology were 123.1 (91.1) million/ml, 43.8 (23.6) %, 27.2(15.9) %, and 7.8(4.6) %, respectively. The mean (SD) percent of apoptosis in ejaculated sperm was 8.3 (6.1) %, with a range from 1.1% to 20.1%. In multivariate linear regression, we found significant inverse associations between percent apoptosis and sperm motility (P=0.01), progressive motility (P =0.02), and morphology (P=0.01).
Conclusion: The present study identified inverse associations between the percent of sperm apoptosis in ejaculated human semen and measures of semen quality, including sperm motility, progressive motility and normal morphology.

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NEW LUBRICATING GEL FOR SEMEN COLLECTION DOES NOT DAMAGE MOUSE EMBRYOS, MUCOSAL MEMBRANES OR MALE FACTOR CONDOMS

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Previous studies have shown no negative impact of His~Seed™ (HS) gel on sperm function *in vitro*, while enhancing the experience and success of semen sample collection by men. Further studies were done to evaluate HS: 1) toxicity to mouse embryos; 2) damage to mucosal membranes (slug model); 3) penile irritation (rabbit); and 4) compatibility with Male Factor Pak™ polyurethane condoms.
1) EmbryoTech: 1-cell mouse embryos (n=42 each) were exposed to 5% HS, KY®, Replens®, or Astroglide® (AG) for 30 min, then cultured and scored for % blastocysts at 96 hours. RESULTS: Control media 96(1); HS 94(1); KY 85(2); Replens 83(2); AG (0). HS showed no embryo toxicity.
2) Ghent University: 100 mg of HS, Replens, or AG was placed on slugs (n=5 each) for 5 days. Membrane irritation & damage were determined by mucus production and protein secretion. RESULTS: Replens- mild irritation, no damage; AG- severe irritation and damage; KY- (previously done) moderate irritation, no damage; and HS- no irritation or damage.
3) Product Safety Labs: Using ISO 10993-10 guidelines, HS or control was applied to protruded rabbit penis (n= 6 each) daily for 10 consecutive days. RESULTS: Macroscopic and microscopic evaluation revealed no inflammatory potential of HS.
4) Nelson Labs: Male Factor condoms (n=13 each) were submerged in HS for 120 min under 37°C, 75% humidity conditions. Samples were then dried for 16 hours, and tensile strength measured using FDA-GLP procedures. RESULTS: HS had no affect on condom integrity.
CONCLUSIONS: His~Seed™ is non-irritating; does not interfere with mouse embryo development; nor polyurethane condom integrity. This new gel can enhance the sperm sample collection.

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ESTIMATION OF NUCLEAR PROTAMINE TO HISTONE RATIO IN THE SPERMATOZOA OF FERTILE AND INFERTILE MEN

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Numerous studies have shown that the infertile men possess high levels of sperm DNA damage. However, there is little known about the etiology of this damage. The objective of this study was to examine and compare the relative proportion of nuclear histones and protamines in the spermatozoa of fertile and infertile men. We conducted a prospective study on consecutive semen samples obtained (after informed consent) from non-azoospermic, asthenospermic infertile men (n=6) and fertile controls (n=5). After standard semen parameters had been recorded, semen samples were stored at -70°C for later assessment of nuclear proteins. Sperm nuclear proteins were extracted and subsequently separated by acid-urea polyacrylamide gel electrophoresis (each sample was run in duplicate and reported as a mean value). The relative protamine 1 to histone and protamine 2 to protamine 1 ratios were estimated by densitometric analysis of the acid urea gels (stained with Coomassie blue). Western blot analysis was used to confirm the detection of protamines 1 and 2 (using previously characterized antibodies – a gift of Dr. Rod Balhorn). In this study, sperm samples from infertile men had a significantly lower relative proportion of protamine 1 to histone than did samples from fertile men (55 vs. 90%, P<0.05)) Sperm samples from infertile men also had a lower relative proportion of protamine 2 to protamine 1 than did samples from fertile men (23 vs. 34%) but the difference was not significant (P=0.06). Our findings indicate that the spermatozoa of infertile men possess a marked deficiency in nuclear protamines. The data also suggest that there may be a further reduction in protamine 2 levels (relative to protamine 1) in the spermatozoa of these men. Taken together with the understanding that protamines play a major role in sperm nuclear compaction, our preliminary data suggest that sperm protamine deficiency may be an important cause of sperm DNA damage in infertile men.

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THE CONCENTRATION OF SPERMS ASSESSED BY 20 MICRON CAPILLARY FILLED SLIDES IS UNDERESTIMATED. A METHOD FOR CORRECTION

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The concentrations of sperm assessed with disposable slides with a fixed cover slip are systematically lower than hemocytometer results. It appeared that after the establishment of a lamellar flow in the capillary, sperms cells are transported to the filling front. This phenomenon can be explained by the Segre-Silberberg effect (SS effect). Theoretical and experimental results have been extensively described by Douglas-Hamilton et al (J Androl, January 2005). In this type of slide (the Leja four chamber slide with a chamber height of 20 micron), flow velocity is the only variable that affects the size of the correction factor for the SS effect. The correction factor was 1.30 for semen suspended in culture medium (viscosity 1 cP), while for very viscous semen the correction factor was close to 1.00. The outcome obtained by using a disposable slide has to be multiplied by this correction factor to obtain the result equivalent to that of the hemocytometer.

The correction factor can be described by an algebraic function f(t)(t = filling time) for a specific slide. There are two asymptotes t_a=2,3 ($f(t_a)$=1.30 for sperms suspended in watery solutions,viscosity 1cP,) seconds) and $f(t_b)$=1.00 for highly viscous semen (filling time $\rightarrow\infty$). Leja four chamber slides were used to determine values between these extremes. Ten human semen samples of different viscosities were analyzed, included highly diluted semen. Each sample was assessed 6 times in a Leja chamber and 6 times in a hemocytometer (improved Neubauer). To each sample 7 µl of 35% formaldehyde was added to immobilize the cells. Samples were stored for several hours and the upper layer was pipetted into a new tube. This tube was carefully mixed. In this way no dilutions were needed (viscosity not affected) and the same mixture could be analyzed both with the Leja chamber and the hemocytometer. Filling time was assessed with a stopwatch.

The best statistical fit was $f(t)= 1 + C_1/t - C_2/t^2$. For a Leja four chamber 20 micron slide, the filling time and the correction factor can be used to estimate the true concentration (as measured in a hemocytometer). Other slides will have corresponding functions. For given chamber type, the correction factor will depend on sample viscosity, which can be estimated by filling time. By measuring the filling time, the sperm concentration correction factor f(t) for each type of capillary filled chamber can be predicted. Concentration can be compared with hemacytometer.

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LECTIN CHARACTERIZATION OF MEMBRANE SURFACE CARBOHYDRATES IN POULTRY SPERMATOZOA

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The surface glycoconjugates of mammalian sperm cells have been shown to be critical components of reproduction, including maintenance of the oviductal sperm reservoir and sperm-egg binding. In contrast, the glycocalyx of poultry spermatozoa has not been well characterized. Our objective was to determine the morphological distribution of sugar residues along the plasma membrane of turkey and chicken spermatozoa. For each species, weekly semen samples from 3 males were pooled. Seminal plasma was removed, and the spermatozoa were fixed in 4% paraformaldehyde (30 min; room temperature) prior to lectin staining. Fixed sperm were air-dried on slides and stained with 1 of 14 different FITC-conjugated lectins (100 µg/mL; 30 min; room temperature) to localize the distribution of 13 different saccharides by epifluorescence. Lectin staining was replicated 3 times for each species, using the same donor males. In both species, α-fucose (*Lotus,* UEA-I lectins), N-acetyllactosamine (ECA lectin), α-mannose and α-glucose (GNA, Con A, PSA lectins) were detected only in the plasma membrane overlying the head region. In contrast, α and β-N-acetylgalactosamine (SBA, WFA lectins), α and β-galactose (RCA-I, GS-I lectins), and both dimers and oligomers of β-N-acetylglucosamine (STA, succinyl-WGA lectins) were distributed mainly along the acrosome region. Sialic acid residues (LFA lectin) were distributed along the entire cell surface in both species. Notable differences between species included: 1) α-galactose and β-N-acetylgalactosamine were not found on the head region of chicken sperm; and 2) α and β-N-acetylglucosamine monomers (GS-II lectin) were present only on the head of chicken sperm (acrosome and head of turkey spermatozoa). These findings suggest that segregation of surface carbohydrates to specific zones of the sperm appears also to exist in birds as reported in mammals.

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IDENTIFICATION OF A DECAPACITATION FACTOR: A CASE FOR PLATELET-ACTIVATING FACTOR-ACETYLHYDROLASE

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Introduction: To become fertilization-competent, sperm must spend sufficient time in the female reproductive tract. This acquired capacity to fertilize was first observed independently by Austin and Chang. This process is referred to as "capacitation" and is associated with biochemical and physical changes in the sperm. Decapacitation factor is present in seminal fluid and it's character is not fully understood. Platelet-activating factor [1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; PAF] is present in human sperm and its' content has a significant and positive relationship with motility. PAF-acetylhydrolase (PAFah), the enzyme responsible for PAF's activity, is present in semen and may serve as a sperm decapacitating factor. There are limited reports on PAFah levels in semen and PAFah's relation to sperm motility. The study objective was to determine the relationship between PAFah content in semen and sperm motility and the effect of PAFah on sperm capacitation and the sperm penetration assay (SPA).

Design: PAFah levels in semen were measured and correlated with sperm motility and the effect of exogenous PAFah on sperm motility (CASA) during capacitation and the SPA was determined.

Materials and Methods: Human semen was obtained from healthy mature males (n=25) and sperm motility (WHO, 1999) recorded prior to measurement of PAFah activity by spectrophotometric analysis (AZWELL Inc, Japan). Sperm washed free of seminal fluid were incubated in test yolk buffer for 48-72 hours at 4°C to induce capacitation with or without exogenous PAFah. Data were analyzed by linear regression, Student's *t*-test and the Chi-squared test.

Results: Seminal PAFah content ranged from a low of 252 IU/L cells to a high of 1,469 IU/L. The overall mean PAFah content in semen was 807.38 IU/L (range 1,217 IU/L). Linear regression analysis revealed a significant ($P<0.001$) relationship [motility=56.76+log(PAFah)] between PAFah content in semen and sperm motility. Semen specimens presenting with a normal (>50%) percent motility (695.06 IU/L) had a significantly ($P<0.01$) lower PAFah content than specimens with an abnormal (<50%) percent motility (1097.11 IU/L). Exogenous PAFah significantly ($P<0.05$) reduced sperm motility and reduced sperm penetration assay scores.

Conclusion: The data confirms the presence of PAFah in human semen and that levels are related to sperm motility. Exogenous PAFah reduces sperm's ability to undergo capacitation (as determined by CASA motility and the SPA). Taken together, the data demonstrates the ability of PAFah to prevent capacitation and, or decapacitate sperm. Therefore, PAFah is identified as a decapacitation factor. Additional studies will elucidate the role of PAFah in semen on sperm motility and the significance PAFah plays in sperm decapacitation and human fertility.

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ESTIMATION OF SPERM DEOXYRIBONUCLEIC ACID DAMAGE BY GENE-SPECIFIC POLYMERASE CHAIN REACTION (PCR) ANALYSIS

Maria San Gabriel, Ph. D., Xiaoyang Zhang, MD and Armand Zini, MD (Presented By: Maria San Gabriel, Ph. D.)

Studies have shown that the spermatozoa of infertile men possess substantially more DNA damage than that of fertile men. However, there is little known about gene-specific sperm DNA damage. As such, the objective of this study was to further characterize human sperm DNA damage by estimation of the relative gene-specific DNA damage by semi-quantitative PCR analysis. We conducted a prospective study on consecutive semen samples obtained (after informed consent) from asthenospermic infertile men (n=4) and fertile controls (n=4). After standard semen parameters had been recorded, semen samples were stored at -70°C for later assessment of DNA damage. Sperm DNA was extracted by phenol/chloroform and exactly 200 ng of DNA was used as template for the PCR reaction (each sample was run in duplicate and reported as a mean value). Probes specific for the ß-globin and IGF-2 genes and for telomeric sequences were used in these experiments and the relative transcript levels were estimated by densitometric analysis. (Semi-quantitative PCR analysis provides a measure of the integrity of the template DNA). In this study population, the mean PCR transcript levels for the ß-globin gene and for telomeric sequences were significantly lower in the sperm samples of infertile compared to fertile men (3352 vs. 6640 and 1232 vs. 5586 pixel counts, respectively, $P<0.05$). The mean PCR transcript levels for the IGF-2 gene was lower in the sperm samples of infertile compared to fertile men but the difference was not significant (data not shown). Our preliminary findings suggest, for the first time, that the spermatozoa of infertile men have higher levels of gene-specific DNA damage than that of fertile men. Further studies are needed to validate these important results.

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β-CYCLODEXTRIN PLUS CHOLESTEROL PROTECTS PORCINE SPERM FROM THE EFFECTS OF COLD SHOCK*

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Porcine sperm are extremely sensitive to the damaging effects of cold shock. It has been shown that cholesterol-binding molecules, such as 2-hydroxypropyl-β-cyclodextrin (HBCD), improve post-cooling porcine sperm viability when added to an egg yolk-based extender, but also enhance sperm capacitation in other species. The objective of this study was to determine the effects of HBCD and cholesterol 3-sulfate (ChS), in a defined medium without egg yolk, on the viability and capacitation of porcine sperm following cold shock (10°C for 10 min). A total of 6 experiments using 3 different semen samples from 2 adult boars were performed. We report here that porcine sperm incubated in medium containing both HBCD and ChS have significantly improved viability following cold shock (77.5 ± 2.1%, mean ± SEM) when compared to cold shocked sperm incubated without HBCD or ChS (28.2± 5.8%), or with either component alone (HBCD: 25.8 ± 4.7%, ChS: 39.5 ± 4.2%). Immunoblots revealed that treatment with HBCD plus ChS completely inhibited the increase in protein tyrosine phosphorylation induced by the cold shock treatment. Two assays of sperm capacitation, the rate of calcium ionophore-induced acrosome reactions and chlortetracycline (CTC) staining, were not significantly altered by HBCD and ChS following cold shock, although there was a trend towards less CTC B (capacitated) pattern sperm following incubation with HBCD plus ChS after cold shock. These results indicate that the manipulation of sperm plasma membrane cholesterol content affects porcine sperm viability and capacitation status and could therefore be useful to protect sperm from cold shock during cryopreservation by improving viability without promoting premature capacitation.

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ANDROGENS/HORMONES

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THE ACCURATE DEFINITION OF REFERENCE INTERVALS FOR THE DIAGNOSIS OF ANDROGEN AND REPRODUCTIVE HORMONE STATUS IN MEN

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The diagnosis of male reproductive disorders demands precise hormonal measurements with appropriate reference intervals, but many current assay protocols fail to meet these requirements, particularly for testosterone (**T**). This study examined the performance of 8 commercial assay platforms in the measurement of total T, LH and FSH in the sera of 124 healthy fertile men (age 21-35 yrs) with normal sperm output in order to define method specific reference intervals using non-parametric analyses. These intervals were then compared both between platforms and with those provided by kit manufacturers or reported by the laboratories. Serum total T reference intervals showed wide variation at the lower end (7.6-12.4 nM) and were up to 6.5 nM (median 2.6 nM) higher than those in current use. Calculated free T levels reflected this variability. Serum FSH and LH values showed better agreement between assays but these new intervals varied widely from those currently in use. We conclude that there are clinically significant differences in existing T assay reporting protocols that would affect the diagnosis of androgen deficiency. The results highlight the need for calibration of T assays against a mass standard to provide uniform results. Gonadotropin assays show less variability between platforms potentially allowing the use of a common reference interval, but current quoted intervals are not optimal for assisting in the diagnosis of azoospermia or androgen deficiency.

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HOW DO AGE-RELATED DECLINES IN TESTOSTERONE AFFECT HIGH DENSITY LIPOPROTEIN CHOLESTEROL? LONGITUDINAL RESULTS FROM THE MASSACHUSETTS MALE AGING STUDY

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Introduction: Prior studies have shown that: (1) low testosterone (T) levels are associated with risk factors for cardiovascular disease (CVD) including increased age, body mass index (BMI), and diabetes; and (2) both T and high density lipoprotein cholesterol (HDL-C) change with age. However, T replacement in elderly, hypogonadal men has little impact on HDL-C. We investigated whether decreases in T were independently associated with changes in HDL-C over time in aging men.

Methods: Data were obtained from the Massachusetts Male Aging Study, a prospective, community-based random sample of men aged 40-70 at T_1 (1987-89). 958 men who had complete data at $\mathbf{T}_1$ and T_2 (1995-97) were analyzed. Serum total **T** (radioimmunoassay), and HDL-C were measured on non-fasting, morning blood samples at T_1 and $\mathbf{T}_2$. Multiple linear regression was used to model change in HDL-C as a function of change in T. We adjusted for: age, diabetes, heart disease, hypertension, BMI, alcohol, smoking, physical activity, and medications.

Results: Mean (standard deviation) T declined from 520 (175) at T_1 to 454 (162) ng/dL at T_2. Mean HDL-C increased: 42.7 (13.1) to 44.0 (12.4) mg/dL. Change in T was not correlated with change in HDL-C (correlation coefficient 0.05; p=0.11). When models were adjusted for baseline confounders, decreases in T were associated with very slight decreases in HDL-C (drop of 0.6 mg/dL per 100 ng/dL decrease in T; p=0.003). However, when models were adjusted for both the baseline and the change in each confounder (T_2-$\mathbf{T}_1$), there was no longer any relationship between change in **T** and change in HDL-C (p=0.09).

Conclusion: These results suggest that changes over time in risk factors for CVD such as BMI may have a greater impact on HDL-C than do declines in T.

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CUBITAL LH AND TESTOSTERONE CONCENTRATIONS ARE NOT CORRELATED TO SCROTAL TESTOSTERONE VALUES IN YOUNG MEN WITH UNILATERAL VARICOCELE

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Introduction: In healthy men the Hypothalamic-Hypophyseal-Gonadal axis regulates the scrotal testosterone production. In this prospective study the connection between peripheral and scrotal LH and Testosterone concentrations in varicocele patients was examined. 102 patients who where treated for unilateral varicocele have been included in this study.
Methods: Varicocele was diagnosed by examination of the spermatic cord and confirmed by Doppler-ultrasound. Men with right sided reflux were ruled out. Prior to the operation the cubital venous values of Testosterone (**T**), LH, FSH, Estradiol (E2) and SHBG had been determined. During the antegrade sclerotherapy the **T**, LH, FSH, E2 and SHBG values in the scrotal venous blood were examined.
Results: Median cubital LH values were significantly correlated with scrotal LH values ($p < 0,001$; $r = 0,705$). No correlation existed between the peripheral LH and scrotal **T** ($p = 0,496$, $r = 0,072$) or E2 ($p = 0,559$, $r = 0,060$) values. No correlation could be found between the cubital and scrotal T ($p = 0,169$, $r = 0,146$) or E2 values ($p = 0,146$, $r = 0,151$). **F**SH as indicator of the relationship between the peripheral and scrotal blood determination was significantly correlated ($p < 0,001$, $r = 0,942$). The scrotal **T** (6,0 – 987,5 µg/l, median 116,5 µg/l) and E2 values showed extremly high variations (10 – 3239 ng/l, median 243 ng/l), which were not found in the cubital vein (**T**: 2,5 – 11,5 µg/l, median 5,6 µg/l, E2: 10 – 65 ng/l, median 65 ng/l).
Conclusion: Cubital venous LH is not correlated with the Leydig cell function as confirmed by the scrotal venous Testosteron and Estradiol concentration in young men with unilateral varicocele. High variations within the testicular **T**estosterone and Estradiol concentration are not found in the peripheral venous blood.

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EXPECTATIONS TOWARDS A NOVEL MALE FERTILITY CONTROL METHOD AND POTENTIAL USER TYPES

Klaas Heinemann, Farid Saad

Introduction: There is little known on the awareness of, or the desires, attitudes, acceptability and complex interactions relating to male fertility control (MFC).
Methods: **T**he objective of this survey was to cross-culturally compare the awareness, attitudes, and acceptability of male fertility control and to evaluate which factors would positively or negatively influence willingness to use such a method of contraception. A survey of over 9,000 males aged 18-50 years was performed in nine countries in four continents between April and June 2002. The data collected were used to determine whether there are different potential user groups who have different views on such a method, who are driven by different collections of concerns and wishes and who also perhaps differ in geographical distribution or socio-economic backgrounds.
Results: The general acceptance of the use of a new hormonal male fertility control varies from fair to high: from 28.5% in Indonesia to 71.4% (Spain) of males expressed their willingness. Out of the participants of the MFC study, three groups of potential users were characterized by multivariate statistical methods. According to their attitudes and for the purposes of a better, more illustrative characterization, they were named as "The Informed", "The Sex-Oriented Narcissist" and "**T**he Religious Refuser". **T**hese names are intended to be of a descriptive nature and by no means neither offensive nor assessing. The clusters differ in their acceptance towards the method, in their geographical distribution and socioeconomic factors, and most importantly in the factors and attributes influencing their decision to use MFC.
Conclusion: The results based on data collected in the course of the MFC study support the view that a market for MFC methods exists. However, market segmentation should be further considered before launching the method. In addition, further studies, particularly among female partners, should follow closer to the launching date i.e. once a method can be better characterized.

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DOES DECLINING CORTISOL CAUSE INCREASED ADIPOSITY? RESULTS FROM THE MASSACHUSETTS MALE AGING STUDY

Amy B. O'Donnell, Beth A. Mohr, Sophia M. Zilber, Andre B. Araujo, John B. McKinlay, Watertown, MA

Introduction: Prior research linking Cortisol with toxic fat has suggested that central adiposity increases with Cortisol. Using this rationale, some have recommended modulating Cortisol levels for weight reduction. In this study we examined whether declining Cortisol levels are associated with increasing adiposity in a large randomly selected community-dwelling, sample of middle-aged and older men.
Methods: Data were obtained from the Massachusetts Male Aging Study, a survey of men who were aged 40-70 at baseline ($\mathbf{T}_1$) and who were observed at 3 time points (T_1: 1987-89; $\mathbf{T}_2$: 1995-97; T_3: 2002-04). Cortisol was measured in morning blood samples by **T**he Endocrine Laboratory, University of Massachusetts, Worcester, MA. Men with Cortisol and waist-to-hip ratio (WHR) and/or body-mass index (BMI) data were included in the analysis ($\mathbf{T}_1$: N=1691, $\mathbf{T}_2$: N=1040, $\mathbf{T}_3$: N=703).
Results: Using the Spearman rank correlation, Cortisol was weakly and negatively correlated with BMI at all time points (T_1: r=-0.09, p=0.0005; T_2: r=-0.15, p=0.0001; $\mathbf{T}_3$: r=-0.09, p=0.02). A similar relationship held between Cortisol and WHR at T_2 (r= -0.11, p=0.0007) but not at T_1 or T_3 (p>0.05 at both). Men who were obese at T_1 (BMI> 30 kg/m2) had significantly lower Cortisol levels than did non-obese men (BMI<30 kg/m²) (median 16 vs. 17 nM, Wilcoxon rank-sum test: p=0.02). The same was true at T_2 (15 vs. 16, p=0.005) but not at T_3 (16 vs. 17, p=0.29).
Conclusion: Cortisol was weakly, inversely correlated with BMI but not consistently with WHR. Differences in median Cortisol level of obese and nonobese were small and inconsistent. These findings suggest that modifying Cortisol levels might not be the most effective way to reduce weight in obese people.

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CORRELATING ANDROGEN AND ESTROGEN STEROID RECEPTOR EXPRESSION WITH CORONARY CALCIFICATION AND ATHEROSCLEROSIS IN MEN WITHOUT KNOWN CORONARY ARTERY DISEASE

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Steroid receptors including the androgen receptor (AR), estrogen receptor alpha (ERα) and beta (ERβ) are expressed in key vascular tissues including endothelial cells and vascular smooth muscle cells (VSMCs). However, the relative abundance and importance of these receptors in the coronary artery is not well defined, particularly in men. We therefore examined AR, ERα and ERβ expression as a function of key components of atherosclerosis, namely plaque and calcium area, in male human coronary arteries. Coronary arteries were obtained at autopsy from 24 men without known coronary artery disease. Coronary calcification was measured by contact microradiography and atherosclerotic plaque area was quantified histologically. Coronary artery cross-sections were immunostained for AR, ERα and ERβ, and then measured semi-quantitatively in each arterial wall layer (intima, adventitia and media). AR, ERβ and ERα were expressed in all artery wall layers, but most avidly in the media ($P<0.001$). ERβ exceeded ERα expression ($P<0.0005$). AR expression in the media correlated negatively with plaque area ($P=0.006$, $R=-0.55$) whereas intimal ERβ expression correlated positively with plaque area ($P=0.012$, $R=0.50$). We conclude that both AR and ERβ are important in relatively early coronary atherosclerosis, but inversely so, since decreasing AR and increasing ERβ expression correlate with more extensive atherosclerosis. ERβ is the predominate estrogen receptor in coronary arteries harvested from men without known coronary artery disease. Interventional studies are required to assess the functional significance of these observations.

FERTILIZATION

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REACTIVE OXYGEN SPECIES PROMOTE THE PHOSPHORYLATION OF MEK-LIKE PROTEINS DURING HUMAN SPERM CAPACITATION

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Capacitation is part of an oxidative process by which spermatozoa acquire fertilizing ability. Reactive oxygen species (ROS), such as superoxide anion ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2) and nitric oxide ($NO\bullet$), as well as protein kinase A (PKA), C (PKC), protein tyrosine kinases (PTK) and the extracellular signal-regulated protein kinase pathway regulate this process. Our aim was to evaluate the role of H_2O_2 and $NO\bullet$ in the phosphorylation of MEK or MEK-like proteins in human sperm capacitation and its modulation by kinases. Immunoblotting using an anti-phospho-MEK antibody indicated that the intensity of 3 protein bands (55, 94 and 115 kDa) increased in spermatozoa treated with fetal cord serum ultrafiltrate (FCSu), bovine serum albumin (BSA) or isobutylmethylxanthine+dibutyryl cAMP (IBMX+dbcAMP) as capacitating agents. This phosphorylation was prevented by PD98059 suggesting that these proteins are MEK-like proteins. The p94 and p115 bands were found in the Triton-soluble fraction whereas p55 band was in the Triton-insoluble fraction of spermatozoa. Immunocytochemistry indicated that phospho-MEK-like proteins are located in the postacrosomal region and all along the flagellum. ROS scavengers prevented, and the addition of H_2O_2 or spermine-NONOate ($NO\bullet$ donor) triggered, the increase of phospho-MEK-like proteins. The capacitation-related increase in phospho-MEK-like proteins induced by FCSu, H_2O_2 and spermine-NONOate was similarly modulated by PKA, PKC and PTK, suggesting H_2O_2 and $NO\bullet$ as mediators in this phenomenon. The phosphorylation of MEK-like proteins probably represents an intermediate step between the early events and the late tyrosine phosphorylation associated with capacitation.

(Supported by the Canadian Institutes of Health Research).

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IMMUNOLOCALIZATION OF N-CADHERIN IN HUMAN SPERM AND EVALUATION OF ITS INVOLVEMENT IN FERTILIZATION

[1]Clara Marín-Briggiler; [1]Ezequiel Lentz; [1]Florencia Veiga; [2]Fernanda Gonzalez-Echeverría; [1]Mónica Vazquez-Levin. [1]IBYME CONICET-UBA; [2]Fertilab. Buenos Aires, Argentina.

Cadherins are membrane glycoproteins involved in cell-to-cell adhesion. Preliminary evidence suggest the presence of neural cadherin (Ncad) in human sperm (*Rufas et al, 2000*), and identification of Ncad sperm transcripts (*Goodwin et al, 2000*).
The aim of the study was to describe the localization of Ncad in human sperm and to assess its involvement in fertilization.
Western immunobloting of sperm proteins with a specific polyclonal antibody towards Ncad domain 5 (Santa Cruz Biotech) revealed the presence of a 113 KDa Ncad form, resistent to sperm dissociation after cell treatment with a buffer containing 1M NaCl. This protein form was also immunodetected in protein extracts from testis and seminal plasma.
Ncad was mainly immunolocalized in the equatorial segment and acrosomal cap of ejaculated (62±7 %; 17±10 %, respectively; mean±SEM) and 18h-capacitated sperm (51±8 %; 30±8 %, respectively) (n=5). In acrosome reacted sperm, Ncad was mainly found in the equatorial segment (84±2 % of sperm) (n=3). In all cases, a specific signal for Ncad on the sperm flagellum was also detected.
Sperm preincubation with the antibody towards Ncad (100 mg/ml) did not affect binding to homologous *zona pellucida* (ZP) (anti Ncad=29±11 vs Control=33±12 bound sperm/hemizona; n=6). However, preincubation of ZP free-hamster eggs with anti Ncad antibody (20 µg/ml) resulted in the decrease of the percentage of sperm penetrations compared to control conditions (anti Ncad=40 % vs Control=100 %; n=2).
The studies describe the presence and localization of Ncad in ejaculated, capacitated and acrosome reacted sperm, and suggest its participation in adhesion events during sperm interaction with the oolema.

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IDENTIFICATION OF EPITHELIAL CADHERIN PROTEIN FORMS IN HUMAN SPERM AND EVALUATION OF THEIR LOCALIZATION IN WHOLE CELLS

Ezequiel Lentz; Clara Marín-Briggiler; Florencia Veiga; Mónica Vazquez-Levin. IBYME CONICET UBA. Bs As Argentina

Epithelial cadherin (Ecad) belongs to a large superfamily of cell adhesion molecules showing a vast structural diversity. Ecad is a 120 KDa glycoprotein organized in five extracellular, a transmembrane, and a cytoplasmic domains. Previous studies from our group described Ecad localization in sperm and suggested its involvement in gamete interaction (*Vazquez-Levin et al, ASA 2003*).

The aim of the study was to perform a biochemical characterization of Ecad protein forms present in human sperm, and evaluate their localization in the whole cell.

Western immunoblotting of protein sperm extracts developed with a specific antibody towards cadherin domain 5 revealed the presence of 4 high Mr Ecad forms of 122, 105, 97 and 86 KDa. While a specific antibody towards cadherin domain 2 recognized Ecad 122, 97 and 86, an antibody towards the Ecad cytoplasmic domain detected Ecad122 and Ecad105; these results suggest that Ecad105 is truncated in the N-*terminus* while Ecad97 and Ecad86 are truncated in the C-*teminus.* Ecad86 was extracted after cell treatment with 1M NaCl, and was detected in *cauda* epididymal fluid and seminal plasma; moreover, its signal was diminished in protein extracts from capacitated sperm, suggesting this is a peripheral Ecad form. Sperm treatment with PLC-PI removed Ecad86 and Ecad97, indicating that Ecad97 would be anchored to the membrane by Glycosyl Phosphatidyl Inositol (GPI). Immunocytochemistry done with the antibodies raised towards the cadherin domains 2 and 5 showed Ecad localization in the sperm head and tail. Contrasting, the antibody towards Ecad cytoplasmic domain only stained the flagellum.

In conclusion, four high Mr Ecad forms were identified in human sperm with a differential association and localization along the cell, which may be indicative of different roles in fertilization.

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SUBCELLULAR DISTRIBUTION OF FUCOSE BINDING SITES ON HUMAN SPERM

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The human sperm membrane associated isoform of alpha-L-fucosidase has previously been purified and characterized in our laboratory. Its unique membrane association in the head region suggests involvements in sperm functions. A fluorescent glycoconjugate (RITC-BSA-fucose) containing bovine serum albumin substituted with fucose residues and linked to the fluorescent tag RITC was used to locate fucose binding sites. Whole semen was washed using a Percoll gradient, enriching for vigorously motile cells. These cells were used to make a variety of preparations, including untreated, detergent treated, capacitated, and acrosome reacted cells. Capacitated cells were acrosome reacted using calcium ionophore A23187. Acrosome status was evaluated following staining with fluoresceinated lectin (FITC-PSA). Each preparation was stained using RITC-BSA-fucose and examined using fluorescence and confocal microscopy. Both FITC-BSA and BSA were used as controls and neither had an observable effect on cell staining patterns. Staining on washed cells revealed faint, diffuse fluorescence covering the entire head region after 30 minutes of staining at 37°C. Very low (0.001%) concentrations of TritonX-100 produce diffuse head staining much like that seen in washed cells; however, staining appears within 5-10 minutes. When treated with 0.01% TritonX-100, bright equatorial band staining and faint posterior head staining results. When capacitated spermatozoa were stained with RITC-BSA-fucose, the majority of cells displayed relatively bright diffuse head staining. When acrosome reacted cells were treated with RITC-BSA-fucose, bright equatorial band and faint posterior head staining was apparent on most cells. Staining is successfully inhibited using fucoidin, a polymer of fucose. These results indicate that fucose binding sites, including fucosidase, may reside on the outer acrosomal membrane.

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SUBCELLULAR IMMUNOLOCALIZATION OF ALPHA-L-FUCOSIDASE IN HUMAN SPERM CELLS

Jennifer Venditti, Kate Donigan, and Barry Bean. Lehigh University, Bethlehem, PA

Sperm-egg interactions in several species, including some mammals, are mediated by sperm receptors that bind carbohydrate containing molecules on oocyte surfaces. Evidence as early as 1982 has suggested that L-fucose is part of the signal recognition between mammalian gametes. Distinctive isoforms of the human alpha-L-fucosidase occur in human seminal plasma and the membrane system of human sperm cells. Although many biochemical properties of alpha-L-fucosidase are known, its role in human reproduction is still unknown. The presence of two novel isoforms of this enzyme in human semen makes it a compelling subject for further investigation. Human sperm cells were evaluated at three stages; washed, capacitated, and acrosome reacted. Cells were fixed on slides and incubated with primary, goat polyclonal antibodies against human liver alpha-L-fucosidase, and secondary, rhodamine labeled rabbit-anti-goat IgG, antibodies. Staining pattern was evaluated using epifluorescence and confocal microscopy. Following acrosome reaction, the brightest signal is revealed. This bright signal is concentrated near the posterior half of the head region. Washed cells display minimal staining and capacitated cells show diffuse head staining. Both the accessibility and subcellular location of fucosidase appear to change as cells progress toward and achieve acrosome reaction. These results suggest that a significant amount of alpha-L-fucosidase protein is associated with the inward facing portion of the outer acrosomal membrane of the human sperm cell.

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INVESTIGATION ON THE CROSS-REACTIVE ANTIGENS BETWEEN UREAPLASMA UREALYTICUM (UU) AND HUMAN SPERM MEMBRANE PROTEINS

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Objective: To investigate the mechanism how UU causes the production of antisperm antibodies (AsAb) and explore the relationship between UU and infertility.
Methods: UU proteins were separated and Western blot analysis was performed to confirm the existence of cross-reactive antigens between UU proteins and human sperm membrane proteins. The cross-reactive antigens of UU were purified using immunoaffinity chromatography. The N- terminal amino acids of the cross-reactive antigens were sequenced. Based on the amino acid sequences, the genes of the cross-reactive antigens were cloned and expressed.
Results: Cross-reactive antigens, which were 61, 50, 27and 10kDa respectively, between UU and human spermatozoa were identified. The 27and 10kDa proteins were purified and the N- terminal amino acids were sequenced. The gene of 27kDa protein was also cloned and expressed.
Conclusion: The cross-reactive antigens between UU and human spermatozoa related closely to the phenomenon that the infertile men who are infected with UU display higher titer of serum and/or semen AsAb compared to non-infected men. Identifying cross-reacting antigens between microorganisms and human sperm may provide useful information for the development of a contraceptive.

ENVIRONMENT/TOXICOLOGY

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HISTONE H4 HYPERACETYLATION IN RAT ZYGOTIC PRONUCLEI FOLLOWING CHRONIC PATERNAL CYCLOPHOSPHAMIDE EXPOSURE

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Preconceptional paternal cyclophosphamide (CPA) exposure leads to advanced male pronuclear formation, dysregulated zygotic gene activation as well as increases in pre- and post-implantation loss. Histone acetylation is intimately associated with transcriptionally competent parental genomes and chromosomal integrity in the zygote. The goal of our study was to determine the effect of paternal CPA exposure on histone H4 acetylation in early preimplantation embryos. Sprague-Dawley rats were given CPA by gavage for 4-5 weeks. Males were mated with control females; 1-cell and 2-cell stage embryos were collected and histone acetylation was assessed using anti-histone H4 (acetyl K5) immunofluorescence. Optical sections were recorded using confocal laser scanning microscopy and images were analyzed quantitatively. Histone H4 acetylation fluorescence intensity of both parental pronuclei in zygotes fertilized by spermatozoa chronically exposed to CPA was significantly disrupted, compared to corresponding controls. Male and female pronuclei were hyperacetylated ($p<0.003$) at pronuclear stage (PN) 2; maintained a hyperacetylated state through PN3 ($p<0.001$), but were not different from controls at PN4-5. Both male and female pronuclear areas, identified by DAPI immunofluorescence, in embryos sired by CPA exposed males were larger ($P\leq0.001$) at PN3 and PN4. Pronuclear stage distribution of embryos sired by CPA-exposed males was also disrupted; fewer ($p<0.001$) zygotes were observed at PN1&2 while a greater number ($p=0.008$) had progressed to PN4, compared to controls. These results suggest that preconceptual paternal CPA treatment produces chromatin modifications very early post-fertilization potentially leading to heritable epigenetic instabilities in later development. Supported by CIHR.

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SERUM TESTOSTERONE LEVELS AND PUBERTY IN RATS TREATED WITH DI(2-THYLHEXYL)PHTHALATE (DEHP)

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Exposures to the plasticizer, diethylhexylphthalate (DEHP), have been linked to the development of male reproductive anomalies. In rats, exposures to DEHP in utero cause decreased serum testosterone (T) levels postnatally while stimulating abnormal increases in Leydig cell numbers. However, in humans, an association was posited between DEHP and precocious puberty, which is presumbably due to increased sex steroid action. Therefore, we tested the effects of prepubertal exposures to DEHP on serum T and preputial separation (an index of puberty). Prepubertal Long-Evans rats aged 21 days were gavaged with 10 (low dose, LD) or 500 (high dose, HD) mg $kg^{-1}day^{-1}$DEHP for 28 days until day 49 postnatally (n = 8/per group). A third group of control rats was gavaged with the vegetable oil vehicle. Serum T concentrations and preputial separation were measured at two and four weeks of treatment. Serum T was unchanged in LD rats by two weeks and became significantly higher at four weeks (3.1 ± 0.6 *vs.* 1.2 ± 0.3 ng/ml in controls, $P < 0.05$). In contrast, T values in HD rats were sharply reduced at two weeks (0.18 ± 0.1 *vs.* 0.73 ± 0.2 ng/ml, $P < 0.05$), and returned to control levels by four weeks. These data from both groups can be explained by compensatory changes in the reproductive axis. Based on previously published results, we infer that decreased T synthesis per cell lowers androgen negative feedback and thereby raises luteinizing hormone (LH) levels leading to offsetting (HD) or increased (LD) T in circulation. Accordingly, the average age of preputial separation was on day 40 in control and HD rats, but was advanced to day 39 ($P < 0.05$) after LD exposures. In summary, this study showed that: 1) low but not high dose DEHP exposures elicited increases in serum T concentrations; 2) low but not high doses of DEHP induced precocious puberty as measured by preputial separation. We conclude that while DEHP is anti-androgenic, causing decreased Leydig cell steroidogenic capacity on a per cell basis, offsetting changes in the male endocrine axis lead to higher androgen levels after LD exposures to the point that precocious puberty is induced. Supported in party by NIH ES10233.

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EFFECTS OF CADMIUM ON UBIQUITIN-PROTEASOME SYSTEM AND STRESS SIGNALING IN A PRIMARY SERTOLI CELL-GONOCYTE CO-CULTURE SYSTEM

X Yu, J Sidhu, J S Hong, E M Faustman, Dept. of Environ. Health, University of Washington

Cadmium (Cd) is a ubiquitous environmental pollutant and has been reported to have male reproductive toxicity both in human and animals. However, its underlying mechanism of toxicity is still unknown. In this study, we hypothesize that Cd-induced alterations to the ubiquitin-proteasome system (UPS) comprise the critical events leading to testicular toxicity. We have employed a recently developed and optimized a three-dimensional primary Sertoli cell-gonocyte co-culture system to examine time and dose-dependent effect of Cd on morphological alteration, apoptosis and activation of stress signaling. We have monitored UPS processing pathway by measuring the accumulation of ubiquitin conjugated proteins (HMW-polyUb) and proteasome activity. We have also evaluated 2 key regulatory proteins, p53 and Nrf2 that are degraded by the UPS dependent pathway. Furthermore, we compared the above changes with two classic proteasomal inhibitors, Lactacystin and MG 132. Our results demonstrated that Cd exposure resulted in a dose- and time dependent morphological changes and these morphological changes were related to the induction of apoptosis. Robust activations of stress responses such as p-SAPK/JNK, p-p38, p-c-Jun and p-ATF 2 were paralleled with the accumulation of HMW-polyUb. The stress responses and accumulation of HWM-polyUb induced by Cd are similar to the non-specific proteasome inhibitor MG 132 but not the specific proteasome inhibitor Lactacystin. In addition, the treatment of Cd dose- and time dependently affected the proteasome activity but inhibition on the proteasome was unique as compared with MG132 and Lactacystin. Taken together, our studies are supportive of our hypothesis that Cd-induced UPS dysfunction is a critical mechanism underlying its *in vivo* testicular toxicity. (Funded by EPA: R826886 and NIEHS: ES09601, ES07033, ES11387 and ES10613)

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ESTROGEN-INDUCED ACCUMULATION OF FAT CELLS IN CORPORA CAVERNOSA PENIS DEPENDS UPON ESTROGEN EXPOSURE DURING CRITICAL PERIOD OF PENILE DEVELOPMENT

HO Goyal[1], TD Braden[3], CS Williams[1], P Dalvi[1], M Mansour[1], and JW Williams[2], Departments of Biomedical Sciences[1], Biology/ CBR/ RCMI[2], Tuskegee University, Tuskegee, AL; and Department of Anatomy, Physiology and Pharmacology[3], Auburn University, Auburn, AL

Previously, we reported that neonatal diethylstilbestrol (DES) or estradiol valerate (EV) exposure at a dose of 0.10-0.12 mg/kg, or higher, per day, on alternate days, from postnatal day 2-12, resulted in abnormal penis development and infertility (J Androl, in print). The objective of this study was to identify a critical developmental period(s) during which EV exposure results in the observed penile abnormalities. Male pups received EV at a dose of 0.10-0.12 mg/kg on postnatal day(s) 1, 1-3, 4-6, 1-6, 7-12, 13-18, 19-24, or 25-30. Fertility was tested at 102-115 days of age and tissues were examined at 117-137 days. Both fertility and penile morphology were unaltered in rats treated with EV after 12 days of age. Conversely, except in rats treated on postnatal day 1 only, none of the males treated prior to postnatal day 12 sired pups, and all had abnormal penises, including varying degrees of accumulation of fat cells in the corpora cavernosa penis, which was maximal in the 1-6-day group. Also, the preputial sheath was partially released or its release was delayed, and the weight of the bulbospongiosus muscle was significantly reduced. Plasma testosterone (T) in the 1-6-day group and testicular T in the 4-6-day group were significantly lower. The testosterone surge, characteristic of controls in the first week of life, was suppressed in the 1-3-day group. Hence, EV exposure prior to 12 days of age (as short as 1-3 days postnatal), but not after 12 postnatal days, results in abnormal penile morphology, characterized by accumulation of fat cells in the corpora cavernosa penis and, consequently, loss of fertility. Supported by NIH/MBRS, NIH/RCMI, and USDA grants.

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GENE EXPRESSION PROFILING TO IDENTIFY MECHANISMS REGULATING TESTICULAR RESISTANCE OR SENSITIVITY TO INORGANIC LEAD (Pb)

Colleen Millan[1], Rebecca Z. Sokol[2], Ian Hurley[1], Joel L. Marmar[3], Susan Benoff[1]
[1]North Shore-LIJ Research Institute, Manhasset, NY; [2]University of Southern California, Los Angeles, CA; [3]Cooper Hospital, Robert Wood Johnson Medical School, Camden, NJ

Increased testicular Pb levels cause a decrease in both fertilization and pregnancy rates, related in part to an increase in testicular apoptosis. However, some men with elevated male tract Pb were fertile and did not exhibit elevated testicular apoptosis. The goal of this study is to determine a mechanistic connection between gene expression and Pb-induced apoptosis leading to hypospermatogenesis, and ultimately infertility. This study compared Pb-resistant Sprague–Dawley (SD) rats to Pb-sensitive Wistar (W) rats. Both groups of rats were given Pb in their drinking water over 24 weeks at varying doses. Animals were sacrificed at 1,4,16, and 24 weeks and the testes were harvested. Apoptosis was analyzed using TUNEL. Gene expression was compared by Affymetrix Genechips followed by analysis with GeneSpring 7.0. Across parameters, strain was the most significant, followed by time and then dose. Between the two strains, 338 genes were significant. In Pb-resistant SD rats, 65 genes were identified as being significantly changed while only 15 were altered in Pb-sensitive W rats, with only one gene common to both lists. The majority of genes were up-regulated. Fold changes were higher in SD vs. W rats (16-fold vs. 4-fold). In SD rats, the majority of gene expression changes were biphasic and affected genes with CREs in their promoters (genes normally modulated by calcium). There is an increase and then a decrease in apoptosis over time, potentially by oxidative stress and inflammatory cytokines. No significant changes in calcium-regulated gene expression were detected in W rats. The relative lack of response to Pb in W rats may be responsible for Pb sensitivity. (Supported by NIH Grant No. ES06100 to SB.)

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EFFECTS OF CHEMOTHERAPY FOR TESTICULAR CANCER ON REPRODUCTIVE ORGANS, SPERM COUNT, AND SPERM MOTILITY IN THE MALE RAT

Adrienne Bieber, Ludovic Marcon, Barbara Hales, Bernard Robaire, McGill University, Montréal, Canada

Testicular cancer is the most common cancer affecting men of reproductive age. Advances in treatment of the disease, which includes the administration of bleomycin, etoposide, and cis-platin (BEP), have brought the cure rate to over 85%. This high cure rate, coupled with the young age of the patients, makes the impact of the treatment on fertility and reproductive function increasingly important. The goal of this study was to determine the effects of BEP, in doses equivalent to that given to humans, on reproductive organs, sperm count, and sperm motility in an animal model. Male Sprague Dawley rats were treated daily with BEP for 3 cycles of 3 weeks, for a total of 9 weeks. Rats were killed and the testes, epididymides, seminal vesicles, and prostate were excised and weighed. Sperm from the cauda epididymidis were analyzed for motility parameters, and sperm from the caput epididymidis were counted hemocytometrically. After 9 weeks of treatment with BEP, treated rats did not gain as much weight as control rats (39% vs 21% weight gain). There was a decrease in testicular weight of 56% when compared to control. The epididymis decreased in weight by 23%, the ventral prostate by 11%, and the seminal vesicles by 8% when compared to control. The sperm count of the treated rats decreased by >10-fold when compared to control ($282x10^6$ vs $17x10^6$ sperm per gram caput epididymidis). The percent motile sperm in the treated rats was > 35% lower than that of control. These results indicate that BEP treatment has significant effects on both sperm numbers and sperm motility. Further studies are required to determine how these changes can affect fertility. Supported by CIHR.

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NEXT DAY DETERMINATION OF SPERM MOTILITY AFTER OVERNIGHT SHIPMENT OF SEMEN

Bennett, D[1], Huszar, G[1], and Janes, M[2]. The Sperm Phys. Lab.,[1]Yale Sch. Med. New Haven, CT, USA, [2]Molecular Probes, Inc. Eugene, OR, USA.

INTRODUCTION: We are reporting on the preservation of sperm motility for next day assessment after shipment of semen. This development follows our previously described methods for preservation of sperm concentration, viability, HspA2 chaperone ratios, chromatin maturity, DNA integrity and sperm shape.
METHODS: We used the fixable MitoTracker Red CM-H_2XRos reagent that detects sperm motility and velocity, as sperm mitochondrial activity and motility are apparently related. We investigated whether differences in the proportions of sperm with high and low fluorescent emission would accurately reflect sperm motility in semen samples. Emission levels of 200-400 individual spermatozoa originating in semen, cooled semen, and swim-up fractions were studied after ejaculation and on the next day using the Metamorph v4.6.9 program (N=28 fractions, 13 men). Motility and velocity were evaluated by the Hamilton-Thorne IVOS system. Statistical analysis was carried out by SigmaStat.
RESULTS: There was a good agreement between sperm motility after ejaculation and mitochondrial activity detected next day. Using a threshold O.D. of 0.7, in samples with 93.8±3.3% of sperm cells brighter than 0.7 the mean motility was 87±1.9% (N=8). Conversely, in samples with 4.4±1.8% of cells brighter than 0.7, the mean sperm motility was only 6.0±2.4% (N=10). The correlations between the proportion of high emission sperm vs. motility and velocity were r=0.93 and 0.86 (both $p<0.001$). The multiple of sperm motility and velocity was also related to the proportion of high emission sperm ($r=0.81$, $p<0.001$).
CONCLUSIONS: Using the MitoTracker reagent and fluorescent imaging, there was a close correlation between mitochondrial activity and sperm motility. We identified a signal threshold that provides the next day prediction of ejaculatory sperm motility. This approach will facilitate the assessment of sperm motility after overnight shipment in men investigated for occupational reproductive toxicity or infertility. (Supp. by the NIH: OH-04061).

POSTER SESSION II
MONDAY, APRIL 4, 2005
10:45 A.M. – 12:00 P.M.
Location: Leonesa Ballroom

ASSISTED REPRODUCTION & INFERTILITY

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PRESENCE OF SPERMATOZOA IN THE RETE TESTIS AND EPIDIDYMIS IN NON-OBSTRUCTED AZOOSPERMIC MEN

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Therapeutic testicular biopsy (TTB; i.e., testicular tissue mincing) is the method to recover spermatozoa from seminiferous tubuli of non-obstructed azoospermic (NOA) men. A previous study has indicated the presence of spermatozoa in the rete testis (RT) of NOA men (Andrologia 35: 89-92, 2003). Our objective was to investigate whether spermatozoa are present in the RT or epididymal lumen of NOA men. Ultrasonographically guided puncture of the RT and microsurgical aspiration of fluid from the vas deferens (VD), epididymal head (EH), epididymal body (EB), and epididymal tail (ET) were performed in 13 NOA men with diagnostic testicular biopsy (DTB; stain of a fragment of testicular tissue) indicating Sertoli cell-only syndrome (group A), in 18 NOA men with DTB indicating arrest at the primary spermatocyte stage (group B), and in 2 NOA men with DTB demonstrating arrest at the round spermatid stage (group C). Then TTB was performed and the TTB-fragment was processed for mincing. All the men from groups A, B, and C had previously demonstrated five semen samples negative for sperms post-centrifugation. Within group C, spermatozoa in VD, ET, EB, EH, RT-sample, and TTB were found in 1, 1, 1, 1, 1, and 2 men, respectively. Within group B, spermatozoa in VD, ET, EB, EH, RT-sample, and TTB were found in 1, 1, 1, 3, 6, and 9 men, respectively. Within group A, spermatozoa in VD, ET, EB, EH, RT-sample, and TTB were found in 0, 0, 0, 0, 3, and 5 men, respectively. Ultrasonographically guided puncture of the RT may be an alternative method for sperm collection in NOA men.

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COMPARING THE OUTCOMES OF IN VITRO FERTILIZATION BY TWO DIFFERENT DENSITY GRADIENT SEPARATION METHODS

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Objective: The purpose of this study was to evaluate SpermGrad as alternative to Percoll for sperm separation of human spermatozoa for use in assisted reproduction.
Material and Method(s): Nineteen semen samples were divided into two fractions. Motile spermatozoa were separated by using two-layer (90 and 45%) SpermGrad gradient and six-layer (100, 90, 80, 70, 60, and 50%) Percoll gradient. Sperm recovery rate, motility, and progressiveness were evaluated before and after separation by CASA system. The prepared motile sperm were used in assisted reproduction (IVF and ICSI) and fertilization rate was compared between two methods.
Result(s): After separation, there was no significant difference in sperm motility by SpermGrad (93.0±8.1%) and Percoll (90.5±10.4%). Sperm progressiveness were not significantly different between two methods: SpermGrad (2.89±0.30) and Percoll (2.88±0.31). The sperm recovery rate using SpermGrad (56.5±65.6%) was significantly higher than that of Percoll (34.4±25.0%) ($p<0.01$). The overall fertilization rate using SpermGrad (76.9±13.8%) was significantly higher than that of Percoll (68.9±20.5%) ($p<0.05$).
Conclusion(s): SpermGrad is a valid alternative to Percoll in assisted reproduction technology (ART).

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COULD BIOTECHNOLOGY IMPROVE OUTCOME AFTER IUI?

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A common perception is that probability of pregnancy after intrauterine insemination (IUI) with mild ovarian hyperstimulation is low and can not be improved. Average per cycle pregnancy rates range from 10% to >20%, depending on clinic as well as selection and management of patient couples. Insufficient research probes sperm therapy to improve outcome. What benefit need biotechnology deliver to make IUI more appealing to subfertile couples?
Assume that a hypothetical "profertility molecule" increases average per cycle pregnancy rate by 20% over a basal rate of 0.15 (15%), to average of 0.18 for all couples electing therapy. As with all drugs, a male is a "*non-responder*" (fertilizing potential of his sperm unaffected) or "*responder*" (fertilizing potential of his sperm substantially increased) in respect to this hypothetical molecule. Further, assume 40% of couples include a responder male. Without such treatment or for couples including a non-responder male, cumulative probabilities of pregnancy would be 0.15, 0.28, and 0.39 after 1, 2 or 3 cycles. For couples including a responder male and electing therapy, cumulative probabilities of pregnancy would be 0.22, 0.40, and 0.54. For entire treated population (all treated couples), they become 0.18, 0.33, and 0.45.
Fact. Exposure of bull sperm to a recombinant sequence representing 62 amino acids in bovine prosaposin (bPSF; J Androl 22:361. 2001), before standard cryopreservation and AI of cattle ($10x10^6$ sperm), shows that this is not wishful thinking. Based on ~1588 dairy cows/group, there was a 3.2 percentage unit increase (19%) above the control rate of 17.1% to 20.3% ($P=0.01$). For the 3 responder bulls (Q, O, M), benefit averaged 25% above their individual bases. Similar improvement of fertilizing potential of human sperm is reasonable.

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IS REACTIVE OXYGEN SPECIES (ROS) AN INDEPENDENT MARKER OF MALE FACTOR INFERTILITY?

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Generation of reactive oxygen species (ROS) in the male reproductive tract has spurred real concern due to their potential toxic effects at high levels on sperm quality and function, leading to male infertility. However, there is no consensus on including measurement of ROS as a routine diagnostic test in the evaluation of male factor infertility (MFI). The purpose of this study was to define the reference values of ROS in normal healthy donors (NHD), and to observe its abnormal pattern in MFI patients at a tertiary care infertility center. We assessed the ROS levels in 60 MFI patients and 71 semen samples from 33 NHD. Among the MFI patients, all three semen parameters (concentration, motility, and morphology) were abnormal in 35 men and normal in 25 men according to WHO criteria. Female factors were ruled out for all of these MFI patients. Men with leukocytospermia were excluded. After simple sperm washing, ROS levels were determined by chemiluminescence assay using luminol. The third percentile value of ROS levels was calculated from NHD. These values are traditionally used as cut-off points of normality in a biological system. In our experiment this value (calculated as 0.48) indicates that ROS levels were below this cut-off in 97% of NHD. We applied this cut-off value to differentiate patients with high ROS levels in MFI group. ROS levels were significantly higher in overall MFI patients as compared to NHD ($P <0.0001$) by Fisher's exact test. Similarly the ROS levels were significantly different between NHD and patients with normal and abnormal semen parameters ($P = 0.002$; $P <0.0001$)). However, the percentage of samples with elevated ROS levels was comparable between the MFI patients with normal and abnormal semen parameters ($P = 0.18$). Furthermore, no significant correlation was observed between different semen parameters and ROS in both these patient groups ($P >0.05$). In conclusion, high ROS levels may be an independent marker of male factor infertility irrespective of the presence or absence of abnormal semen parameters. We suggest that measurement of ROS levels be included as part of infertility evaluation of male patients. Patients with high ROS levels may be treated for underlying pathology or considered for antioxidant therapy.

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INTRAINDIVIDUAL VARIATIONS AND THE EFFECT OF ANTIBIOTIC THERAPY ON HUMAN SEMINAL PLASMA IGA ANTIBODIES AGAINST CHLAMYDIA TRACHOMATIS

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The clinical relevance of male urogenital infections with Chlamydia trachomatis (Ch.tr.) has been dicussed controversially. Since direct determination of Ch.tr. in seminal plasma is difficult, detection of specific IgA antibodies has been introduced into semen anylysis. Data about intraindividual variations of these antibodies and the effects of antibiotic treatment are not yet available.

Andrological patients with increased seminal plasma antibodies against Ch.tr. and more than one analysis of these antibodies were identified and differentiated into two groups: group A included men (n=35) without antibiotic treatment, group B those men (n=34) with analyses before and after antibiotic treatment. Increased IgA antibodies (cut-off index > 1.1) were determined by ELISA in 157/1978 (7.9%) seminal plasma samples.

In group A 20/35 (57%) patients showed a decrease of IgA antibodies in the second analyses with 6/35 (17%) men having normal antibodies. The mean (± SD; median) values of cut-off indices were not significantly (p=0.86) different between the first (5.8±7.0; 2.6) and second (6.0±8.5; 2.9) analysis.

Patients in group B demonstrated a decrease of IgA antibodies after antibiotic therapy in 24/34 (71%) with a significantly ($p<0.05$) higher percentage of normalisation of the cut-off index (13/34 (38%)). The cut-off indices were significantly ($p>0.05$) lower after antibiotic therapy (4.2±6.1; 1.7 vs. 6.0±6.2; 3.9).

In summary, male infertile patients show a wide range of intraindividual variations of seminal plasma IgA antibodies against Ch.tr.. Since antibiotic treatment results in significantly reduced seminal plasma antibodies and a higher percentage of men with normal cut-off indices, the determination of seminal plasma chlamydial antibodies seems to be of clinical relevance.

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ETIOLOGY OF MALE INFERTILITY AS DIAGNOSED WITHIN A COMPREHENSIVE INFERTILITY SERVICE PROGRAM

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The advent of assisted reproductive technology (ART) has radically changed the management of infertile couples. The repercussion of this change on the activity of a diagnostic andrology laboratory has not been previously assessed. This study aims to chart the profile of patients referred to a clinical andrology laboratory for diagnostic purposes within a comprehensive infertility program in a teaching hospital with services for conventional therapies and ART to resolve couples' infertility. We examined data from 805 men assessed for their fertility potential in a tertiary care center between 1998 and 2003. The parameters considered were: male race, insurance status, referring physician, and the working diagnosis. Among the 805 patients, 783 had documented ethnicity (white - 82%, African-American - 7%, Asian – 2%, Hispanic – 2%, other – 7%) and 85% had medical coverage for their infertility evaluation. The proportion of patients who were covered by health insurance was similar among different ethnic groups ($X^2 = 14.3$, $P = 0.09$). Thirty one percent of patients were referred by an in-house gynecologist or urologist, 48% by primary healthcare practitioners, and 21% had no documented source of referral. There was no significant difference in the incidence of different causes of infertility among different ethnic groups ($X^2 = 28.45$, $P = 0.77$). However, the proportion of patients diagnosed with post-vasectomy obstruction or idiopathic infertility was markedly lower than previously reported in literature. On the other hand, the proportions of men presenting with other etiologies were in agreement with previous reports. Our results demonstrated a redistribution of patient activity within a comprehensive fertility program. The low percentage of patients diagnosed with post-vasectomy obstruction may be attributed to patients directly undergoing ART to resolve their infertility. In our experience, having a specialized andrology laboratory with an in-house reproductive endocrinologist and urologist (with training in male infertility) is important in establishing the correct diagnosis for couples seeking help, thus reducing the number of patients categorized as idiopathic infertility. This shift should be of interest to healthcare planners.

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SELECTIVE SEROTONIN REUPTAKE INHIBITOR (SSRI) CAUSES SPERM CHROMATIN DAMAGE IN SMOKING MEN

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Numerous studies have shown an association between fertility outcomes & sperm DNA (chromatin) fragmentation. Over 50% of men taking SSRI therapy for depression exhibit sexual dysfunction. This may be due to SSRI induced hormone changes (elevated prolactin) or increased intracellular oxidative stress. This study evaluated a potential relationship between SSRIs & sperm DNA damage in men, through similar mechanisms.

Methods: Healthy male volunteers: 6 smokers (S), & 12 non (NS), received 20 mg fluoxetine (Prozac) for 120 days followed by a 120 day wash-out. Measurements included: % sperm with high DNA damage (DFI) using SCSA^R; sperm motility (CASA); and sexual function (Arizona Sex score). Sampling occurred at Time 0, 60 & 120 days w/ 20 mg daily fluoxetine; and 60 & 120 days after fluoxetine was stopped.

Results: Data in Table are x ± sd. Fluoxetine therapy caused: 1) no motility change (though S had poorer motility; $p<0.05$); 2) a decline in sexual function the first 60 days of treatment (*$p<0.05$); & 3) a profound increase in DFI for S men (*$p<0.001$) during treatment which never returned to baseline. Also, 2 of 12 NS had DFI doubling on fluoxetine.

Men	Days	Fluoxetine	%DFI	% PMot	Asex
NS	0	0	13 (6)	55 (22)	11 (2)
NS	60 d	yes	12 (9)	58 (22)	13 (3)#
NS	120 d	yes	16 (12)	58 (23)	12 (3)
NS	180 d	no	13 (6)	65 (19)	11 (2)
NS	240 d	no	13 (7)	68 (12)	11 (2)
S	0	0	12 (7)	39 (18)	10 (2)
S	60 d	yes	29 (19)*	40 (28)	12 (4)#
S	120 d	yes	18 (11)*	50 (31)	10 (3)
S	180 d	no	20 (12)*	52 (33)	11 (3)
S	240 d	no	15 (10)	56 (24)	11 (3)

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CYTOGENETIC DAMAGE IN MALE INFERTILITY

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The aim of the current study was to evaluate the genetic fragility in infertile men. The methodology of sister chromatid exchanges was applied in cultures of peripheral blood lymphocytes and the levels of sister chromatid exchanges were evaluated as a quantitative marker of genotoxicity. In addition the values of mitotic index (MI) and the proliferation rate index (PRI) as qualitative indices of cytotoxicity and cytostaticity, respectively, were assessed. The genotoxic and antineoplastic agent mitomycin C (MMC) and the caffeine (CAF), a well-known inhibitor of DNA repair mechanisms, were used in order to attempt to induce chromosomal fragility in infertile men (n=11) and fertile men (n=5). Thus we attempted to detect a probable underlying damage on the DNA. These experiments revealed that infertile men compared to fertile men demonstrate a statistically significant DNA fragility in peripheral blood lymphocytes after being exposed simultaneously to MMC and CAF. The current study provides experimental evidence that there is a genetic fragility in infertile men probably contributing to the development of impaired reproductive capacity.

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THE EFFECT OF TREATING SPERM WITH AUTOANTIBODIES WITH A PROTEIN DIGESTIVE ENZYME PRIOR TO INTRAUTERINE INSEMINATION ON PREGNANCY OUTCOME

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A previous randomized study found that treating sperm coated with antisperm antibodies with the protein digesztive enzyme chymotrypsin resulted in a significantly higher pregnancy rate following intrauterine insemination (IUI) vs. IUI with sperm ejaculated into medium alone. There have been no subsequent studies refuting or corroborating these data. The observational study presented here was aimed at determining the pregnancy rate per cycle following IUI with at least 80% of the sperm coated with antisperm antibody as determined by the direct immunobead assay. There were 30 women treated in cycle one. There were 15 males with 100% IgG and IgA; 7 with both IgG and IgA >80% but not 100%, and 8 with either IgG or IgA >80%. There were 4 live births following IUI in cycle 1 (live birth rate 13.3%). The live birth rate for cycle 2 was 2 of 20 (10%) and was 1 of 14 for cycle 3 (7.1%). The cumulative pregnancy rate after 2 cycles was 22.0% and was 27.5% after 3 cycles. There were 9, 7 and 1 woman treated in cycles 4-6. Overall there were 9 live births in 81 IUI cycles (an 11.1% live birth rate per cycle). These women had their IUI cycles without purposeful superovulation. The median age of the women was 34. Though there were no controls, the expected live birth rate per cycle at this age would be approximately 17%. Considering the severity of infertility associated with sperm autoimmunity problems even following IUI, the treatment of the sperm with chymotrypsin seems to provide a reasonable alternative to treating with IVF with intracytoplasmic sperm injection. Though the latter is likely to be more successful, the former is far less expensive and safer.

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REPRODUCTIVE POTENTIAL OF SPERM WITH CHROMATIN DEFECTS DECLINES DURING EPIDIDYMAL PASSAGE AS REVEALED BY INTRACYTOPLASMIC SPERM INJECTION

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Previously we showed that injection of cauda epididymal spermatozoa from mice with no transition nuclear proteins (TPs) into oocytes was less effective at fertilization and supporting embryo development than ICSI with spermatozoa from the testes of these mice. To further investigate this possible decline in genomic integrity of spermatozoa during epididymal passage, we performed ICSI with testicular, caput, and cauda epididymal spermatozoa from *Tnp1-/-Tnp2+/-* mice, which have a minimal level of TPs. Whereas there was no increase in sperm chromosome abnormalities at the cleavage division after oocyte injection of motile spermatozoa from the testis and caput epididymis of mutant males, compared to wild-type, mutant spermatozoa from the cauda epididymis resulted in a significant increase in chromosomal aberrations. Compared to wild type, embryonic and fetal development after ICSI were not different for motile testicular and caput epididymal spermatozoa from mutant males, but there was a reduced level of fertilization for cauda spermatozoa from these mutant mice with abnormal head morphology, and reduced implantation rates for cauda epididymal spermatozoa with either normal or abnormal head morphology. The overall yield of live born offspring per oocyte injected was about 20% with mutant cauda spermatozoa *vs.* about 50% using caput epididymal spermatozoa, which was similar to wild-type. These results suggest that testicular or caput epididymal spermatozoa may be better than ejaculated spermatozoa for use in ICSI when the sperm chromatin is defective. Thus, in certain cases of human male infertility in which ICSI with ejaculated spermatozoa is unsuccessful, better results might be obtained with testicular or caput epididymal sperm extraction in conjunction with ICSI.

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INSUFFICIENT RESPONSE TO VENOUS SURGERY: IS PENILE VEIN RECURRENT OR RESIDUAL?

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In order to elucidate the possible interpretation of an insufficient responsiveness of penile venous surgery in an attempt at restoring erectile function— the contention of recurrent or residual veins, we report on those patients who failed to respond to the first venous surgery and subsequently underwent a second operation. From July 1996 to July 2003, a total of 45 patients, aged from 25 to 83 years, who were unsatisfied with their first venous surgery and were later diagnosed with persistent veno-occlusive dysfunction via our dual cavernosography, subsequently underwent penile venous stripping surgery for a second time. They were evaluated with the abridged five-item version of international index of erectile function (IIEF-5) scoring every 6 months for one to five years and cavernosogram if necessary. Their preoperative IIEF-5 score was 10.1±3.7 that increased to 17.1±3.2 ($p<0.001$) after first surgery and further increased to 20.7±3.1 ($p<0.0001$) after a second venous surgery of the cavernosal vein that was consistently demonstrated on the cavernosogram. The follow-up period ranged from 12 months to 72 months with an average of 37.0±11.5 months. Overall 41 men (91.1%) reported a positive response to venous surgery with more satisfactory coitus consistently encountered. They showed improvements of at least 3 scores of IIEF-5 and reported that they believed the venous stripping procedure to be a worthy treatment modality. We may refer to a clinical relapse of erectile dysfunction as a result of "residual" veins rather than "recurrent" ones.

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REDOX SIGNALING MECHANISMS AND APOPTOTIC RESPONSE IN HUMAN CAVERNOSA UNDER OXIDATIVE STRESS

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Introduction/Objective: Increased free radical generation and oxidative stress have been implicated in diabetes-induced erectile dysfunction as well as in Peyronie's plaque formation. High doses of vitamin **E** have shown limited success. We have shown earlier that nitric oxide (NO), though known to mediate penile erection, can induce cytotoxicity to cavernosal cells at high levels (*J Androl.* 2001;22:34-39,). The present study evaluates cavernosal cell apoptosis due to induced oxidative stress in order to understand the molecular/cellular basis of disease progression and possible prevention.

Methods: Our well-established human cavernosal cell-culture model was used for monitoring cell growth, mitochondrial activity and apoptosis. Primary culture cells (passage 2-3) were subjected to oxidative insult for 24-48 hours by hydrogen peroxide and sodium nitroprusside [0.3 nM to 3 mM] in the absence and presence of various doses [10-100 µM] of vitamin E and L-NAME. Kinetics of cell growth and mitochondrial activity was monitored by WST-8 assay and expressed as a percent of control. Cellular apoptosis was monitored by DNA fragmentation assay.

Results: These data indicate a differential cell-growth/inhibition pattern by different primary cells in response to various doses of H_2O_2 and sodium nitroprusside (as a source of NO). To our surprise, low doses of H_2O_2, L-NAME and even vitamin E were proliferative. Higher doses [>30 µM] of H_2O_2 and SNP-inhibited cell growth by non-apoptotic signaling pathway.

Conclusions: We conclude that differential cell growth and apoptotic response pattern exist in various cavernosal cells to extraneous oxidative stress in the presence of vitamin E. This may also explain why limited success is achieved by various antioxidant modalities.

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TIMING OF SEXUAL INTERCOURSE POST TADALAFIL IN VARIOUS POPULATIONS OF MEN IN MOMENTUS (MULTIPLE OBSERVATIONS IN MEN WITH ED IN NATIONAL TADALAFIL STUDY IN THE US)

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Tadalafil has been shown to be effective up to 36 hours after dosing for the treatment of erectile dysfunction (ED). A retrospective analysis of an open-label US study of 12 weeks of treatment with tadalafil 20 mg (a maximum of 1 tablet per day prior to sexual activity), examined the time from dosing to sexual intercourse attempt in 1911 men with ED. Patients were enrolled in 8 predefined groups: (1-3) Caucasian, Black, or Hispanic men, £ 65 years, with no diabetes or depression; (4-5) men £ 65 years with depression (no diabetes), or diabetes (no depression); (6) men > 65 years, with no diabetes or depression; (7) men who met enrollment criteria but were not included in any other group; and (8) men with ED due to spinal cord injury. Patients were instructed regarding the 36-hour post-dose efficacy of the drug and were asked to record the time of dose, time of intercourse attempt, and whether the attempt was successful. The analysis showed that during the 12 weeks, 91% (range: 82-94%) and 78% (range: 64-83%) of men in the various groups attempted intercourse at least once in the 4- to 36-hr and 12- to 36-hr time intervals, respectively, after dosing with tadalafil. From these, mean per patient successful attempts were 75% in both the 4- to 36-hr (range: 50-84%) and the 12- to 36-hr (range: 55-81%) time intervals, respectively. Overall, 46% (range: 36-49%) of attempts occurred in the 4- to 36-hr time period. Patients on tadalafil in all the groups studied had the flexibility to engage in sexual activity over a broad time period up to 36 hrs post dose.

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CHRONIC INHIBITION OF PHOSPHODIESTERASE-5 INDUCES LONG-TERM POTENTIATION OF ERECTILE FUNCTION IN AGED BUT NOT YOUNG RATS

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Despite demonstrated clinical efficacy of phosphodiesterase (PDE)-5 inhibition for the treatment of erectile dysfunction, the possibility that PDE5 inhibitor used long-term may durably augment erectile ability remains unclear. We investigated whether long-term administration of sildenafil at therapeutically relevant levels to aged rats "primes" the penis for improved erectile ability and involves nitric oxide or Rho-kinase signaling. Male Fischer 344 "young" (4 month-old) and "aged" (19 month-old) rats were injected with sildenafil mesylate (20 mg/kg) or saline s.c. every 8 hours for 3 weeks. After a 1, 3, or 7 day wash-out period, electrical stimulation of the cavernous nerve (CNS) was performed to assess penile erection, penes were excised, and blood was collected for plasma sildenafil measurement. In aged rats sildenafil increased post-CNS detumescence up to 3 day wash-out, while the improvement in young rats was minimal. Phosphorylated endothelial nitric oxide synthase (eNOS) and Akt (eNOS activator) expressions were less in the aged relative to young penis at baseline but were increased by sildenafil through day 3 wash-out to similar levels found in penes of young rats. Phospho-MYPT1 (marker of Rho-kinase activity) expression was increased by sildenafil in penes of young rats through day 1 wash-out only. Sildenafil inhibited PDE5 activity and increased PDE5 expression in penes of young rats through day 7 wash-out. Animals had sildenafil free plasma levels through day 3 wash-out equivalent to the therapeutic range in men after sildenafil dosing. This study suggests that erections can be enhanced under preconditions of erectile impairment by chronic inhibition of PDE-5, and the effect is mediated by Akt-dependent eNOS phosphorylation. The lack of erection enhancement by chronic PDE5 inhibition in the young "healthy" penis may relate to restrained nitric oxide signaling by PDE5 upregulation.

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SILDENAFIL CITRATE TREAT ERECTILE DYSFUNCTION AFTER KIDNEY TRANSPLANTATION

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Introduction To analyze the morbidity of organic erectile dysfunction (ED) in kidney-transplanted patients, and to evaluate the efficacy and reliability of sildenafil citrate treatment.
Methods: 65 ED patients with normal grafts function after kidney transplantation for 3-12 months were involved in our study. ED was diagnosed in all the patients by International Index of Erectile Disfunction (IIEF). Among them, 10 patients were in Light degree, 32 patients in Moderate degree and 23 patients in Severe degree according to IIEF score. To each patient, the IIEF score, blood urea nitrogen, creatinine and the trough concentration of cyclosporin were compared before and after taking sildenafil citrate at initial dose of 50 mg every night.
Results: 26 patients without ED before transplantation suffered from ED after the operation, and 32 patients with ED before transplantation got worse. Taking sildenafil was effective in 53 patients (81.54%). There were no statistically differences in BUN, creatinine and the trough concentration of cyclosporin of the patients before and after sildenafil treatment.
Conclusions: The morbidity of organic ED increased after transplantation. Sildenafil citrate treatment in ED of kidney-transplanted patients was effective and safe. The graft function and the trough concentration of cyclosporin were not affected by taking sildenafil.

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RESPONSE TO SILDENAFIL CITRATE THERAPY FOLLOWING NERVE SPARING RADICAL PROSTATECTOMY: IMPACT OF RISK FACTORS

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Systemic diseases like hypertension (HTN), diabetes (DM) and hyperlipidemias are important risk factors for erectile dysfunction in normal population, however their effect on response to treatment after RP has not been reported in the literature. We evaluated the relation between various systemic risk factors and treatment response to sildenafil. We identified 69 patients, who underwent bilateral nerve-sparing radical prostatectomy from 2001- 2003. All patients received sildenafil citrate 50-mg dose initially, later increased to 100 mg if no response was observed. 31 (44.9%) responded to oral sildenafil (Group 1) and 38 (55.1%) did not respond to sildenafil (Group 2). Variables analyzed include diabetes mellitus (DM), hypertension (HTN), hyperlipidemias, smoking and alcohol. Binary logistic regression was used to calculate the significance between the variables and treatment outcome. Results: In Group 1 (responders); 11/31 (35.5%) patients had one or more risk factors and 20/31 (64.5%) did not have any risk factors. The specific risk factors in Group 1 included: 3/31 from DM, 3/31 from HTN, 1/31 hyperlipidemias, 2/31 smokers and 3/31 were alcoholics. In Group 2 (non-responders), 25/38 (65.8%) had either one or more risk factors and 13/38 (34.2%) did not have any risk factors. The specific risk factors in Group 2 included: 11/38 from DM, 14/38 from HTN, 3/38 from hyperlipidemias, 5/38 were chronic smokers and 6/38 were alcoholics. Comparing Group 1 to Group 2, there was a difference in the incidence of risk factors with DM and HTN ($P<0.05$). No significant association was found between the two groups in the other risk factors (hyperlipidemia, smoking, and alcohol) .The presence of risk factors - hypertension and diabetes - significantly reduces the response rate to sildenafil.

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EFFICACY AND TREATMENT SATISFACTION OF PDE-5 INHIBITORS IN MANAGEMNT OF ERECTILE DYSFUNCTION FOLLWING RADICAL PROSTATECTOMY: SHIM ANALYSIS

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Our centre has reported comparably efficacy in the abridged 5-item version of the International Index of Erectile Function questionnaire referred to as the Sexual Health Inventory of Men (SHIM) between phosphodiesterase 5 (PDE5) inhibitors, yet patients with same SHIM scores, have often reported a difference in efficacy. Our objective was to assess the effectiveness of SHIM questionnaire, in comparing efficacy of PDE5 inhibitor, and if an additional rigidity question, increased its sensitivity. In this prospective study, 23 men with erectile dysfunction after nerve sparing radical prostatectomy who responded efficacy to 100mg sildenafil were given 20mg tadalalfil for 5 weeks and then 20mg vardenafil for 5 weeks. After 5 weeks of each PDE5 inhibitor, patients had one week without a PDE5 inhibitor. After 5 weeks of each PDE5 inhibitor, each patient completed a SHIM questionnaire. An additional question assessing rigidity of erection was obtained, using a scale of 0-4. Patients were asked if one drug was more potent than others.Of the 23 sildenafil responding patients, 20 had completed a five week course of tadalafil, and 13 completed a five week course of vardenafil. Of the 20 patients that completed 20mg tadalafil, 12 patients had equal SHIM scores for efficacy of sildenafil and tadalalfil. When the rigidity score results where added to the SHIM scores, only 4 patients had equal scores, the 8 patients that previous had equal SHIM scores now had scores that reflected one drug more efficacy, which was consistent with patients selection of the more potent medication. Of the 13 patients that completed 20mg of vardenafil, 9 patients had equal SHIM scores. After adding the rigidity score results to the SHIM score, 4 of the 9 patients showed one drug more efficacy than other, which was consistent with the patients choice of most potent medication.A rigidity question when added to the SHIM questionnaire was more effective at differentiating potency of PDE5 inhibitor medication, than SHIM score alone.

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EFFECTIVE TREATMENT WITH VIAGRA® (SILDENAFIL CITRATE) IS ASSOCIATED WITH REDUCED BOTHER IN MEN WITH ERECTILE DYSFUNCTION

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Introduction: The impact of erectile dysfunction (ED) frequently extends beyond the initial erectile problem. Consequently, an important aspect of successful and long-term continuation of therapy for ED is an improvement in other psychosocial factors, often referred to as "distress" or "bother," which can affect a patient's life.

Methods: Combined data from 5 randomized, double-blind, placebo-controlled, multicenter, flexible-dose (50 mg initial dose, adjustable to 25 or 100 mg), 12-week studies in 1128 men with ED were analyzed. The International Index of Erectile Function (IIEF) and the 5-item ED-specific questionnaire, the Erection Distress Scale (EDS), were administered at baseline and end of treatment (EOT). The EDS assesses the patient's concern with his erection problem (frustrated about, weighed down by, discouraged by, felt despair over, a worry), and is graded on a scale from 1 (all of the time) to 6 (none of the time).

Results: At EOT, men receiving Viagra showed significant improvement in IIEF Erectile Function (EF) domain score (mean±SD 10.5±6.5, baseline; 20.3±9.0, EOT) compared with men receiving placebo (10.3±6.4, baseline; 11.3±7.4, EOT; $P<0.0001$). Significantly greater improvements in each of the 5 EDS questions were also observed with Viagra compared with placebo ($P<0.0001$). Increases in individual EDS question scores ranged from 23% to 64% with Viagra compared with 7% to 12% with placebo; scores at baseline were lowest and improvements with Viagra highest for "frustrated about" and "discouraged by" erection problems. Greater EDS score improvements with Viagra were noted for men with moderate (26%–85%) or severe ED (19%–67%) compared with men with mild ED (13%–41%). Changes in EF domain scores were positively correlated with changes in EDS scores ($P<0.0001$).

Conclusions: In men with ED, Viagra treatment resulted in significant improvements in erectile function that correlated significantly with improvements in ED-specific bother.

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LONG-TERM EFFICACY OF SILDENAFIL CITRATE FOR ERECTILE DYSFUNCTION AFTER RADICAL PROSTATECTOMY: 5 YEAR FOLLOW-UP

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We evaluated the 5-year efficacy and side effects of sildenafil after RP. We identified 68 patients with erectile dysfunction (ED) who were initial sildenafil responders following RP and had a minimum follow-up of 5 years. Using a self-administered questionnaire, we surveyed these 68 patients at one and five years to determine patient response/efficacy, compliance and side effects. Data were collected from a self-administered questionnaire using the 5 question Sexual Health Inventory of Men (SHIM). At 5 years, 31/68 (45.6%) were still responding to sildenafil, but 37/68 (54.4%) were not responding satisfactory and either discontinued the drug, switched to another therapy or used sildenafil in combination therapy. Specifically, in the 37 unsatisfied patients, 14/37 (37.8%) found sildenafil nonresponsive, with 8 patients discontinuing the drug and 6 switching to other forms of treatment (3 vacuum compression device (VCD), 3 intracavernosal injections (IC). Twelve of 37 patients (32.4%) developed a suboptimal response and used combination therapy (VCD, IC injection, and MUSE). Eleven of the 37 (29.7%) discontinued the treatment due to side effects (2), change in the personal circumstances (4), and cardiovascular comorbidities (5). The most common side effects at 5 years were headache (4/25, 16%), flushing (2, 8%), and blurred vision (2, 8%). The vast majority of patients (88.8%) still responding to sildenafil at 5 years had nerve-sparing procedures, 22/25). At 5 years, 50% of initial sildenafil responders continue to do well but required bilateral nerve-sparing procedures. Conversely, 50% of the patients become dissatisfied with the response and switched or added other erectaids, or discontinued therapy due to comorbidities, loss of partner, or side effects.

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PHOSPHODIESTERASE TYPE 5 INHIBITOR TREATMENT OF ERECTILE DYSFUNCTION MAY IMPART LONG-TERM BENEFITS FOR PATIENTS WITH DEPRESSION

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Introduction: Depression and antidepressant drug use are factors that may cause erectile dysfunction (ED). Here, we review 5 reports evaluating the efficacy of sildenafil in the treatment of ED in men with depression.

Methods: Men diagnosed with depression and ED were randomized to placebo or sildenafil treatment for 6 to 26 weeks. In 3 of the 5 studies, depression was in remission prior to randomization, and in 2 studies, ED was associated with serotonin reuptake inhibitor (SRI) treatment. The International Index of Erectile Function (IIEF) was used to quantify changes in sexual symptoms, while the Montgomery-Asberg Depression Rating Scale (MADRS) or Hamilton Rating Scale for Depression (HAM-D) was used to assess severity of depression.

Results: IIEF scores indicated that erectile function improved significantly with sildenafil treatment ($P<0.001$) (Seidman et al., 2001; Nurnberg et al. 2001; Fava et al. 2003; Nurnberg et al. 2003; Tignol et al. 2004). Furthermore, patients whose depression was in remission prior to randomization remained in remission through the end of the studies, as confirmed by MADRS and HAM-D scores (Fava et al. 2003; Nurnberg et al. 2003; Tignol et al. 2004). One report demonstrated that sildenafil responders with minor depression (ie, "Depressive Disorder Not Otherwise Specified") exhibited significant improvements in HAM-D scores compared with nonresponders (Seidman et al. 2001; Nurnberg, 2003).

Conclusions: Evidence from these 5 reports suggests that sildenafil is an effective treatment for ED associated with depression and antidepressant therapy. While direct antidepressant actions of sildenafil have not been demonstrated, successful treatment of ED may be associated with modest improvement in depression severity scores. Most important, treatment of ED may enhance long-term SRI compliance in men with depression- or SRI-associated ED.

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COMPARISON OF THE EFFICACY OF SILDENAFIL CITRATE TADALAFIL AND VARDENAFIL FOR THE TREATMENT OF ERECTILE DYSFUNCTION (ED) AFTER RADICAL PROSTATECTOMY (RP)

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In this prospective study, 23 men with ED after nerve –sparing (NS) RP who had responded to 100mg of sildenafil were given 20 mg tadalafil for 5 weeks, then 20 mg vardenafil for 5 weeks. After 5 weeks of each phosphodiesterase 5 (PDE5) inhibitor, patients had a 1-week washout period without a PDE5 inhibitor. 20/23 patients completed 20 mg of tadalafil for 5 weeks and 13 patients completed 20 mg of vardenafil for 5 weeks, an additional 10 patients completed both 20-mg of tadalafil for 5 weeks and 20 mg of vardenafil for 5 weeks. After 5 weeks of each PDE5 inhibitor, each patient completed a SHIM questionnaire, a rigidity score, and a side effects profile measuring frequency, duration, and severity of side effects. Of the 23 patients, 20/23 (87%) patients completed 20 mg of tadalafil for 5 weeks, 3/23 (13%) patients discontinued use of tadalafil due to side effects with a mean SHIM score for tadalafil of 18.7 [vs. sildenafil (n = 20) 19.85]. In comparing individual SHIM scores 6/20 had greater sildenafil SHIM scores, 2/20 had greater tadalafil SHIM scores, and 12/20 had equal SHIM scores. Of the 23 patients, 13/23 (57%) patients completed 20mg of vardenafil for 5 weeks, 0/13 discontinued use because of side effects, with a mean SHIM score for vardenafil of 19.53 [vs. sildenafil (n = 13) 19.85]. In comparing individual SHIM scores 2/13 had greater sildenafil SHIM scores, 2/12 had greater vardenafil scores, and 9/12 had equal SHIM scores. Ten patients completed both 20mg of tadalafil for 5 weeks and 20mg of vardenafil for 5 weeks, with mean SHIM scores for tadalafil of 18.2 and vardenafil of 19.9 [verse sildenafil (n = 10) 20.3]. Based on SHIM scores, sildenafil, tadalafil, and vardenafil, are an equally efficacious treatment in patients with ED following NS RP.

SPERMATOGENESIS/TESTIS

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INTERLEUKIN-FOUR INDUCED GENE-1 VARIANT (IL4I1-L) IS ASSOCIATED WITH SPERMATOGENESIS

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In testes, a longer 2.3 kb mRNA variant of interleukin-four induced gene-1 (IL4I1 L) is highly and uniquely expressed. IL4I1 L is no longer regulated by interleukin-four because it has a different upstream promoter that lacks STAT binding sites. IL4I1 L alternatively splices four 5' testes-specific exons to replace two 5' immune-specific exons, resulting in the loss of the signal peptide required for lysosomal targeting. Thus, IL4I1-L protein should target to the cytoplasm. Anti-IL4I1-L staining of testes sections from obstructive azoospermia (OA) patient biopsies reveal high protein expression of IL4I1-L throughout the seminiferous tubules, including the basement membrane, cytoplasm of tubule wall cells, spermatagonia, spermatocytes, and reduced levels in spermatids. Sperm samples have IL4I1-L protein limited to cytoplasmic droplets and no detectable IL4I1-L mRNA. Anti-IL4I1-L staining of testes sections from Sertoli cell only and hypospermatogenesis (HS) patient biopsies was markedly reduced, whereas maturation arrest (MA) patient biopsies (with and without varicocele) were similar to OA. Thus, IL4I1-L expression correlates with spermatogenesis, but not necessarily spermiogenesis. IL4I1-L expression positively correlates with apoptosis (TUNEL, r=0.423) and parameters previously shown to regulate apoptosis (testicular cadmium levels, basement membrane thickness, and FasL immunostaining; r=0.500-0.657). The hydrogen peroxide produced by IL4I1-L enzyme activity may be required for apoptosis in spermatogenesis. Further, as IL4I1-L is not equivalently expressed in testicular pathologies with elevated apoptosis (MA vs. HS), it may be developed as a diagnostic marker.

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CLONING AND CHARACTERIZATION OF SPERMATID SPECIFIC GENE ALPHA 131 CODING A NOVEL MANCHETTE PROTEIN

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Development of spermatozoa is a complex process involving specific morphological formation of flagella, mitochondria and nucleus. Although detailed morphological observations of these events are available, the molecular mechanisms remain to be fully elucidated. We report here the molecular cloning and characterization of alpha 131 coding a novel manchette protein from a haploid germ cell-specific subtracted cDNA library of mouse testis. Isolated cDNA clones contain 3 variants of alpha131. They lie on one locus of chromosome 4 of mouse genome and are composed from 7 exons. Western blotting using antisera specific to each translated product reveals that only one type of alpha 131 protein is detectable in mouse testis. Other two mRNAs are not translated, or translated under the detectable level. Immunohistochemistry shows that alpha 131 protein is localized at the cytoplasm of the step 9-12 elongated spermatids. The signals appear like a cap formation which covers the caudal sides of the elongated nucleus. Manchette is known as a structure forming a similar appearance at step 8-13 spermatids. Alpha 131 may play a role as a structure protein coordinated with manchette proteins, including actin or tubulin.

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A NON-INVASIVE ISOTOPE ASSAY TO MEASURE HUMAN SPERMATOGENESIS KINETICS IN VIVO

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Objective: Our current understanding of the kinetics of normal spermatogenesis in humans is limited and derived mainly from analyses of testis histology from men of different ages. We have developed a non-toxic method of assessing cell turnover in vivo using stable isotope labeling and gas chromatographic /mass spectrometry. In this study, we sought to define the parameters and variables associated with this assay system in healthy men.
Methods: In this prospective study in healthy men with normal and abnormal semen quality, subjects ingested heavy water (^2H_2O) daily for 3 weeks. Saliva and semen samples were collected bi-monthly for 90 days for analysis. Label incorporation into the deoxyribose moiety of sperm DNA was quantified by mass spectrometry. Since DNA synthesis (label incorporation) is equivalent to cell division, the percentage of new cells was calculated. Sperm production kinetics were defined as lag time until labeled (new) sperm appeared in the ejaculate.
Results: 12 subjects completed the study. By 60 days, 11/12 subjects had appreciable (>20%) new sperm in their ejaculates. The average rate of new sperm in the ejaculate (n=6 men with sufficient follow-up) was 2.4 +/- 0.8% new cells/day. Men with normal semen quality (n=6) had labeled sperm appearing in the ejaculate between 50-60 days. Men with abnormal semen quality (n=6) had labeled sperm appear <50 days in 2 cases, between 50-60 days in 3 cases and after 60 days in 1 case.
Conclusion: Spermatogenesis has been estimated to require approximately 60-75 days in normal men. This study suggests that sperm production may be quicker, but wide interindividual variability may exist. This method holds promise for furthering our understanding of the physiologic basis of human male infertility.
Support: This study was funded by KineMed, Inc.

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ROLE OF ETHNICITY IN MALE REPRODUCTIVE FUNCTION

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Male reproductive function is influenced by multiple factors like hereditary factors, ethnicity, and geographical background. Examples of ethnic differences in susceptibility to disease and responsiveness to treatment include the observed differences in the incidence of clinical and advanced prostate cancer. The goal of this study was to assess if ethnicity has any affect on reproductive function of men. Eight hundred eighty-nine vasectomies were performed for voluntary sterilization purposes from January 2000 to July 2003 at a teaching hospital. Patients were divided based on ethnic origin into Group I (Caucasian, n = 397) and Group II (African-Brazilian, n = 492). Testicular volume, semen parameters, follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone and prolactin were assessed. There was no difference in mean age (33.1 ± 5.1 *vs.* 34.5 ± 4.2; $P = 0.28$), hormone levels, and semen parameters (table). In addition, sperm motion characteristics showed no difference. While reported ethnic differences such as spermatogenic capacity and apoptosis rate may play a significant role, environmental factors must also be considered as important interacting factors for ethnic differences. Our study supports this statement, since it found a lack of difference in reproductive function between fertile Brazilian men of Caucasian and African origin. Further studies are needed to confirm these results.

Variable	Group I	Group II	*P* value
Right volume (mL)	20.32 ± 6.2	19.83 ± 5.9	0.144
Left volume (mL)	19.06 ± 8.85	18.97 ± 6.21	0.45
Concentration (10^6/mL)	111.8 ± 84.4	110.0 ± 77.0	0.801
Motility (%)	59.0 ± 15.4	59.9 ± 16.0	0.522
FSH (IU/L)	3.7 ± 2.2	3.5 ± 1.9	0.778
LH (IU/L)	3.2 ± 1.5	3.3 ± 1.4	0.124
Testosterone(nmol/L)	563.5 ± 185.6	578.4 ± 180.5	0.831
Prolactin (mIU/L)	6.6 ± 5.9	6.4 ± 4.7	0.143

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ATP PRODUCTION AND PYRIDINE NUCLEOTIDES IN HUMAN MALE GERM CELL DEATH

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INTRODUCTION: Testicular energy metabolism and cell death have been suggested to play roles in fertility issues. ATP production and pyridine nucleotides (PNs; NAD and NADP) are regulators of and are regulated by mitochondrial functions and cell death. As their involvement in testicular cell apoptosis is not well known we studied the role of mitochondrial ATP machinery and PNs in human male germ death.

RESULTS (and methods): Incubation of human seminiferous tubule segments under serum and hormone free conditions induced germ cell apoptosis (Southern blot analysis of DNA fragmentation, TUNEL and EM), and decreased the levels of ATP and PNs (HPLC). Inhibiting complexes of mitochondrial respiration, or ATP synthase (oligomycin) dropped ATP levels further, but did not significantly affect the levels of PNs. All these compounds suppressed apoptosis at 4h. A combination of an uncoupler (DNP) and oligomycin also suppressed death at 4h, as did the DNP by itself. Inhibiting glycolysis by 2-DG neither suppressed apoptosis nor altered the results obtained with the mitochondrial modulators. After 24h, many cells underwent delayed apoptosis despite ATP-depletion (mitochondrial inhibitors+/-2-DG) and some of cells showed signs of necrosis or toxicity.

WE CONCLUDE that: 1) in the human testis, the apoptotic process, triggered by the culture conditions, affects ATP and PN metabolism, and that 2) the mitochondrial ATP production plays an important role in primary pathways of male germ cell death. 3) None of the studied distinct mitochondrial components alone, nor significantly the levels of PNs, appear to explain this regulatory role. 4) We also conclude that there seem to be secondary pathways of human testicular apoptosis that do not require mitochondrial ATP production and possibly not ATP either.

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EFFICACY AND SAFETY OF PERCUTANEOUS TESTICULAR ASPIRATION IN EVALUATION OF AZOOSPERMIC OR SEVERE OLIGOSPERMIC PATIENTS

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The histopathological study of the testis is used in the evaluation of infertile men with azoospermia or severe oligospermia. We evaluated needle aspiration of the testis as a method to obtain testicular tissue in these patients. Twenty-four patients with azoospermia or severe oligospermia were included in our prospective study. Follicle-stimulating hormone (FSH) level and an ultrasound of the testicles were performed in all patients. All patients underwent bilateral testicular aspiration with a 21G needle. Forty of the 48 testicular aspirations yielded tissue fragments, macroscopically represented by filiform cylinders. Thirty-six of these resulted in 5 or more transversal sections of seminiferous tubules (19.9 ± 12.8), with a specimen quality adequate for histopathological purposes. Of these 36, maturation arrest at the spermatocyte level was detected in 55.6%, germ cell aplasia in 19.4%, normal spermatogenesis with signs of obstruction in 22.2% and atrophy of the seminiferous tubules in 2.8%. In the same 36 samples, spermatozoa was found in 6% of the transversal sections of seminiferous tubules in cases of maturation arrest at the spermatocyte level, and 91.3% in the specimens with diagnosis of normal spermatogenesis with obstruction signs. No differences were seen in the mean testicular volume (P = 0.239) or FSH levels (P = 0.263) between patients with a sample adequate and inadequate for examination. The only clinical complication was ecchymosis of the scrotal skin in 3 cases. The ultrasound performed 7 and 14 days after the procedure revealed minimal hydrocele in 8.3%, hyperechogenic spots in 4.2% and hypoechogenic area with 7mm on the longest axis in 2.1%. This technique was associated with low morbidity and all samples macroscopically represented by filiform cylinders resulted in the presence of seminiferous tubules. Testicular volume or FSH levels did not constitute predictive factors for obtaining adequate testicular tissue samples.

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IS POOR SEMEN QUALITY IN MEN WITH MALIGNANCIES DUE TO PRE-EXISTING DEFECTS IN SPERMATOGENESIS?

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It is well known that sperm quality is often poor in men with malignancies at the time of their cancer diagnoses. The low sperm density may be an indirect effect of surgery and anesthesia or it may be due to a decrease in sperm production after orchiectomy. It is possible that the stress of discovering that one has a potential fatal disease requiring unpleasant treatment might be sufficient to lower semen qualities. Physical and mental stress, genetic factors, and hormonal imbalances may all be responsible for the altered spermatogenesis. The goal of our study was to evaluate the histology of multiple biopsies performed in orchiectomy specimens due to testicular cancer. Thirty-two patients with testicular cancer (seminoma) were included in this retrospective study at a teaching hospital. We evaluated the testicular histology surrounding or associated with the seminoma. Testicular histology was divided into five different patterns: maturation arrest, germ cell aplasia, atrophy, hypospermatogenesis, or normal histology. Maturation arrest was found in 15 testes, germ cell aplasia in 4, atrophy in 7, atrophy plus germ cell aplasia in 5, and only 1 case of normal spermatogenesis. Our results show that most of the testicular cancer patients (97%) have defects in spermatogenesis. Abnormal testicular histology in patients with testicular tumor might indicate pre-existing spermatogenesis defects or local tumor effects that are responsible for the impaired semen analyses usually observed in patients with seminoma. Testicular histology when performed in contra-lateral testis should help in discriminating between these two pathologies. Sperm cryopreservation should therefore, be recommended for all patients with testis cancer who may desire to have children in the future.

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THE EFFECTS OF GN-RH ON SEMINIFEROUS TUBULES IN IMMATURE RATS

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Seminiferous tubules (ST) are dedicated to the production of spermatozoa. The somniferous tubules (ST) are composed of a thick epithelium. The somniferous epithelium is several cell layers and is composed of two types of cells: Sertoli cells or supporting cells and spermatogenesis cells. The cells of spermatogenesis are stacked in *4-8* layers that occupy the space between the basal lamina and the lumen of the tubule. These cells divide several times and finally differentiate; producing spermatozoa. The latter cells are in various stages of maturation. For increasing animal productions, we need a lot of animals. For this reason, we have studied effect of Gn-RH on somniferous tubules (ST) function. Gonadotropin-releasing hormone (Gn-RH) is a peptide composed of ten amino acids which are synthesized in the hypothalamus and produced and released from the hypothalamus. Gn-RH release stimulates the secretion of both follicle-stimulating hormone (FSH) and lutenizing hormone (LH) from the pituitary gland. This hormone travels in the bloodstream to the anterior pituitary, where it causes the release of the gonadotropic hormones this hormone in males; LH stimulates the testis to release testosterone.
In this study, male immature rats at 3 weeks of age were divided into two groups of experimental (*n=20*) and control (*n=10*). Two groups were injected with *0.1* ml Gn-RH and *0.1* distilled water one time for *5* days respectively. After that, on *30, 35, 40*, and *45* days, testis samples from two groups referred to pathology laboratory and were fixed in formalin buffered (*10 %*) and used routine H&E staining then processed For light microscope (LM). Spermatozoa productions (spermatogenesis) were observed in seminiferous tubules (ST), of experimental group. But seminiferous tubules (ST) in control group couldn't product spermatozoa. It is concluded that Gn-RH was caused spermatogenesis and early maturation in male immature rats.

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EFFECTS OF LIGHT ON LEYDIG CELL DIFFERENTIATION IN THE PREPUBERTAL HAMSTER TESTIS

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Leydig cell differentiation in the postnatal mammalian testis is crucial to establish the adult population of Leydig cells, which are the primary source of androgens in the adult male mammal. Hamsters are seasonal breeders and Leydig cells of adult hamsters regress under short day lengths, however, recrudescence is seen when re-exposed to longer days. To our knowledge, information on regulation of postnatal Leydig cell differentiation in the hamster testis is sparse. In the present investigation, we hypothesized that Leydig stem cell differentiation in the pre-pubertal hamster testis is regulated by light. We tested this hypothesis by using two groups of male Syrian Golden hamsters. Hamsters in Group I were raised under short day length (23h:1hr Dark:Light) and hamsters in Group II were raised under regular light conditions (12h:12h Dark:Light), respectively, from birth to 28 days of age. At postnatal day 28, testicles of hamsters in the two experimental groups were fixed by whole body perfusion, processed and embedded in epon-aradite. Using 1 µm sections stained with Methylene Blue-Azure II, Leydig cell number per testis in the two experimental groups was quantified by state-of-the-art stereological techniques. Identification of Leydig cells was based on their location in the testis and characteristic morphology. The results revealed that Leydig cell number per testis in 28-day-old hamsters raised in the dark (Group I) was significantly lower than those raised under regular light regime (Group II). These findings suggested that Leydig stem cell differentiation in the prepubertal hamster testis is positively regulated by light. (Funded by UT Center of excellence and WHO).

SEMEN ANALYSIS/SPERM

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UBIQUITINATION OF DIVERSE SEMEN COMPONENTS DIFFERENTLY CORRELATES WITH STANDARD SEMEN PARAMETERS

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Global ubiquitination in human semen has been found to negatively correlate with parameters of routine semen analysis, indicating that ubiquitination can be considered a marker of poor semen quality. However, the inclusion of all semen components in the analysis may be misleading on the biological significance of ubiquitination of sperm cells. We have recently demonstrated that bodies of different size are present, in variable amounts, in semen from different pathological conditions, with the highest concentration in oligoasthenoteratozoospermia. The purpose of the present study was to evaluate the relationship between ubiquitination and standard semen parameters, after distinguishing between ubiquitinated sperm and bodies in each sample. Ubiquitination was evaluated by flow cytometric sperm ubiquitin tag immunoassay (SUTI) in sperm samples from 14 normozoospermic, 9 astheno-teratozoospermic, 11 teratozoospermic and 11 oligo-asthenoteratozoospermic men. Semen analysis was performed according to WHO. When correlation was evaluated considering the percentage of ubiquitinated bodies a negative correlation was found with good quality of semen ($r=-0.50$, $P<0.001$ with count, $r=-0.45$, $P<0.005$ with progressive motility and $r=-0.47$, $P<0.001$ with normal morphology, P<). Conversely, when only ubiquitinated sperm were considered, a positive correlation with number ($r=0.36$, $P<0.05$) motility ($r=0.52$, $p<0.001$) and normal morphology ($r=0.58$, $p<0.001$) was found.
Our results indicate that the negative correlations previously found between global semen ubiquitination and parameters of semen analysis are mainly driven by components other than sperm. The positive correlation between sperm ubiquitination and good quality parameters suggests a novel, previously unrecognized, role for sperm ubiquitination besides marking defective sperm for their elimination in the epididymis.

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WORLD HEALTH ORGANIZATION STANDARDS OF TRADITIONAL SEMEN ANALYSIS: REVISITED

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Semen analysis including concentration, motility, and morphology remains the cornerstone of assessing male fertility potential. The World Health Organization (WHO) has published manuals in 1980, 1987, 1992, and 1999 with the objective to standardize the practice of andrology laboratories worldwide when assessing these criteria. The purpose of this study was to evaluate the appropriateness of the reference limits in a large infertility program in a teaching hospital. Sixty-two semen samples from 47 healthy donors with proven natural fertility and single ejaculates of 406 patients evaluated for infertility were assessed using the 1999 WHO standards. The third percentile values of sperm concentration, motility, and morphology were calculated for the fertile donors. These values are traditionally used as cut-off points of normality in a biological system. The normality of all semen samples studied was reclassified using these derived values. Applying the 1999 WHO standards, 30 (48%) fertile donors and 331 (81.5%) men assessed for infertility had one or more abnormal sperm parameters. The third percentile values for sperm concentration, motility, and normal morphology in fertile semen samples were $11x10^6$/mL, 27%, and 12%, respectively. The application of these values has resulted in a significant reduction in the number of samples diagnosed with abnormal semen parameter(s) among fertile donors (19% vs. 48%, $X^2 = 17.6$, $P < 0.0001$) and men assessed for infertility (45% vs. 81.5%, $X^2 = 28.7$, $P < 0.0001$). The results of this study demonstrate that, traditionally, assessed sperm parameters are not a reliable indicator of fertility potential even among a fertile population. The reference values set by the WHO need to be constantly revised in order to enhance its predictive power. Fertility laboratories may utilize the 3rd percentile values of semen variables from a fertile population as an alternative reliable method to evaluate the status of male fertility. This may be further validated by wider multicenter studies.

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ADDING CHOLESTEROL TO STALLION SPERM IMPROVES CRYOSURVIVAL AND OSMOTIC TOLERANCE LIMITS

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Cryopreservation induces partially irreversible damage to equine sperm membranes. One way to prevent this damage is to increase the membrane fluidity at low temperatures. These experiments were designed to determine if adding cholesterol to stallion sperm prior to cryopreservation would improve cryosurvival and increase the osmotic tolerance limits of the sperm. In experiment 1, semen from 15 stallions was collected and diluted to 120 x 10^6 sperm/mL in a modified Tyrode's media (TALP). The sperm were divided into 7 groups and treated with different levels of cholesterol-loaded cyclodextrins (CLC's; 0, 0.5, 1.5, 3.0, 4.5, 6.0 or 7.5 mg CLC/120 x 10^6 sperm) for 15 min at 22°C and then diluted 1:1 with a skim milk, glucose diluent. Samples were then centrifuged at 600g for 10 min, the cells resuspended to 200 x 10^6 sperm/ml in Lactose-EDTA freezing extender and the samples frozen. Straws were thawed in 37°C water for 30 sec and the percentages of motile and viable sperm determined. The addition of 1.5 mg of CLC's to stallions considered to be "poor" freezers resulted in higher total (67%) and progressively (45%) motile sperm compared to control samples (50 and 32%, respectively; $P<0.05$). Addition of CLC's also increased the percentages of viable sperm surviving cryopreservation for all stallions ($P<0.05$). In experiment 2, semen from 9 stallions was collected, diluted to 120 x 10^6 sperm/mL in TALP, divided into three groups and treated with 0, 1.5 or 6.0 mg CLC/120 x 10^6 sperm as described above. Cells were then diluted 1:1 with TALP, centrifuged, resuspended to 1 x 10^9 sperm/ml in TALP and aliquots placed into anisosmotic solutions (75, 150, 225, 270, 300, 350, 370, 425, 600, 1200 or 2400 mOsm), for five min before sperm motility was assessed. Samples were then returned to isosmotic conditions and the sperm motility again determined. Samples treated with 1.5 mg or 6.0 mg CLC exhibited higher motility at 75, 150, 225 and 600 mOsm when the sperm were returned to isosmolality ($P<0.05$). In conclusion, adding CLC's to stallion sperm improves cell cryosurvival and the osmotic tolerance of cells. This is a simple procedure that can increase the cryosurvival of equine sperm.

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DOES EJACULATORY FREQUENCY AFFECT SEMEN PARAMETERS?

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The aim of this study is to investigate the effect of ejaculatory frequency (EF) on semen parameters.

Information was collected from a continuously updated database of semen analysis of subjects undergoing initial screening for infertility. Only the first specimen provided by each patient in our Centre was considered. Morphology assessment was performed on fresh samples according to Kruger's criteria (1986); the other parameters were evaluated according to the WHO criteria (1992). The Mann-Whitney U test and the Spearman rank correlation test were used; a multivariate analysis of covariance was performed with adjustment for age and days of abstinence.

8097 men were included in the study. The median number of sexual intercourses per week was 2 (range: 0.5-10). EF had no influence on volume of ejaculate, sperm concentration and total sperm count. The percentage of motile spermatozoa was significantly affected by the EF ($p<0.0001$); the percentage of motile spermatozoa (mean±SEM) ranged from 50.2±0.5% in subjects having 1 intercourse/week to 39.8±0.7% in those having e 8 intercourses/ week. However, when different types of sperm motility were considered, EF had no influence on the percentage of rapid progressive motility, while it affected the percentages of slow progressive ($p<0.0001$) and non-progressive motility ($p<0.0001$). EF had a significant effect on the percentage of normal spermatozoa ($p<0.0001$), which ranged from 29.4±0.5% in subjects having 1 intercourse/week to 41.1±0.6% in those having e 8 intercourses/ week; EF significantly affected the percentage of head, midpiece, and tail abnormalities. Total motile sperm count and total progressively motile sperm count were not significantly correlated with EF.

EF affects sperm motility and morphology.

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RELATIONSHIP BETWEEN SPERM CHROMATIN STRUCTURE AND CAPACITATION

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Introduction: Sperm chromatin is a highly organized and compact structure that maintains genetic integrity. Correlation studies have demonstrated the predictive nature of sperm chromatin structure on IVF and IUI outcomes. Since the ability of sperm to undergo capacitation is also related to IVF and IUI outcomes, we investigated the relationship between the sperm cell's capacitation index and chromatin structure integrity.
Design: Comparison of sperm capacitation index (SCI; as determined by the sperm penetration assay) with sperm chromatin fragmentation index (SDFA; as defined by the flow cytometric-based sperm degree of DNA fragmentation chromatin structure assay).
Setting: Private medical center-based andrology laboratory and infertility program.
Patients: Forty (40) men who presented for routine semen analysis.
Main Outcome Measures: SCI scores were determined by the optimized sperm penetration assay (number of penetrations per ova). SDFA scores were determined by the protamine dissociation and fluorescent flow cytometry (Reprodmedix, Woburn, MA).
Results: Linear regression analysis revealed a significant relationship (P=0.01) between sperm chromatin integrity and the sperm capacitation (R^2=0.162). There was a significant difference (P=0.005) of SDFA scores between specimens that passed (SCI $\geq$5.0; SDFA mean 17.69) the SCI versus those patients that failed (SCI , 5.0; SDFA mean 29.32) the SCI.
Conclusion: Sperm chromatin structure integrity and capacitation are interrelated, however, the causative relationship requires further study. Sperm capacitation as defined by the sperm penetration assay can be predicted by assessing sperm chromatin integrity.

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SPERM COUNT DISTRIBUTIONS IN FERTILE MEN.

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Sperm concentration and count are often used as indicators of environmental impacts on male reproductive health. Existing clinical databases may be biased towards subfertile men with low sperm counts and less is known about expected sperm count distributions in cohorts of fertile men. We are surveying semen quality in a selected cohort of presumed fertile men (n=228) who are resident partners of pregnant women. These couples live in the metropolitan area of 1 of 3 mid-sized US cities selected for availability of environmental monitoring data. Semen was collected at home and cold-shipped to a central lab for analysis the following morning. For sperm concentration (10^6/ml semen determined by hemacytometer), mean = 147 ± 115.6 (SD), median = 114 and % < 20 (the WHO reference value) = 3.9%. For volume (ml), mean = 3.3 ± 1.7 and median = 3.0. For total sperm count (10^6), mean = 463 ± 410, median = 325, and % < 40 = 2.2%. Compared with another cohort of 156 fertile US men reported by Zinamen et al. (J. Androl 21:145, 2000), these values are relatively high. Differences in mean concentrations and counts by city of residence were statistically significant overall, p<0.01 (log-transformed, PROC GLM, SAS, controlling for abstinence). The city with the highest mean sperm concentration (169 ± 136, n=90) was different from that with the lowest (126 ± 92, n=46), p<0.02. These data will be used, in combination with other semen outcomes, to examine associations with other factors including lifestyle and environmental/occupational exposures. This preliminary analysis suggests that recently fecund men may have higher than expected sperm concentrations and counts, and that geographic differences in these outcomes may be detectable in relatively small cohorts of fertile men. This abstract does not represent EPA policy.

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EXTENT OF OVERLAP OF SPERM PARAMETERS BETWEEN FERTILE MEN AND PATIENTS EVALUATED FOR INFERTILITY

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Semen analysis constitutes the initial and most essential step in the evaluation of male infertility. However in clinical practice, men undergoing infertility evaluation may present with normal semen parameters. This indicates that male factor alone may not be responsible and other etiologies may be the cause of infertility in the couple. The purpose of this study was to evaluate the extent of overlap of sperm parameter values in fertile donors and men undergoing infertility evaluation in a large infertility program in a teaching hospital. Analysis included 406 semen samples of men undergoing infertility evaluation and 62 samples from proven fertile donors. We determined the range of overlap of sperm parameters [concentration, motility, and morphology (WHO and Kruger's)] in the study population. The higher set points of this derived range were established wherein 90% of men evaluated for infertility were below this established value. Similarly, 90% of fertile donors were above the established lower set point. The percentages of study population within the established range are shown in the below table. Despite significantly higher values of sperm parameters in fertile donors compared to men evaluated for infertility, the table revealed that most of the study population fell within the established range of overlap. Also, there was a broader range of overlap for concentration and motility parameters compared to morphology criteria (WHO & Kruger's).

		Range		Proven fertile (%)	Patients (%)
		Lower	Higher		
Concentration (M/ml)		24.3	132	61	50
Motility (%)		45	86	68	49
Morphology (%)	WHO	20	35	34	35
	Kruger's	4	12	48	52

Very high degree of overlap of sperm parameter values was observed in our study population. This overlap underscores the importance of ruling out female factor, idiopathic infertility, and performing other specific investigations in the male before characterizing an individual as infertile based on semen parameters.

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***IN VITRO* EVALUATION OF CRYOPRESERVED BOAR SEMEN**

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Motility or the percentage of living cells after thawing are typically used in the study of frozen boar semen, but whether the information they provide is sufficiently accurate warrants investigation. The aim of our work was to address this question. Twenty-two samples were evaluated for motility in the absence (MOT) or presence (MOTc) of 5 mM caffeine, as well as for the percentage of living cells (LV) using Propidium iodide. Semen quality groups were then established (cluster analysis), and representative ejaculates incubated at 37°C for 4h, monitoring the viability of cells throughout the period. MOT values (8-56%) were significantly lower than those of LV (11-67%) in half the ejaculates, but no significant difference between MOTc (15-71%) and LV was observed. Over the course of incubation, viability significantly decreased in all samples, although not in a quality dependent manner, with motility revealing thermoresistance differences between ejaculates in a more reliable way (Figure 1). Therefore, LV and MOTc (unlike MOT) indicate the proportion of cells resisting cryopreservation, but do not indicate the ability of cells to survive in the female genital tract, which might be a more interesting aspect of quality. Monitoring sperm motility for several hours after thawing, rather than the percentage of living cells, could give more accurate knowledge on frozen semen quality.

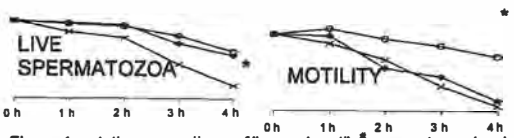

Figure 1: relative proportions of live and motile* spermatozoa in ejaculates of high (-X-), good (-○-) and low (-●-) quality (*:the highest of two values, with or without caffeine, is shown in each point; *: significantly different from the other two samples).

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EFFECT OF EGG YOLK, COOLING/THAWING RATES AND CHOLESTEROL ON CRYOSURVIVAL OF RABBIT SPERM

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Several diluents and protocols have been used to freeze rabbit sperm, however, the fertility of frozen-thawed rabbit sperm is still low. Experiments evaluated the effect of egg yolk (EY), cooling (fast or slow) and thawing temperatures (39 or 50°C) and the addition of cholesterol to rabbit sperm on sperm cryosurvival. Rabbit sperm were diluted to 120 x10^6 sperm/ml in a Tris-based diluent containing no cryoprotectants and divided into 8 aliquots. One aliquot was then treated with cyclodextrin alone, two aliquots with 5mg of cyclodextrin pre-loaded with cholesterol (CLC, Purdy and Graham, 2004), one aliquot with 10mg of CLC's and 4 aliquots were not treated with cyclodextrin (controls). Samples were incubated for 15min at 23°C, and then diluted to 60x10^6 sperm/ml with tris diluent as follows: two of the control samples, with Tris having no EY, and all other samples with Tris containing 33% EY. All samples were then diluted 1:1 with freezing diluent (3.5M DMSO and 0.1M sucrose, Vicente and Viudes de Castro, 1996), and packaged into 0.25ml straws. Samples were cooled to 5°C either slowly (2h), or quickly (30min), and then frozen in liquid nitrogen vapor. The straws were then stored in liquid nitrogen, thawed at two different temperatures (39 or 50°C) and sperm motility and viability assessed. The percentages of motile and live sperm were similar ($p>0.05$) for: cooling rates (14-15% motile and 30-32% live cells); CLC levels (14-17% motile and 25-33% live cells); and thawing temperatures (14-16 % motile and 30-32% live cells). The presence of EY did not affect the percentage of motile cells (14-15%), but did result in fewer viable sperm post-thaw (29%) than diluent with no EY (36%, $p=0.02$). In conclusion, CLC addition, cooling rate and thawing temperature had no effect on sperm cryosurvival, while the presence of EY reduced cell viability.

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LOW HYPO-OSMOTIC SWELLING TEST SCORES SIGNIFICANTLY CORRELATE INVERSELY WITH HIGH DNA FRAGMENTATION INDICES WHEN PERFORMING THE SPERM CHROMATIN STRUCTURE ASSAY

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The hypo-osmotic swelling test (HOST) score when <50% rarely allows a successful pregnancy to occur following intercourse or intrauterine insemination (IUI). Interestingly following in vitro fertilization (IVF) with conventional oocyte insemination oocytes fertilize and form morphologically normal embryos but they rarely implant. The abnormality is obviated by performing intracytoplasmic sperm injection (ICSI). Similar statements can also be made about males with DNA fragmentation indices >30% when performing the sperm chromatin structure assay. The present study evaluated whether there is an association of low HOST scores with high DFI scores. The SCSA test was performed by Dr. Evenson's laboratory in South Dakota. The HOST and sperm chromatin structural assay were performed on initial semen analysis not the one on the day of IVF. The studies were performed on refractory IVF patients. There were 192 patients having SCSA tests and 168 had HOST scores ≥50% and 24 were <50%. Only 20.8% (n=5) of the low HOST scores were found in males with DFI ≤15%, compared to 45.8% (n=11) for the range 16-30%, and 33.3% (n=8) for males with DFI >30%. The Pearson chi-square (2 sided) found p=.011. These data suggest a significant inverse correlation between low HOST score and high DFI score. Further studies are needed to determine if subfertility increases for males with both abnormalities, or possibly vice versa, maybe only the male with high DFI and low HOST is able to achieve viable pregnancies by ICSI. Perhaps there are two different etiologies for males with high DFI with and without low HOST scores. Further studies are needed.

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USING ATOMIC FORCE MICROSCOPY TO STUDY THE SPERM ULTRASTRUCTURE OF THE RAT MODEL

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Objective: To investigate ultrastructural changes, particularly at the surface, that occur in pathological spermatozoa by using atomic force microscopy (AFM) and to examine the morphological alterations responsible for infertile sperm.
Materials and Methods: Normal fertile and pathological spermatozoa were examined by using a conventional AFM in a noncontact mode. Sperm of rat with ligoasthenoteratozoospermia (OAT) and asthenozoospermia were obtained from the animal model.
Result(s): Morphological details, topological information, and three-dimensional images of the head, neck, and flagellum are presented for both normal and pathological sperm. The obtained images clearly show dramatic alterations in the morphology of the head, neck, and flagellum of pathological sperm. Even the ultrastructure at the top of the flagellum and the region of the acrosome cap are clearly distinguishable.
Conclusion(s): This study has significant importance not only for identifying spermatozoa alterations but also for understanding morphological defects and their effects on infertility. By this experiment, AFM should be applied to study the human sperm as an important novel tool.
Key Words: Ultrastructure, fertile sperm, atomic force microscopy, oligoastenoterato-zoospermic(OAT)

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DETECTION OF SUB-FERTILE RABBITS BY MEANS OF SPERM HEAD MORPHOMETRIC ANALYSIS

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Sperm morphometry analysis can be useful in the prediction of sperm fertility. In this work, 35 ejaculates from 7 rabbits were used for the analysis of sperm head morphometry (length, width, area and perimeter) with the software Sperm-Class Analyzer® (SCA). The morphometry of sperm head was significantly different between ejaculates from the same male (due to the low coefficient of variation, around 6%) and between males. Since morphometry is a multidimensional character and a correlation exists between the four parameters analyzed, an analysis of Principal Components was performed. This analysis establishes new components not directly measurable and independent between them (Jobson, 1992) and the interpretation of the results is easier. The first component was a general size component and differentiated big from small sperm heads. Thus, ejaculates were classified by this component on a basis of sperm head size. The size of sperm heads from one of the males was significantly lower than the sperm size from the other males (score=-1.08 *vs* −0.09 to 0.66, respectively). Females were inseminated with sperm from each of these males, and a significant decrease in fertility rate was observed when sperm from the male with small sperm heads was used to perform the inseminations, compared to the results observed for the other males (45.0% (n=20) *vs* 77.9% (n=89), respectively, $p<0.01$). In conclusion, analysis of sperm head morphometric parameters can be used to identify sub-fertile males, avoiding fertility problems.
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QUALITY EVALUATION OF THREE DIFFERENT SPERM COUNTING CHAMBERS

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Objective: To assess the accuracy of three commercially available sperm counting chambers. Hemacytometer (Qiujing, Shanghai), Makler (Sefi-Medical, Haifa), and Cell-VU (Millennium Sciences, New York) sperm counting chambers were used.
Methods: Two pre-calibrated standard latex beads solutions (Hamilton Thorne Biosciences) were used as the standard quality control (QC) solution for each sperm counting chamber. Bead counts were compared and variability for each chamber was determined.
Results: Mean bead concentration for the Cell-VU chamber was consistently close to the standard QC bead solution, while those for both Hemacytometer and Makler chambers were largely overestimated. The average coefficients of variation for the Cell-VU chamber were 7.51% and 1.22% for the high and the low bead QC standard solutions; while those for Makler chamber were 52.91% and 38.72% and those for the Hemacytometer were 22.11% and 13.78%.
Conclusion: A reliable and accurate sperm counting chamber is essential for male fertility evaluation. Study results underscore the importance of standardization and QC in semen analysis. Of the three chambers tested, Cell-VU was the most accurate. Both the Makler and the Hemacytometer chambers overestimated counts.

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MALES AGE 50 OR GREATER ARE LIKELY TO HAVE A GREATER CHANCE OF SUBFERTILITY RELATED TO LOW HYPO-OSMOTIC SWELLING TEST SCORES

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Subnormal hypoosmotic swelling test (HOST) scores (<50%) have been associated with a marked decrease in embryo implantation rates and pregnancy rates. The present study evaluated whether this defect is more common in males that are in the older reproductive age range. HOST scores were evaluated according to 4 different age categories: <40; 40-45; 46-49; ≥50 years. The initial semen specimens were evaluated for HOST scores, volume, count, % motility, total motile sperm, and motile density. Since these variables tend to have skewed distribution due to the occurrence of extreme values, the summary statistics used were the median and range. Medians were compared by age groups using Kruscal-Wallis analysis of variance. The percentage of low HOST scores in those males ≥50% vs. <50% according to age were compared by Fisher's exact test. The median HOST score was significantly lower ($p<.001$) in males age ≥50 (53.5 vs. 70.4, 70, and 62.0, respectively). Semen volume was also significantly lower in this age group (1.8 vs. 2.7, 2.3 and 3.0ml) with $p=.036$. The sperm count for ≥50 was $40.1x10^6$/mL, % motility 53.0%, total motile sperm $41.3x10^6$/mL, and motile density of $20.4x10^6$/mL and these levels were not different than the other age groups. Eight of 24 males ≥50 (33.3%) had subnormal HOST scores. In contrast only 8.3% (29/351), 8.9% (11/123), and 16.0% (4/25) of the other 3 age groups had HOST scores <50% ($p<.001$, Fisher's exact test). These data suggest that males age ≥50 have lower semen volumes and lower HOST scores than younger males. The former is not likely to be clinically important. However, we have found that a low HOST score may be the best predictor of subfertile sperm.

ANDROGENS/HORMONES

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THE RELATIVE INFLUENCE OF AGE, LIFESTYLE, AND HEALTH STATUS ON THE DECLINE IN TOTAL TESTOSTERONE IN NORMALLY AGING MEN: LONGITUDINAL RESULTS FROM THE MASSACHUSETTS MALE AGING STUDY (MMAS)

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Introduction: Concentrations of serum total testosterone (TT) in men have been found to decline with age in cross-sectional studies. The purpose of this analysis was to use longitudinal data to characterize TT trends over a 15-year period, and to examine the relative contribution of personal and behavioral characteristics to these trends.

Methods: Analyses were based on data from the Massachusetts Male Aging Study (MMAS), a longitudinal study of men randomly sampled from the Boston metropolitan area. TT values were measured at three time points: a baseline interview in 1987-89 (when men were aged 40-79 years) and follow-up interviews 9 years and 15 years later. Latent trajectory models (LTMs) were fitted to the longitudinal data to characterize the initial (baseline) TT levels and slopes over time. The effects of age, lifestyle factors, and health problems on both levels and slopes were tested.

Results: A total of 634 men had TT values for all three interviews. A linear LTM model provided a nearly perfect fit to the repeated measures data (RMSEA=0.000; CFI=1.00). Mean TT values decreased from 525.5 to 416.8 ng/dL, a decline of 1.4% per year of follow-up. Baseline TT levels were significantly correlated with body mass index (r=-0.32), cigarette smoking (r=0.27), and age (r=-0.13). Trajectory slopes were age-invariant. The only factor affecting TT slopes was smoking status, with slopes declining more rapidly for smokers than for non-smokers.

Conclusions: The results suggest that TT concentrations decline at a linear rate and that the trends do not vary by age. While several lifestyle factors are associated with TT levels at any point in time, only smoking status was found to affect the rate of decline over time.

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ANDROGEN REPLACEMENT THERAPY IN PATIENTS PREVIOUSLY TREATED FOR PROSTATE CANCER

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Introduction: Historically, prostate cancer has prevented patients from ever being placed on androgen replacement. We review a select group of patients previously treated for prostate cancer and who were subsequently placed on testosterone and/or DHEA.

Methods: A retrospective chart review was performed of patients treated for sexual dysfunction who had received therapy for prostate cancer. Men who were relatively hypogonadal after their therapy and then chose androgen replacement were reviewed.

Results: Seven patients seen for sexual dysfunction who had been treated for localized prostate cancer were treated with androgens including testosterone (T) and/or DHEA. Age of the 7 patients ranged from 53 - 68. The mean length of time on hormones was 8.3 months. The mean pre and post T levels were 337 and 528 ng/dL respectively. Finally, the mean time since initial prostate treatment was 56 months. Of the 6 patients after radical prostatectomy placed on hormones, 1 patient terminated his therapy of DHEA after 1 month when his PSA rose from <0.3 to 0.4. There was no rise in the PSA of the 5 other radical prostatectomy patients after T and DHEA. The 1 patient treated with radioactive seeds started testosterone and DHEA 42 months after treatment with a stable PSA of 0.1. After 2 months his PSA has risen to 0.32 and he is still currently on hormones.

Conclusions: It is possible to give men treated for prostate cancer hormone therapy. However, it is important to carefully scrutinize which patients are treated. Guidelines need to be established to assist the urologist in making this decision. These guidelines should take into account length of time since treatment of prostate cancer, treatment modality used, and PSA.

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LARGE DOSE AND LONG TIME DEPOTMEDROXY PROGESTERONE ACETATE(DMPA) AND TESTOSTERONE UNDECNOATE(TU) INDUCED APOPTOSIS OF GERM CELLS IN RAT TESTIS

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The objectives of the present study were to document the effect of DMPA with or without TU on male rats reproductive function and to study the apoptosis of testicular cells. Groups of adult male rats(n=5, each group) were given DMPA(75mg/Kg) with or without TU(25mg/Kg) for 12 weeks. At the end of this study each male rat was paired with one adult female SD rat to estimate male fertility. After treatment with DMPA(Group P) or TU(Group T) or DMPA+TU(Group PT), compared with control animal(Group C), the ratios of testes/body and epididymis/body weights were all found decreased ($P<0.01$) while the ratios of prostate/body weights were similar. Serum testosterone decreased in the P group, but not changed in T or PT group, compared with group C. Epididymal sperm counts(681.83±87.44, 407.33±63.58, 310.83±69.81, 169.33±61.31, Group C, P, T, PT, respectively $\times 10^6$/ml), sperm motility (79.40±2.01%, 40.01±5.79%, 37.68±6.75%, 26.05±3.34%, Group C, P, T, PT, respectively) decreased markedly ($P<0.01$) as sperm morphology abnormality increased ($P<0.01$). Fertility as assessed by the number of fetuses in uterus of paired female rat was also found after P or TU treatment but most markedly reduced in the combination group (12.7±1.2, 5.7±3.3, 4.4±4.6, 0.6±0.8, Group C, P, T, PT, respectively). In comparison with control, in all treatment groups, the proportion of apoptosis spermatogenetic cells (1.96±0.40%, 9.82±1.30%, 9.52±0.74%, 26.23±3.30%, Group C, P, T, PT, respectively) increased dramatically ($P<0.01$). Our results showed that combined DMPA and TU resulted in more marked suppression of spermatogenesis than each treatment alone. Studies are in progress to examine whether DMPA has any direct effects on spermatogenesis in addition to the effects due to suppression of gonadotropins.

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PREVALENCE OF PRESCRIPTION HORMONE AND ERECTILE DYSFUNCTION MEDICATION USAGE: RESULTS FROM THE MASSACHUSETTS MALE AGING STUDY

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Introduction: Direct-to-consumer advertising of hormone replacement therapies and erectile dysfunction (ED) medications has increased dramatically in the last 10 years. We estimated the prevalence of prescription hormone and ED medication usage in a community-based sample of older men.

Methods: Data were obtained from the Massachusetts Male Aging Study, a randomly selected sample of men aged 40-70 at baseline (T_1) and observed 3 times (T_1: 1987-89; T_2: 1995-97; T_3: 2002-04). Full sample sizes were used for all time points (T_1: N=1709; T_2: N=1156; T_3: N=853). During in-home interviews, trained interviewers asked subjects whether they were taking prescription medications for particular conditions and verified the medication names against the prescription bottle. At all 3 time points, men were asked if they were taking androgens or estrogens (hereafter referred to as hormones). At T_3, men were asked if they were taking any prescription drugs for ED.

Results: At baseline, the sample was predominantly white (96%), employed (78%), and most had at least a high school education (89%). At T_1, no men were taking prescribed hormones. During 1995-97 (T_2), only 6 men (0.5%) reported taking prescribed hormones, but by 2002-04 (T_3), the number rose to 26 (3.1%), a six-fold increase. At T_3, 84 participants (9.9%) were taking a prescription medication for ED.

Conclusion: Although the prevalences of prescribed hormones at both T_2 and T_3 are very low, prescription hormone usage was 6 times more prevalent at T_3 than T_2. The disjunction between the prevalence of both hypogonadism (androgen deficiency) and erectile dysfunction and the use of medications for these conditions warrants further investigation.

EPIDIDYMIS

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THE MOUSE EPIDIDYMAL TRANSCRIPTOME: ANALYSIS OF SEGMENTAL GENE EXPRESSION IN THE EPIDIDYMIS

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As mammalian spermatozoa pass from the testis into the epididymis they possess a specialized and distinct morphology, but are infertile. Maturation of spermatozoa occurs during transit through the dynamic environment of the epididymis. The specific microenvironments created along the length of the epididymal tubule are crucial to the modifications that result in fertile spermatozoa. We hypothesize that the changing environments of the epididymal lumen are established by gene expression profiles that are regulated differentially among these segments. In the current study, ten distinct segments of the mouse epididymis were identified by microdissection. RNA isolated from each of the ten segments was subjected to microarray analysis using the Affymetrix MOE430 chipset. Analysis of the microarrays from each of the segments demonstrated that over 17,000 genes are expressed in the mouse epididymis. This study identifies 1969 genes that are up- or down-regulated by greater than 4-fold between at least two different segments. The expression patterns of these genes identify distinct patterns of segmentally regulated gene expression. Using principle component analysis, we determined the 10 segments form six different "functional units". These analyses elucidate the changes in gene expression along the length of the epididymis for 17,000 transcripts for which the expression data will be placed in a public database. This resource for the research community will aid in the understanding of male infertility, identification of novel contraceptive targets, and elucidation of biological factors that mediate sperm maturation in the epididymis. Supported by DK45179 (TTT).

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PROTEINS IN THE ACCESSORY SEX GLAND AND CAUDA EPIDIDYMIS FLUID AS RELATED TO DAIRY BULL FERTILITY

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We evaluated the relationships between accessory sex gland (AGF) and cauda epididymis (CEF) fluid proteins and bull fertility. Fertility was normalized as the percentage point deviation of their non-return rates (PD) from the average fertility of all bulls at a given artificial insemination center. Services for each sire ranged from 269 to 77,321 and PD values, from + 7.7 % to – 18.1 %. CEF and AGF, from 26 and 37 bulls, respectively, were obtained from vasa deferentia cannulae and artificial vagina. Samples were evaluated by 2D SDS-PAGE and gel images, by PDQuest software. Total integrated optical density of protein spots was used as independent variables in linear regression analysis. An average of 113 spots was detected in the CEF and 47 spots in the AGF polypeptide maps. No spot was unique to either high (PD ³ 0) or low fertility (PD < 0) bulls. However, PD values related to protein quantities in the CEF gels (R^2 = 0.66, with 9 spots), AGF gels (R^2 = 0.44, with 4 spots) and when spots from both 2D maps (n = 21) were included in the same regression analysis (R^2 = 0.83, with 3 AGF and 4 CEF spots; R^2 = 0.87, with 3 AGF and 5 CEF spots). Considering only bulls with at least 1,000 services, there was an increase in R^2 when CEF (n = 18; R^2 = 0.73, with 4 spots) and AGF maps (n = 23; R^2 = 0.61, with 6 spots) were analyzed separately, but not when spots from both data sets (n = 12) were evaluated together (R^2 = 0.74 to 0.83). Osteopontin, a cell adhesion component that may also stimulate embryo growth, and BSP-30 kDa, a sperm capacitation inducer, were identified by Western blots as some of the proteins related to fertility. Thus, interactions among proteins from the accessory gland and cauda epididymal fluid explain a significant proportion of the variation in bull reproductive performance. Supported in part by USDA grant 2003-34437-13460 and a Fellowship* from the Brazilian Research Council (CAPES).

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EXPRESSION OF EPITHELIAL CADHERIN IN MOUSE EPIDIDYMIS, SPERM LOCALIZATION, AND ASSESSMENT OF ITS PARTICIPATION IN FERTILIZATION

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During epididymal transit, sperm acquire the ability to fertilize the oocyte; the epididymis provides with proteins that confer such function. Epithelial cadherin (Ecad) is an adhesion molecule described to be expressed in the human and rat epididymis. Studies from our group have shown presence of Ecad in human sperm and oocytes, and suggested its participation in gamete interaction (*Vazquez-Levin et al, ASA 2003*).

The aim of this study was to evaluate: 1) the expression of Ecad in the mouse epididymis, 2) its presence and localization in epididymal sperm, and 3) its participation in fertilization. Studies were done with a specific polyclonal antibody towards Ecad (Santa Cruz Biotech., Santa Cruz, USA).

Expression of Ecad in the epithelium of *caput*, *corpus* and *cauda* epididymis was confirmed by immunohistochemistry; Western immunoblotting of epididymal protein extracts revealed a 127 KDa form, also found in sperm extracts from *cauda* epididymis. RT-PCR analysis confirmed the presence of epididymal Ecad transcripts. Immunocytochemistry of epididymal sperm showed a strong signal in the acrosomal and postacrosomal region in a variable percentage of cells (*caput*=25-78%, *corpus*=50-84%, *cauda*=16-79%; n=3); immuno-electron microscopy analysis confirmed Ecad localization in the plasma membrane of the acrosomal region. In addition, Ecad localized to the sperm tail in all cells evaluated. Sperm preincubation with anti Ecad (20 mg/ml) resulted in the inhibition of *in vitro* fertilization (anti Ecad: 49±8 % fertilized oocytes relative to control; n=5).

The studies describe the expression of Ecad in mouse epididymis and its presence and localization in the sperm head and tail; in addition, they provide preliminary evidence suggesting its involvement in mouse fertilization.

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MACROPHAGE MIGRATION INHIBITORY FACTOR IN THE HUMAN EPIDIDYMIS AND SEMEN AND ITS POTENTIAL ROLE IN SPERM MOTILITY

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During the epididymal transit, the mammalian spermatozoa acquire new proteins involved in the acquisition of the male gamete fertilizing ability. We have previously shown that membranous vesicles called epididymosomes are involved in the transfer of epididymal originating proteins to spermatozoa. The cytokine Macrophage migration Inhibitory Factor (MIF) has been identified as one of these proteins. In this study we searched for the presence of MIF along the epididymis and in different compartment of semen in human. Northern and Western blots analysis as well as immunohistochemical studies show that MIF is expressed all along the epididymis with a higher level of transcript in the proximal segment. MIF is associated with epididymosomes as well as to prostasomes, the latter being membranous vesicles present in the semen. In semen, MIF is associated to spermatozoa, prostasomes as well as to the soluble fraction. The amount of MIF in the seminal fluid varies from one individual to the other but does not correlated with the amount of MIF associated to ejaculated spermatozoa. There is a negative correlation between the amount of sperm associated MIF and the percentage of motility in different semen samples. Determination of MIF quantities associated to spermatozoa separated on a discontinuous Percoll gradient shows that the amount of MIF associated to spermatozoa with normal morphology is much higher in poorly motile spermatozoa when compared to high density spermatozoa characterized by vigorous motility. These results are discussed with regards to the possible involvement of MIF in sperm motility acquisition during the epididymal transit. Supported by CIHR and NSERC grants to RS.

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ANTIMICROBIAL PROPERTIES OF THE HUMAN AND MACAQUE EPIDIDYMIS PROTEIN 2 (EP2) ISOFORMS

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The role of epididymal sperm binding proteins is not well understood. Besides their role in sperm maturation, recent evidence suggests that they may play a role in epididymal innate immunity. We demonstrate that human and macaque epididymal recombinant proteins of the EP2 (referred to as HE2 in humans) family exhibit potent antibacterial activity against *E. coli* in a dose and time dependent manner. Using colony forming unit (CFU) assay we observed that incubation of *E. coli* with human EP2C for 2 h resulted in 3 logs of bacterial killing, where as the macaque EP2C showed more than 4 logs of bacterial killing. Similarly, the macaque EP2K and EP2 L isoforms caused 3 logs of bacterial killing when incubated for 2 h. However, the C-terminal peptides of both human and macaque EP2C isoforms did not exhibit any bacterial killing. The antimicrobial activities of the human and macaque EP2 isoforms was slightly affected by NaCl concentrations of up to 300 mM. Their mechanism of action appears to be membrane dependent as evidenced by their ability to permeabilize the outer and inner *E. coli* membranes. Scanning electron microscopic studies show that these proteins at a concentration of 50 µg/ml induce significant structural changes in *E. coli* membrane morphology when incubated for 1 h. EP2 protein isoforms failed to damage erythrocyte membranes suggesting their specificity to bacterial membranes.

UROLOGY

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PEROXIDE IONS PLAY A KEY ROLE AS NEW SIGNALING MECHANISM OF TUMOR DEVELOPMENT IN AGING HUMAN PROSTATE

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Introduction and Objective: Free radicals play a significant yet paradoxical role in normal health and in many diseases. How oxidative stress and certain antioxidants affect prostate cells in aging and tumor development is unknown. The present study evaluates proliferation of various prostate cells exposed to hydrogen peroxide as a source of reactive oxygen species (ROS).
Methods: We compared growth of established prostate stromal (PS30), epithelial (benign BPH-1 & normal RWPE-1), and cancer (LNCaP, PC3, DU145 and C4-2B) cells under oxidative stress by hydrogen peroxide (0.03 nM to 3.0 mM). Cell growth kinetics and apoptotic signaling mechanisms were monitored by caspase activation.
Results: We observed a dose-dependent differential cell growth and inhibition pattern in response to low and high doses of peroxide treatment. PS30 (48%), BPH-1 (44%), and PC3 cells (50%) showed significant proliferative response over control at very low H_2O_2 concentrations. Other prostate cells (RWPE, LNCaP, DU145 and C4-2B) did not demonstrate such proliferative response. Antioxidant treatment (vitamin E plus selenium) inhibited this proliferative response. High H_2O_2 (>30µM) showed growth inhibition in all cells. Apoptotic response (caspase 3 and 9 activation) also showed differential pattern especially in the presence of antioxidants. This was supported by DNA fragmentation data.
Conclusions: We conclude: 1) this differential growth and inhibition response pattern to peroxide ions is due to the existence of differential signaling pathways in normal and diseased cells; 2) low oxidative stress stimulates growth of benign tumor; and 3) an individualized interventional approach by antioxidants toward disease prevention may be necessary.

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THE EFFECT OF SHORT-TERM MEDICAL CASTRATION ON HORMONES, PSA AND PROSTATE SIZE IN NORMAL, MIDDLE-AGED MEN

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Introduction: Most male hormonal contraceptive strategies rely on gonadotropin deprivation supplemented by exogenous testosterone (T) administration to maintain serum T levels in the normal range. Moreover, there has been increasing interest in treating older men with low T levels with T replacement. The effects of exogenous T administration on normal prostate tissue growth, differentiation, and prostate cancer risk are unknown. We have developed a novel *in vivo* model to examine the effects of **T** deprivation on the prostate in healthy, middle aged men.
Methods: 12 healthy, male volunteers ages 35-55 were treated with either placebo, a novel, long-acting GnRH antagonist, acyline (which results in castrate T levels within 24 hours) every 2 weeks, or acyline plus T gel (10 mg/day) for 28 days. Weekly serum hormone and PSA levels, prostate volume by transrectal ultrasound at baseline and on day 28, and prostate biopsies on day 28 were collected.
Results: 9/12 subjects have completed the prostate biopsy. Study procedures were well tolerated. At Day 28 (mean ± SEM):

	Serum T(nmol/L)	PSA(ng/ml)	Prostate Vol.(cm^3)
Placebo (2)	13.9±5.2	0.73±0.1	24±1.0
Acy alone (3)	0.6±0.2	0.29±0.1*	16±0.1
Acy + T (4)	19.3±5.7	0.77±0.1	25±2.5

Gonadotropins were significantly suppressed in both groups receiving acyline compared to placebo. PSA and prostate volume were decreased in the acyline-alone group compared to placebo and acyline + T.
Conclusion: The GnRH antagonist acyline rapidly suppresses serum T, gonadotropins and PSA in normal, middle aged men. This model will allow us to examine effects circulating androgens on intraprostatic cell turnover, gene expression and hormone concentration.

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DIFFERENTIAL EXPRESSION OF THE NOTCH SIGNALING SYSTEM IN TESTICULAR GERM CELL TUMORS

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The expression of Notch 1 and its ligand Jagged 2, critical factors in cell type specification, in testicular germ cell tumors was examined in order to evaluate its possible relationship with their pathogenesis. Northern blot analysis and immunohistochemical staining for Notch 1 and Jagged 2 were done in 139 samples of testicular germ cell tumors. Notch 1 and Jagged 2 transcripts were expressed in non-cancerous testicular tissues and Notch 1 and Jagged 2 proteins were positive in the spermatids. However, the expressions of transcript and protein were negative for both Jagged 2 and Notch 1 in seminomas, they were negative for Jagged 2 but were positive for Notch 1 in embryonal carcinomas and choriocarcinomas, and they were positive for Jagged 2 and Notch 1 in teratomas. Our results offer, for the first time, the possibility that the activity of the Notch signaling system, one of the cell proliferation and differentiation pathways, correlates with the different histological subtypes of germ cell tumor, which may be responsible for characteristics of cancer cells such as responses to chemotherapy and/or irradiation.

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IMPACT OF NEUROVASCULAR PRESERVATION ON FEMALE SEXUAL DYSFUNCTION FOLLOWING ORTHOPTIC RADICAL CYSTECTOMY

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Previous reports in the literature regarding female orthotopic cystectomy have focused primarily on urethral recurrence and urinary incontinence/continence. Recently, in the new era of female sexuality, the issue of evaluating postoperative sexual outcomes has become a new surgical endpoint.

This retrospective study compared a subgroup of female patients that underwent neurovascular preservation with orthotopic diversion (n =6) versus a contemporary series of non nerve-sparing female orthotopic diversion (n=7). All 13 patients were sexually active, and baseline Female Sexual Function Index (FSFI) scores where obtained. All patients where evaluated 6, and 12 months following cystectomy, with a mean follow-up of 14 months. No patient had any adjuvant chemo or radiotherapy and all patients had no evidence of disease at time of evaluation. Post-op evaluations included were a self-administered validated FSFI questionnaire. This retrospective study compared a subgroup of female patients that underwent neurovascular preservation with orthotopic diversion (n =6) versus a contemporary series of non nerve-sparing female orthotopic diversion (n=7). All 13 patients were sexually active, and baseline Female Sexual Function Index (FSFI) where obtained. All patients where evaluated 6, and 12 months following cystectomy, with a mean follow-up of 14 months. No patient had any adjuvant chemo or radiotherapy and all patients had no evidence of disease at time of evaluation. Post-op evaluations included were a self-administered FSFI questionnaire. Neurovascular preservation in the orthotopic female cystectomy significantly improves sexual function in the postoperative period. This improvement is seen in all six domains of the Female Sexual Function Index.

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ASSESSMENT OF RETURN OF PARTIAL AND COMPLETE ERECTILE FUNCTION FOLLOWING NIGHTLY ADMINISTRATION OF SILDENAFIL CITRATE AFTER BILATERAL NERVE-SPARING RADICAL PROSTATECTOMY

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Introduction: We previously examined the effect of nightly sildenafil administration on the return of normal erectile function following bilateral nerve-sparing retropubic radical prostatectomy (BNSRRP) in a double-blind, placebo-controlled, prospective study. Here we examine the return of any degree of erectile function in this study population.

Methods: Patients had normal preoperative erectile function, defined as a combined score of ≥8 for International Index of Erectile Function (IIEF) question 3 (Q3, ability to achieve) and Q4 (ability to maintain) and normal nocturnal penile tumescence (NPT; ≥10 continuous minutes of ≥55% base rigidity). Four weeks post-BNSRRP surgery, 76 patients were randomized to 36 weeks of double-blind treatment with sildenafil (50 mg, n=23; 100 mg, n=28) or placebo (n=25) taken nightly. Erectile function was assessed 8 weeks after discontinuation of drug treatment with the question "Over the past 4 weeks, have your erections been good enough for satisfactory sexual activity?" and by IIEF and NPT. Responders were defined as those having a combined score ≥8 for IIEF Q3 + Q4 and a "yes" response to the question. A subgroup analysis of patients reporting some erectile function (IIEF Q3 + Q4 ≥4) was conducted.

Results: 48 weeks after BNSRRP, 14/51 (27%) patients receiving sildenafil and 1/25 (4%) patients receiving placebo were responders (P=0.0156). Q3 + Q4 scores ≥4, indicating some erectile function, were achieved by 18/51 (34%) sildenafil- and 6/25 (24%) placebo-treated patients. Of these, 14/18 patients receiving sildenafil but only 2/6 patients receiving placebo had combined scores ≥8.

Conclusion: At 48 weeks post-BNSRRP, a higher percentage of erectile function return, the majority in the normal range, was achieved with sildenafil.

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CONTINENCE RATE FOLLOWING NERVE SPARING (NS) RADICAL PROSTATECTOMY (RP): 5 Yr. FOLLOW-UP

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The reported true and stress incontinence rates following RP vary considerably depending on age and institution. Baseline and follow-up data from 152 patients (mean age 64.06 ± 6.72) who underwent RP (1994-1998) were obtained. The mean F/U was 7.8 ± 1.3 yr.; and median PSA was 8.7. Outcome analysis included: pre-operative PSA levels, nerve-sparing status (bilat -NS, unilat-NS, non-NS), age (<65 yr. or >65 yr.) and the current QOL (on the Linkert scale of 0-6). Overall, the true and stress only incontinence (IC) rates were 17.7% (27/152) and 11.4% (17/152), respectively. When stratified by nerve-sparing status, the true and stress only IC rates for BNS was 9%, 3.1%; UNS 12%, 12% and NNS 29.5%, 20%. True and stress IC rates were significantly higher in NNS group (18/61) compared to BNS group (6/66) (P<0.05). No difference in the true and stress IC rates between the other two groups (BNS vs. UNS; UNS vs. NNS) was seen (P>0.05). In regaining urinary control (defined as pad free), continence was achieved in 35/152 (23.1%) at the end of 1 month, 77/152 (50.1%) at 3 months, 106/152 (69.8%) at 6 mo. and 115/ 142 (75.7%) at 12 months. The association between the age and true IC was significant (P <0.05): <65-yr. (7/75) vs. > 65 yr. (20/77). The association between pre-operative PSA levels and true IC was nearly significant P= 0.053, (incontinence group - PSA 8.75, continence group- PSA 5.9). Regarding the QOL, 84.8% (129/152) patients were "mostly satisfied", 11.2% (17/152) had "mixed satisfaction" and only 4% (6/152) were "mostly unsatisfied". NS-RP significantly improves the long term true and stress incontinence rates after RP. Younger patients (< 65) with lower PSA levels are more likely to regain total continence after RP.

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WORLDWIDE POTENCY RESULTS FOLLOWING RADICAL PROSTATECTOMY

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Potency is a major quality of life issues after radical prostatectomy (RP). The definition of potency is hardly universal. Lack of suitable criterion to define the variables in the reporting of potency rates is a universal problem and may explain the discrepancies in the literature. Comprehensive literature review was performed to evaluate these discrepancies and to suggest modifications. We conducted a Medline Search, reviewing all published reports since 1993. Any reports RP including patients who received neoadjuvant/ adjuvant therapies were excluded. Results were summarized with the following variables: author/ institution, year of report, mean follow-up period, type of prostatectomy, nerve-sparing status, number of subjects, and reported potency rates. This review summarized total 40 publications and most of them are from single institutions. Potency rates following radical retropubic and perineal prostatectomy ranged from 11-86%. This literature review suggests that the best potency reports include: 1) a single surgeon's series, 2) a mean interval of 18 months or more, 3) greater that 200 subjects and 4) a higher percentage of bilateral nerve-sparing procedures. Thus, future reporting standards should include a universal definition of potency, documentation of the nerve-sparing status, a minimum number subjects, and a standard time interval of assessment.

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INFORMATION ABOUT LEYDIG-CELL FUNCTION IN YOUNG MEN WITH UNILATERAL VARICOCELE CANNOT BE PROVIDED BY THE GNRH-TEST

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Introduction: The GnRH-Test is thought to uncover a testicular malfunction by exaggerated response and to reveal patients who benefit from varicocele repair. In this prospective study the results of GnRH-Tests and hormone values of 102 young men with unilateral varicocele were analysed and compared to the hormone concentration within the spermatic vein.
Material: The assessment of the testicular volume was perfomed by ultrasound, reflux was proven by Doppler-ultrasound. After determination of the baseline values of LH, FSH, Testosterone (T), SHBG and Estradiol (E) 100 µg of LH-RH were administrated intravenously. The analysis of the test results was carried out analogue to Kass EJ et al., Urology 42:179-181, 1993. During antegrade sclerotherapy a blood sample was collected from a dilated vein of the pampiniform plexus and analysed for T and E concentration.
Results: 50 of 102 patients had an exaggerated GnRH-Test response, whereas only 6/50 patients showed an elevated LH response. No significant differences could be found concerning the scrotal T (p = 0,908) or E (p = 0,737) concentration within the groups with or without exaggerated GnRH-Test. No correlations could be shown between the scrotal T or E concentration and the peripheral values of LH, T or E in either group. In contrast to the above findings, the testicular volume of both testis was significantly lower in the group with exaggerated GnRH response (p < 0,01).
Conclusion: The GnRH-Test does not provide any information about the Leydig cell function in terms of T and E production in young men with unilateral medium or large varicocele, whereas the testicular volume of both testis is found to be significantly decreased in patients with exaggerated GnRH-Test.