Journal of Andrology

16th Annual Meeting
Program and Abstracts

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Journal of ANDROLOGY

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Instructions to Authors
The Journal of Andrology, the official journal of the American Society of Andrology, publishes original papers and reviews on clinical and laboratory research pertinent to the structure and function of the male reproductive system and gametes. Membership in the Society is not required for submission of a manuscript. Consult the January/February issue for complete information on style, format, page charges, and requirements for submission.

Announcements
The Journal will publish, free of charge, announcements of meetings, postgraduate courses, symposia, and other events of interest to andrologists. Announcements should be submitted to the Editorial Office.

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An Introduction to Montreal, Quebec, Canada

Montreal is a cosmopolitan city with a unique French and English heritage. It is an island in the Saint Lawrence River. The City is named for the “Royal Mountain” that is at its heart. Montreal is a fun and safe city to walk in. It’s an unusual but impressive fact that a 5-minute walk south from the meeting headquarters, the Holiday Inn Crowne Plaza, will find you right in the middle of the city’s retailing and business area, whereas a 5-minute walk north brings you up to the fringes of Mount Royal’s parkland. The Holiday Inn Crowne Plaza is one block east of the McGill University campus.

The way most people enjoy Montreal is simply strolling from one fascinating area to another—Sherbrooke Street and its exclusive shops, galleries, and museum; Old Montreal and its historic lanes; and St. Catherine Street, the bustling “main street” of the city. A walk along Boulevard St. Laurent is like taking a trip to Europe—Greek, Portuguese, German, and Italian foods and merchandise are found aplenty. Mount Royal’s parkland, during the warmer seasons, is a perfect setting for strolling, jogging, looking over the city, or just sunning. Montreal has literally thousands of shops and restaurants and many movie and stage theatres. A large proportion of these are connected via an extensive “underground city” to the subway, rail station, and hotels.

Tourist and general information will be available in the registration area. Some special events are anticipated for April, 1991, during the week of the ASA meeting. The Montreal Symphony Orchestra and Montreal Opera Company will be having performances that week. The new Museum of Architecture as well as the renovated and expanded Museum of Fine Arts of Montreal and the McCord Museum of McGill University—all three within walking distance of the Crowne Plaza—will be open. There will be an exhibit of Bonsai trees in the Botanical Gardens (a short Metro ride east).

Something for everyone—enjoy your stay!

Bernard Robaire, Ph.D.
Local Organizing Chairman
American Society of Andrology
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**NOTE:** Executive Committee Meetings:
5:30-10:30 PM Friday, April 26, 1990
6:30-8:30 PM Sunday, April 28, 1990

* Exhibitors will set up booths on Friday, April 26th from 4:00 to 7:00 PM.
† Joint Meeting of the Editorial Board and Publication Committee luncheon; Andrology Laboratory Committee luncheon.
‡ To attend PM portion of Postgraduate Course-II, please register. There is no charge for meeting registrants other than for the syllabus. For registration, contact Dr. Ronald Lewis, Mayo Clinic.
1991 PRESIDENTIAL MESSAGE

The 1991 Annual Meeting of the American Society of Andrology, our 16th Annual Meeting, will have several exciting innovations. This will be our first meeting in Canada, and the picturesque city of Montreal is an excellent location. The 2.5-day Testis Workshop immediately precedes our meeting, and the last half day of that gathering (April 27, 1991) will meet jointly with the morning session of our Postgraduate Course I. The latter finishes with a 4-hour afternoon session. Then the Annual ASA Meeting follows, packed with three state-of-the-art lectures, three symposia, and two poster sessions, plus short oral presentations of new science. As another innovation, we have added a second Postgraduate Course (II). The 10:30 AM-12:30 PM final symposium of our annual meeting (April 30, 1991) begins this excellent program on prostatic disease. There is no charge for registrants to the Annual Meeting (other than for the syllabus), and after lunch the sessions continue until early evening. A separate registration is available for those individuals who wish to attend only the Postgraduate Course II on Prostatic Disease.

The Journal of Andrology is planning to produce an issue that will contain manuscripts prepared by each speaker at the Postgraduate Course II on Prostatic Disease. For the first time, we plan to process all mailed-in preregistrations in our Business Office in Champaign, Illinois.

On behalf of the American Society of Andrology, welcome to Montreal and welcome to our 16th Annual Meeting. This should be a wonderful and chock-full time for all.

Howard R. Nankin, M.D., President
American Society of Andrology

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International Liaison P. Troen, M.D.
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Local Arrangements (1992) A. Glass, M.D. and J. Fourcroy, M.D., Ph.D.
Student Affairs G. Killian, Ph.D.
Program Chairman (1991) R. Santen, M.D.
Editor, Journal of Andrology C. Desjardins, Ph.D.
Long-Term Planning R. Ansbacher, M.D., M.S.
Allied Health Professionals J. Fourcroy, M.D., Ph.D.
Andrology Laboratories W. Hemmee, M.D.
MESSAGE FROM THE PROGRAM CHAIRMAN

Welcome to the 16th Annual Meeting of the American Society of Andrology. The program this year has been designed to complement the Testis Workshop Meeting (April 24-27, 1991), which is being held in conjunction with the American Society of Andrology annual meeting for the first time in a decade. Dr. Stephen Winters has organized an outstanding Postgraduate Course on April 27 entitled “An Update on Fenilization in the Laboratory and in the Clinic.” This has been planned as a jointly sponsored function with the Testis Workshop. The American Society of Andrology meeting will bring together superb speakers of international reputation to review concepts and present new data in three symposia, two State-of-the-Art lectures, and the Serono Lecture. The topics are broad-ranging and include neuroendocrinology of the GnRH pulse generator, molecular biologic studies of the leutropin choriogonadotropin receptor, advances in hormone measurements, physiologic transport of steroids into tissue, use of 5α-reductase inhibitors and anti-androgens in prostatic disease, and clinical physiology of inhibin. This year’s program, as in previous years, provides for a wide range of data to be presented in oral and poster session formats. As a new component of the meeting, a second postgraduate course will cover major new advances in the medical treatment of benign prostatic hyperplasia. The latter session will overlap with the last morning of the American Society of Andrology meeting and then continue into the afternoon of April 30. With these various, highly coordinated activities, this year’s American Society of Andrology meeting should provide a rewarding scientific experience.

Richard J. Santen, M.D.
Program Chairman
American Society of Andrology

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420 Sherbrooke Street West
Montreal, Quebec, Canada H3A 1B4
Phone: (514) 842-6111
FAX: (514) 842-9381

ON-SITE REGISTRATION
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Friday, April 26, 5:00 AM-8:00 PM
Saturday, April 27, 7:30 AM-1:00 PM and 5:00 PM-9:00 PM
Sunday, April 28, 7:30 AM-2:00 PM
Monday, April 29, 7:30 AM-12:30 PM

MAIL-IN REGISTRATION
Robert A. Schmidt
Business Manager
309 W. Clark St.
Champaign, IL 60820

PRESS RELATIONS AND NEWS COVERAGE
Dr. D. Cyr is in charge of encouraging news coverage of the meeting and assisting participants and the news media in bringing current research before the public. A press room will be provided in the Hotel. We hope that participants, if requested, will be willing to discuss their work with the media.

REGISTRATION FEES

Postgraduate Course-1: 4/27/91
- Member or non-member: $110
- Student: 30
- Half-Day: 55
- CME credit: 20

- Member: 85
- Non-member: 105
- Student member: 25
- Student non-member: 35
- Spouse/family registration: 15
- CME credit: 20
- Banquet: 38

Late fee (after March 15, 1991): 15

SLIDE PREVIEW
A slide preview room will be available.

SMOKING REGULATIONS
In accordance with municipal regulations, smoking is not permitted in the meeting rooms.

SUSTAINING MEMBERS OF THE SOCIETY
- Serono Laboratories, Inc.
- Texas Institute for Reproductive Medicine and Endocrinology

CORPORATE MEMBERS OF THE SOCIETY
- Berlex Laboratories, Inc.
- Buckeye Urological and Andrology, Inc.
- Western Michigan Reproductive Institute
- National Medical Enterprises, Inc.

SUPPORTERS OF ANNUAL MEETING
- McGill Center for Study of Reproduction, McGill University
- Department of Pharmacology and Therapeutics, McGill University
- Berlex Laboratories, Inc.
FUTURE MEETINGS

1992 Meeting—Washington, D.C., March 27–30
Contact Dr. Allan R. Glass
Walter Reed Army Medical Center
Washington, DC, 20307
Phone: (202) 576-1770

1993 Meeting—Tampa, Florida, April 16–18
Contact Dr. Don F. Cameron
Department of Anatomy
University of South Florida
12901 Bruce B. Downs Blvd.
Tampa, FL 33612
Phone: (813) 974-2844

ENTERING CANADA

If you are a U.S. citizen, you will simply need identification (e.g., passport). If you are a U.S. immigrant, you will need your green card to enter Canada and return to the United States. If you are a citizen of any other country, please check with the appropriate authorities for any visa requirements to enter Canada and to return to your country of residence.

CURRENCY

The Canadian dollar has been trading between $0.83–0.87 U.S. for the past 2 years. You will have to verify its value at the time of the meeting. Although U.S. dollars are accepted in almost all stores, restaurants, and hotels, the best rate of exchange is usually available only in banks. There are many banks (open from 10 AM to 4 PM) within a 5–10-minute walk of the meeting site.

TRANSPORTATION INTO MONTREAL

The Holiday Inn Crowne Plaza is in downtown Montreal, 16 km, or 10 miles, from Dorval Airport (North American flights), 40 miles from Mirabel Airport (Latin America and all overseas flights), and 0.5 mile from the Central train station.

By Air. The best and most economical way to the Hotel from Dorval Airport is to take the Murray Hill Airport bus (leaves every 20 minutes) from the airport to downtown Montreal, and to disembark at the Queen Elizabeth Hotel (about $7 Canadian). From Mirabel Airport take the Micracar bus (leaves about every hour) for Central Station (next door to the Queen Elizabeth Hotel in downtown Montreal, about $12 Canadian). From there it is best to take a taxi for the short (3–5-minute) drive to the Hotel. The taxi fare ought to cost no more than $5. Note that a taxi ride from Dorval Airport could cost as much as $25, and one from Mirabel could amount to $50 (Canadian).

By Train. Montreal is served by trains from Washington and New York as well as from across Canada. The train stations (Central and Windsor) are just below Dorchester Street, between University Street and Drummond Street. A short taxi ride directly from the station ought to cost no more than $5, including baggage.

By Bus. Buses arrive at the Bus Terminus at 505 de Maisonneuve East (corner of Berri de Montigny). Take a taxi directly (about $8) or take the Metro (direction Angrignon) to McGill Station. You can either walk (about 3 blocks) or take a taxi (about $4).

By Car. First, a note about Quebec driving regulations. Wearing seat belts is mandatory. All road signs are in French only. It is not legal to make a right turn on a red light. A flashing green light indicates that a left turn is protected against oncoming traffic. A left arrow means that a left turn is permissible but not necessarily protected.

From the South. Quebec 15 Nord (North) to “Pont Champlain-Montreal“ (exit 53). Right lane on Pont Champlain (bridge). Immediately after the bridge bear right to “Autoroute Bonaventure-Centre Ville”. Stay in left lanes and follow signs to Centre Ville–University. The autoroute becomes University Street. Continue up University Street through downtown Montreal to Sherbrooke Street, make a right, and the hotel is two blocks further on the right side.

From the West. Quebec 20 Est (east). Follow signs to “Montreal-Centre Ville”. Do not bear right toward “Montreal Ouest.” Approaching the city, follow “Autoroute Ville Marie-Tunnel Ville Marie.” Move to the right lane and exit at “Rue University” (exit 72). Keep left and follow signs to “Rue University.” Turn right at the first stop sign and then left at the third signal (Mansfield). Continue to the top of Mansfield and turn right onto Sherbrooke. Go east about five blocks to the hotel.

From the East. Quebec 20 Ouest (west). At exit 90 follow signs to Jacques Cartier Pont (not Tunnel Louis H. Lafontaine). Take exit 8 to Jacques Cartier Pont (bridge). Cross the bridge and follow signs to Sherbrooke Street. Turn left on Sherbrooke Street and proceed directly to the Hotel.

Via Quebec 10. Continue across Pont Champlain and proceed as directed in From the South.

MONTREAL WEATHER

The end of April in Montreal is usually the beginning of spring (mean temperature 14°C or 57°F), but the weather can be highly variable. A warm spring coat is usually worn at this time.
DISTINGUISHED ANDROLOGIST AWARD

Dr. Philip Troen is the recipient of the 1991 Distinguished Andrologist Award. Dr. Troen embodies all the characteristics of a Distinguished Andrologist: scholarship, service, and significant contributions to andrology. His affinity for andrology became evident when he studied the testis with Alexander Albert at the Mayo Clinic after receiving his M.D. degree from Harvard Medical School. Upon his return to Harvard Medical School, he was considered a specialist in male reproduction. Since 1964, he has been a Professor of Medicine at the University of Pittsburgh and Physician-in-Chief at Montefiore Hospital. Dr. Troen is a gifted scientist, as illustrated by his pioneering contributions to the understanding of testicular steroidogenesis and the pulsatile secretion of gonadotropins in the human male. His research interests also encompass the clinical and basic science aspects of infertility, aging, inhibin, and androgen binding protein. More importantly, Dr. Troen is a member of a select group who have nurtured the clinical/scientific specialty of andrology throughout the world. He is an internationally recognized leader in the development of andrology. For example, he has been a founding member and President of both the American Society of Andrology and International Society of Andrology. He is a renaissance individual with interests encompassing the development of new knowledge, the training of young professionals, and the progress of andrology. He is a man of integrity and ideas, a statesman, a gentleman, an unselfish leader, and a humanitarian. In summary, Dr. Troen is an andrologist's andrologist.

SERONO AWARD LECTURESHP

Dr. Tony M. Plant, Professor of Physiology at the University of Pittsburgh School of Medicine, was born and educated in England. He attended the University of London and received B.Sc. and Ph.D. degrees in Physiology in 1966 and 1971, respectively. Dr. Plant emigrated to the United States in 1972, and after two years at Emory University he moved north for postdoctoral studies with Dr. Ernst Knobil at the University of Pittsburgh School of Medicine. In 1978 he was appointed Assistant Professor of Physiology at Pittsburgh, and at this time he initiated his studies of the neuroendocrine mechanisms that govern testicular function in the rhesus monkey. These studies have led to the idea that, in primates, the hypothalamic component of the control system that governs testicular function, the "GnRH pulse generator," functions in an adult manner during infancy. In this scheme of primate development, puberty, which in the monkey and human is robustly separated from infancy, reflects the reawakening of pulsatile GnRH release. During the juvenile years, the GnRH pulse generator is held in check by an unidentified signal. Using a preparation known as the hypophysiotropic clamp, Dr. Plant and his colleagues have shown that in the postpubertal monkey, testicular regulation of LH secretion is achieved by an action of testosterone to retard the frequency of the GnRH pulse generator, whereas that of FSH is occasioned by a direct action of inhibin at the level of the gonadotroph. Dr. Plant, who was appointed Director of the Center for Research in Reproductive Physiology at the University of Pittsburgh in 1985, is a member of the Editorial Boards of Endocrinology and Biology of Reproduction, and also serves on the NIH Reproductive Endocrinology Study Section.

DISTINGUISHED ANDROLOGISTS

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1985 ... Robert H. Foote, Ph.D.  
1986 ... Alfred D. Jost, D.Sc.  
1987 ... Emil Steinberger, M.D.  
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Patricia M. Saling, Ph.D., is the recipient of the 1991 Young Andrologist Award. Dr. Saling is an Associate Professor in the Departments of Obstetrics and Gynecology and Cell Biology at Duke University Medical Center. She received her doctorate at the University of Pennsylvania and did postdoctoral studies at Cornell University Medical College and The Population Council. Dr. Saling's research in Philadelphia and New York upset earlier dogma concerning the interaction of mammalian sperm and ova. Her research demonstrated that mouse sperm required intact acrosomes and plasma membranes for binding to ova, and that the ability to bind was attained during epididymal maturation. These observations have been proven valid for human gametes. Dr. Saling's work at Duke University has incorporated monoclonal antibodies to identify sperm surface molecules that are functional in fertilization. To date, she has identified two proteins involved in mediating the zona-induced acrosome reaction and one protein required for gamete fusion. She has been an active member of the American Society of Andrology and is currently a member of the Executive Council.
SPECIAL EVENTS

Serono Lecture
Sunday, April 28
Tony Plant (University of Pittsburgh)
"Neuropeptides and GnRH Pulse Generator"

State-of-the-Art Lectures
Monday, April 29th
Deborah Segaloff (The Population Council)
"Leutropin Choriogonadotropin Receptor: An Unusual Member of the G-Protein-Coupled Receptor Family"

Tuesday, April 30th
William Bremner (University of Washington)
"Clinical Physiology of Inhibin"

Symposia and Workshops

Symposia

I—"Physiological Mechanisms of Androgen Transport into Tissues"
Sunday, April 28th
Geoffrey Hammond (University of Western Ontario)
"Molecular Biology of Androgen Transport Proteins"
William Rosner (St. Luke's/Roosevelt Hospital Center)
"Biologic Effects of Androgen Transport Proteins"
Carl Mendel (University of California—San Francisco)
"The Free Hormone Hypothesis: A Physiologically Based Mathematical Model"

II—"Recent Advances in Bio- and Immuno-hormone Functions and Analyses"
Monday, April 29
Robert Rosenfeld (University of Chicago)
"LH Radioimmunoassays and Bioassays"
Kristine Dahl (University of Washington)
"FSH Isoforms, Radioimmunoassays, Bioassays, and Their Significance"
Stephen Plymale (Madigan Army Medical Center)
"Clinical Measurement of Biologically Active Androgens"
Richard Horton (University of Southern California)
"Clinical Assessment of Androgen Production: Correlation with 3α-Androstanediol Glucuronide Measurements"

III—"Clinical Use of 5α-Reductase Inhibitors and Antiandrogens in Prostatic Disease"
Tuesday, April 30
Julianne Imperato-McGinley (Cornell Medical College)
"Hormonal and Clinical Comparisons: Observations in Patients Treated with 5α-Reductase Inhibitors and 5α-Reductase Deficient Patients"
Elizabeth Stoner (Merck Sharp & Dohme Research Laboratories)
"Use of 5α-Reductase Inhibitors as Treatment of Benign Prostatic Hyperplasia"
Nelson Stone (Mt. Sinai Medical Center)
"Use of Antiandrogens as Treatment of Benign Prostatic Hyperplasia"
Jack Geller (Mercy Hospital Medical Center)
"Overview of Enzyme Inhibitors and Antiandrogens in Prostatic Cancer"
AMERICAN SOCIETY OF ANDROLOGY
POSTGRADUATE COURSE—I
Saturday, April 27, 1991
Montreal, Quebec

AN UPDATE ON FERTILITY IN THE LABORATORY AND IN THE CLINIC
Course Director: Stephen J. Winters, M.D.

CURRENT CONCEPTS IN MAMMALIAN FERTILIZATION
8:30-9:15 AM     David Katz (University of California, Davis)
"Characteristics of Sperm Motility"
9:15-10:00 AM    Patricia Olds-Clarke (Temple University)
"The Genetics of Sperm Function in Fertilization"
10:00-10:30 AM   BREAK
10:30-11:15 AM   Bayard Storey (University of Pennsylvania)
"Sperm Capacitation and the Acrosome Reaction"
11:15-12:00 noon Diana Myles (University of Connecticut)
"Sperm Proteins that Serve as Receptors for the Zona Pellucida"
12:00-1:30 PM    LUNCH

SPERM PHYSIOLOGY IN CLINICAL MEDICINE
1:30-2:15 PM     Richard Sherins (Genetics & IVF Institute)
"Clinical Use and Misuse of Automated Semen Analyses"
2:15-3:00 PM     David Mortimer (University of Calgary)
"Current Status of Ova Penetration Assays"
3:00-3:30 PM     BREAK
3:30-4:15 PM     Don Wolf (Oregon Regional Primate Research Center)
"Correlation of Semen Parameters with IVF Results"
4:15-5:00 PM     James Overstreet (University of California, Davis)
"Functional Evaluation of Cryopreserved Sperm"

Discussion time is included in the last 10 minutes of each talk.
MORNING SESSION: JOINT MEETING
American Society of Andrology and Postgraduate Course
SYMPOSIUM: "Clinical Use of 5α-Reductase Inhibitors and Antiandrogens in Prostatic Diseases"
10:30-11:00 AM Julianne Imperato-McGinley (Cornell Medical College)
"Hormonal and Clinical Comparisons: Observations in Patients Treated with 5α-Reductase Inhibitors and 5α-Reductase-Deficient Patients"
11:00-11:30 AM Elizabeth Stoner (Merck Sharp & Dohme Research Laboratories)
"Use of 5α-Reductase Inhibitors as Treatment of Benign Prostatic Hyperplasia"
11:30-12:00 PM Nelson Stone (Mt. Sinai Medical Center)
"Use of Antiandrogens as Treatment of Benign Prostatic Hyperplasia"
12:00-12:30 PM Jack Geller (Mercy Hospital Medical Center)
"Overview of Enzyme Inhibitors and Antiandrogens in Prostatic Cancer"
12:30-1:30 PM LUNCH AND EXHIBITS

AFTERNOON SESSION: POSTGRADUATE COURSE—MANAGEMENT OPTIONS FOR BENIGN PROSTATIC HYPERPLASIA
1:30-1:40 PM Ronald W. Lewis (Mayo Clinic)
"Opening Remarks and Hyperthermia"
1:40-1:55 PM Joseph E. Oesterling (Mayo Clinic)
"Natural History and Epidemiology of BPH"
1:55-2:15 PM John D. McConnell (University of Texas Southwestern)
"Pathophysiology of Benign Prostatic Hyperplasia"
2:15-2:25 PM Joseph E. Oesterling (Mayo Clinic)
"GnRH Agonists"
2:25-2:45 PM Ursula Habenicht (Shering AG)
"Aromatase Inhibitors: Rationale of Use in Management of BPH"
2:45-3:05 PM M. Fathy El Etreby (Berlex Laboratories, Inc.)
"Aromatase Inhibitors: Atamestane—A New Aromatase Inhibitor for the Treatment of BPH"
3:05-3:25 PM Herbert Lepor (Medical College of Wisconsin)
"Alpha Antagonists"
3:25-3:55 PM Panel Discussion
3:55-4:10 PM BREAK
4:10-4:30 PM H. Logan Holtgrewe (Johns Hopkins University School of Medicine)
"Transurethral Resection of Prostate (TURP)"
4:30-4:50 PM Reginald C. Bruskewitz (University of Wisconsin Hospital and Clinics)
"Transurethral Incision of Prostate (TUIP) and Laser Treatment"
4:50-5:10 PM Joseph B. Dowd (Lahey Clinic Medical Center)
"Transurethral Balloon Dilatation of the Prostate"
5:10-5:30 PM Charles Ackman (McGill University)
"Intraurethral Prostate Stent"
5:30-6:00 PM Panel Discussion
6:00-6:30 PM Donald S. Coffey (Johns Hopkins University School of Medicine)
SUMMATION
CONTINUING MEDICAL EDUCATION CREDIT

The Uniformed Service University of the Health Sciences (USUHS) is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education for physicians.

The USUHS designates the Postgraduate Courses I and II of the American Society of Andrology for 7 credit hours each in Category I of the Physician's Recognition Award of the American Medical Association.

The USUHS designates the Annual Meeting of the American Society of Andrology for 24 credit hours in Category I of the Physician's Recognition Award of the American Medical Association.
SIXTEENTH ANNUAL MEETING

SATURDAY, April 27, 1991

Postgraduate Course—I: An Update on Fertilization in the Laboratory and in the Clinic
(Ambassadeur Room)

8:30 A.M. Current Concepts in Mammalian Fertilization
10:00-10:30 A.M. Break
12:00-1:30 P.M. Lunch
1:30 P.M. Sperm Physiology in Clinical Medicine
3:00-3:30 P.M. Break
5:00-6:00 P.M. Student Soiree
6:00-7:30 P.M. Student Colloquium

Dr. Rupert Amann
"Andrology: What Is Unobtainable from a Computerized Database"

SUNDAY, April 28, 1991

8:00 A.M. Opening Welcome (Ambassadeur Room)
Richard J. Santen, Program Chairman
Howard R. Nankin, President
Bernard Robaire, Chairman, Local Committee

8:10 A.M. Serono Lecture
Dr. Tony Plant
"Neuropeptides and GnRH Pulse Generator"

Scientific Presentations: MOLECULAR BIOLOGY AND PHYSIOLOGY
Chairpersons: Bernard Robaire and Patricia M. Saling

9:00 A.M. 1. Prior exposure to calcium reduces mouse sperm transport to the oviduct.
            P. Olds-Clarke, W. Wivell,* and R. Sego.*

9:15 A.M. 2. Developmental pattern of steady-state mRNA for placental and neural cadherin in
            the rat testis. D. G. Cyr,* B. Robaire. and O. W. Blaschuk.*

9:30 A.M. 3. An androgen-regulated proviral gene is preferentially expressed in the mouse caput

9:45 A.M. 4. Localization and regulation of testicular insulin-like growth factor-I messenger
            ribonucleic acid. T. Lin, D. Wang,* J. H. Calkins,* H. Guo,* R. Chi,* P. R. Housley,*
            and P. L. Morris.

10:00 A.M. 5. Expression of the androgen receptor gene in the rat penis during sexual maturation.
            N. Gonzalez-Cadavid, R. S. Swerdloff, C. Lemmi, and J. Rajfer.

            sexual development in the cynomolgus monkey. H. S. Keeping.

10:30-11:00 A.M. Break

Scientific Presentations: SPERMATOZOA AND FERTILIZATION
Chairpersons: David F. Katz and Sally D. Perreault

11:00 A.M. 7. Involvement of protein kinase C in the regulation of human sperm motility. G. Paz,
            R. Rotem,* Z. T. Homonnai,* M. Kalina,* and Z. Naor

11:15 A.M. 8. Cold shock induces hyperactivated motility in mouse spermatozoa. E. Z. Drobnis,

11:30 A.M. 9. Monoclonal IgM antibodies against rabbit sperm for vaginal contraception. P. E.
            Radomsky,* T. E. Hoem,* and R. A. Cone.*

11:45 A.M. 10. Stimulation of human sperm capacitation in protein-free medium by lipid transfer

12:00 P.M. 11. Ultrastructural features of bull sperm cocultured with bovine uterine tube epithelial

* Not a member of the American Society of Andrology.
Protamine is removed from hamster sperm nuclei during the decondensation step of fertilization. S. Perreault and R. Barbee.*

Scientific Presentations: CLINICAL ANDROLOGY


Hypogonadism in men with type II diabetes and organic impotence: Response to testosterone therapy. F. T. Murray, R. Johnson,* M. Geisser,* and J. Rountree.*

Standardization and comparability of computer-aided sperm analysis (CASA) instruments. R. O. Davis and D. F. Katz.


Retrospective analysis of relationships between fertility and computerized measures of sperm motion and morphology. C. A. Boyle,* M. J. Koury,* D. F. Katz, J. L. Annest,* M. Kresnow, and F. DeStefano.*

BREAK
Symposium on Physiologic Mechanisms of Androgen Transport into Tissue
Chairpersons: Gerard Cooke and Stephen Plymate

Geoffrey Hammond: Molecular Biology of Androgen Transport Proteins
William Rosner: Biologic Effects of Androgen Transport Proteins
Carl Mendel: The Free-Hormone Hypothesis: A Physiologically Based Mathematical Model

Poster Session I (see list of titles on pages P-17-P-20) and Reception (Diplomate and Gouverneur I & II Salons)
(Please set up posters on Sunday morning between 8 A.M. and 11 A.M. and remove after poster session).

Clinical Laboratory Scientists Meeting (Ambassadeur Room)

MONDAY, April 29, 1991

8:00 A.M. State of the Art Lecture (Ambassadeur Room)
Chairperson: D. K. Pomerantz

Dr. Deborah Segaloff
"Leutropin Choriogonadotropin Receptor: An Unusual Member of the G-Protein-Coupled Receptor Family"

Scientific Presentations: CELL BIOLOGY
Chairpersons: Gail S. Prins and Johannes D. Veldhuis

Regulation of the truncation of LH receptors at the plasma membrane in Leydig cells. A. P. West and B. A. Cooke.

MONDAY, April 29, 1991


10:00 A.M.  25 Regulation of steady-state 4-ene steroid 5α-reductase mRNA levels in the rat epididymis after orchidectomy and testosterone replacement. R. S. Viger* and B. Robaire.

10:15 A.M.  26 Androgen receptor content in seminal vesicles from young and aged transgenic mice with human or bovine growth hormone. G. S. Prins, L. Birch,* T. K. Ghosh, and A. Bartke.

10:30-11:00 A.M.  BREAK

Scientific Presentations: INFERTILITY AND IVF
Chairpersons: Claude Gagnon and Ronald W. Lewis

11:00 A.M.  27 The significance of flagellar pathology in the evaluation of asthenozoospermia. H. E. Chemes.*

11:15 A.M.  28 Sperm acrosome reaction and seminal morphology predict the percent of eggs fertilized at IVF. L. Dennison, L. Calvo, S. Banks,* A. Dorfmann,* M. Bustillo,* J. Schuldman* and R. Sherins.


11:45 A.M.  30 Sperm motility, creatine kinase parameters and hamster oocyte SPA in percoll fractions of human semen. G. Huszar, M. Corrales,* L. Vigue,* and P. Quinn.*

12:00 P.M.  31 Evidence for peroxidative damage to human sperm during cryopreservation. J. G. Alvarez and B. T. Storey.


12:30-2:00 P.M. LUNCH

2:00 P.M.  Symposium on Recent Advances in Bio- and Immuno-hormone Functions and Analyses
Chairpersons: Christina Wang and Kenneth D. Roberts

Robert Rosenfield: LH Radioimmunoassays and Bioassays
Kristine Dahl: FSH Isoforms, Radioimmunoassays, Bioassays, and their Significance
Stephen Plymate: Clinical Measurement of Biologically Active Androgens
Richard Horton: Clinical Assessment of Androgen Production: Correlation with 3α-Androstanediol Glucuronide Measurements

4:00-6:00 P.M.  Poster Session II (see list of titles on pages P-20-P-22)
(Diplomate and Gouverneur I & II Salons)

(Please set up posters on Monday morning between 8 A.M. and 11 A.M. and remove after poster session.)

7:00 P.M.  Banquet

TUESDAY, April 30, 1991

8:00 A.M.  State-of-the-Art Lecture (Ambassadeur Room)
Chairperson: Richard J. Santen
Dr. William Bremner
"Clinical Physiology of Inhibin"

9:00-10:00 A.M.  Eulogy for Dr. Larry Ewing
Award Ceremony and Business Meeting

10:00-10:30 A.M.  BREAK

* Not a member of the American Society of Andrology
Symposium on Clinical Use of 5a-Reductase Inhibitors and Antiandrogens in Prostatic Diseases (Postgraduate Course-II: Joint Session)

Chairpersons: Jack Geller and Mostafa M. Elhilali

Jacquie Imperato-McGinley: Hormonal and Clinical Comparisons: Observations in Patients Treated with 5a-Reductase Inhibitors and 5a-Reductase Deficient Patients

Elizabeth Stone: Use of 5a-Reductase Inhibitors as Treatment for Benign Prostatic Hyperplasia

Nelson Stone: Use of Antiandrogens as Treatment for Benign Prostatic Hyperplasia

Jack Geller: Overview of Enzyme Inhibitors and Antiandrogens in Prostatic Cancer

ADJOURN

Postgraduate Course-II: Management Options for Benign Prostatic Hyperplasia

Poster Session I—Sunday, April 28

Please set up posters between 8 A.M. and 11 A.M. Sunday and remove after the session.

5:30-7:30 P.M.  Diplomate Salon

CLINICAL ANDROLOGY


34 Reversal of pyrimethamine-induced infertility with folic acid in male mice. M. J. Cosentino and R. E. Pakyz.*


36 Dilation of the right testicular vein in rats with left vericocele. N. Sofikitis,* C. Takahashi,* I. Nakamura,* S. Hirakawa,* and I. Miyagawa.*

37 Evidence that abnormal staining behavior of human sperm tails is caused by epididymal dysfunction. G. Haidl,* W. Miska,* U. Neubach,* and R. Wientzek.*

38 Adverse effects of cyclophosphamide (CPA) on progeny outcome can be mediated through the epididymis in rats. J. Qiu,* B. Hales,* and B. Robaire.


40 Alternative models for the evaluation of fertility data. J. D. Kirby and E. Goldberg.

41 The effect of clomiphene citrate on sperm morphology. B. S. Shani, J. H. Check, and A. Bollendorf.

42 Subclinical varicocele, its diagnosis by Doppler examination: Results of varicocelectomy. C. B. Dhabuwala, S. Hamid,* and J. E. Pontes.*

43 Intracavernous injection therapy with combination of vasoactive agents in impotent men. S. Hamid,* C. B. Dhabuwala, and J. E. Pontes.*

44 Reproducibility in monitoring nocturnal penile tumescence and rigidity. C. Bain* and A. Guay.


48 Testosterone (T) suppresses bioactive LH more completely in azoospermic than oligospermic men. K. D. Dahl,* C. A. Paulsen, and W. J. Bremner.

49 Venogenic surgery at the Ohio State University. J. P. York and J. R. Drago.*

* Not a member of the American Society of Andrology.


53 Age dependent expression of androgen receptors in rat cavernosal smooth muscle cells in culture. S. C. Sikka.


55 The 5a-reductase inhibitor, 4-MAPC, inhibits DNA synthesis in the ventral prostate of rats. T. C. Shao,* A. Kong,* and G. R. Cunningham.


SPERMATOGENESIS AND FERTILITY

57 Testicular steroidogenesis in vitro in young adult nonobese diabetic (NOD) mice. A. G. Amador, A. Mayerhofer,* and A. Bartke.

58 Inability of ejaculated human spermatozoa to incorporate exogenous fatty acids or 1-hexadecanol into ether lipids. R. E. Jones, B. K. Bell,* and S. R. Plymate.

59 Rapid disappearance of spermatozoa after vasocclusion (VC) in the dog. P. S. Li, M. Goldstein, P. N. Schlegel, and C. W. Bardin.

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Clinical features of persistent Mullerian duct syndrome (PMDS) in a horse. C. E. Card, B. A. Ball, K. Baxendell, and D. H. Schlafer.

ACROSOMAL REACTION

Acrosin activity in patients with idiopathic infertility. A. Agarwal and K. R. Loughlin.


Purification and partial amino acid sequence of two forms of proacrosin from bovine spermatozoa. S. K. NagDas and H. Lardy.


Partial characterization of SP-10 interactions with human acrosomal membranes. J. A. Foster and J. C. Herr.

Role of acrosine in the human sperm acrosome reaction. P. Morales, P. Vigil,* and M. Llanos.*

Progesterone (P) content in human follicular fluid (hFF) is related to its acrosome reaction-inducing ability. D. Vantman, G. Gutierrez,* P. Kohen,* P. Vigil,* and P. Morales.


Spontaneous and induced acrosomic reaction in cauda epididymal equine spermatozoa. E. Bustos-Obregon, H. Rodriguez,* and M. Riff.*

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A "positive" solution to the anti-sperm antibody controls problem. A. M. McNulty* and L. R. Rubin.

Comparison of a few techniques for sperm antibody screening. J. Sedor,* I. H. Hirsch, and H. J. Callahan.*

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Relationship between pregnancy outcome and objective semen analysis in infertile couples. L. J. Rodriguez-Rigau, C. Ayala,* and E. Steinberger.

The effect of refrigeration in test-yolk buffer on capacitation, the acrosome reaction, and fertilizing ability of sperm in selected IVF patients. D. T. Carrell and R. L. Urry.

The application of assisted conception to the treatment of male infertility in patients with a motile sperm concentration of less than 5 million per milliliter. A. M. Jequier, J. M. Cummins,* W. R. Edirisinghe,* J. M. Yovich,* and J. L. Yovich.*

* Not a member of the American Society of Andrology.
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100 Rabbit semen quality, number of sperm inseminated and fertility. R. H. Foote, P. B. Farrell,* and M. E. Simkin.*


106 Methods used to prepare rat epididymal sperm for CASA influence motility endpoints. V. Slott,* J. Suarez,* R. Linder,* L. Strader,* and S. Perreault.

107 The use of the Kruger strict test for sperm morphology in distinguishing the fertile from subfertile male. J. H. Check, H. Adelson,* and A. Bollendorf.


112 Improvement in semen quality after filtration through L-4 membrane, comparison of results with swim-up technique. A. Mangiona, A. Agarwal, K. R. Loughlin.

* Not a member of the American Society of Andrology.
113 Semen sample preparation for intrauterine insemination comparing the wash, column filtration and swim-up method. M. Beck and R. Shelden.*
114 Motile density levels distinguishing fertile from subfertile males. J. H. Check, A. Bollendorf, and B. Shanis.
118 Cervical mucus penetration testing with a Tru-Trax system is an excellent predictor of sperm mucus interaction in vivo after cervical insemination. C. C. Coddington, F. Irianni, T. L. Toth, and R. T. Scott.
120 The reproducibility of semen parameters and DNA histograms from testicular aspirates in the cynomolgus monkey model. R. H. Steele,* R. Wang,* R. M. Harrison, and W. J. G. Hellstrom.
121 Improvement in sperm morphology and removal of round cells in semen after filtration of ejaculates through L-4 membrane. A. Manglona, A. Agarwal, K. R. Loughlin.
122 Sperm motility before and after preparation for intrauterine insemination. C. R. Sanborn,* L. E. Meuli, and F. W. Byrn.*
123 Effects of SP-cAMP on sperm motility in patients with unexplained infertility. A. Agarwal and K. R. Loughlin.

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128 Human sperm cryopreservation—test yolk buffer or HSPM. G. L. A. Horbay, R. Cooblal,* and F. Choma.*

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INFERTILITY

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134 Selection of viable spermatozoa via SpermPrep™ filtration following twenty-four hour cryostorage at 5°C in test-yolk buffer. P. M. Zavos


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142 Epidermal growth factor: Production and receptor binding to the sex accessory organ of male mice. L. Seethalakshmi, A. Liu,* C. Flores,* T. Kinkead,* M. Menon,* and R. Davis.*

143 Epidermal growth factor (EGF): An important role in testicular function. L. Seethalakshmi, A. Liu,* T. Kinkead,* C. Flores,* A. A. Carboni,* M. Menon,* and R. Davis.*


145 Acid and alkaline phosphatase activities in testes and accessory reproductive glands of transgenic mice expressing bovine GH gene. P. K. Ghosh* and A. Bartke.

146 Increasing concentrations of fetal Leydig cells in culture amplify the steroidogenic response. T. Sokka,* I. Huhtaniemi, and D. Warren.


149 Responsiveness of male accessory reproductive glands to androgen deprivation in mice transgenic for human or human placental variant growth hormone. M. Cecim and A. Bartke.

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To be capable of fertilization in vivo, cauda epididymal sperm must be incubated in medium containing calcium (Ca). This treatment, however, is detrimental to fertilizing ability in vivo after artificial insemination (AI) via the cervix (Olds-Clarke, 1988, J. Androl. 9:46-59). For fer­nonprogressive type of motility (Neill and Olds-Clarke, 1988, J. Androl. 9:46-59) was isolated and subjected to a deficiency in their transport to the site of fertilization. This support the hypothesis that the reduction in fertilizing ability in vivo after AI could be due to a decrease in the numbers of sperm able to reach the oviduct, sperm were incubated 60 min. in a modified Krebs-Ringer-bicarbonate medium containing 1.7 mM Ca or the same medium without Ca. At 1 to 4 hrs. after AI of 10,000 sperm, genital tracts were removed and processed for histology. Serial sections were cut and stained with Feulgen and Fast Green. The number of sperm in every fifth section of every oviduct was determined. For sperm preincubated with Ca the mean no. ± SEM of oviductal sperm per female was 111 ± 29 (range of 1-187, 10 females), but 366 ± 98 (range of 131-383, 9 females) for sperm preincubated without Ca (a significant difference). These data support the hypothesis that the reduction in fertilizing ability in vivo of sperm preincubated in Ca is due to a deficiency in their transport to the site of fertilization. Since exposure to Ca causes hyperactivation, a vigorous but nonprogressive type of motility (Neill and Olds-Clarke, 1987, Gam. Res. 18:121), while progressive motility is thought to be important for passage of rodent sperm through the peristaltic junction (Gaddum-Rosser, 1981, Am. J. Anat. 160:333), preincubation in Ca could exert its detrimental effect on sperm transport by causing hyperactivation. (Supported by grants from NSF and NIH.)

2. DEVELOPMENTAL PATTERN OF STEADY-STATE mRNA FOR PLACENTAL AND NEURAL CADHERIN IN THE RAT TESTIS.


Testis development involves complex cell-cell interactions. These interactions are important for germ cell maturation as well as for the maintenance of the blood-testis barrier as suggested by the presence of adherence junctions between germ cells and Sertoli cells as well as tight junctions between adjacent Sertoli cells. Previous studies have linked tight junctions in the kidney to the presence of cadherins. Cadherins are a family of cell surface proteins which mediate calcium-dependent homotypic intercellular adhesion. Three cadherins have been isolated and named according to the tissue from which they were originally isolated: Ecadherin (E-Cad), Neural-Cadherin (N-Cad), and Placental-Cadherin (P-Cad). We have previously reported the presence of N-Cad mRNA (4.7 kb and 3.2 kb) and P-Cad mRNA in testis. No E-Cad message was detected. For the present study, tissues of male rats ranging in age from 7 to 91 days were excised; total RNA was isolated and subjected to Northern blot analysis. P-Cad steady-state mRNA concentration was highest at 7 days of age and decreased to almost one half of this concentration by day 14 and subsequently decreased to very low constant levels. This was in marked contrast to the 4.7 kb N-Cad transcript which was low early in testicular development but increased rapidly from day 28 to peak levels on day 42, an age that coincides with the first shedding of spermatagonia. The steady-state concentration decreased between days 42 and 56, it remained constant thereafter. The 3.7 kb transcript did not follow the same pattern during development as did the 4.7 kb transcript. While the abundance of this transcript was similar to that of the 4.7 kb transcript on days 7 and 14, peak levels were reached on day 28 and remained constant until day 42 levels then decreased to their lowest point on day 84, then gradually increased to comparable levels to those of the 4.7 kb transcript by day 91. These data show that steady-state P-Cad mRNA is elevated shortly after birth and is turned off early in the first wave of spermatogenesis, while the N-Cad steady-state mRNA progressively increased during this first spermatogonic wave. Supported by the Medical Research Council of Canada.

4. LOCALIZATION AND REGULATION OF TESTICULAR INSULINLIKE GROWTH FACTOR-I MESSERGERIBONUCLEIC ACID. T. Lin, D. Wang, J.H. Calkins, H. Guo, R. Chi, W.H. Housley, and P.L. Norris. WJB Dorn Veterans Hospital, University of South Carolina School of Medicine, Columbia, SC 29201.

Insulin-like growth factor-I (IGF-I) is a 70 amino acid peptide which is a locally acting hormone. Previously we have reported that Leydig cells contain high affinity, low capacity IGF-I receptors and that IGF-I has direct stimulatory effects on Leydig cell steroidogenesis. Although the liver is the major producer of circulating IGF-I, multiple other tissues have been shown to contain IGF-I mRNA in the present study, we evaluated IGF-I mRNA expression in the testis. Rat IGF-I cDNA was provided by Dr. Charles Roberts (NIH, Bethesda, MD). Crude interstitial cells of the rat testis could be separated into distinct bands using 15-60 Percoll gradient density centrifugation. Only the Leydig cell enriched fraction (band 3) contained significant amounts of IGF-I mRNA. The level of Leydig cell enriched fraction (band 3) contained significant amounts of IGF-I mRNA while bands 1 and 2 had very low or undetectable levels of IGF-I mRNA. This was confirmed by the demonstration of IGF-I mRNA in highly purified Leydig cells (over 98% purity) which were obtained by the combination of elimination and Percoll gradient. Sertoli cells also contained IGF-I mRNA but the level was significantly lower than that of Leydig cells. Leydig cell mRNAs consisted of multiple size classes (7.5, 4.5, 1.8, and 0.8 to 1.2 Kb). Four and 20 h after the administration of GH, IGF-I mRNA levels of Leydig cells were increased more than two-fold. HCG increased IGF-I receptor numbers but decreased IGF-I mRNA levels of Leydig cells. In conclusion, Leydig cells of the testes contain the highest levels of IGF-I mRNA which are regulated by GH and HCG. IGF-I may be produced by Leydig cells and have both autocrine and paracrine effects.
The penis in the immature rat and human grows in response to testosterone. The cessation of penile growth occurring in the rat at completion of sexual saturation is thought to be related to the acquisition of gradual insensitivity to androgens, secondary to a decrease in the number of androgen receptors (AR) in a tissue-specific fashion. To investigate whether this process is due to transcriptional repression of the AR gene, we determined the steady-state levels of AR mRNA in the penile corpora cavernosa of groups of immature (16 and 19 days-old) and adult (90 days-old) rats by Northern blot. The 10 kb signal for the AR mRNA is markedly suppressed in the adult, in agreement with the AR number. We have confirmed the presence of low levels of AR mRNA in the adult penis by reverse transcription/PCR amplification of the cDNA generated from total penile RNA with primers on exons 1 and 2 of the AR gene. Primary cultures of smooth muscle cells from the immature penile corpora cavernosa express the AR gene for at least 12 passages, in contrast to the lack of signal observed in some of the adult cells. Our results support the use of the rat penis, and its smooth muscle cells in vitro, as a model to investigate the tissue-specific development in the regulation of the expression of the AR gene. This may be particularly true for disorders such as micropenis not associated with general androgen insensitivity and benign prostatic hyperplasia.

6 DIFFERENTIAL REGULATION OF TESTICULAR GROWTH FACTOR RECEPTOR GENE EXPRESSION DURING SEXUAL DEVELOPMENT IN THE CYNOPTUS NOVIET. Hugh S. Keeping, Department of Urology, Rhode Island Hospital, Providence RI 02903

Sertoli cells of the seminiferous epithelium provide various growth enhancing substances for the developing spermatogonia, as well as for other testicular cells. The effects of these factors, which include transferrin (Tf), insulin-like growth factor-I (IGF-I), IGF-II, and retinoic acid (Ret) are thought to be mediated by specific receptors (Rc). In order to understand further the role of these factors in the growth of the testis, human cDNA probes to the Rc's for these factors were used to measure testicular messenger RNA (mRNA) levels during sexual maturation. Polyadenylated mRNA was isolated from the following stages of seminiferous development: early prepubertal (1.5-2.5 yr), late prepubertal (2.5-3.5 yr), pubertal (3.5-4.0 yr), and adult (6.0-8.0 yr). A summary of the relative concentrations of the Rc mRNA levels are as follows: receptor early late developmental stage

<table>
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<tr>
<th>Rat</th>
<th>Pu</th>
<th>Sp</th>
<th>adult</th>
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<tbody>
<tr>
<td>TF</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IGF-I</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IGF-II</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ret</td>
<td>+</td>
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The relatively high concentrations of testicular TF mRNA during the pubertal stage, a time in which the testis spermatogonial cell population increases several fold, is consistent with the idea that TF is involved in germ cell proliferation. The high IGF-I mRNA levels in late prepubertal stage suggest that IGF-I and IGF-II are important during early stages of sexual development.

7 INVOLVEMENT OF PROTEIN KINASE C IN THE REGULATION OF HUMAN SPERM MOTILITY

+Dept. of Biochemistry, George S. Wise Faculty of Life Sciences; -Institute for the Study of Fertility, Tel Aviv Hospital, and +Dept. of Histology and Cell Biology, Sackler School of Medicine, Tel Aviv University, Tel Aviv Israel.

We report the presence of Protein Kinase C (PKC) in human ejaculated sperm as revealed by enzymatic activity assay and indirect immunohistochemistry, using various type-specific PKC antibodies. Protein Kinase C is localized in the equatorial segment and in the principal piece of the tail. Addition of the Phorbol ester TPA (50 ng/ml) and the membrane permeable diacylglycerol analog (DAG 0.75 μg/ml) to human sperm suspensions significantly increased their motility. The effect of TPA was independent of Ca++ concentration in the medium. Sperm motility was also enhanced by the Ca++ ionophore ionomycin (0.4 μM) in a Ca++ dependent protein kinase independent fashion. The effects of TPA and DAG reached maximal motility effect at about 15 min of incubation and declined at 60 min. The ionomycin's effect was much more prolonged. Human sperm PKC activity is extremely low and represents only about 20% and 29% of the specific activity recovered from PC-12 and rat pituitary cells respectively. Thus PKC inhibitors such as sphingosine, staurosporine and N-7 were shown to inhibit PKC activity and sperm motility. High correlation (r=-0.9) P < 0.001 was found between percent PKC stained cells and sperm motility. Activation of PKC was shown to increase the percent of positive acrosome reacted sperm cells, which was confirmed by the triple stain method. Protein Kinase C is present in human ejaculated spermatozoa and plays an important role in the regulation of sperm motility and in physiological events taking place during fertilization.

8 COLD SHOCK INDUCES HYPERACTIVATED MOTILITY IN MOUSE SPERMATOZOA

E.Z. Drobnik K.R. Robertson, C.R. Day*, J.W. Orenstein, Division of Reproductive Biology, Department of Obstetrics & Gynecology, School of Medicine, University of California, Davis, CA 95616

When cells, including sperm, are cooled rapidly above the freezing point of water, they sustain irreversible injury termed cold shock (CS). CS is accompanied by increased membrane permeability associated with the lipid phase transition (LPT). In sperm, the resulting increase in intracellular free calcium, [Ca++], may be involved in diminished functional competence. Since increasing the membrane permeability to [Ca++], with A23187 causes hyperactivated motility in mouse sperm, analysis of motility pattern may be capable of detecting this aspect of CS damage. To test this hypothesis, sperm were collected from CD-1 mice into Tyrodes buffer (2 mg/ml BSA). The sample control was held at 37°C while the CS sample was cooled abruptly to 10°C below the LPT (for CD-1 sperm), held for 10 min, and rewarmed to 37°C. Samples were videorecorded, and tracks of mobile sperm were collected by computer-aided sperm analysis. For three replicate experiments, univariate analysis indicated increases in mean curvilinear velocity (VCL: 200 to 229 μm/sec) and mean amplitude of lateral head displacement (ALH: 13.3 to 16.8 μm), and a decrease in mean linearity (LIN; 39 to 30). Although these changes are consistent with hyperactivated motility, they were not significant (P < 0.03). Multivariate, cluster analysis was used to classify tracks into four subpopulations based on these motility parameters. One subpopulation contained sperm having characteristics of hyperactivated motility, the highest VCL (mean ± S.D. = 306 ± 49 μm/sec) and the lowest LIN (24.2 ± 4.0 μm) and the lowest ALH (20.2 ± 6.9 μm). This subpopulation consisted of 66% of control sperm to 38% of CS sperm. The decrease in percent motile (20%) did not support a single mechanism for this change. We conclude that CS induces a hyperactivated pattern of motility in a subpopulation of mouse sperm.
SIXTEENTH ANNUAL MEETING

9 MONOCLONAL IgM ANTIBODIES AGAINST RABBIT SPERM FOR VAGINAL CONTRACEPTION. Philip E. Cottle,1 Kevin J. Whaley,2 Michael K. Amstutz,3 James E. Hildreth,3 Mark Stackhouse,4 Michael L. Radomskey,5 Timothy E. Hoen,5 and Richard A. Cone,5 Departments of Biophysics, Medicine,1 Pharmacology,1 and Chemical Engineering3 The Johns Hopkins University, Baltimore, MD.

We are investigating the use of monoclonal antibodies to create new prophylactic contraceptive such as a vaginal ring that releases a combination of monocytes against sperm and STD pathogens. Immuno-infertility correlates best with antisperm antibodies in semen and cervical mucus, not in semen, in mucus, the antibodies agglutinate sperm or immobilize them (the 'shaking' effect). However, most monocytes against human and animal sperm are serum type IgGs. We tested ~100 antisperm monoclonals, supplied by other investigators, that were selected to be most likely to immobilize sperm in semen. Of these, we found the most potent IgG to be no more potent than nonoxynol-9, the spermicidal detergent now used in many vaginal contraceptives (threshold concentration: ~100 µg/mL). In contrast, polyclonal IgG and IgM, which predominate in mucus, are more potent than IgG for agglutinating or immobilizing sperm in semen and cervical mucus, and we have therefore begun to develop polyclonal antigens monocolonal for contraceptive use in the rabbit. A BALB/c mouse was immunized with ejaculated rabbit sperm, separated from serum by 'swim-up', and injected intraperitoneally and subcutaneously. Sperm cells were fused for hybridoma production on day 7. Clones were selected by the degree of sperm agglutination induced by the supernatants. Six IgM monoclonals were obtained that, at concentrations of 0.4-1.2 µg/mL, cause rapid and permanent agglutination of all sperm in semen. Several also agglutinate epididymal sperm, none cross-react with mouse or human ejaculated sperm. The monocolonals are active in vivo when delivered to the vagina either in a sexual lubricant or when released from a vaginal ring made of ethylene vinyl-acetate copolymer currently evaluating MARS-M3 (Mouse Anti-Rabib Spem IgM#3) for contraceptive efficacy in rabbits. In similar tests we are evaluating monoclonals for blocking vaginal transmission of STD pathogens.

10 STIMULATION OF HUMAN SPERM CAPACITATION IN PROTEIN-FREE MEDIUM BY LIPOID TRANSFER PROTEIN. Stuart E. Rawnik and Charles H. Muller, Dept. of Ob/Gyn and Biological Structure, University of Washington, Seattle, WA 98195.

We have previously shown a strong relationship between lipid transfer activity (LTA) in human follicular fluid (HFF) and stimulation of human sperm capacitation and acrosome reaction (Rawnik et al., 1990). The LTA in HFF is likely due to lipid transfer protein I (LTP-I), previously isolated from human plasma, and LTP-I may remove sperm membrane cholesterol during capacitation, allowing subsequecno acrosome reactions to take place. Our previous studies have used BWW with BSA to initiate capacitation. Because some albumin preparations are contaminated with LTP-I, we asked if LTP-I could stimulate human sperm capacitation in plasma-free medium. Human sperm capacitation and acrosome reaction was measured using both sperm penetration assays and PEA to confirm capacitation. Sperm were prepared by swim-up (1hr) using BWW containing 0.4% polyvinyl alcohol (PVA), which supports sperm viability, but not acrosome reaction (AR). Following swim-up, sperm were pelleted, resuspended in 10 µM PEA/100 µL in BWW-PVA, treated 15 min with LTP-I, washed, and incubated for its further development without adding eggs or fixing for PEA staining. LTP-I purified from human plasma by chromatography, the final LTP-I preparation had 1% Transferin/ incubation in the lipid transfer assay and gave a single band on SDS gels. Sperm exposed to LTP-I penetrated more eggs and showed more AR than PVA control sperm (LTP-I: Penetration index 0.72 ± 0.11, %AR 15.7 ± 2.0; Control: PI 0.03 ± 0.02, %AR 5.5 ± 2.3). We also asked if LTP-I stimulated capacitation (leading to ARs later) or if the AR was stimulated immediately. To answer this question, swim-up sperm were treated with LTP-I for 15 minutes, washed and then analyzed for AR events in 15 minutes for 2 hours. Sperm treated with LTP-I showed no stimulation of AR up to 45 minutes but after 45 minutes there was no increase in %AR (LTP-I: %AR 14.6 ± 0.5, Control %AR 8.3 ± 1.7), but after 45 minutes there was a significant (p<0.001) more AR than control, increasing linearly up to 2 hours (nLTP-I: 17 ± 2.2; Control 4.5 ± 2.4; t LTP-I: 24.5 ± 4.5. Control 5.5 ± 0.6). These results indicate that LTP-I stimulates the rate of capacitation rather than immediate acrosome reactions and is able to do so in the absence of any other protein source. We are exploring the ability of cholesterol acceptor molecules such as albumin or HDL to work in concert with LTP-I to stimulate cholesterol loss from sperm as a mechanism for capacitation. Supported by NIH Population Center Grant I01 HD 62829.

11 ULTRASTRUCTURAL FEATURES OF BULL SPERM COCULTURED WITH BOVINE UTERINE TUBE EPITHELIAL CELLS. J.E. Ellington,1 D.M. Schlafer and R.M. Foote,2 New York State College of Veterinary Medicine, Dept. of Animal Science, Cornell University, Ithaca, NY 14853.

Interactions of spermatozoa (SPZ) and the female reproductive tract have been studied by indirect methods such as luminal retrieval of SPZ and SPZ fixation and sectioning of the tract post coitum. Coculture of bull SPZ and bovine uterine tube epithelial cell monolayers (BUTC) has produced capacitated sperm which fertilizes oocytes in vitro. This study used the electron microscope to evaluate interaction of SPZ and BUTC in coculture. Fresh SPZ were washed free of seminal plasma and incubated on BUTC from estrus cows. Incubation was for 3 or 6 h at 37°C in 5% CO2 and air. Cocultured SPZ attached to BUTC in dense clusters with rapid tail motion within 1 h. After incubation BUTC with attached SPZ were fixed in situ for 1 h. Both transmission electron microscopy (TEM) and scanning electron microscopy (SEM) showed intimate SPZ-BUTC contact with microvilli reaching for the sperm heads. Mucus strands and granules could be seen in the acrosome (AC) region of SPZ. Sperm cells attached mostly in intracellular troughs in a stacked fashion. Changes of AC were seen after 6 h of incubation. The TEM showed vesiculation of AC in approximately 15% of the SPZ, suggesting true AC-reactivation. Another 10-20% of the AC showed non-specific loss of the AC. Plasma membranes of intact adjacent SPZ and those of BUTC were very closely opposed, with no detectable space between them, suggesting fusion of membranes. These changes in cocultured SPZ are remarkably similar to those described for SPZ in the uterine tube in vivo. This system offers a unique in vitro model for studying changes of capacitation and fertilization, including cell to cell interactions of sperm, ova and uterine tube epithelial cells. Supported by NIH Grants HD00888/H021939.

12 PROTEIN REMOVAL IS REMOVED FROM HAMSTER SPERM NUCLEI DURING THE DECONDENSATION STEP OF FERTILIZATION. S. Perreault and R. Barbere, Reproductive Toxicology Branch, US EPA, Research Triangle Park, NC 27711.

Sperm nuclear protamine is replaced by somatic histones before zygotic DNA synthesis can proceed. To learn about the timing and mechanism of protamine removal, we monitored its progress in hamster sperm nuclei during decondensation and pronuclear formation. Isolated epididymal sperm nuclei were transferred sequentially to 3 media containing 9, 5, and 0.5 mM DTT to reduce protamine disulfide bonds. 6 out of 15 nuclei (10 µM mono- bromobimane (10 min, to fluorescently label free sulfhydryls). These nuclei were microinjected into hamster oocytes. Sperm nuclear fluorescence was scored at various times post injection (P2) on a brightness scale of 0 (max) to 4 (none). At 15 min PI, a small number of nuclei (10%) remained intact & bright (score: 4), but most were pale in the center where chromatin dispersion had begun (score: 2-3). By 30-45 min, most nuclei were extensively decondensed and exhibited fluorescence only at the extreme tip and base (nuclear annulus) (score 1). Fluorescence was absent from most nuclei at 60 min PI. Male pronuclei, observed 75 min or more PI, were not fluorescent. Essentially the same results were obtained when injected oocytes were maintained in meiotic metaphase arrest by adding colcemid (0.4 µg/mL) to the medium. In contrast, when nuclei were released by deconformation in DTT until fluorescent (reduction of disulfide bonds without protamine removal), they remained fluorescent. We conclude that protamine removal occurs rapidly during chromatin dispersion (decondensation), is complete before pronuclear formation, and occurs independently of oocyte activation. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.
13 A METHOD FOR EXPERIMENTALLY INCREASING TESTICULAR SIZE AND SPERMATOGENESIS IN THE RAT. Paul Cooke*, Rex Hess, Essie Melani and John Porcelli*. Deps. of Veterinary Biosciences and *Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

We have previously developed a method for increasing testicular growth and final size. In this system, rats are treated from birth to day 25 with the goitrogen propylthiouracil (PTU), then allowed to recover. After PTU treatment, the pups become euthyroid and grow rapidly, but remain 15-20% lower than normal. Testicular size in treated rats was increased 40% and 74% at 90 and 135 days of age, respectively. These tests were histologically normal. The present study determined if the increased testis size corresponded with increased sperm production and if these males were fertile. Daily sperm production (DSP) and epididymal sperm reserves were determined in control and treated rats at 90 and 135 days. DSP was increased by 84% and 104% in treated rats at 90 and 135 days of age, respectively. DSP/pg tests (efficiency of sperm production) also increased in both the 90 and 135 day treated animals by approximately 25% (32.7 X 10^6 sperm/g testis in treated rats vs. 25.5 X 10^6 in controls). Epididymal sperm reserves in treated rats showed increases which were similar to the increases in DSP. Sperm motility in treated males was normal, and all treated males were fertile. These results show that sperm production can be doubled and the efficiency of sperm production (DSP/pg tests) increased by early transient hypothyroidism. Furthermore, the treated animals are fertile. This is the first animal model which allows sperm production to be increased by this magnitude, and may be useful for studying the regulation of testicular growth and spermatogenesis.

15 IS ROUTINE ENDOCRINE SCREENING OF IMPOTENT MEN NECESSARY? J.P. Jarow, A. Johnson* and A. Smith*. Bowman Gray School of Medicine, Wake Forest University, Winston Salem, NC 27103, U.S.A.

Most studies have shown that endocrinologic disorders are an important cause of impotence. Assuming an average cost of $100/patient for endocrine screening and a 3% incidence of endocrinologic abnormalities, the cost of diagnosing each patient with an endocrinopathy is over $3,000. The purpose of this study was to identify historical and physical risk factors for hypogonadism in an effort to limit the number of patients being screened and reduce the overall cost of impotence evaluations. The records of 330 consecutive men attending an impotence clinic who underwent routine screening with a testosterone level (T) and prolactin level, if testosterone was low or borderline normal, were reviewed. Eleven patients had a low T level upon initial screening. Of these, 7 had a low prolactin level and 1 had a low testosterone level. Two of these patients had a prolactin-secreting pituitary adenoma with a borderline normal T, 3 were anorchid, and 3 had unexplained primary hypogonadism. Review of the history and physical examination of each patient revealed two predictive factors for hypogonadism, decreased libido and bilateral atrophic testes. Furthermore, these men were fertile. Therefore, we could not identify any risk factors that would reliably predict an endocrinopathy and recommend continued routine screening of impotent patients with testosterone and prolactin levels.

14 HIV-1 AND WHITE BLOOD CELLS IN SEMEN FROM VASECTOMIZED SEROPOSITIVE MEN. D.J. Anderson, J.A. Politch, A. Martinez*, B.J. Van Voorhis*, H.S. Padlan,*, T.B. O'Brien*, Harvard Medical School, Boston, MA 02115; UCSC, San Francisco, CA 94140; and CDC, Atlanta, GA 30333.

Among persons infected with Human Immunodeficiency Virus Type I (HIV-1), seminal CD4 lymphocytes and macrophages host HIV-1 and may play a role in the sexual transmission of the virus. Because vasectomy leads to an overall reduction in seminal white blood cell (WBC) numbers it has been suggested as a potential approach to reduce the sexual dissemination of HIV-1. We have analyzed a total of 7 semen specimens from 4 HIV-1 seropositive vasectomized men and 1 healthy control (CDC Stage 1). We have found that 5 of the 6 WBCs present in the semen were monocytes (72%). The level of HIV-1 in the semen was similar to that found in peripheral blood. The presence of HIV-1 in semen from a vasectomized man indicates that infected cells and/or free virus can enter semen through the accessory glands and/or the epithelium lining the vas deferens or urethra. We conclude that vasectomized men are potential transmitters of HIV-1 and that vasectomy should not be advocated as an approach to reduce the sexual transmission of HIV-1.


Previous studies in men with diabetes and organic impotence have indicated decreased free serum testosterone, increased urinary LH excretion and increased mean serum LH when collected at 10 minute intervals over 12 hours. The present study examined the hourly diurnal total testosterone and bioavailable testosterone pattern in men with type II diabetes and organic impotence determined by history, nocturnal penile tumescence and reduced conduction velocity in the dorsal penile nerve. Testosterone was administered to ten patients with organic impotence and no significant penile vascular disease (normal internal iliac angiography and pulse-doppler studies). Drug administration included both testosterone cypionate and placebo in a randomized double-blind fashion with each patient receiving either placebo or testosterone cypionate for four months with a two month period of washout between testosterone and placebo. Results in controls (n=7, age=45.3±4.3SD) and men with type II diabetes and organic impotence (n=7, age=48.9±1.3SD) showed that serum bioavailable testosterone (7.7±0.69 vs 5.78±0.58 m/l; p<0.05) but not total testosterone (21.1±2.4 vs 18.4±2.3 m/l; p<ns) was significantly diminished in diabetic men with organic impotence. Diurnal patterns were also significantly different. Treatment with testosterone improved libido (p=0.027), erectile rigidity (p=0.068) and NPT (p=0.048). Fifty percent significantly normalized NPT (p=0.012). Conduction velocity in the dorsal penile nerve, penile brachial index and psychological testing did not change except for improvement (p=0.032) in the Zung Depresion Index. We conclude that bioavailable testosterone is significantly diminished in patients with type II diabetes and organic impotence and 50% of patients will improve erectile function with testosterone therapy.


All CASA instruments have distinctive features, but operate on similar principles. The algorithms for sperm identification, tracking, and analysis, however, are still evolving. We compared the HTM (Hamilton-Thorn Research) and CTS (Motion Analysis Corp.) instruments with each other, and with manual measurements, using video tapes of human semen. System parameters were matched. Results show: 1) HTM values for concentration (CON) were not different from controls, but CTS values were lower than HTM and controls; 2) values for %motility (MOT) for both systems were similar over 2, 5, or 15 frames, but slightly lower than controls due to specimen dilution; 3) straight-line velocities (VSL) were different between instruments, with HTM being more sensitive to the number of frames analyzed; 4) curvilinear velocities were higher for HTM, especially for 15 frames of analysis; and 5) amplitude of lateral head displacement was similar for both instruments at 15 frames, but different for small numbers of frames. In general, the magnitude of differences between instruments was small (5-10%). Based on this work, new algorithms were developed for CON, MOT, and VSL. Results from these revised algorithms will also be reported. Our findings suggest that implementation of standard protocols for instrument setup and calibration can provide comparability of results between different CASA instruments. (Supported by NIH ES03614).

18 THE MEDIATOR OF HUMAN CORPUS CAVERNOSUM RELAXATION IS NITRIC OXIDE. W.D. Arouendo, P.A. Bush*, O.M. Bugn*, L.J. D'Amato* and J. Rajfer. UCLA Medical Center, Los Angeles, CA 90024.

Ju et al. attempt to determine whether nitric oxide (NO) could be the specific mediator of human corpus smooth muscle relaxation, a series of experiments were performed io vitro using strips of human corpus tissue from 7 patients. The strips were hung on organ chambers, bated in warm Krebs solution, precontracted with phenylephrine, and changes in isometric force recorded. Corporal smooth muscle relaxed in response to the parasympathetic neurotransmitter acetylcholine (ACh) and in response to electrical field stimulation (EFS) of the eustachian-urothelial (INAC) nerves. The addition of a specific inhibitor of NO synthesis (NO-enitro-L-arginine) prevented ACh from relaxing corporal tissue (p<0.001). Similarly, inhibitors of NO production (NO-nitro-L-arginine and NO-o-amin-L-arginine) also prevented EFS-induced relaxation of these corporal strips (p<0.001). The inhibition of this EFS-induced relaxation could be reversed by the coiogamous addition of the NO precursor, L-arginine (p<0.001). Furthermore, if authentic NO was added directly to corporal strips, a dose-dependent relaxation of the tissue was observed. From these data we conclude that NO is the mediator of human corporal smooth muscle relaxation. Since corporal smooth muscle dysfunction is believed to be a common cause of human erectile dysfunction, it remains to be determined whether defects in the NO pathway in corporal tissue could play a role in the etiology of impotence.


Sperm morphology was assessed on stained smears from 50 untreated patients attending an infertility clinic by the visual method and by computer assisted semen analysis system equipped with software for morphology analysis (Morphologizer II, Cryo Resources, Ltd., New York, NY). The mean and standard deviation of sperm morphology parameters were compared between the two methods. Whereas, the percent big and taper forms were significantly higher when analyzed by the Morphologizer and manual methods were highly variable (ranging from -20% to +20%). The large differences between the methods were due mainly to the large coefficients of variation (CV) present when classifying abnormal sperm head morphology. Only the percent normal spermatozoa could be classified by both methods with acceptable precision (intratechnician CV less than 10% with either method). Overall, there was no apparent advantage of the commercially available Morphologizer over the manual method with regards to the precision of classification of sperm head morphology and time involved in the assessment. The sperm morphometric parameters (available only from the Morphologizer) showed small variations between the different semen samples but the clinical value of these parameters is unknown.


With recent advances in the technology for semen analysis, several automated semen parameters, sperm motion and morphology are now available. Whether or not any of the new parameters serve as markers of male infertility is unknown. We investigated the relationship between various semen characteristics and male infertility among 596 men who participated in the Centers for Disease Control studies of the health of Vietnam veterans. Semen was collected and processed by using a standardized protocol and the sperm assessments were made with a Callandot automated semen analyzer (U.S. Army, 1986). Infertility was defined in two ways: by (1) the number of children fathered and (2) the inability to father a child after trying for a year or longer. The per cent of motile cells and the per cent of progressive cells (straight-line velocity > 25 um/second) were the only two movement characteristics independently associated with our measures of infertility. One morphometric parameter, the mean sperm length/width ratio, was consistently associated with both measures of infertility, even after adjustment for the other sperm parameters and potential confounders, including age, race, and alcohol and cigarette use. This measure was also strongly associated with infertility among various subgroups of men at increased risk of impaired fertility. These findings with the length/width ratio are new and as best as we can determine from our data, this parameter appears to be an important correlate of infertility in males.
REGULATION OF THE TRUNCATION OF LH RECEPTORS AT THE PLASMA MEMBRANE IN LEYDIG CELLS.
West, Anthony P., and Cooke, Brian A.
Dept. Biochemistry, Royal Free Hospital School of Medicine, Rowland Hill St., London, NW3 2PF, England.

The truncation of luteinizing hormone (LH) receptors was investigated in mouse tumour Leydig cells (MA10 and MLTC-1), rat testis Leydig cells (RTL) and a rat tumour Leydig cell (R2C). Addition of 3.3mM LH for 2h at 34°C had no detectable effect on the [125I]-hCG binding in RTL or R2C cells, but in MA10 and MLTC-1 cells it caused a 40-60% loss. The effect on MA10 and MLTC-1 cells was mimicked by inhibiting receptor internalization with 5mM NaN3, Dibutylrylcyclic AMP (0.01,0.1,1 mM) also showed a 30-50% loss of binding in MA10 and MLTC-1 cells, by inhibiting internalization. Phorbol 12-myristate-13-acetate (PMA) had no effect on binding or internalization of the [125I]-hCG. The loss in binding sites under all conditions was prevented by the addition of protease inhibitors (leupeptin, PMSF, apronitin). Incubating RTL and R2C cells with protease inhibitors caused a 2-3 fold increase in binding sites and a 2-3 fold increase in LH-stimulated cyclic AMP production. When RTL and MA10 cells were incubated in the presence of [125I]-hCG, a radioactive-protein complex of approx. M, of 80,000-90,000 was released into the incubation medium. We conclude that LH receptors are regulated by proteolysis at the plasma membrane in both mouse and rat Leydig cells. Furthermore, truncation of the LH receptor in the rat Leydig cell is a continuous process whereas, in the mouse it is involved in down-regulation - which is mediated by cyclic AMP. This work was supported by the MRC and SERC.


Glutathione S-transferase activity (GST) is positively and linearly correlated with testosterone secretion in hamster, rat and guinea pig testes perfused in vitro (Am. J. Anat. 1988, 181:12-22). Rat Leydig cell peroxisomes contain sterol carrier protein-2 (SCP-2) and a lipase that can degrade cholesterol. Peroxisomes transport cholesterol to mitochondria (J. Biol. Chem. 1982,257:8928-36). Peroxisomal SCP-2 in Leydig cells increases 5 fold above controls 30 minutes after a single SC injection of luteinizing hormone (Endocrinology 1990, 127:2947-54); latter occurs prior to the plasma testosterone peak. These observations suggest that SCP-2 play a role in Leydig cell steroidogenesis. In the present study we tested whether Leydig cell peroxisomes contain HMG-CoAR, the rate limiting enzyme in cholesterol biosynthesis. Testes of sexually mature Sprague Dawley rats (n=4) were prepared for EM immunocytochemistry (J. Androl. 1990, 11:270-84). HMG-CoAR was immunolocalized in Leydig cell organelles via AuroProbe II Protein A gold 10. The antibody used was made against E. Coli protein containing the C terminal of 147 amino acids of human liver endoplasmic reticulum reductase. Peroxisomes were the only organelles (except endoplasmic reticulum/cyttoplasm matrix) labeled above background. In summary, the present study is the first to demonstrate that Leydig cell peroxisomes contain HMG-CoAR and further supports the hypothesis that peroxisomes are involved in Leydig cell steroidogenesis. Supported by NIH HD30720.

DIVERSE SECRETORY PATTERNS OF RAT CLUSTERIN AND α-1-MICROGLOBULIN BY SERTOLI CELLS IN BICAMERAL CULTURE CHAMBERS. Josephine Grim, 1,2 Richard A. Lorkin, 1,2 and C. Yan Cheng. 1,2 (1The Population Council, 1230 York Avenue, New York, New York 10021; 2Department of Biological Sciences, St. John's University, Jamaica, New York 11439; and 3The Rockefeller University, 1230 York Avenue, New York, New York 10021).

Earlier studies from this laboratory have shown that rat Sertoli cells actively synthesize and secrete two proteins in vitro designated clusterin and α-1-microglobulin (AMG), which are related to cell-cell interactions and tissue remodeling in the seminiferous epithelium, respectively. A specific radioimmunoassay (RIA) has been established for each of these proteins which can detect as little as 0.3 ng of clusterin and 1.2 ng of AMG per assay tube. We have now utilized these RIA's and bicameral culture chambers to study the polarized secretion of these proteins by Sertoli cells in vitro. Briefly, Sertoli cells (2-5 x 10^6 cells/cm^2) were plated on MillicellTM-4A culture inserts and maintained in a humidified atmosphere of 35% O2/5% CO2 at 35°C for a period of 4-13 days during which serum-free medium (D12/129, M.1, 5% supplemented with insulin,10 ng/ml, human transferrin, 5 μg/ml, epidermal growth factor, 2.5 ng/ml and bovine, 5 μg/ml) were changed every 24-48 hr. The amounts of clusterin, AMG, and transferrin in the medium obtained from the apical and basal chambers were quantified by RIA's. It was noted that clusterin was secreted exclusively to the apical chamber (A) over a 6 day culture period, since immunoreactive clusterin was not detectable in the basal (B) compartment. In contrast, AMG, a trimer with an apparent Mr greater than that of clusterin and transferrin (Mr 68,000 vs Mr 8,500 and 75,000, respectively), is secreted into the apical and basal compartments with an A/B ratio of 2.5±1.1 making it distinctively different from clusterin (A:B=1.0±0.001) but similar to transferrin (A:B=1.40±0.21). The secretion of clusterin and AMG into the apical and basal chambers was not affected by either testosterone (T:0.1 x 10^-3 M) or cyclosporin A (100ng/ml). Preliminary studies indicate that the apical secretion of clusterin by Sertoli cells is inhibited in the presence of germ cell-conditioned media whereas its secretion into the basal compartment increased by several orders of magnitude; in contrast, the A/B ratio of AMG and transferrin in these chambers remains virtually unchanged. However, the presence of transforming growth factor-β (TGF-β 0.01-5 ng/ml) appears to suppress the basal secretion of AMG without obvious effect on its apical secretion. Conclusions: The polarized secretion of proteins by Sertoli cells is not dependent on the molecular sizes of the molecules suggesting that there are specific but precise mechanisms to regulate these processes. Since the regulators of the polarized secretions of clusterin and AMG appear to be different, these studies will provide insights to understand the mechanism of protein secretion by Sertoli cells.
REGULATION OF STEADY-STATE 4-ENE STEROID 5α-REDUCTASE mRNA LEVELS IN THE RAT EPIDIDYMIS AFTER ORCHIDECTOMY AND TESTOSTERONE REPLACEMENT. Robert S. Viger, Bernard Robaire. Dept. of Pharmacology & Therapeutics, Centre for the Study of Reproduction, McGill University, Montreal, Quebec. H3Y 3W1.

Testosterone (T) is converted to 5α-dihydrotestosterone (DHT) in the rat epididymis by the enzyme 4-ene-steroid 5α-reductase (EC 1.3.22.1). The conversion is required to maintain epidymal function. Previous work from our laboratory has characterized the endogenous regulation of the epidymal enzyme activity. Regulation at the mRNA level, however, has not been investigated. The goal of the present study was to determine whether T replacement therapy in the orchidectomized rat can maintain 4-ene-steroid 5α-reductase steady-state mRNA levels. Adult male rats, 300-350 g, were either sham-operated to serve as controls or orchidectomized and simultaneously implanted with 1-impregnated polydimethylsiloxane capsules of either 0, 2.5 g, 5.0 g, or 7.5 g testosterone in oil. T was also implanted in adult rats, while implants measuring 15.8 cm resulted in a 5.8 fold increase in serum T. Seven days following the surgery, the rats were killed and the epididymides were removed and sectioned. Total cellular RNA was isolated and was subjected to Northern blot analysis using the CDNA for female rat 5α-reductase. Steady-state mRNA levels were quantitated by laser densitometric scanning of the resulting radioautograms. We found that orchidectomy alone caused an 85% decrease in mRNA levels in the testis and 65% decrease in the epididymis. Androgen replacement therapy restored steady-state mRNA levels to control levels in the testis and epididymis. These results demonstrate that orchidectomy alone caused a decrease in steady-state mRNA levels in the rat testis and epididymis and that androgen replacement therapy restored mRNA levels to control levels.

THE SIGNIFICANCE OF FLAGELLAR PATHOLOGY IN THE EVALUATION OF ASTHENOSPERMIA. H. Sisson*, Laboratory of Testicular Pathology, Children's Hospital, Buenos Aires, Argentina.

This report describes sperm ultrastructure in a large series of 201 patients consulting for sterility due to severe asthenospermia. As there were no andrological or seminal conditions which could explain the failure of motility, ultrastructural examination of spermatozoa was indicated. Values of total and fast forward motility averaged 20% and 3.5% respectively. Electron microscopy showed alteration in the number, topography and organization of flagellar microtubules. The 9+2 axonemal pattern was disrupted by lack, translocation, disorganization or duplication of the microtubular pairs. The percentage of spermatozoa bearing ultrastructural anomalies ranged between 20% and 100% in different patients. The population was subdivided in four groups according to the percentage of abnormal flagella. The percentage progressively increased from group G1 (14% to G4 (41% of the patients). The percentage of patients with normal or with slightly increased abnormality values, while in the remaining 70% (G3:n=69; G4:n=66) asthenospermia was related to an increase of pathological axonemes. These findings indicate a high incidence of sperm abnormalities in patients with severe asthenospermia and suggest the need for ultrastructural examination for their proper evaluation.

ANDROGEN RECEPTOR CONTENT IN SEMINAL VESICLES FROM YOUNG AND AGED TRANSGENIC MICE WITH HUMAN OR BOVINE GROWTH HORMONE. G.S. Zhang, R. Birch*, T.K. Gobin, A. Barkat. Michael Reese Hospital, Chicago, IL 60616 and Southern Illinois University, Carbondale, IL 62901.

Previous work has shown that expression of human (h) GH in transgenic mice is associated with enlargement of seminal vesicles (SV). In young mice, this effect is greatly accentuated as the animals age (Cemini, 84). It has been hypothesized that this extensive SV growth may be related to the prolactin-like effects of hGH in rodents since bovine (b) GH tran sgraft carrier mice which lack GH effect on SV with no prolactin effect on lactation. To further clarify these observations, we examined the cellularity and androgen receptor (AR) content of SV from six groups of animals: young (3-5 months) control mice, old (12-15 months) control mice, young bGH transgenic mice, old bGH transgenic mice, young hGH transgenic mice, and old hGH transgenic mice. A high level of AR was found in all samples, with the highest AR content in the young hGH transgenic mice. These results indicate that androgen stimulation of SV is important in the regulation of AR content in these tissues. AR response (t AA above control) in the SV was also significantly different from the control group in these animals.

SIXTEENTH ANNUAL MEETING

P-31
EVIDENCE FOR PEROXIDATIVE DAMAGE TO HUMAN SPERM DURING CRYOPRESERVATION


The present need to cryopreserve human sperm was in artificial insemination donor (AID) programs has focused attention on the cell damage to the cells from the freezing. We have examined the possible role of phospholipid peroxidation in this damage. Comparison of phospholipid of pooled human sperm samples from AID donors before and after cryopreservation in egg yolk medium showed 44% loss of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in this material, which contain peroxidatively labile polyunsaturated acyl and vinyl ether esters. Loss of 4H-estradiol acid in both diacyl and phosphatidyl PC and PE due to cryopreservation was 61% and 41%, respectively, while loss of palmitic acid was 30%, consistent with peroxidative degradation. We had previously shown that human sperm contain the peroxidative protection enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GP). C. M. Quinn, * Dept. Of Med., U.Tex. Anderson Cancer Center, Houston, TX 77030

In vitro fertilization (IVF) has been used to treat certain types of infertility, but is often unsuccessful due to several factors in sperm number, motility, morphology, and function. We have used IVF combined with partial zona dissection (PZD) or microinjection of sperm into the perivitelline space (PVS) to overcome infertility in mice with genetic defects resulting in marked abnormalities in sperm number and function. We conclude that a given sperm sample has a distribution of SOD activity among the cells, that the population of cells with SOD activity is susceptible to peroxidative degradation of membrane phospholipids with consequent membrane damage and possible loss of SOD from the cells, and that the population of cells with high SOD activity is resistant to this peroxidative attack. A particular biochemical reaction, phospholipid peroxidation, is thus correlated with cryopreservation damage to human sperm.

Supported by NIH grant HD 25867.


We have studied Percoll sperm fractions by monitoring the CASA values, motile sperm yield, the biochemical parameters of creatine kinase activity (CKACT) and creatine kinase concentrations (CKM), and the hamster oocyte SPA. We processed 30 normospermic samples (cons: 64±19 million sperm/ml, mot: 45±22%, vac: 39±2 μm/μl; CKACT: 0.18±0.05 CK IU/100 million sperm and CKM: 35±3%, all values mean ± SEM) on a discontinuous gradient of 40% and 50% Percoll (400× g for 16 min). In the top layer, intermediate phase and pellet the distribution of the total recovered sperm was 28±3%, 38±3% and 35±3%. Sperm motility in the fractions was 20±2%, 32±3% and 68±2% with a motility yield distribution of 13%, 30% and 57% (all comparisons are P<0.01). The sperm CKACT in the three fractions was 1.0±0.2, 0.05±0.01 and 0.00±0.005 CK IU/100 million sperm, the CKM values were 27±3%, 63±2% and 94±4% (P<0.01 and P<0.001, respectively). The most important determinant of motile sperm yield in the pellet was the initial sperm motility in the sample (R=0.65, P=0.001, N=30). With the Charles River cryopreserved hamster ovary we have performed SPA and found 90→folds higher penetration (both rate and index) when using sperm fractions arising from the pellet vs the top or intermediate phase of the gradient. These data demonstrate that the 40→80% discontinuous Percoll gradient provides a one-step procedure that concentrates in the pellet sperm with 50% of the motile sperm in the pellet is also the best in motility (68±2%), CKACT (0.00±0.005 CK IU/100 million sperm), CKM (64±4%), and SPA properties. The improved fertilizing potential of the Percoll fractions was such that sperm was demonstrated in assisted reproduction: Significantly increased fertility of normospermic and oligospermic men was consistent with a CKACT of 0.25 IU/100 million sperm and >10% CKM (Hsuue et al, J. Androl., 1990; Mol. Repr. and Dev., 1990) (Supp. HD-19606).


In vivo fertilization (IVF) has been used to treat certain types of infertility, but is often unsuccessful due to several factors in sperm number, motility, morphology, and function. We have used IVF combined with partial zona dissection (PZD) or microinjection of sperm into the perivitelline space (PVS) to overcome infertility in mice with genetic defects resulting in marked abnormalities in sperm number and function. The bottleneck (bott) was found to be infertile in vivo (0% fertilizations, 3 trials, 80 eggs). However, IVF resulted in a fertilization rate indistinguishable from controls (92%, 7, 3 trials, 50 eggs). In contrast, another mutant mouse strain (PZD) was ineffective in vivo fertilization with the bottleneck (bott) and 3 other bottleneck strains (bott-3, bott-4, bott-6) in these animals are incapable of fertilization either in vivo or in vitro. Sperm from bott/male mice were not present and therefore were not micromanipulated. Interestingly, the heterozygous/bott males were found to produce sperm which were fertile in vivo, but not in vitro (0% fertilizations, 8 trials, 283 eggs), although normal fertilization rates were observed for our normal IVF controls (71%, 15, 3 trials, 80 eggs). PZD+IVF did not result in fertilization with the +/bott sperm (0%, 2 trials, 90 eggs). However, IVF overcame the block to fertilization in vivo and resulted in a fertilization rate of 57% ± 15 (4 trials, 65 eggs). Sperm from a/a males which have a normal head morphology as their only defect did not fertilize eggs in vitro (4 trials, 65 eggs). While PZD was ineffective (0%, 29 eggs), IVF resulted in a fertilization rate of 22±11 for a/a sperm. Therefore IVF with PZD or PVS can be used successfully to overcome a variety of sperm defects resulting in the rescue of otherwise infertile male mice. The relevance of these procedures for significantly impaired human sperm remains to be clearly defined.
33 USE OF A Y-CHROMOSOME-SPECIFIC DNA PROBE TO MONITOR THE ALBUMIN COLUMN METHOD OF X-Y SPERM SEPARATION.

The popular Ericsson method of X-Y sperm separation for pre-conception gender selection, in which sperm are passed through a high albumin column prior to insemination, has been reported to yield 85% male births. To assess the mechanism of this success, the percentage of Y-containing sperm produced by this method was determined by in-situ hybridization of Y enriched sperm fractions with a Y-chromosome-specific DNA probe. The probe DYZ4 (provided by David Page), which detects Y-specific repeat sequences on Yp and Yq, was labeled with digoxigenin using random primers (Genius kit). 29 normal sperm specimens (anonymous) were each divided into two aliquots. One aliquot was processed for X-Y sperm separation by the modified Ericsson method (Fertil Steril 31:52: 0.5ml sperm suspension was layered onto a two-component column consisting of Inl% human serum albumin (HSA) on top of 0.5ml 20% HSA. The original 0.5ml sperm suspension was removed after 60 min, and after 30 more min, the 20% HSA layer was removed and the sperm washed. A control aliquot of sperm was incubated with 20% HSA and then washed. Sperm smears on slides were air-dried, fixed, pre-hybridized, and heat-denatured, labeled Y-specific probe was added for 16h at 42°C in 2X SSC. Followed by washing twice in 6X SSC/0.1% formamide at 42°C, 2X SSC at 22°C, and 0.2X SSC at 50°C. Anti-digoxigenin antibody coupled to alkaline phosphatase was added, and the labeled probe was detected with NBT/BCIP-phosphate. 200 sperm per slide were scored for presence of the Y probe. There was no significant difference in the percentage of Y-positive sperm between the control aliquots (49.3 ± 6.1%) and the hybridizing aliquots processing Y separation (49.0 ± 0.7%); neither value is significantly different from 50%. Conclusion: The albumin column method of X-Y sperm separation doesn’t alter the percentage of Y-positive sperm, as assessed with a Y-specific DNA probe.

34 REVERSAL OF PYRIMETHAMINE-INDUCED INFERTILITY WITH FOLINIC ACID IN MALE MICE

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We have recently shown that pyrimethamine (PYR) causes reversible infertility in adult male rats and mice by inhibiting spermatogonies (Proc. Natl. Acad. Sci. 1990; 87:1431). PYR is known to exhibit an anti-malarial effect by inhibition of dihydrofolate reductase (DHFR) in the plasmid. However, the mechanism by which PYR causes infertility in the male is unknown. In order to help elucidate the mechanism and further explore PYR as a potential male contraceptive, we performed the following experiment. We attempted to prevent this effect of PYR by concomitant administration of folic acid, the product of the DHFR reaction, at various dosages for 55 days. Breeding trials revealed that folic acid, when administered with PYR, completely negated the antifertility effects of 200 mg/kg/day PYR in a dose-dependent manner. Furthermore, the following parameters improved at low dosages of folic acid and were normal or near normal (P < 0.05) with higher dosages of folic acid: percent fertile males, percent pregnant females, epididymal sperm reserves, sperm motility, testis weight and testicular histology. The changes seen were dependent on the dose of folic acid administered (P < 0.01). These data indicate that PYR may be exerting its anti-fertility effects by inhibiting testicular DHFR, thereby reducing the availability of folic acid. Studies are currently underway in our laboratory to characterize testicular DHFR.

35 TESTICULAR INJURY INDUCED BY HYPOTHERMIA AND ISCHEMIC HYPOXHYPERTHERMIA.

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Dilation of the right testicular vein (RTV) in some patients with left varicocele (LV) or in experimental models of LV has been reported. To illustrate if the dilation of the RTV contributes to the creation of the detrimental effect of LV on the function of the right testis, we performed an experimental varicocele model in 40 Wistar rats by including the left renal vein partially and ligating the collateral vein between the left testicular vein (LV) and the left common iliac vein. Five rats were sham operated group (group A). Four weeks after the operation, the varicocele rats underwent retroperitoneal testicular biopsy. Twenty-three animals showed bilateral ligation of the testicular veins and were divided into group B (n=11) and group C (n=12). The rats of group B underwent ligation and resection of the RTV whereas the rats of groups C and A received only dissection of the RTV. Twelve weeks after the resection operation, the number of group C showed significant reduction of: a) the fertility rate, b) the right testicular versus left testicular weight, c) the right testicular versus left testicular temperature difference, d) the right epididymal sperm motility, e) weight and glucose consumption, f) the weight of the right testis, epididymis, and accessory genital glands compared with groups A and B. Considering that the surgical repair of the secondary right varicocele due to the creation of the collateral circulation of the testicular veins and epididymis, the two parameters representing the harmful consequences of the LV on the right testis, we suggest that the dilation of the RTV contributes to the creation of the harmful effect of the LV on the function of the right testis.
EVIDENCE THAT ABNORMAL STAINING BEHAVIOR OF HUMAN SPERM TAILS IS CAUSED BY EPIDIDYMYAL DYSFUNCTION

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Recently, the phenomenon of abnormal staining behavior of human sperm flagella was reported. Such spermatozoa were shown to be immotile, the motility disturbance being referred to epididymal dysfunction. This hypothesis was demonstrated by histologic examination of tissues from the rete testis and from different epididymal sections and by smears of epididymal fluid from the caput epididymidis. It was shown that the process of normal staining, which indicates progressive motility, occurs in the distal caput epididymidis and that staining of spermatozoa is mediated in the caput and corpus epididymidis. This observation is of great clinical relevance, as the diagnosis of chronic epididymitis, where a highly significant correlation between abnormal staining of flagella and impaired motility, occurrence of macrophages in the seminal plasma (demonstrated by monoclonal antibodies) and activity of α-galactosidase was found. These parameters improved after antiphlogistic/antiinflammatory therapy. The diagnosis of epididymal disorders is greatly facilitated by this observation; future studies will elucidate the underlying membrane-physiological substrate of this descriptive phenomenon.

ADVERSE EFFECTS OF CYCLOPHOSHAMIDE (CPA) ON PROGENY OUTCOME CAN BE MEDIATED THROUGH THE EPIDIDYMIS IN RATS. J. Qi*, R. Haile and B. Rosane, Dept. of Pharmacology and Therapeutics, Centre for the Study of Reproduction, McGill University, Montréal, Québec, Canada

CPA is an anticancer and immunosuppressive agent commonly used in men of reproductive age. Previous studies from our laboratory have indicated that CPA may have an adverse effect on fetal progeny outcome by affecting epididymal spermatozoa. To elaborate on this epididymal effect and to determine the site(s) which is the most sensitive to CPA treatment, three experiments were under way. (1) Male rats were treated with saline (group A: control) or CPA (group B: 6.6 mg/kg/day; group C: 10.0 mg/kg/day) by gavage for 1, 4, or 7 days. Each male was mated overnight with two females in proestrus. The females were killed and pregnancy outcome was assessed on day 20 of gestation. No marked effects was observed on pre-implantation loss at any treatment time among the groups, but there was a time-dependent dose-related increase in post-implantation loss. Post-implantation loss was increased after 4 days of treatment and reached a maximum of 40% in group C after 7 days of drug exposure (A: 4.8±1.9%, B: 28.8±2.8%, C: 39.2±4.4%). (2) Bilateral ligation of the efferent duct for 7 days did not affect male progeny outcome. The post-implantation loss produced by the combination of ligation and administration of CPA in group C (10, 30, 70 mg/kg/day) for 7 days was not different from that produced by CPA treatment alone (Control: 3.6±3.2%, Ligation: 3.9±3.9%, CPA: 3.6±3.2%; CPA plus ligation: 35.9±15.3%). (3) Treatment of male rats with a single dose of CPA (0, 10, 30, 70 mg/kg) four days prior to breeding resulted in a significant increase in post-implantation loss in the two high-dose groups (0 mg/kg: 3.0±2.3; 10 mg/kg: 4.6±1.9; 30 mg/kg: 21.4±5.0%; 70 mg/kg: 26.5±2.7%). Thus, treatment with CPA for 4 or 7 days has an adverse effect on progeny outcome, specifically on post-implantation loss. This effect occurs during spermatogenesis at the caput through the epididymis and is mediated in the caput and corpus epididymidis, but not in the cauda epididymidis. Together these studies provide conclusive evidence that spermatozoa, as they pass through the epididymis, are subject to the adverse influence of drugs. Supported by the Medical Research Council of Canada.

SUPPRESSION OF SPERMATOGENESIS BY A GnRH ANTAGONIST PLUS TESTOSTERONE IN NORMAL MEN DOES NOT AFFECT SPERM MOTION PARAMETERS (SMP)

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Administration of GnRH antagonists plus testosterone supplementation is proposed as a potential male contraceptive. This study evaluated whether sensitive SMP can be affected by the chronic administration of this treatment. Six normospermic men received daily subcutaneous injections of 10 mg GnRH antagonist (Nal-Glu) for a period of 20 weeks. Testosterone enanthate, 25 mg/week, was added to this regimen 2 weeks later. Computerized sperm motion (SM) analysis was performed in the control period, during antagonist plus testosterone administration until sperm counts decreased below 1 million/ml and then during the recovery period. After 30 minutes liquefaction semen samples were diluted with their own seminal plasma to 20 million/ml. Sperm motility (MOT), curvilinear velocity (VEL), linearity (LIN), amplitude of lateral head displacement (ALH), and beat/cross frequency (BCF) were measured using a computerized video system (CellSoft, Version 3.0). Sperm concentration started decreasing on week 4, following suppression of the LH and FSH serum levels below assay detectability. Mean MOT also decreased from 51.8±2.1% at baseline to 34.0% on week 4 to 21.1% on week 5. In contrast, VEL, LIN, ALH and BCF did not change in any of the subjects. Azoosperma was reached within 6 to 12 weeks after initiation of the treatment. During recovery, SMP were still unaltered in spite of reduced overall MOT and sperm density. These data suggest that combined administration of a GnRH antagonist plus testosterone causes a parallel decline in sperm MOT and counts, following suppression of serum gonadotrophins, with no changes in SMP. Sperm motion of residual sperm during suppression or recovery of spermatogenesis seem to remain unaffected by an overall decline in sperm motility.

ALTERNATIVE MODELS FOR THE EVALUATION OF FERTILITY DATA

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The ultimate test for determining the effectiveness of a treatment on germ cell function is fertilizing capacity. Whether these tests are performed in vivo, as with a fertility trial, or in vitro, using fertilization or zona-free egg penetration as an endpoint, a great deal of useful information can be obtained. However, as these types of trials only infrequently provide pure dichotomies in response, an appropriate statistic must be chosen to provide insight into potential differences between groups. Traditionally, investigators have chosen a procedure such as the t or χ2 test to differentiate between two samples. However, as the data may not be adequate to apply standard procedures, a form of ANOVA, or, for more complex designs, a form of ANCOVA. Any of these procedures may be appropriate when their respective underlying assumptions have been met. A problem frequently encountered with fertility data lies in the distribution of error variances, even following arc-sine transformation of percentages. In an effort to restore the homoscedastic distribution of variances, a log-linear transformation of fertility data, coupled to a weighting paradigm is proposed as an alternative methodology for evaluating these data in a linear model. Under these conditions a log odds model: \( p(x) = 1\left[1 + e^{-x}\right] \), where \( x = x_1 + x_2 + \ldots + x_n \), weighted as \( n \) = the number of the total fertilization events, is evaluated using a general linear model procedure. Even greater power can be obtained from fertility trials through the measurement of fertilizing capacity as a function of time. For example, in the fowl a single insemination leads to the production of fertile eggs over a number of days. The change in fertility over a given time is not constant, thus a nonlinear model is implied. This relationship can be effectively modeled utilizing an iterative least squares regression model: \( y = \frac{1}{1 + e^{-x}} \), which approximates a logistic function. These parameters are easily interpreted and can be useful measures of sperm fertilizing ability. Further comparisons relating to the duration of fertility are made using an extra-sums of squares F-test. The use of these types of models may enhance the interpretation and thus increase the utility of fertility data.
THE EFFECT OF CLOMIPHENE CITRATE ON SPERM MORPHOLOGY.
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Much controversy exists regarding the beneficial effects of clomiphene citrate (CC) in treating male infertility. In order to determine the effects of CC on sperm morphology, the semen analysis on 74 men were evaluated for intervals between 3 and 18 months of treatment. The changes were compared with 70 patients who received no medication. The patients in each category were divided into groups: A (1-3 months), B (4-6 months), and C (18 months). Morphology was evaluated using Kruger's strict criteria. Differences between the groups were compared using analysis of variance. A p value <0.05 was required for statistical significance. In the control group, there were no significant differences in % of normal forms (volume groups (5% normal/00%) between baseline and repeat analysis at any interval. Treatment with CC for 1 to 3 months did not adversely affect % normal forms (7.4 ± 4.3% before vs 7.1 ± 4.4% after). Treatment for greater than three months caused a highly significant decrease in % normal forms (group B 53% before treatment as compared with 4.9 ± 3.5% after treatment, group C 12.5 ± 5.5% vs 67 ± 4.3%). Three months of CC caused no change in the absolute number (expressed in 10¹²) of morphologically normal sperm (80 ± 18 before and 87 ± 9.5 sperm after treatment) but longer periods were associated with a decreasing trend (group B 10.1 ± 13.2 before and 5.6 ± 6.0 after, group C 2.5 ± 5.6 before and 10.3 ± 10.3 after). This data indicates a deterioration of sperm function as measured by percent normal forms in patients treated with CC for four to eighteen months. The clinical significance remains to be determined.

42. SUBCLINICAL VARICOCELE; ITS DIAGNOSIS BY DOPPLER EXAMINATION: RESULTS OF VARICOCECTOMY. C.B. Dhabuwala, Sayeed Hamid*, L.E. Perlmunt, Department of Urology, Wayne State University & The Detroit Medical Center Hospitals, Detroit, MI.

We define subclinical varicocele as the varicocele diagnosed by doppler examination of scrotum in patients where no varicocele was detected on clinical examination. The association of varicocele and infertility is well established. The significance of subclinical varicocele and its impact on fertility remains controversial. Doppler examination permits diagnosis of subclinical varicocele. We present our experience of treatment of varicocele diagnosed by doppler examination.

78 men were available for this study. 48 of the varicoceles were unilateral, 30 (79%) were diagnosed clinically (confirmed by doppler examination) group 1. In 30 (21%) patients the varicocele was diagnosed by doppler examination, group 2. All these patients underwent internal spermatic vein ligation. Repeated measures of multivariable analysis of variance show a significant improvement in sperm density and sperm morphology in both the groups, following varicocelectomy (p <0.01 & p <0.001), the same analysis does not show a significant improvement in forward motility (p =.446). 19 out of 38 (50%) men in group 1 became fertile, 3 out of 10 (30%) men in group 2 became fertile. There was no significant difference in fertility between the groups (p >0.05). Pre operative sperm density of > 20 mil/ml and spouse under 30 years of age were found to be good prognostic indicators of fertility.

43. INTRACAVERNOUS INJECTION THERAPY WITH COMBINATION OF VASAOCATIVE AGENTS IN IMPOTENT MEN. Sayeed Hamid*, C.B. Dhabuwala, J.P. Pontes*, Department of Urology, Wayne State University and Detroit Medical Center Hospitals, Detroit, MI.

We report our experience in combination intracavernosal pharmacotherapy (CIP) for the treatment of erectile dysfunction. We started using CIP due to development of increasing resistance to papaverine alone or papaverine and phentolamine combination. The rationale for using CIP was different vasoactive mechanisms of the agents will produce better erection and decrease the complication rate. Papaverine is a smooth muscle relaxant, phentolamine an alpha blocker, alprostadil (PGE1) is a vasodilator and smooth muscle relaxant.

The combination we use has 2.5 cc. of papaverine hydrochloride (30mg/cc), 0.55 cc. of phentolamine (5mg/cc), 0.05 cc. of alprostadil (500ucg/cc) and 1.2 cc of 0.9 normal saline mixed together to produce a dial of 4.5 cc. The dose range is 0.05 cc - 0.35 cc. of the above combination.

We have used CIP in 70 patients in the age range of 22-60 yrs. In 32 men erectile dysfunction was secondary to vascular insufficiency, in 20 to diabetes, in 5 to psychological factors and in 3 to mixed factors. There were only two patients (2.85%) in whom the erection at maximum dose of 0.35 cc was not of enough rigidity to achieve vaginal penetration.

We compare our experience in 100 patients, in whom we were using papaverine or papaverine, phentolamine combination, 66 patients (60%) did not have erections rigid enough for vaginal penetration at 60 mg. of papaverine or 30 mg. of papaverine and 1 mg. of phentolamine. Both the groups were comparable as per age and etiology of impotence.

At the present time 20 patients are using CIP in auto injection program. They are all satisfied with CIP. There have been no complications.

44. REPRODUCIBILITY IN MONITORING NOCTURNAL PENILE TUMESCENCE AND RIGIDITY. C. Bain*, A. Guay, Endo. Sect., Lahey Clinic Medical Center, Burlington, MA 01805

The evaluation of sexual dysfunction has improved with the advent of nocturnal penile tumescence (NPT) testing. This method measures penile rigidity (RigiScan®) by Dacomed) (NPTTR). Previous techniques may not have noted abnormal rigidity despite normal tumescence.

To test the reproducibility of NPTTR monitoring results, initial and repeat tracings done between 8-129 days (mean 39d) were compared in 17 patients ages 38-72 (mean 61). Three nocturnal patterns were identified. Group A (n=4) showed a normal pattern in patients felt to have psychological impotence. Group B (n=6) showed near total absence of tumescence and rigidity. Group C (n=7) showed decreased tumescence and/or rigidity along with dissociation between base and tip rigidity. Fifteen of 17 patients reproduced their initial NPTR pattern on repeat testing. Patterns which were not reproduced in the other two patients (Group A & C) were explained by (1) alcohol ingestion and (2) a febrile illness during the monitored period. Third NPTR tracings on these patients did reproduce their initial patterns. The RigiScan® is a useful and reproducible tool in the evaluation of male sexual dysfunction sensitive to even minor alterations affecting sexual dysfunction.
Quantitative Testicular Biopsy in Spinal Cord Injured Men: Comparison of Quantitative Micrometric and Cytometric Analysis


Spermatozoal abnormalities have been reported in the majority of men following spinal cord injury and contribute to the multifactorial etiology of their reproductive dysfunction. Thus far, few have studied seminiferous epithelium in this group by objective quantitative techniques. While both quantitative micrometric (QM) and flow cytometric analysis (FCA) are accurate and reproducible methods of quantitating spermatogenesis, the latter is simpler and permits needle aspiration as a method of tissue recovery. The objective of this study is to determine the concordance of QM and FCA as objective techniques in quantitating spermatogenesis. Incisional testicular biopsy was performed in 8 spinal cord injured men and the tissue specimens divided for QM and FCA. QM consisted of determining the mean tubular diameter, tubular wall thickness, and mean tubular concentration of Sertoli cells, and mature spermatids in ≥10 round seminiferous tubules/patient. Specimens were prepared for FCA by mechanical disruption of tissue specimens in Hank’s balanced salt solution. The prepared cell suspension was adjusted to a cell count of 3x10⁶/mL. Nuclear DNA staining was accomplished by the Vindelov method (Cytometry 3.31, 1983). A minimum of 10⁵ filtered cells were analyzed by a Becton-Dickinson flow cytometer and the DNA histograms analyzed to determine the percentage of cells in each ploidy compartment. In 4/6 men, the mean spermatid tubule concentration exceeded 10 and all 4 showed haploid dominant DNA histograms. We conclude that FCA compares favorably to the highly accurate but laborious quantitative technique of QM and is suited as a screening method for eligibility of spinal cord injured men for semen recovery techniques.
SIXTEENTH ANNUAL MEETING

VENOCULAR SURGERY AT THE OHIO STATE UNIVERSITY
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Venogenic incompetence has become an accepted etiology for erectile dysfunction. Surgical correction of this abnormality has been attempted with varying results. This is a report on twelve men having venogenic surgery at the Ohio State University. All men were evaluated for at least twelve months. Ages range from thirty-seven to fifty-two with a mean of forty-five years. Six men were diabetic and eight were hypertensive. Evaluation included hormonal studies, non invasive vascular studies, nocturnal penile tumescence testing, and dynamic infusion cavernosometry.

At one month following surgery eleven men claimed good erections. By one year six men were still spontaneously obtaining erections satisfactory for vaginal intercourse, while three men required self injection with pharmacologic therapy to accomplish sexual intercourse.

We believe venogenic surgery may be an alternative to penile prosthesis in selected patients.

PARATESTICULAR MYOSARCOMA-REPORT OF 2 CASES
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We report 2 cases of paratesticular myosarcoma. Case A, an 18 year old boy had a 4 cm scrotal mass removed surgically with the clinical impression of a hydrocele. Grossly, it showed the typical look of hemorrhage and solid white neoplastic tissue. Sheets of large polygonal cells were noted on histologic examination and there was infiltration of the epididymis. Transmission electron microscopy showed the presence of polygonal and occasional spindle cells. There was some evidence of basal lamina formation. Alternating thick and thin filaments were present in many of the cells and abortive Z disc material was seen in some of them. Occasional sarcomatous areas were present, supporting the diagnosis of rhabdomyosarcoma.

Case B, a 58 year old man, presented with a 6 mm cyst-like nodule in the upper scrotum. Within a month it had enlarged to 1.0 cm. It was movable and somewhat tender. 10 weeks later a 25 cm oval mass was removed. It weighed 28 gm and was firm, although there were some yellowish, myxoid areas. Histology showed a pleomorphic sarcoma with a predominantly spindle cell pattern. Immunohistochemical studies were positive for desmin and vimentin. Spindle cells in a loose focullent stroma were noted on electron microscopy. There was an external membrane and pericyclic vesicles. The cytoplasm contained some fine filaments and there were occasional dense bodies on the plasma membrane. The ultrastructural diagnosis was a poorly differentiated mesenchymal tumor with some evidence of smooth muscle cell differentiation. Removal of the mass was followed by a radical orchietomy.

Histological examination was instrumental in establishing the diagnosis in both patients. This was of particular importance in the case of rhabdomyosarcoma as a combination of surgery, radio- and chemotherapy can effect a cure. Therefore, a definitive diagnosis should be established as early as possible.

DIMODULATION OF HUMAN SPERM ANTIGENS BY LIGHT AND ELECTRON MICROSCOPY. H. Raedt and V.D. Rara, University of Virginia School of Medicine, Charlottesville, VA 22908.

We describe immunofluorescent and EM immunogold localization of cognate antigens exemplified by a panel of monoclonal antibodies (mAb), S19, S69, S71, S72, S75, and S77. Examination of the mouse testis by mAb S71 and S72 revealed a cell type, which did not react with S19 or S69. Em immunolocalization of the sperm with 95% ethanol followed by staining with the mAb and a FITC conjugated secondary antibody revealed that S69 recognized an internal tail antigen, while S71, S72, S75 and S77 recognized acrosomal proteins. Retention of immunoreactivity after fixation in various combinations of glutaraldehyde and paraformaldehyde and tannic acid was tested for using S69 and S77 by immunofluorescence microscopy. Little or no staining was observed, indicating that the epitopes recognized by these mAb were adversely affected by these fixatives. We therefore employed a pre-embedding immunogold staining technique prior to fixation with Karnovsky's buffer, osmium, dehydration and embeddng in Araldite. With mAb S19, gold particles were observed over the head and tail of the intact sperm. mAb S69 was observed associated with the fibrous sheath. The mAb S71, S72, S75 and S77, which required spera permeabilization to show their acrosomal loci by EM, did not immunoreact with the plasma膜ma at the EM level. Ultrastructural examination of sperm lacking acrosomes revealed association of S71 and S72 with the inner acrosomal membrane and with acrosomal matrix material. The mAb S75 and S77 appeared to be associated with the inner acrosomal membrane. Supported by WHO Anti-Sperm Tolerance Vimescence Steering Committee, NIH 222 HD03502 and HD03769.

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AGE DEPENDENT EXPRESSION OF ANDROGEN RECEPTORS IN RAT CAVERNOSAL SMOOTH MUSCLE CELLS IN CULTURE.

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Normal penile growth until puberty is an androgen dependent event that requires the presence of an androgen receptor. As the animal approaches sexual maturity, this androgen dependent event may become down-regulated resulting in cessation of further growth of the penis. In an attempt to determine in vitro, the correlation between cavernosal growth and the androgen receptor activity, rat cavernosal cells obtained from the corpora cavernosa of 21 (immature), 42 (pubertal) and 60 day (adult) old rats were grown in monolayer culture. Cells from the fourth passage were transferred to 6-well clusters and grown using choral threon serum until 7 more days. At confluency, cells were washed and incubated for 60 minutes at 37°C with various concentrations (0.1-2 nM) of [3H] R-1881 in absence and presence of excess of cold R-1881 (500 nM). The amount of bound steroid (fmol/mg protein) was determined and specific binding (the difference between total and NSB at a particular ligand concentration) calculated. No saturable R-1881 binding was observed for the cells from 42 and 60 day old rats while for the cells from the 21 day old rats the Bmax was 32 fmol/mg protein with a KD of 0.27 nM. The affinity of binding (half-maximal saturation) was 6.9 pM, 0.4 pM and 1.0 fM for the cavernosal cells from the 21, 42 and 60 day old rats, respectively. These data indicate that the measurable androgen receptor activity in the cavernosal cells of the rat penis progressively decreases from a high level during immaturity to non detectable levels at adulthood. This supports the use of such a cell culture as an appropriate model to investigate the biochemical events related to penile development and function.

...MEAN SEM (cpm/mg DNA)

INTACT SESAME OIL TP TP+4-MAPC TP+CA
15.483 10.18 106.744 30.248 17.871

Both 4-MAPC and CA inhibited 3H-thymidine incorporation when compared with TP alone (p < 0.001); however, there was no significant difference between the two inhibitors. Total DNA and total protein, were similar in the TP, 4-MAPC, and CA groups. A second study used the same experimental model and groups to examine the effect of 14 days treatment. 4-MAPC inhibited ventral prostate weight by 27% (p<0.002); whereas, CA inhibited it 74% (p<0.001).

These results indicate that 4-MAPC inhibits DNA synthesis in the rat ventral prostate. In castrated rats treated with TP (1 mg/kg/d) a high dose of CA appears to inhibit regrowth of the ventral prostate more than a high dose of 4-MAPC.

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MEMBRANE LIPID PEROXIDATION IN CAVERNOSAL SMOOTH MUSCLE CELLS FROM IMPOTENT MEN.

S.C. Sikka and W.J.G. Hellstrom. Tulane University School of Medicine, New Orleans, LA 70112.

Impairment of cavernosal smooth muscle function (relaxation) causes significant reduction in blood flow to corpora cavernosa resulting in organic impotence. In order to delineate the biochemical mechanisms in the pathogenesis of cavernosal smooth muscle damage leading to impotence, an in-vitro cell culture model was developed and characterized. Two to three pieces (4mm³) of human corpora cavernosa from men with arteriogenic impotence were removed at the time of prostate implantation. These were immediately placed in cold Dulbecco medium (DMEM) in presence and absence of butylated hydroxytoluene (BHT), which prevents further lipid peroxidation, and processed for (i) cell culture, (ii) morphological evaluations, and (iii) lipid peroxidation analysis. Cultured cells (80% confluence after passage one/wo) and tissue samples were studied for (a) expression of smooth muscle cell-specific structural filaments, desmin and alpha-actin (b) morphological evaluations by light and electron microscopy and (c) lipid peroxidation changes. Cavernosal cells, before and during culture, expressed desmin and alpha actin by both immunofluorescence and immunoperoxidase methods. Morphological evaluations showed the presence of smooth, reticulum, microchondria, thickened basement membrane incroded collagen and some degenerative changes in arteries and vacuolated appearance in smooth muscle cells. Lipid peroxidation (n moles malonylaldehyde/mg protein) in non-BHT treated samples (0.4 ± 0.1 in cavernous tissue and 0.3 ± 0.1 in cultured cells, n=6) was 3 to 4 fold more when compared to BHT treated tissue and cultured cells (0.1 ± 0.1 n=6). These studies indicated that smooth muscle cells from human cavernosa can be successfully grown and maintained in cell culture and that the biochemical mechanisms which may alter smooth muscle functions can be studied in this model.
THE incubation buffer consisted of 20mM ATP, 20mM MgCl2, 0.1mM coenzyme A, 5mM dithiothreitol, 380mM Triis [pH 8.4] and 30mM Fatty acid or lysophosphatidylcholine. Phospholipids were extracted with chloroform and methanol and spotted on washed LBS TLC plates which were run in a mobile phase consisting of chloroform:trichloroacetic:acetic:water [30:30:30:8 w/v]. Spots corresponding to standards were scraped and counted. Blank incubations were performed in the absence of acyl substrates. When 3H-16:0-1 was included in the incubation with unlabelled lysophosphatidylcholine or PC, a labelled PC product could not be isolated. Similarly, when 3H-lyso-PC was incubated with unlabelled 16:0 or 22:6, a choline containing ether lipid could not be detected in levels above the blank incubations. In contrast, both 16:0 and 22:6 were readily incorporated into ester-linked PC as has been previously noted. From these data we conclude that, under the conditions employed in our assays, ether lipids cannot be synthesized by ejaculated human sperm.

Sixteenth Annual Meeting

57 TESTICULAR STEROIDGENESIS IN VITRO IN YOUNG ADULT NON-OBSE DIABETIC (NOD) MICE
A.G. Amador1, A. McArthur1,2* and A. Bartke3.
Dept. OB/GYN, SIU School of Medicine, Springfield, IL 62794-9230; 2Abt. Anatomie & Zellbiologie, Universiteit U15, 0-7900, U1m, F.R.G.; 3Dept. Physiology, SIU School of Medicine, Carbondale, IL 62901-6512, U.S.A.

We have previously shown that male mice with inherited non-insulin-dependent diabetes, due to different alleles, have a partial resistance to the action of HCG (Amador et al., Horm. Res. 1986; Proc. Int. Cong. Androl. 1989). This defect appears to be an integral part of the pathology present in those mice. The present study was designed to determine if mice that have an inherited predisposition to develop insulin-dependent diabetes, would have similar testicular alterations. Thus, 3-4 month old NOD mice were compared to ICR mice of the same age. Histemites from each mouse were incubated with or without 12.5mM HCO3/m1, and media testosterone (1) was determined by RIA. Body and testes weights, but not seminal vesicle weight, were significantly reduced in NOD vs. ICR mice. Histemites from NOD mice produced less T than those of ICR mice. Also, stimulation with HCG was significantly less effective in NOD than in ICR mice. The present results indicate that mice with insulin-dependent diabetes have a partial resistance to the steroidogenic action of HCG, and this occurs prior to the onset of clinical disease.

58 INABILITY OF EJACULATED HUMAN SPERMATOZOA TO INCORPORATE EXOGENOUS FATTY ACIDS OR 1-HEXADECANOL INTO ETHER LIPIDS
R.E. Jones, B.K. Balle, S.R. Plymate, Madigan Army Medical Center, Tacoma, WA 98431-5000.

Over 40% of the phospholipids present in ejaculated spermatozoa are ether lipids. In nongermline tissues, ether lipids have been demonstrated to be synthesized from PC or lysyl-PGAC and long chain fatty acids. Alternatively, ether lipids can be produced by condensing lysyl-ether lipids. This study was undertaken to determine whether ejaculated sperm could synthesize ether lipids from a variety of precursors such as 3H-1-1-0-alkyl-6-glyceryl-3-phosphorylcholine (lysophosphatidylcholine - lysophosphatidylcholine - PAF), 1-4Cl-l-1-1-hexadecanol [16:0-0.1], phosphatidylcholine [PC], or glyceryl-3-phosphate (glyceryl-3-phosphate [GPI]). Ejaculates were obtained from fertile volunteers and were washed to yield approximately 107 sperm per assay. The incubation buffer consisted of 20mM ATP, 20mM MgCl2, 0.1mM coenzyme A, 5mM dithiothreitol, 380mM Triis [pH 8.4] and 30mM Fatty acid or lysophosphatidylcholine. Phospholipids were extracted with chloroform and methanol and spotted on washed LBS TLC plates which were run in a mobile phase consisting of chloroform:trichloroacetic:acetic:water [30:30:30:8 w/v]. Spots corresponding to standards were scraped and counted. Blank incubations were performed in the absence of acyl substrates. When 3H-16:0-1 was included in the incubation with unlabelled lysophosphatidylcholine or PC, a labelled PC product could not be isolated. Similarly, when 3H-lyso-PC was incubated with unlabelled 16:0 or 22:6, a choline containing ether lipid could not be detected in levels above the blank incubations. In contrast, both 16:0 and 22:6 were readily incorporated into ester-linked PC as has been previously noted. From these data we conclude that, under the conditions employed in our assays, ether lipids cannot be synthesized by ejaculated human sperm.


The reproductive system of the male dog is unique in that seminal vesicles and bulbourethral glands are the major site of sperm production. Thirty adult male Beagle dogs providing ejaculates containing at least 500 X 106 sperm with 90% motility were studied. The dogs' vasa were occluded percutaneously using a Vasoclude clip applying device through a small scrotal puncture hole. Dogs were sacrificed and semen analysis performed. The first 24 dogs were completely azoospermic 1 week following VC. In view of these unexpected results, we studied six more dogs to determine the specific course of sperm disappearance after VC. Semen was collected at 1, 3, 5, and 7 days post-VC. The results revealed that spermatozoa were almost completely absent 24 hours after VC.

N=6
Vol.m1 Count (x10^6/ml) Motility %
PRE-VC + SEM 4.01±0.41 510±66±0.5 90±7
POST-VC 1 DAY 3.69±0.10 1±1 0
3 DAYS 3.00±0.71 0 0
5 DAYS 6.03±0.33 0 0
7 DAYS 5.81±0.22 0 0

This observation indicates that sperm disappearance from the dog's reproductive tract following VC is much faster than in humans, where azoospermia often takes 2 to 3 months or 15 ejaculations to achieve. It is assumed that the seminal vesicle is an extra-epididymal site for storage of spermatozoa in several species. The absence of a seminal vesicle in the dog, coupled with the rapid clearance of sperm cells from the reproductive tract following VC is consistent with this hypothesis. (Supported by USAIID-DPE3050-A-00-8059-0015)

60 FAILURE OF PLATELET ACTIVATING FACTOR (PAF) ANTAGONISTS (BN52012 and WE82086) ON BLOOMER IN COUCHE TRICRANIAL PREGNANCY IN THE DOG. R.D. McClure1, R.A. Tom1, J.S. Pape2, and M. Aguilar3. Virginia Mason Clinic, Seattle, WA 98101; 2Dept. of Pathology, University of California, San Diego, CA 92103; 3Women's Hospital, IVF Program, Las Vegas, NV 89104.

PAF antagonists have been shown to affect gamete interaction and fertilization. We have examined 2 PAF antagonists, WEB2012 and BN52012, for their ability to block PAF induced changes in sperm function.

Media 10mM were aspirated from semen using a 150/450, 150.500.500.500 micropipet. After separation, sperm was washed 2X in BWW containing 0.2% FSA fraction and incubated for 30 min to affect capacitation. The sperm were equilpated to the antagonist (500 nM) followed by addition of PAF (5 min), washed in PSS, fixed in 1:3.7% formaldehyde solution (FA) and fixed in 45 min at 4°C, and acrosome reaction (AR) assayed with PFA labeled (PAF antibodies (Table 1). Alternatively, sperm was treated with anagolipid (AP) or HSA (CON) prior to PAF treatment, then labeled with PFA prior to the SPA such that different sperm populations (AP and CON) could be distinguished when the SPA was assessed using the same cell sorter employed (Table 2). ANOVA and Duncan's multiple range tests were also evaluated for sperm attachment and fusion to zona free oocytes. Oocytes were perfused with Rhodamine dye and were co-incubated for 30 min before evaluation (Table 2).

Table 1

<table>
<thead>
<tr>
<th>Effect of antagonists on PAF induced AR</th>
<th>10^4 M</th>
<th>10^5 M</th>
<th>10^6 M</th>
</tr>
</thead>
<tbody>
<tr>
<td>% AR (IC50/IC100)</td>
<td>50/25</td>
<td>15/15</td>
<td>10/10</td>
</tr>
<tr>
<td>% Motility</td>
<td>30/25</td>
<td>30/25</td>
<td>30/25</td>
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<tr>
<td>(PAF concentration 10^-7 M)</td>
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Table 2

<table>
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<tr>
<th>Sperm-ovocyte interaction HxA</th>
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<tbody>
<tr>
<td>CON</td>
</tr>
<tr>
<td>% sperm penetrated</td>
</tr>
<tr>
<td>% motile</td>
</tr>
<tr>
<td>% fusion/pair</td>
</tr>
<tr>
<td>% attachment</td>
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<td>% sperm penetrated</td>
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<td>% motile</td>
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<tr>
<td>% fusion/pair</td>
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<tr>
<td>% attachment</td>
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<tr>
<td>(anagolipid concentration 10^-7 M)</td>
</tr>
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</table>

A reduction in sperm motility was observed for both antagonists at 10^-7 M (Table 1), this was also noted for 10^-6 M PAF. BN52012 and WEB2012 were unable to block PAF induced AR, or affect sperm-oocyte fusion and penetration of zona free hamster oocytes in vitro under the conditions used in our mixed gamete interaction system.
Sertoli cells form junctional specializations with step B spermatids during puberty, an event presumed necessary for completion of spermatid differentiation. To determine if testosterone plays a role in the formation and/or maintenance of the Sertoli-spermatid junctional complex and the subsequent production of the attached spermatids, daily spermatid numbers were determined and number of maturation phase spermatids were counted by phase contrast cytometry of testicular homogenates. Daily sperm production/testis (DSP/T) was determined by dividing the number of spermatid per testis by the testicular parenchymal weight. Over both treatments, testicular and parenchymal weights were significantly decreased with age. Both DSP/T were highest at 21 months of age, but were significantly decreased at 26 months of age. DSP/T was uniform in adult mice, but increased to 18-21 months and decreased thereafter. Over all ages, DSP/T decreased in EDS-treated rats but returned to normal by three weeks post-treatment.

EFFECT OF AGE AND DIETARY RESTRICTION ON SPERMATOGENESIS IN MICE L. Johnson, M.B. May and D.L. Busbee. Department of Veterinary Anatomy and Public Health, Texas A&M University, College Station, TX 77843.

Dietary restriction (DR) extends the DNA repair capacity and life span of mice. Age-related changes in spermatogenesis and the effect of DR on these changes were evaluated. At weaning, mice were fed NIH-31 diet ad libitum or restricted to 60% of the ad libitum caloric intake and sacrificed at 6, 12, 18, 21, 25, or 26 months of age. Testicular and parenchymal weights were determined and number of maturation phase spermatids were counted by phase contrast cytometry of testicular homogenates. Daily sperm production/testis (DSP/T) was determined by dividing the number of spermatid per testis by the testicular parenchymal weight. Over both treatments, testicular and parenchymal weights were significantly decreased with age. Both DSP/T and DSP/T were highest at 21 months of age, but were significantly decreased at 26 months of age. DSP/T was uniform in adult mice, but increased to 18-21 months and decreased thereafter. Over all ages, DSP/T decreased in EDS-treated rats but returned to normal by three weeks post-treatment.

DAILY SPERM PRODUCTION, TESTOSTERONE AND TRANSIT TIME OF EPIIDIDYMAL SPERM. Over all ages, DR reduced epididymal weight (33.5 ± 1.3 mg vs. 22.7 ± 0.5 mg; P < 0.01), number of sperm (28.4 ± 1.3 x 10⁶ vs. 20.5 ± 0.9 x 10⁶; P < 0.01) and transit time (15.5 ± 1.9 days vs. 10.6 ± 1.0 days; P < 0.05). There was an age x diet interaction (P < 0.05) for number of epididymal sperm. The DR group had fewer epididymal sperm at all ages, but the difference between groups was greater in younger mice when DSP had not reached maximum levels in the DR group. Transit time of sperm in the mouse epididymis was very long, could vary widely, and was reduced by age and DR. Supported by Grants AG02260 and AG07734.


Aberrant or blind-ending tubules (BET) are found in the head of the epididymis of most large mammals (Henrich et al., 1978), but have not been documented in rodent species. Even in large mammals, the histology of these tubules has not been reported. Microdissection of the efferent ductules of the testis was performed on 20 adult Sprague-Dawley rats to determine the branching pattern of the ductules and the location and structure of blind-ending tubules. Four efferent ductules were found in the rat (mean = 6). The ductules formed as individual tubules from channels of the rete testis and merged with the ductules at the rete testis. Most ductules continued until a single ductule (terminus) was formed in the capsule of the epididymal head. Length to the first junction ranged from 20-41 mm. Length of the terminus ranged from 10-32 mm. The number of junctions ranged from 4-11. At least one BET was found in 60% of the testes and the greatest number found was 3. Most BET were diverticul in the conus region, but one originated at the rete testis and another at the terminus. BET were recognized histologically by the following features: large amounts of dense connective tissue surrounding the epithelium, smaller luminal diameters, lack of sperm in the lumen, reduced epithelial height, and the lack of lysosomes in the cytoplasm of noniliated cells. In conclusion, care must be taken not to confuse BET with pathology of regressed efferent ductules. In contrast to what is found in large mammals, it is doubtful that BET are normal in the epididymis and become deformed in the formation of sperm granulomas, because the ductules are very small and the epithelium contains actively beating cilia that beat in the direction of the intact tubules.
were noted while in Kreb's Ringer bicarbonate (KRB) media in a humid environment. It was found that there was an increase in specific activity (Aitken, et al., 1965). Data on different PG effects on motility is currently being analyzed. Differences were noted between all treatments, but none were significant. PGE1 was used in this study, whereas PGD2 was used in the previous study (Aitken, et al., 1965). Washed sperm (a 10 x 10^6 sperm/ml) were incubated at 37°C in 5% CO2 up to 4 h in a protein-free modified Tyrode medium (Parish, et al., 1988, BORJ 38:117) containing 1 mg Penicillin (MTM), 10% serum, and human follicular fluid. Daily samples of ODF collected during the 15-minute coincubation of swim-up sperm with PG were subjected to gel filtration (Sephadex G-25). The amount of ODF recovered in the fraction above 10,000 molecular weight was determined. The motility of sperm was not destroyed by the incubation. A total lipid fraction of follicular fluid containing oviduct fluid that stimulates sperm capacitation (Dickman, et al., 1968, PNAS 58:4000) was used in this study. Analysis after a 15-minute coincubation of swim-up sperm with PG showed that the fraction above 10,000 molecular weight was not destroyed. The activity of sperm was not destroyed by the incubation. A total lipid fraction of follicular fluid containing oviduct fluid that stimulates sperm capacitation was used in this study. Analysis after a 15-minute coincubation of swim-up sperm with PG showed that the fraction above 10,000 molecular weight was not destroyed. The activity of sperm was not destroyed by the incubation.
DNA UPTAKE BY MAMMALIAN SPERMATOZOA


Mouse epididymal and washed ejaculated rabbit spermatozoa were preincubated (63°C, 37°C) in capacitation medium (30 mg/ml BSA) before incubation (30-60 min; 5°C, 37°C) with 25-labeled pcdNA mouse met cDNA (9.0 kb), and the radioactivity determined after 3 washings. Uptake averaged about 2100 molecules DNA per mouse spermatozoon and 2700 molecules DNA per rabbit spermatozoon. Autoradiography performed with mouse and rabbit spermatozoa treated in the same fashion but with 25-labeled pcdNA met cDNA, revealed that DNA binding occurred primarily in the postacrosomal cap region although sometimes binding was also observed at the anterior portion of the sperm head and the tail. Anti-digoxigenin antibody only reacted strongly with mouse, rabbit and human spermatozoa treated with digoxigenin-labeled pcdNA met cDNA, after the membranes were permeabilized with digitonin, suggesting the intracellular localization of the DNA. Little or no uptake of 3H-labeled pcdNA met cDNA occurred when added to immotile/dead spermatozoa obtained by long term incubation in the absence of substrate or 30 paraformaldehyde-killed spermatozoa. Addition of 3H-labeled pcdNA met cDNA to mouse spermatozoa incubated for different lengths of time, showed a close relationship between the percentage of motile spermatozoa and DNA binding. These results imply that DNA is primarily taken up by motile spermatozoa. Supported in part by E. Boughnon Trust.

COLUMN SEPARATION OF SPERMATOZOA FROM SUBFERTILE STALLIONS. P. J. Casey*, R. R. Robertson*, L. M. Liu*, S. Boita-Espinosa* & E. Z. Drobnis, Dept. Reproduction, School of Veterinary Medicine andDiv. Reproductive Biology, Dep. Obstetrics & Gynecology, School of Medicine, University of California, Davis, CA 95616

A study was performed to investigate the ability of column separation techniques to isolate motile sperm from stallion ejaculates. Sperm from 5 stallions was extended with Texas A&M clean extender. Original motility used from 24% -80%. A 4 X 3 X 4 factorial design (n = 5) was used to compare column materials: Sephadex (hydrated in extender), glass wool, nylon wool, glass beads: column length: 2 cm, 3 cm, 4 cm; and centrifugation times: 30, 60, 90, and 140 sec. Columns were pre- treated with extender, centrifuged 60 sec (300 X g). 1 ml of extended semen was added to the top, and columns were centrifuged for the allotted time. The motility (M%), volume, and concentration were assessed to determine the percentage recovery of motile sperm (PR%) and percentage change in motility (PRC%). There were significant differences in PR for column type (p < 0.0001) and length (p < 0.0001); and in M% improvement (p < 0.0001) and PRC (p < 0.0001) for column type. Type Column | %R | %M | Length | %R
--- | --- | --- | --- | ---
Sephadex 45 ± 5 | 35 ± 5 | 2 cm | 63 ± 5
Glass Wool 25 ± 2 | 65 ± 5 | 3 cm | 47 ± 5
Nylon Wool 25 ± 3 | 54 ± 5 | 4 cm | 41 ± 3
Glass Beads 25 ± 3 | 44 ± 5 | 5 cm | 38 ± 5

A. B. C. Means in columns with different superscripts are different (p < 0.05). Centrifugation time and all first order interactions were not significant (p > 0.05). Between stallions, %M was different (p < 0.0001), with greater improvement for subfertile stallions with poor motility. We conclude that glass bead columns of 2 cm may be suitable for selection of motile sperm from semen of subfertile stallions.


Because live sperm change size and density depending on osmolality of the medium and associated water flux, it was hypothesized that the highly variable results reported for bull sperm density could be due partly to osmotic properties of the gradients. Nycodenz, a non-ionic, non-toxic, water soluble material was chosen because it is one of the few non-toxic materials available to form gradients of relatively low viscosity more dense than bull sperm. Following preliminary experiments, two ejaculates from each of two bulls were washed and diluted with 10 mM Ca++ and 10 mM sperum/m. Three fractions, 100% fresh (75-80 motile), 100% killed and and 50:50 mixture of sperm were prepared and 5 ml of these suspensions were layered on discontinuous gradients of 23, 24, 25 and 44% Nycodenz prepared with distilled water (220, 231, 237, and 407 mosmols respectively), and 24, 29, 29 (prepared with a Ty rods solution to give 342, 345, and 343 mosmols respectively) and 44% Nycodenz (as above). Sperm were centrifuged at 100,000 X g, 30°C, for 60 minutes. In the 23-25% hyponic gradients, 82% of the sperm in the fresh sperm treatment was recovered from the 23-25 layers (density=1.121-1.132 g/ml), and were 90% motile. In the killed sperm treatment 100% were in the 44% layer (density=1.123 g/ml). The 50:50 treatment was intermediate. In the hypotonic series motile sperm layered differently, but the dead sperm always penetrated to the 44% Nycodenz layer. These results indicate that the percentage of motile cells and osmolality greatly affect the estimated density of sperm cells and are important factors to consider in separation experiments.

CLINICAL FEATURES OF PERSISTENT MULLERIAN DUCT SYNDROME (PMDS) IN A HORSE. C. C. Card, R. A. Ball, H. M. Wendell, D. S. Schaffer, New York State College of Veterinary Medicine, Ithaca, NY 14853.

A 10-year-old, Thoroughbred horse was donated to the NYSVM for progressive neurologic disease, with a history of urination in the prepuce. The acquired neurologic disease was unrelated to the genitl abnormalities. Scrotum and palpable testes were absent; no secondary sex characteristics were present. A small penis with a normal urethral opening was palpated in the prepuce. A small penis was identified on necropsy. Two linear firm structures and a soft mass were felt in the pelvis per rectum. Accessory sex glands could not be palpated. Transrectal ultrasonography (5 mHz probe) confirmed the presence of 2 tubular (1.5 cm diam.) structures which converged at a small (4 x 1.5 cm), ovale, echogenic region near the neck of the bladder. A small glans penis, urethra, and urethral fossa were identified during endoscopy of the prepuce. Plasma testosterone (T) levels were undetectable initially as well as undetectable at 2 and 4 h after injection of 10,000 IU HCG. Resting plasma LH levels were 0.28 ng/ml, estrone sulfate levels were 397 ng/ml initially and 535 ng/ml 1 h and 272 ng/ml 4 h post HCG. Laporoscopy was used to visualize the sperm in the fresh semen treatment was recovered from the 23-25 layers (density=1.121-1.132 g/ml), and were 90% motile. In the killed sperm treatment 100% were in the 44% layer (density=1.123 g/ml). The 50:50 treatment was intermediate. In the hypotonic series motile sperm layered differently, but the dead sperm always penetrated to the 44% Nycodenz layer. These results indicate that the percentage of motile cells and osmolality greatly affect the estimated density of sperm cells and are important factors to consider in separation experiments.

A grey, 8-yr-old, Thoroughbred horse was donated to the NYSVM for progressive neurologic disease, with a history of urination in the prepuce. The acquired neurologic disease was unrelated to the genital abnormalities. Scrotum and palpable testes were absent; no secondary sex characteristics were present. A small penis with a normal urethral opening was palpated in the prepuce. A small penis was identified on necropsy. Two linear firm structures and a soft mass were felt in the pelvis per rectum. Accessory sex glands could not be palpated. Transrectal ultrasonography (5 mHz probe) confirmed the presence of 2 tubular (1.5 cm diam.) structures which converged at a small (4 x 1.5 cm), ovale, echogenic region near the neck of the bladder. A small glans penis, urethra, and urethral fossa were identified during endoscopy of the prepuce. Plasma testosterone (T) levels were undetectable initially as well as undetectable at 2 and 4 h after injection of 10,000 IU HCG. Resting plasma LH levels were 0.28 ng/ml, estrone sulfate levels were 397 ng/ml initially and 535 ng/ml 1 h and 272 ng/ml 4 h post HCG. Laporoscopy was used to visualize the sperm in the fresh semen treatment was recovered from the 23-25 layers (density=1.121-1.132 g/ml), and were 90% motile. In the killed sperm treatment 100% were in the 44% layer (density=1.123 g/ml). The 50:50 treatment was intermediate. In the hypotonic series motile sperm layered differently, but the dead sperm always penetrated to the 44% Nycodenz layer. These results indicate that the percentage of motile cells and osmolality greatly affect the estimated density of sperm cells and are important factors to consider in separation experiments.
ACROSIN ACTIVITY IN PATIENTS WITH IDIOPATHIC INFERTILITY. *Ashok Agarwal* and Kevin R. Loughlin, Boston, MA (Presentation to be made by Dr. Agarwal).

Acrosin is a sperm acrosomal enzyme that is involved in the acrosome reaction, the binding of spermatozoa to the zona pellucida and fertilization. This study was designed to determine whether sperm acrosin measurements can identify subgroups of infertile or subfertile patients that are not recognized by routine semen analyses. We measured the total acrosin activity of ejaculates in a group of 22 men (18 suspected subfertile patients and 4 fertile donors). The acrosin activity was measured in liquefied semen specimens using the methodology described by Kennedy et al. (J. Androl, 10:221, 1989). The acrosin assay consists of three main steps. First the spermatozoa were washed free of seminal plasma by centrifugation over Ficoll to remove the soluble proteinase inhibitors in human semen that can interfere with acrosin activity. The sperm pellet was subsequently suspended in buffer that has: 1) a detergent that facilitates disruption of the acrosome and releases the acrosomal enzymes; 2) a basic pH that allows activation of proacrosin into enzymatically active acrosin; and 3) a synthetic arginine amide substrate that, when hydrolyzed, releases a chromophoric product.

Finally the total amount of color developed after a 3-hour incubation period was measured spectrophotometrically. Thirteen patients in the suspected subfertile group had a mean acrosin value of 90.0 units/million sperm which is clearly in the abnormal range (<14 units/10^6 sperm). Three patients had a mean acrosin value of 20.1 units/10^6 sperm which is in the indeterminate range (16.0-25 units/10^6 sperm). Two other patients and four proven fertile donors had mean acrosin values in the normal range (<5.6 units/10^6 sperm). The mean normal controls had a mean acrosin value of 32.5 units/10^6 sperm.

The acrosin measurement results did not correlate with routine semen analysis parameters: count, motility and morphology. Our results suggest that acrosin measurements can provide an additional marker for assessing the functional competence of human spermatozoa and may clarify the cause of some cases of idiopathic infertility.

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Studies have implicated γ-proteins and protein kinase C pathways as signal transducing mechanisms of the human acrosome reaction (AR; Biol Reprod 42 (Suppl) 87, 1990; J Androl, in press). In somatic cells, enzymes within these pathways are modulated by receptor-linked GTP-binding proteins. C3a, Cholera toxin (CTX, 1 μμM), or pertussis toxin (PTX, 5 μμM NaF/10 μM ATP, (fluorooruminate) were used to evaluate γ-protein involvement in the human AR. The AR of washed (no albumin added) uncapsulated ejaculated spermatozoa was measured. 15-20 min after additions, by light microscopy (Biol Reprod 42 (Suppl) 87, 1990; Gamete Res 23:387, 1989).

Values are % AR above baseline (no additions) of 19.0 (18.4-19.6; 90% confidence limits given in ). 1 μμM A23187 and fluorooruminate combined gave an AR of 21.6 (19.1-24.2; higher than the maximal AR (ΔAR) induced by either A23187 (17,3 (14.8-19.9) or fluorooruminate (15.1 (12.7-17.7). Neither NaF alone or CTX, AR induction by CTX, but not PTX, required activation with dihydrothreitol. PTX-induced AR was 22.2% (from linear regression of transformed data) (PTX, 0.5 μμM, EDP = 5.3 μμM. 1 μμM A23187 increased the EDP for PTX (10μM); the curve changed from rectangular/hyperbolic to sigmoidal. AR was 52% greater than the A23187-induced AR. CTX-induced AR was 15.2%; EDP = 6.5 μμM. A small response, 5.4 (3.6-7.3), was produced by the CTX B subunit (0.35 μμM). The A subunit had no effect. PTX (0.95 μμM) decreased the EDP for CTX (2.3 μμM) with no change in AR (15.9%). Unlike the A23187-induced AR, neither PTX- nor CTX-induced AR was a first-order interaction. The above findings suggest that human AR may be modulated by multiple γ-proteins and may occur via separate, though intersecting, Ca-dependent and -independent pathways. Supported by NIH grant HD 15555.
INTERACTIONS WITH ACROSOMAL MEMBRANES

One of the crucial steps in the process of fertilization in mammals is the sperm-zona pellucida (ZP) interaction. In this interaction it is possible to distinguish two events, sperm-ZP binding and sperm-ZP penetration. It has been suggested that a serine protease located in the acrosome, acrosin, could be involved in either of these processes. In the present work we have evaluated the effect of three protease inhibitors upon human sperm zona binding and penetration. Mobile sperm were selected by a two-step Percoll gradient and then resuspended in modified Tyrode’s medium supplemented with 26 mg/ml BSA. The sperm concentration was adjusted to 1x10^7 cells/ml and the cells were incubated at 37°C for 4.5 h. After incubation, spermatozoa were treated with 100 µM of P-aminoazbenzamide (pAB) or with DPBS (control) for 30 min. Then, 2-3 x 10^6 spermatozoa/ml were added to each zona pellucida. Twenty minutes after addition, spermatozoa were evaluated by transmission electron microscopy for acrosomal status and location. Twenty spermatozoa were counted at magnification of 8000X. All evaluated spermatozoa were sperm-ZP bound and their acrosomes were swollen.

To test the ability of hFF to induce the AR, spermatozoa from a fertile man were treated with 0.5 mg/ml of charcoal charcoal treated with 10 µg/ml of P to the Charcoal-treated hFF improved AR-inducing activity (7±1% charcoal-treated vs 20±1% untreated hFF). The addition of 10 µg/ml of P to the Charcoal-treated hFF improved AR-inducing activity (7±1% charcoal-treated vs 16±1% charcoal-treated plus P). These data support the idea that P in hFF is responsible for inducing the AR in human sperm.

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ACROSIN ACTIVITY CORRELATES WITH FERTILIZATION IN VITRO

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Recognition of male reproductive disorders is determined by limitations of diagnostic tests. Each test may provide information about a portion of the seminal requirements for fertility. Acrosin, a protease constituent of the acrosomal space, affects sperm-oocyte interaction. The purpose of the present study was to evaluate the possible relationship between acrosin activity and fertilization in vitro. Semen samples were obtained from 70 couples undergoing IVF. Acrosin activity was assayed using a miniaturized, simplified enzymatic method on fresh and processed sperm. This commercially available method (ACCU-SPERM, OEM Concepts, Tom's River, New Jersey) calculates a relative value for acrosin activity and was measured by an observer unaware of the fertilization status. Acrosin activity did not correlate with parameters of standard semen analysis. The mean (± SD) acrosin activity index was highest when 1 or more mature oocytes fertilized (10.5 ± 6.0), compared to 5.5 ± 4.7 when no mature oocytes fertilized (P=0.001). Acrosin activity before sperm processing also correlated with the proportion of mature oocytes that were fertilized (r=0.55, P=0.001). Sensitivity of the assay was 62%, specificity was 82%. The positive likelihood ratio was 5.7, suggesting that acrosin activity is a suitable parameter to measure for the biochemical evaluation of one aspect of sperm function.

IMPROVED METHOD FOR DETECTION OF THE ACROSOME REACTION

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Sperm must undergo the acrosome reaction (AR) prior to fertilization. Current methods for detection of the AR include long incubation times for capacitating sperm prior to the addition of calcium ionophore (CI). We have developed a method for rapid detection of the AR that reduces capacitance time to 15 minutes. The process involves treatment with specific proteolytic enzymes followed by addition of CI. Liquefied, washed sperm samples were incubated at 37°C in supplemented medium with the enzymes for 15 minutes, after which time CI was added. Control samples with CI alone show an AR rate of ≤30%. With enzyme treatment, the percentage of sperm undergoing the AR upon addition of CI increases to ≥80%. After CI addition the samples were incubated at 37°C for 15, 30 and 60 min. No difference in the rate of the AR was found with time of incubation with CI. Treatment with the enzymes alone does not cause the sperm to undergo the AR. Computer assisted semen analysis found motility patterns typical of capacitated sperm after the enzyme treatment with no decrease in the percent motile. Probes used to detect the AR have been adapted to a "dot-blot" format using peroxidase labeling, eliminating the necessity of microscopic analysis. The assay can also be used with fluorescence microscopy if precise counts are required. Supported by NIH Grant AI433459682-01.

SPONTANEOUS INDUCED ACROSOMAL REACTION IN CAUCA

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In humans, the acrosome reaction (AR) is an event elicited by capacitation and sperm-oocyte interaction. In various species, capacitation may occur with or without sperm-oocyte interaction. In view of the species differences in capacitation, we have used a novel in vitro AR assay to test sperm from various bovine species. The assay is based on the hypothesis that the capacitation of sperm is associated with the appearance of an antigen, which will bind with a monoclonal antibody, in a manner similar to the AR. This study determined whether capacitated sperm from various bovine species could react with our monoclonal antibody, 3E10. The antibody is directed against the bovine acrosin (N. J. Alexander, personal communication). We found that capacitated sperm from all species tested showed a positive reaction with the antibody, as did capacitated sperm from Capra hircus, M. Bernard, R. M. Stein, and M. P. Stein.

THE SLOTH (Bradypus variegatus) IS A NON-HUMAN PRIMATE

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The sloth is a non-human primate that has been studied intensively to understand the evolution of primate behavior. The sloth is unique in that it spends most of its life in the tree tops. The purpose of this study was to determine the effect of arboreal life on the development of the brain in the sloth. We found that the sloth has a large cerebellum, which is thought to be involved in fine motor control. This is consistent with the sloth's arboreal lifestyle. The sloth also has a large brainstem, which is thought to be involved in the control of respiration and heart rate. This is consistent with the sloth's arboreal lifestyle. The sloth also has a large amygdala, which is thought to be involved in the control of aggression and fear. This is consistent with the sloth's arboreal lifestyle. The sloth also has a large hippocampus, which is thought to be involved in the control of memory and learning. This is consistent with the sloth's arboreal lifestyle.

SPONTANEOUS INDUCED ACROSOMAL REACTION IN CAUCA

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The male mammalian gamete undergoes changes known as capacitation of which, the acrosome reaction (AR) is a key event before being able to fertilize the egg. In vitro eliciting of these changes has not been successful with stallion sperm epididymal maturation seems completed in this species before cauda. Hence, it become of interest to elicit AR in stallion sperm taken from the cauda and characterize it by the triple staining technique modified from Talbot and Chacon (1981).

Samples were taken from 5 healthy stallions after castration and used if progressive motility was over 60%. One stallion was kept in captive medium and another had BSA added to a final concentration of 5 mg/ml. Sperm samples were incubated for 0, 2, 4 and 6 min at 57°C with 10% humidity, 5% CO2 and 95% air. Half an hour before and at 4 and 6 h of incubation 50 μg/ml of IF (lyophosphatidylcholine) was added to the BSA containing samples. Spontaneous AR was observed after 10 min of incubation in AR observed in 11.7% of living sperm when BSA was added, 18%, 20%, 5% and 20% of AR were found after 2, 4 and 6 h. IF induced 53.5% of AR in active sperm by 4 h.

In conclusion, cauda epididymal sperm have spontaneous AR. It can be induced by addition of BSA to the incubation medium and synchronized if LFC is added. Thus, in vitro reacted, capacitated living spermatozoans are obtained. They could eventually be used for interaction with oocytes and represent a new and promising tool for in vitro fertilization.
The incidence of antisperm antibodies in semen, serum and cervical mucous has been implicated as a cause of both male and female infertility. This retrospective analysis was done to determine the incidence of sperm antibodies in infertile patients presenting to our service. The indications for testing were significant sperm immobilization or agglutination, repetitive poor post-coital test or unexplained infertility. Semen samples were fixed and tested by indirect immunofluorescence and fluorescein-conjugated anti-human IgG antibody. Semen specimens were positive after dilution (1:10 and 1:100) for IgG and IgA. The IgG was primarily directed at the tail in the semen and the head and tail of menotropin induction and luteinizing hormone induced infertility. Antisperm antibody testing should be performed in both male and female infertility. This retrospective analysis of 24 sera from both male and female infertility, was performed to determine the incidence of sperm antibodies in infertile patients presenting to our service. Indications for testing were significant sperm immobilization or agglutination, repetitive poor post-coital test or unexplained infertility. Semen samples were fixed and tested by indirect immunofluorescence and fluorescein-conjugated anti-human IgG antibody. Positive controls were obtained by fixing semen and testing with enzyme conjugated anti-human IgG. Absorbance values were read on a spectrophotometer and values > 0.1 were considered positive. In IIBT, SP dilutions were incubated with fertile donor swim-up sperm, mixed with immunobead suspensions. Immunobead binding of 20% were considered positive. LA was performed by mixing 10μl of antigen-coated bead suspension with 200μl of SP and observing for variable agglutination after 2 min. Positive results were defined as > 20% immobilization or agglutination. Positive results were considered positive. In IIBT, SP dilutions were incubated with fertile donor swim-up sperm, mixed with immunobead suspensions. Immunobead binding of 20% were considered positive. LA was performed by mixing 10μl of antigen-coated bead suspension with 200μl of SP and observing for variable agglutination after 2 min. Positive results were defined as > 20% immobilization or agglutination. Positive results were considered positive.

A "POSITIVE" SOLUTION TO THE ANTI-SPERM ANTIBODY CONTROLS PROBLEM. A.M. McKain*, L.R. Rubin, Department of Urology and Obstetrics and Gynecology, Indiana University School of Medicine, 926 W. Michigan Ave., Indianapolis, IN 46223.

Patient anti-sperm antibody (ASA) status in Immunofluorescence ASA assays are generally determined against reference ASA positive and negative controls. Positive controls for this assay system are frequently unavailable, and often unreliable. Our laboratory has thus developed an artificially engineered positive control. Aggregated IgG was prepared by heating a 1 mg/ml solution to 63°C for 30 min and will nonspecifically bind to human sperm. Positive sera were performed studying 5 and 10 million sperm. All specimens were from previously studied ASA negative controls and washed twice with excess phosphate buffered saline (PBS) before addition of PFS (5%). Fifty microliters of aggregated IgG was added to each specimen and incubated 2 hours. The percent positive sperm was estimated using negative control as baseline in fluorescence microscopy, and flow cytometry. Ten million sperm resulted in 52.2% positive (+/ - 10.8) and 34.9% positive (+/ - 5.5) by the two techniques, respectively. Five million sperm resulted in 54.5% positive (+/ - 12.4) and 39.6% positive (+/ - 8.6) when studied the same day. Sensitization of sperm by this technique provides a means for monitoring the interaction of the fluorescence labeled secondary antibody with its primary antibody target. The inter and intra assay reliability demonstrated by this technique is critical to the proper diagnosis of asbestos status of infertility patients. Studies to determine the long term stability of aggregated IgG at -60°C are being pursued.

88 OUTCOMES OF SPERM BANKING FOR HODGKIN'S DISEASE

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Seventy men (age 17-43 years) with Hodgkin's Disease had pre-treatment sperm banking during the last 6 years. Sperm counts ranged from 2.4-274,3 million post-thaw, and inversely correlated with serum FSH and LH. Semen was cryopreserved using 6-7% glycerol (usually in egg-yolk buffer) and staged freezing, then stored under liquid nitrogen. Accumulated motile sperm post-thaw were used to project fertility potential and anticipated reproductive potential. Seventeen men had over 150 million total sperm post-thaw (55% recovery), suitable for at least one IVF cycle. Thirty-three men had 15-150 million motile sperm post-thaw (47% recovery) suitable for at least one cycle of IUI, IVF, or GIFT. Twenty men had less than 15 million total motile sperm post-thaw (33% recovery), requiring IVF and in some cases micromanipulation. All patients received multigent chemotherapy (usually ABVD-MOPP). One patient was lost to follow-up. Ninety percent of the patients have survived. Five men used stored sperm, and one pregnancy was achieved using IVF. Sperm quality did not correlate with disease stage and symptoms, suggesting that all men with Hodgkin's Disease should be evaluated for sperm banking. Progress in fertilization technology should improve the utilization of suboptimal cryopreserved sperm from Hodgkin's Disease patients.
90 THE EFFECT OF REFRIGERATION IN TEST-YOLK BUFFER ON CAPACITATION, THE ACROSOME REACTION, AND FERTILIZING ABILITY OF SPERM IN SELECTED IVF PATIENTS.

J. Carroll R.L. Urry, Division of Urology, University of Utah School of Medicine, Salt Lake City, UTA 84132.

We have previously reported that fertilization rates in selected IVF patients were significantly improved using sperm refrigerated in TEST-yolk buffer, as compared to sperm prepared by serum swim-up. While other studies have shown similar results, the data have been limited and varied in their effect on the fertilization rate and chromosomal abnormalities. In this study, we have analyzed sperm penetration capacity on 49% successful IVF patients and identified 147 patients with decreased penetration ability as measured by the SPA. These patients were divided into two IVF treatment groups, in which fertilization was compared. The results showed that the percentage of viable, acrosome-reacted sperm was increased after addition of cycle homospermatozoa R2187. The mean percent fertilization was 66.2 ± 0.9 % for refrigerated and fresh samples, and 47.1 ± 3.9 for the control group. The pregnancy rate was 28.0% compared to 11.3% for the control group. The percentage of viable, acrosome-reacted sperm was 20.0 ± 1.4 (mean ± SEM) for the control group and 30.3 ± 2.0 for the treatment group. The percentage increased significantly in both groups after addition of homospermatozoa R2187. We conclude that the percentage of capacitated and acrosome-reacted sperm increases in selected IVF patients and that the increase may contribute to increased fertilization rates in IVF.

91 RELATIONSHIP BETWEEN PREGNANCY OUTCOME AND OBJECTIVE SEMEN ANALYSIS IN INFERTILE COUPLES.

L.L. Rodriguez-Rigau, C. Ayala, E. Steinberger, Texas Foundation for Research in Reproductive Medicine, 7800 Fannin, Houston, TX 77054.

In a study of 136 couples, we evaluated the relationship between the presence of sperm motility, morphology, percent viability, and incidence of early pregnancy wastage in couples undergoing gamete intra-fallopian transfer (GIFT). The incidence of early pregnancy wastage was significantly related to the presence of sperm motility and when the total number of spermatozoa with good linear motility was decreased. Since in the GIFT procedure spermatozoa and ova are placed in the fallopian tube, the spermatozoa do not have to travel through the female reproductive tract to reach the site of fertilization. Therefore, the postulated role of a decrease in the number of spermatozoa with good motility in the incidence of pregnancy wastage is supported.

92 THE APPLICATION OF ASSISTED CONCEPTION TO THE TREATMENT OF MALE INFERTILITY IN PATIENTS WITH A MOTILE SPERM CONCENTRATION OF LESS THAN 5 MILLION PER MILLILITER.


These patients were divided into two groups based on the presence of motile spermatozoa. The group with 0-5 million/million and the group with >5 million/million. The group with >5 million/million had a pregnancy rate of 17%, while the group with 0-5 million/million had a pregnancy rate of 12%. These results suggest that the presence of motile spermatozoa is a significant factor in the success of assisted conception methods in treating male infertility.

93 EFFECT OF LUTEINIZING HORMONE (BOVINE AND EQUINE) ORIGIN ON IN VITRO MATURATION OF EQUINE OOCYTES.

P. Willott, A.R. Crickx, and B.A. Farquharson.

The effects of bovine and equine LTH on in vitro maturation of equine oocytes were investigated. The results showed that bovine LTH was more effective than equine LTH in promoting maturation. The difference in efficacy was attributed to the differences in the origin of the hormones.

94 SIXTEENTH ANNUAL MEETING
PREDICTION OF HUMAN IN-VITRO FERTILIZATION OUTCOME USING A MODIFIED SPERM PENETRATION ASSAY

M.C. Barlas, C.K. Gibson*, H.Kamilo*, M.R. Freeman*, C.M. Herbert, Department of OB/GYN, Vanderbilt University, Nashville, TN 37232.

Numerous methodologies have been proposed over the past 10 years to improve the ability of the sperm penetration assay (SPA) to predict male fertility potential. Our patients with low or no sperm penetration in the SPA want to know their prognosis in IVF. To answer this question, a prospective study was carried out in the same ejaculate. Semen from 67 men were divided into two aliquots. For IVF, the sperm were washed twice with Ham's F10 + 10% maternal serum, prior to doing a swim-up procedure. Mature oocytes were inseminated with 125,000 motile sperm/ml. For the SPA, each aliquot was diluted 1:1 with Test York Buffer (TYB). The tube was then refrigerated in a water-filled container for 42 hours at 4°C. Half of this sperm suspension was warmed up slowly by placing the container with the tube in a 37°C air incubator for 1 hour, then washed twice with BWW medium (SPA-SW). The other half was rapidly warmed up to 37°C by washing the sperm twice with warm BWW (SPA-RW). Both aliquots were adjusted to 10 million sperm/ml and 25 zona-free hamster eggs were placed in 100 µl droplets. Our SPA protocol correctly predicted IVF fertilization or its failure in 55 patients (80%) using SPA-SW protocol and in 61 patients (81%) using SPA-RW protocol. Six patients (5%) failed to fertilize human oocytes, but had penetration in the SPA. One of them had oocytes inseminated with donor sperm and achieved 100% fertilization. It is concluded that SPA results using the SPA-RW protocol accurately predict IVF outcome. There is still a small group of patients with false positive SPA results that cannot be diagnosed because this test does not measure the sperm's ability to bind, enter and traverse the zona pellucida.

SOURCES OF VARIATION OF THE SPERM PENETRATION ASSAY UNDER FIELD STUDY CONDITIONS


As part of a longitudinal study of human semen characteristics of unexposed workers, six monthly semen samples from 26 men were assayed using the sperm penetration assay (SPA). The semen was diluted in TEST-YOLK buffer and cooled in a refrigerated water bath. The samples on the water bath were packed in ice and shipped via next day delivery to a contract laboratory for the SPA analysis. The percent of eggs penetrated, the penetration index (PI = # swollen sperm heads/# penetrated eggs), and the fertility index (FI = # swollen sperm heads/# viewed eggs) were calculated for each sample. The mean, standard deviation both between (SD1) and within worker (SDw), interclass correlation (Rw), and the coefficient of variation for both total (CVw) and within worker (CVw) were determined. The Rw for the variables were ranged from 43% to 63% indicating good repeatability, but the CV ranged from 46 to 98 and the CVw ranged from 23 to 74 indicating poor precision. Unless the effect of a toxicant on sperm penetration is very pronounced, SPA under the conditions reported lacks the precision to provide significant additional information in the occupational field study setting.

SPERM HEAD MORPHOMETRY: FITTING POPULATIONS INTO TOLERANCE INTERVALS


Tolerance intervals having a 95% confidence that 75% of the observations will be included have been reported previously for sperm head morphometric parameters (7 Androl 11:32). These tolerance intervals were calculated from data collected during a longitudinal study of 45 unexposed workers (Cincinnati, OH: June 1986 – February 1987) using mean values per ejaculate. Sperm head morphometry was determined for six other unexposed worker populations using the identical methodology. These results were compared to the reported tolerance intervals. The populations were from Dillon, CO (n=15; May 1983), Hilo, HI (n=44; December 1983), Portland, OR (n=38; June 1984), Groton, CT (n=41; December 1984), Laurel, MD (n=33; June 1988), and Killeen, TX (n=29; July 1990). The tolerance intervals for each parameter included at least 75% of the ejaculates for most of the populations. The TX samples contained less than 15% of the ejaculates for area (52%), length (74%), and width (61%). This was apparently due to cells being larger in this population of men. The samples from CO and CT contained less than 75% of the cells for the perimeter measurement (63% and 68% respectively). With the noted exceptions, sperm head morphometry appears to be reasonably consistent across studies.

AN ANALYSIS OF THE FUNCTIONAL ABILITY OF SPERM FROM SEVEN SPERM MORPHOLOGY CATEGORIES

D.I. Carrell, P.L. Zebell, A.L. Ulivo, S.J. Coleman, A.L. Urry, Division of Urology, University of Utah School of Medicine, Salt Lake City, UT 84132.

Little is known about the differences between normal and various classes of abnormally shaped sperm to respond in assays which assess the functional ability of sperm. We have analyzed semen samples from 10 semen donors and 31 infertile patients to characterize the ability of sperm from seven sperm morphology categories (normal, large, small, tapered, immature, duplicate and amorphous) to undergo spontaneous acrosome reaction, acrosome reaction after incubation with calcium ionophore A23187, respond to the HOS test, and maintain viability over a 24 hour period in vitro. Samples were washed and resuspended in Ham's F-10 media supplemented with 8% heat-inactivated serum and analyzed at 0, 3, 6 and 24 hours for each of the above assays. Each assay was repeated for each individual class of morphology. Morphologically normal sperm were significantly increased in each assay compared to other morphology classes with the exception of sperm viability compared to tapered sperm and the percentage of viable, tapered sperm undergoing the acrosome reaction after exposure to A23187. The large, small, immature, duplicate, and amorphous sperm were significantly decreased (p<0.05) in all assays compared to tapered sperm. The HOS assay showed the greatest disparity between morphology classes. The percentage reacted was 49.2 ± 5.1 (mean ± SEM) for normal morphology, 26.3 ± 4.0 for tapered, 18.2 ± 2.5 for immature, and less for all other types. These data indicate a decreased functional ability for all types of abnormal sperm morphology classes analyzed, however, they indicate a need to characterize the classes of sperm morphology present in a sample and the role they may play in the fertilization process.
SIXTEENTH ANNUAL MEETING

97 COMPARISON OF FLOW CYTOMETRIC ANALYSES WITH CLASSICAL VIABILITY AND MORPHOLOGIC PARAMETERS OF BOVINE SPERMATOZOA. Scott A. Ericsson1, Duane L. Garner2, Troy W. Downing1 and Clifton E. Marshall3. Departments of Animal Science and Biolo, University of Nevada, Reno, NV 89557. 1Select Sires, Plain City, OH 43064.

Flow cytometric analyses of spermatozoa were compared with the classical assessments of motility, head abnormality, acrosomal status and the presence of vacuoles/agglutinates. The flow cytometric analyses, which utilized two different fluorescent staining protocols (1 and 2), were used to estimate spermatozoal viability, morphology and morphology. These staining combinations assessed the functional capabilities of spermatozoal plasma membranes, mitochondria, and acrosomes. Plasma membrane integrity was assessed with dihydroethidium (HED), carboxydimidylfluorescein diacetate (CDMFD) or propidium iodide (PI). Acrosomal membrane integrity was examined using fluorescein isothiocyanate-labeled Pterum sativum agglutinin (PSA). Mitochondrial function was estimated by the level of rhodamine 123 (R123) staining. Protocol 1 consisted of PI, R123 and CDMFD; and protocol 2 consisted of HED, R123 and PSA. Thawed, cryopreserved samples from 8 bulls were fluorescently stained and incubated for 1.5 hr prior to flow cytometric analyses. These analyses were repeated after 4 hr. These flow cytometric analyses were compared with classical sperm quality assessments. Specific fluorescent staining patterns indicated that the percentage of viable spermatozoa was negatively correlated with the percentage of abnormal heads (r=-0.8, P<0.05). The percentage of dead spermatozoa was positively correlated with the percentage of abnormal heads (r=0.73; P=0.05) and negatively correlated with intact acrosomes at both incubation times (r=-0.73; P<0.05). Tri-functional staining systems 1 and 2 provided quantitative information relative the quality of cryopreserved bovine spermatozoa. Supported, in part, by grants USDA-89-37240-4735, NSF-DMB 8518021.

96 TRI-FUNCTIONAL CYTOMETRIC ANALYSES OF FERTILITY-TESTED CRYOPRESERVED BOVINE SPERMATOZOA. Scott A. Ericsson1, Duane L. Garner2, Troy W. Downing1 and Clifton E. Marshall3. Departments of Animal Science and Biolo, University of Nevada, Reno, NV 89557. 1Select Sires, Plain City, OH 43064.

Two fluorescent staining protocols (1 and 2) were used to estimate the potential fertilizing capacity of spermatozoa. The integrity of plasma membranes was assessed using dihydroethidium (HED), carboxydimidylfluorescein diacetate (CDMFD) or propidium iodide (PI). Also, acrosomal membrane integrity was assessed with fluorescein isothiocyanate-labeled Pterum sativum agglutinin (PSA) and mitochondrial function was determined using rhodamine 123 (R123). Protocol 1 consisted of PI, R123 and CDMFD; and protocol 2 consisted of HED, R123 and PSA. Cryopreserved samples from 8 bulls were thawed, stained and incubated for 1.5 hr prior to flow cytometric quantification. Duplicate aliquots were analyzed 4 hr after thawing. Fertility information on the ejaculate nonreturn (ENR), which was acquired from one ejaculate for each bull, and cumulative nonreturn rates (CNR), which were from many different ejaculates for each bull, were obtained. The relative green and red fluorescent intensities of individual spermatozoa were quantified using FACS Quadrant analyses to identify the percentage of viable, morphubd and dead spermatozoa and the cellular debris. Multiple regression analyses were used to measure the relationship between these parameters with ENR and CNR. Analysis of protocol 1 stained spermatozoa showed that ENR could be predicted by the percentage of viable and dead spermatozoa at 1.5 hr and all phenotypes at 4 hr (R2=0.99, P<0.05) and the CNR by the percentage of dead cells at 1.5 hr and morphubd spermatozoa at 4 hr (R2=0.75; P<0.05). Analysis of protocol 2 stained spermatozoa showed that ENR could be predicted by the percentage of viable and morphubd spermatozoa at both incubation times (R2=0.39; P<0.05) and the CNR by the percentage of viable and morphubd spermatozoa at 1.5 hr and viable spermatozoa at 4 hr (R2=0.99, P<0.05). Tri-functional staining provided valuable information predictive of the fertility potential of cryopreserved bovine spermatozoa. Supported, in part, by grants USDA-89-37240-4735, NSF-DMB 8518021.


This study compared the results for sperm density from Cell Trak (Molecular Analysis Corp.) to those from a manual system using a hemocytometer and WHO methodology. Although similar comparisons have been undertaken, none have thus far validated Cell Trak. Phase I assessed repeatability of results by each method. Phase II assessed agreement in results between the two methods. Method: Sperm from Fertility Clinic and donors, collected into sterile containers via masturbation after 48 hrs abstinence, was allowed to liquefy and then was evaluated for density. In Phase I each of 52 samples was counted twice manually, and twice on the Cell Trak using a Makler Chamber by an experienced lab tech. In Phase II each semen sample was counted manually, then counted on Cell Trak to compare different calibration setups: (1) 15 frames (F) at 15 frames per second (FPS); (2) 60 FPS at 60 FPS; (3) 15 F at 30 F/S; (4) 2 F at 30 F/S; and (5) 2 F at 15 F/S. All data were analyzed after log conversion by the statistical methods of Bland and Altman. Results: In Phase I both manual and automated methods were found to have good repeatability. Manual densities were in agreement for 95% of the samples, and automated densities were repeatable in 98% when agreement is defined as at least 95% of differences between 2 measurements on the same sample fall within ± 25% of the mean of differences for all samples. However, analysis of agreement between the 2 methods revealed that the automated density could be up to 180% higher or 57% lower than the manual density. In Phase II analysis for densities between the 2 methods found that automated setting 1 produces results from 88% above to 5% below the manual method; setting 2 20% more to 72% fewer; setting 3 100% more to 61% fewer; setting 4 84% more to 61% fewer, and setting 5 92% more to 44% fewer than the manual density. These data suggest that additional calibration of Cell Trak is needed to achieve agreement with the currently accepted WHO method of deriving sperm density.


Rabbits were chosen as a test species for computer automated semen analysis because of the ease of collecting semen and controlling both ovulation time and insemination dose, so as to relate sperm characteristics to fertility. However, granules in rabbit semen can interfere with the analysis. To establish critical sperm numbers, superovulated does were inseminated with 1000 x 106, 500 x 106 and 100 x 106 sperm. Fertilization rate was 66, 81 and 64% respectively (n=8, P<0.05). Another trial, with 100 x 106, 50 x 106 and 25 x 106 sperm, resulted in 63, 68 and 41% Fertilization (n=119, P<0.05). For the automated semen analyses a Hamilton-Thorn 2030 unit was used to analyze sperm recorded on 3/4" video tape, using a DX negative phase objective and a 6X photo objective. Granule interference observed initially was reduced by recording with less light so that the brightest granule in the field did not exceed the limit of the HT-2030 to discriminate on the basis of brightness. A variety of sort functions were used to reduce granules detected as sperm. The percentage of progressively motile sperm was defined as those sperm with a velocity of >50 µ/sec and a straightness of >80%. Three Makler Chambers and nine fields per chamber were replicated (S4 fields) in analyzing a single ejaculate used in fertility trial 2. The range in progressively motile sperm was 42 to 85%. Progressively motile sperm and fertility were correlated (r=0.33). Further refinements in instrumentation and sperm separations with low numbers of sperm are planned. Supported by EPA contract CR815428-01.

Our studies reveal that: 1) teratospemia in androgen-deficient domestic cats compromises zona pellucida (ZP) binding and penetration; and 2) swim-up separation to increase the number of normal sperm does not increase sperm-ZP interaction. Many nondomestic fields, including the cheetah, ejaculate high proportions of morphologically abnormal sperm. This study examined the utility of ZP-inclined, salt-stored domestic cat oocytes (1) assessing the ability of cheetah sperm to undergo capacitation and/or the acrosome reaction and penetrate ZP in vitro, and (2) determining the influence of sperm morphology on ZP binding and penetration. Donors were 21.5±1.6 yr old and ranged in age from 18 to 27 yr. Samples were collected from 10 females. Sperm were washed, pre-incubated (4x10^6/ml spermatorr) for 1 h, then co-incubated (2x10^6/ml spermatorr) with ZP for 10 h at 38°C. Oocytes (n=360) were assessed for % ZP penetration (defined as the proportion of oocytes containing sperm within or through the inner ZP). Mean (+SEM) number of ZP-permeated sperm and the morphology of all bound and penetrated sperm were assessed. earrings ejaculated averaged high sperm motility (70.5±5.9%) with low numbers of normal sperm (24.9±3.4%). Abnormal sperm forms included head (8.3%), midpiece (27.0%) and flagellum (39.3%) defects. Cheetah sperm contained ZP in the presence of BSA (24.5±5.8%) and FCS (49.1±9.4%), however, penetration was lower (P<0.00) when sperm were incubated in protein-free (PVA) medium (7.4±3.4%). The number of ZP-permeated sperm was 3.8±1.1-fold higher (P<0.05) in BSA (0.62±1.3) and FCS (0.60±0.9) compared to PVA (0.07±0.03). The morphology of bound sperm included normal forms (41.5%) and sperm with midpiece (43.6%) and flagellum (14.9%) defects, but every sperm within or through the inner ZP was morphologically normal. These results indicate: 1) abnormalities of spermatozoa compromised zona pellucida (ZP) binding and penetration; 2) ZP binding and penetration of sperm to oocytes is a useful tool for evaluating factors influencing capacitation and acrosomal reaction; and 3) this heterologous zona pellucida (ZP) assay may be useful for assessing factors influencing the capacitation of other species.
COMPARISON OF GLYCEROL AND 1,2-PROPANEDIOL AS CRYOPROTECTANT AGENTS FOR HUMAN SPERM


To achieve acceptable post-thaw motility of human sperm, cells must be frozen in the presence of cryoprotective compounds. In part due to historical protocols, and in part due to their proven efficacy, the standard cryoprotectant for human sperm has been glycerol; in spite of its apparent adverse effects on motility. Although 1,2-propandiol (PrOH) is effectively used as a cryoprotectant for other cell types (eg, embryos) its use has not been reported for the cryopreservation of spermatozoa. Therefore, this study was designed to test the hypotheses that human sperm cryopreserved using PrOH would possess greater (1) post-thaw motility and (2) acrosomal integrity than cells frozen with glycerol. Washed human sperm was aliquotted into non-frozen controls or fractions diluted 1:1 with media containing 1M glycerol, 2M, 1M, 0.5M, 0.1M PrOH, or no cryoprotectant. Diluted fractions were frozen in a programmable freezer and stored in liquid nitrogen. Sperm samples were obtained from the distal cauda epididymidis and analyzed for (1) motility, using CASA, and (2) plasma and acrosomal membrane integrity, using indirect immunofluorescence (76 mononuclear antibody). Results indicate that cells frozen in glycerol had greater (P<0.01) post-thaw motility than those in PrOH [motility: 43.8 ± 8 (SE) SEM vs 19.1 ± 2.8, 18.244 ± 7.1, 15.246 ± 0.0, 5.3 ± 2.1, and 5.0 ± 2.2 for 1M glycerol, 2M, 1M, 0.5M, 0.1M, and no cryoprotectants, respectively]. The percent sperm without acrosomal integrity was not different (P>0.01) among control (28% ± 15.6) or 1M (34.9 ± 3.7) and 0.5M (14.2 ± 3.7) PrOH treatments; while 1M glycerol treated cells (41.5 ± 4.0) had a higher frequency of acrosomal damage than controls. These data do not support the hypothesis that PrOH is an effective cryoprotectant for human sperm motility; but do support the hypothesis that PrOH may serve as an effective cryoprotectant for acrosomal integrity. This work was supported, in part, by a grant from the NIH. (R01 HD25949-01). 76 mononuclear antibody was graciously supplied by D. P. Wolf, Oregon Regional Primate Research Center, Beaverton, OR.


The objective of these studies was to optimize the preparation of rat sperm samples for computer-assisted sperm analysis (CASA). Methodological issues investigated included sample collection technique, type of diluent used, and sample chamber depth. Sperm samples were obtained from the distal cauda epididymis and analyzed with theHamilton HTM-2000 motility analyzer (Hamilton Thorne Research, Danvers, MA) for 13 frames at 19 frames/sec, software version 6.5R. When small cuts were made into the tubules, and the sperm allowed to disperse into a petri dish of medium, the sperm exhibited significantly higher and less variable motility than sperm collected by an aspiration method; 87 and 56%, respectively. Transferring a small amount of expressed epididymal fluid to a medium-filled Vial also resulted in high motility. Of the four media examined, all maintained a high percentage of motile sperm (27±4%) over an hour long incubation period. However, sperm maintained in Medium-199 or modified HBSS had significantly greater straight-line velocity (96 and 103 μm/sec, respectively) compared to sperm maintained in Dulbecco’s PBS with or without glucose (70 μm/sec). The motility and velocity endpoints of sperm in 200, 100, or 40 μm-deep chambers were not significantly different but the percentage motile, straight-line velocity, and average path velocity of sperm in 20 μm-deep chambers were 71%, 61%, and 74 μm/sec, respectively, of values obtained for sperm in 200 μm-deep chambers. Since these and other variables in the preparation and analysis of rat sperm influence the motility endpoints, it is important to know the conditions under which the rat sperm samples were prepared and consider these issues when interpreting CASA data.

This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

107 THE USE OF THE KRUGER STRICT TEST FOR SPERM MORPHOLOGY IN DISTINGUISHING THE FERTILE FROM SUBFERTILE MALE. E.J.H. Cheek, H. Adelson*, A. Bollendorf, The University Of Medicine And Dentistry Of New Jersey, Robert Wood Johnson Medical School At Camden, Cooper Hospital/University Medical Center, Department OB/GYN, Division Of Reproductive Endocrinology & Infertility, Camden, New Jersey.

There are reports suggesting that the Kruger strict test for sperm morphology may be a very reliable predictor of poor fertilization during in vitro fertilization. Since it is even more difficult to fertilize in vivo, a priori, one would expect a poor Kruger test to even better predict the subfertile male trying to achieve a pregnancy in vivo. If <4% predicted poor fertilization in vitro, perhaps an even higher level might still predict a subfertile male trying a natural pregnancy. A retrospective study of 236 couples (with at least 1 year of infertility where 2 baseline Kruger tests were performed on the male partner) was performed to evaluate the correlation of the strict morphologic sperm test and in vivo conception. Pregnancy rates in 6 months were determined in couples where all female infertility factors were considered corrected and were correlated with the Kruger scores in the males. The data was further subdivided according to whether the motile densities were <10x10^6/ml or ≥10x10^6/ml. When the Kruger test was ≥4% 14 of 28 (50%) conceived versus 46 of 77 (58%) when the level was <4%. Interestingly, 3 of 4 couples where the male had a level of 1 and the motile density was <10x10^6/ml conceived as did 2 of 3 with low motile densities and Kruger’s of ≥4%. The data prompted a prospective study where any patient with a Kruger score ≥4% was matched with the next infertile couple where the Kruger score was ≥4%. There were 14 of 34 (41%) pregnancies in 6 months with Kruger tests ≥4% compared to 30 of 34 (90%) with Kruger scores ≥14%. Though in this group not all female factors were corrected the >14% group did not seem to have a disproportionate amount of very severe female factors. Unfortunately, we were not able to share the same enthusiasm about the Kruger test in distinguishing fertile from subfertile donors no matter whether the motile density was <10x10^6/ml or greater.
110 PROGRESSIVE MOTILITY OF PROCESSED SPERM PERMITS IMPROVED PREDICTION OF SPERM PENETRATION ASSAY OUTCOME.

WE Nolten, WR Boone*, R Abdoulaev*, SP Viosca, SG Kraymen and SS Shapiro*, Departments of Medicine and Obstetrics, Gynecology, University of Wisconsin, Madison, WI 53792 and Department of Medicine, UCLA, Los Angeles, CA 90024.

We examined semen samples from 52 infertile couples to determine whether computer-assisted semen analysis parameters from unprocessed ejaculate (RAW) or from sperm which had undergone washing and swim-up procedure (WASH) would predict the outcome of the sperm penetration assay (SPA). Of 30 evaluated parameters the following were found to discriminate best between abnormal (<10%, n=30) and normal (≥10%, n=22) SP: percent total (incl. rapid linear, slow/ nonlinear and unclassified) progressively motile sperm (T), percent unclassified progressively motile sperm (U), and percent immotile sperm (I), as defined by WHO (1987).

RAM: abnormal normal P

\[
\begin{array}{ccc}
T & 31.3±2.5 & 42.0±2.9 \times 0.008 \times 4.8±2.8 \times 7.4±2.6 \times 0.005 \\
U & 10.1±1.1 & 16.1±2.5 \times 0.020 \times 24.6±2.3 \times 41.3±3.9 \times 0.003 \\
I & 59.0±2.9 & 48.0±2.4 \times 0.026 \times 28.6±2.6 \times 16.6±2.1 \times 0.014 \\
\end{array}
\]

The following correlations were found: 1) SP vs. T: (RAM) r=0.405, p=0.0029; (WASH) r=0.423, p=0.0018. 2) SP vs. U: (RAW) r=0.46, p=0.0005; (WASH) r=0.623, p=0.001. 3) SP vs. I: (RAM) r=0.390, p=0.042; (WASH) r=0.428, p=0.015. Proportions of T, U and I showed significant differences in normal and abnormal sperm function and correlated with SP. Discrimination between sperm with normal/abnormal SP was improved by the inclusion of measurements of progressive motility following WASH, a procedure that mimics the natural sperm selection prior to in vivo fertilization. These parameters provided stronger predictability of SP than did the traditional semen analysis of RAW ejaculate, but still could not substitute for the SP assay.

112 IMPROVEMENT IN SEEN QUALITY AFTER FILTRATION THROUGH L4 MEMBRANE, COMPARISON OF RESULTS WITH SWIM-UP TECHNIQUE.

Ann Mangione, Ashok Agarwal, Kevin R. Laughlin, Boston, MA (Presentation to be made by Dr. Agarwal).

A leucocyte adsorption medium (PLH Biosupport Corp., NY) is a fibrous polyester sheet used to separate white blood cells from blood by their adsorption to the fiber surfaces. These filters were used in our laboratory to filter semen specimens (10 suspected subfertile patients and 5 fertile donors) to assess changes in semen quality after routine sperm washing and swim-up versus filtration through the L4 membrane. Liquefied specimens were analyzed by a Cell-Soft semen analyzer. Seven specimens were divided equally and one aliquot was processed by sperm washing and swim-up technique and the second aliquot was used for filtration. The L4 membrane was cut into 16 mm diameter discs to fit in 12.5 ml Eppendorf pipette tip. Two discs were placed at the bottom of the pipette tip. Sperm and media were mixed with 5.0 ml of human tubal fluid medium by vortex and passed through a 0.25 mm diameter disc by gravity. The filtrate was collected in 15 ml polystyrene centrifuge tubes. The L4 membrane was rinsed with 4.0 ml of fresh medium to collect remaining spermatogonia from its surface. Filtered specimens were centrifuged at 500g for 5 minutes. Supernatants were carefully aspirated by a Pasteur pipette and discarded. Sperm pellets from both aliquots (L4 and swim-up) were suspended separately in 0.5 ml medium and a complete semen analysis was performed on final resuspended specimens.

The comparison of semen analysis results between the two techniques showed significant improvement in sperm quality after filtration through the L4 membrane. The percentage of total and motile sperm count recovered after filtration showed a small increase as compared to the swim-up method. Total sperm count remained at 25% (swim-up) to 28% (L4) and motile sperm count increased from 34% (swim-up) to 40% (L4). Sperm motility on the other hand showed an increase from 63% (swim-up) to 68% (L4) (P<0.002). The percentage of amino acid increased from 47% (swim-up) to 52% (L4) (P<0.048). Our results demonstrate that sperm filtration through L4 membrane gives superior results as compared to traditional swim-up technique.
SIXTEENTH ANNUAL MEETING


A recently introduced column filtration system (Sperm Prep, Polymedco, Yorktown Heights, N.Y.) was compared to the widely used swim-up method and simple sperm washing. Thirty-nine frozen-thawed samples were used in this study to examine whether the column filtration method would improve percent recovery and the total motile sperm for insemination with frozen-thawed samples. After thawing, each sample was divided into three aliquots, one was washed, the second was subjected to column filtration and the third was prepared by the swim-up method. Results are expressed as means with standard errors in parentheses.

**TOTAL MOTILE RECOVERY VELOCITY NORMAL**

<table>
<thead>
<tr>
<th>Method</th>
<th>Percentage Recovery</th>
<th>Velocity (v/sec)</th>
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<tbody>
<tr>
<td>Wash</td>
<td>30.2 ± 1.8</td>
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</tr>
<tr>
<td>Column</td>
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<td>71.5 ± 1.5</td>
</tr>
<tr>
<td>Swim-Up</td>
<td>61.9 ± 1.5</td>
<td>83.0 ± 1.5</td>
</tr>
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</table>

115 THE USE OF A SPERM "SWIM-DOWN" PROCEDURE FOR PROCESSING FROZEN-THAWED HUMAN SEMEN. Mark S. Siegel and Judy W. Wang. Dept OB/GYN, University of Southern California, Los Angeles, CA 90033.

It was previously reported in our laboratory that the recovery rate of motile sperm obtained from frozen-thawed semen following swim-up was very poor (5-7%). In the present study, a "swim-down" or self-migration/sedimentation method was developed and the recovery rate of motile sperm from frozen-thawed semen was assessed. Frozen semen from 9 donors was thawed at 37°C for 20 min. Thawed semen (0.4 ml) was mixed with Hart's F-10 medium (1% HSA) in a 15 ml graduated polystyrene centrifuge tube and allowed to swim-down for 0.5 or 1.5 hr. Seven 200 ul aliquots of the sperm mixture were sequentially removed from the bottom fraction (top 1) to the top fraction (1/4). Aliquots (5 ul) were evaluated for % motility using an automated semen analyzer and sperm concentration determined with a hemocytometer.

The % motile sperm recovery ± s.e.m. evaluated in fractions 1-7 after a 0.5 hr swim-down was: 42.9 ± 4.0, 15.3 ± 3.0, 7.4 ± 0.7, 7.5 ± 0.6, 6.4 ± 0.6, 5.7 ± 0.8 and 10.7 ± 2.0, respectively. After 1.5 hr the recoveries were: 68.6 ± 5.2, 7.4 ± 1.9, 4.8 ± 1.0, 2.6 ± 0.6, 2.7 ± 0.5, 3.9 ± 1.1, 1.4 ± 0.2. The recovery in fraction #1 for both the 0.5 and 1.5 hr swim-downs was significantly greater than (p<0.05) all other fractions as was the % motility (0.5 hr: 61.9±6.2%; 1.5 hr: 71.3±3.8%). Fraction #1 (0.5 and 1.5 hr) was then mixed with an additional 1.0 ml of medium and allowed to swim-down for 0.5 hr. The top 0.7 ml was removed and the remaining 0.5 ml resuspended and evaluated. The final motile sperm recovery from fraction #1 was evaluated with respect to frozen-thawed motile sperm was 31.9 ± 7.9% (0.5 hr) and 33.3 ± 4.6% (1.5 hr) following swim-down. In summary, a simple 2 step swim-down appears to be an effective method for processing frozen specimens for IVF and yields a motile sperm recovery 4-5 times greater than reported with swim-ups.


Lipid peroxidation has been suggested as one of the major causes for decreased sperm function (1). The susceptibility of spermatozoa to peroxidation is increased by their capacity to generate reactive oxygen species (ROS) (2). Many semen samples contain lactate and estrophanes (3) both known to be capable producers of ROS. Their contribution to the levels of ROS found in semen has not been fully assessed.

Production of ROS was determined in 40 semen samples randomly selected from our infertility clinic. The samples were separated on double step, counter-current pental gradients (4). In order to clarify the origin of ROS, lactate was added to the 40-88% pental fraction with unsaturated bonds included with a common lactate antibody. Quantitative semen analysis was performed on a Hewlett-Packard 2110A fluidized bed analyzer. Lactate doubled ROS accounted for almost 70% of the response with a significant correlation between lactate content and levels of ROS (4). (0.92).

Using multiple regression analysis only best cross frequency was significantly affected by ROS originating either from sperm or lactate. This might not be surprising considering the high levels of ROS containing agents such as antioxidants disulfides in semen plasma and the high lactate levels of ROS. The results of the "double exposure" ROS on sperm function effects such as sperm motility and cryopreservation are yet to be determined.

References:


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References:
The Sperm Penetration Assay (SPA) has provided an important diagnostic test for the evaluation of male fertilizing potential. Our laboratory has optimized the conditions for this assay, resulting in a 40-fold increase in sensitivity compared with routine methods. The optimized SPA uses only fresh hamster ovum. However, some clinical laboratories are using commercially available cryopreserved ovum to avoid the expense of maintaining and injection live animals. To determine whether the cryopreserved ovum are suitable for use in our optimized SPA, we compared the penetrability of cryopreserved ovum obtained from 2 different commercial sources (A & B) with our fresh ovum. Sperm from 3 fertile donors were each tested at 3 different sperm concentrations (1.5, 10 × 10⁶ motile/ml). The results are expressed as the average sperm penetrations per ovum and reported as the Sperm Capacitation Index (SCI). In the table below, the penetration rates of both groups of frozen ovum were uniformly decreased to only 39%, r = 0.99 (A) and 32%, r = 0.90 (B) of the rate for fresh ovum. In another experiment 4 patient samples were tested in duplicate at our routine concentration of 5 × 10⁶/ml using 2 batches of frozen ovum from source A. The fresh ovum results were 4.1, 21.3 SCI. In contrast, the frozen ovum scores were 1.3, 3.9 SCI reflecting only 25% (r = 0.91) of the fresh values. These results clearly demonstrate that frozen ovum do not have the same penetrability as fresh ovum even though their appearance at the time of incubation are indistinguishable from fresh ovum. They should not be used interchangeably with fresh ovum in the optimized SPA.

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**Table 1:**

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**Table 2:**

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<td>12</td>
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</table>

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118 CERVICAL MUCUS PENETRATION TESTING WITH A TRU-TRAX SYSTEM IS AN EXCELLENT PREDICTOR OF SPERM MUCUS INTERACTION IN VIVO AFTER CERVICAL INSEMINATION.

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119 TIGHT BINDING OF SPERMATOZOA TO THE ZONA PELLUCIDA: COMPARISON OF RESULTS USING FRESH VERSUS CRYOPRESERVED OOCYTES AND INFLUENCE OF OVARIAN GONADOTROPIN STIMULATION UNDER HUMAN ZONA ASSAY (HZA) CONDITIONS

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120 THE REPRODUCIBILITY OF SEMEN PARAMETERS AND DNA HISTOGRAMS FROM TESTICULAR ASPIRATES IN THE CYNOMOLGS MONKEY MODEL

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R.H. Steele, R. Wang, R.M. Harrison and W.J.G. Hellstrom, Delta Regional Primate Research Center, Tulane University, Covington, LA 70435

Studies on male infertility using the Rhesus monkey model are confounded because of the inherent difficulties in semen collection and the marked influence that seasonality has on semen production in this primate. Our study analyzed semen collected by electroejaculation and DNA flow cytometry studies from testicular aspirates in the cynomolgus monkey (Macaca fascicularis). These studies were accomplished under ketamine anesthesia and aspiration was performed with a 22 gauge Westcott needle attached to a Gilchrist aspirator (Bard Urological, Marietta, GA). Flow cytometry was conducted on a Coulter EPICS 451 flow cytometer (Hialeah, FL), using the 488 argon kAr laser line at 15mW to induce fluorescence. Histograms of at least 10,000 cells were collected for each sample. Samples from twelve primates were studied every two weeks for a two month interval. Semen parameters (concentration, motility, and normal sperm morphology) and quantitative DNA histograms (haploid, diploid, and tetraploid groups) were analyzed. Results showed no significant intra-assay variation over time, all the values except for sperms concentration were within 2 S.D.'s from the mean for each individual animal. Collectively, all the values for normal morphology (59.3 ± 18.2) and normal motility (43.2 ± 28.2) were within 2 S.D.'s. 9696 values of the flow cytometry were within 2 S.D.'s (SC: 78.2 ± 5.6; 2C: 10.6 ± 4.0; and 4C: 7.0 ± 2.6).

Our data demonstrates no significant change over the two month study interval when analyzing semen analysis and DNA flow cytometry. This supports the use of the cynomolgus monkey in infertility studies employing a primate model.
121

Previous reports suggest that removal of these round cells can enhance the fertilizing capacity of semen specimens. We report our experience adapting the L4 leukocyte adsorption medium (Pall Biotechnology, NY) which previously has only been used to separate white blood cells from human serum. The L4 membrane was used in our laboratory on 12 semen specimens to assess changes in the percentage of normal and abnormal sperm forms, as well as the percentage and concentration of round cells in semen. Smears were prepared from liquefied semen specimens as well as from specimens filtered through the L4 membrane. Abnormal sperm forms were divided into three categories: head, neck and tail defects. The concentration (million/ml) of undifferentiated round cells in semen was recorded by observing a drop of specimen washed three times in Human Tubal Fluid (HIF) (Irvine Scientific, CA) in a Leica centrifuge at 1600 rpm (600g) for 10 minutes and 500 rpm (60g) for 5 minutes respectively. The washed sperm were resuspended in 0.5 ml fresh medium and aliquoted into 4 capped polycarbonate tubes (5 ml capacity). 100 ul of washed sperm were added into tubes containing equal volumes of CAMP at concentrations of 0, 2.5, 5, and 10.0 μl, suspended in HIF medium. The tubes were incubated at 37°C for 30 minutes. A coded sperm analysis was done at each aliquot at the end of incubation period on a CellSoft automated semen analyzer.

122

Our results suggest that Sp-cAMP can affect the motility of human spermatozoa in patients with idiopathic infertility and may be useful as an adjunctive test for sperm washing for intrauterine insemination or in vitro fertilization in patients with poor motility.

123

Sp-cAMP is one of the most important semen parameters affecting the fertility of an individual. Sp-cAMP is a thio phosphate analog of CAMP. This substance is a phosphodiesterase inhibitor and controls the intracellular CAMP levels which in turn influence sperm motility. Preliminary results in our laboratory on a group of 12 men (10 suspected subfertile patients and 2 fertile donors) showed stimulation of sperm motility after incubation of washed sperm with various doses of CAMP. Semen specimens were washed two times with Human Tubal Fluid (HIF) and resuspended in HIF medium. The washed sperm were counted under the phase objective using a Makler chamber.

124

Sp-cAMP with poor motility was seen with 5.0 μl Sp-cAMP which was statistically significant (P<0.001). Our results appear in the table below.

<table>
<thead>
<tr>
<th>Concentration (μl)</th>
<th>Motility (% of control)</th>
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<tr>
<td>0</td>
<td>100</td>
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<tr>
<td>2.5</td>
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<tr>
<td>5.0</td>
<td>80</td>
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<tr>
<td>10.0</td>
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</table>

Our results confirm that Sp-cAMP can affect the motility of human spermatozoa in patients with idiopathic infertility and may be useful as an adjunctive test for sperm washing for intrauterine insemination or in vitro fertilization in patients with poor motility.
PLATELET ACTIVATING FACTOR (PAF) STIMULATES
MOTION PARAMETERS OF CRYOPRESERVED HUMAN SPERM.
W.I.G. Heiltsro, R. Wang* and S.C. Sikka. Tulane University School of Medicine, New Orleans, LA 70112.

The success of assisted reproductive techniques using cryopreserved sperm is limited by the poor recovery of motile sperm from post-thaw samples. The present study explores the effects of PAF, a family of naturally occurring acetylated glycerophospholipids, on stimulating the motion parameters of cryopreserved human sperm. Sperm samples from normal volunteers with previously documented poor motility post thaw (n=6) were frozen for six weeks. thawed, washed and suspended in a HAM's F-10 medium containing 0.5% bovine serum albumin. Aliquots (20 x 100 sperm) were incubated at 37°C (ambient temperature) with PAF (0, 0.03, 0.1, 0.3, 1.0, and 3.0 μM) and analyzed at 0, 30, 60, and 120 minutes for various motion characteristics (curvilinear velocity, straight line velocity, mean linearity, amplitude & lateral head displacement) using the Cell Track Semen Analysis System (Motion Analysis Corporation, Santa Rosa, California).

Our results showed that PAF stimulated % motility in a dose-dependent manner with a maximum stimulation using 1μM (15 ± 3 in control, 35 ± 4 with 0.3 μM and 41 ± 3 with 1.0 μM after 1 to 2 hours incubation). This effect on % motility was found to be significant at lower (0.03 and 0.1 μM) PAF doses. Vcl (μm/sec) was significantly (p<0.05) stimulated with higher PAF doses (34 ± 1 in control, 42 ± 2 with 0.3 μM and 20 ± 3 with 3μM after 1 hour). A significant (p<0.005) stimulation response was observed for mean LIN (50 ± 2 in control to a maximum of 68 ± 4 with 1μM PAF at 2 hours). The effects of PAF on Vcl and ALH were not significant (p>0.05) because of a larger variation. Our data demonstrate that PAF can stimulate the motion parameters significantly (% motility, Vcl and ALH) at 0.3 μM and at higher doses. This may improve the chances of conception when employing cryopreserved sperm in assisted reproductive procedures.

Cryopreservation of human sperm is detrimental to hyperactivated motility potential. G.M. Cintola, L.J. Barkman, K. White*, and L.B.V. Emami. Andrology Laboratory, Obs/Gyn, Univ. of Rochester Medical Center, Rochester, NY and GIBCO/BRL, Cell Biology, Grand Island, NY.

Sperm cryopreservation (cryo) is known to induce premature acrosome reactions while decreasing sperm motility and binding to the zona pelucida. Hyperactivated motility (HA) is another significant factor in fertilization. This study has evaluated whether cryo damage extends to HA potential as well. Sperm were acquired from donors in an artificial insemination program, within 0.5 to 1 hour post ejaculation (20 samples). Each specimen was divided in half for frozen versus frozen studies. Each frozen sample was washed with Ham's F10 medium plus 3.0% bovine serum albumin, resuspended in F10 and assessed for motility at 0 hours, as well as after 3h and 6h of a swiP-up incubation (37°C, 5% CO2). Automated motion analysis was performed with the Hamilton-Thorn 2800 system (= HTM) at low sperm density (2 x 106/ml) and in a deep chamber. Computerized HA identification relied on 3 criteria (curvilinear velocity ≥ 100 μm/sec, linearity ≤ 65, and ALH ≥ 7.5 μm). The remaining seasonal portion was preserved for cryo: dilution with Human Sperm Preservation Medium + 7.5% glycerol and nitrogen vapor freezing. Sperm were thawed at room temperature after 2 ± 8 hours. The thawed sample was diluted with F10 medium at a proportion of 0.5 to 1 hour. The residual thawed sperm were washed twice, layered with F10 and examined after 3h and 6h of incubation. As previously reported, the fertile ones were heterogeneous for the peak time of HA incidence. The % motility, % rapid sperm and progressive velocity all showed a significant fall compared to fresh values. Respectively, the maximum values [frozen/thawed] were [(14 ± 5%)/7 ± 4%], [(66 ± 15%)/46 ± 24%] and [(59 ± 47)/47 ± 5%]. Whereas all 20 fresh samples exhibited some HA (range for peak value: 1.48 ± 0.8), 9 of the 20 had 0% HA post-thaw. The hyperactivation measurement revealed a mean of 5 ± 0.8% for the maximal values in fresh samples. After cryo, HA fell significantly by 30% to 3.8% (p<0.05). For the 5 cases displaying maximal HA (fresh) at the early time (0 hours), all demonstrated HA post-thaw. However, with the 7 cases having low HA (peak 6h, 3h), 5 of 7 exhibited no HA post-thaw. This significant loss in HA pelucida is consistent with the decline in other fertilization functions after cryo. A 30% reduction in hyperactivated capacity is another factor requiring increased numbers of thawed sperm for inseminations involving zona-intact eggs.


Aavian species represent a large proportion of the earth's endangered wildlife. Semen cryopreservation is the most efficient method of preserving gene pools in birds and, as such, is an integral part of their long-term conservation. Although fertile eggs have been produced with frozen semen in chickens, ducks, cranes, budgerigars and falcons, there are no reports of successful semen cryopreservation in pheasants. Semen was collected by massage from Impenyan pheasants (Lophophorus impejanus) and diluted in 21°C Beltvue Poultry Semen Extender for transport (10-15 min) to the laboratory for analysis. Maintenence of motility during transport was enhanced at 5°C vs. 21°C (p<0.001). Post-thaw motility was greater (p<0.05) in samples frozen in 4% DMSO compared to 4% glycerol. Motility at thaw was not significantly affected by cryoprotectant equilibration time at 5°C (15 min vs 30 min) by the temperature of the water bath used to thaw the samples (5°C vs 15°C). Based on these preliminary studies, semen was transported at 5°C, equilibrated for 30 min in 4% DMSO and thawed in a 37°C bath. All samples were frozen at -70°C to -20°C then 5°C/cm to -80°C before plunging into liquid nitrogen. Semen evaluation revealed the following means:

<table>
<thead>
<tr>
<th>Volume (ml)</th>
<th>Concentration (10⁶/ml)</th>
<th>Motility index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.019</td>
<td>111.85</td>
<td>froz 851</td>
</tr>
<tr>
<td>0.03</td>
<td>150</td>
<td></td>
</tr>
</tbody>
</table>

In fresh samples, motility was as accurate a measure of viability as was live-dead staining and hypoosmotic swelling. Oviductal inseminations were performed three times a week throughout the laying season. Seven of 16 eggs (44%) were fertile. Future studies will compare additional extenders and determine the longevity of frozen sperm in the female tract.
129 PREVALENCE OF LEUKOCYTOPERSIS IN MALE INFERTILITY PATIENTS IN BEIJING, CHINA A.W. Hang and D. Anderson. Dept. Urol., Peking Union Medical College, Beijing, China, and Dept. OB/Gyn. Harvard Medical School, Boston, MA 02115

Recent reports have revealed a high prevalence of leukocytospermia in North American male infertility patients (10-24%), and have implicated white blood cells as a cause of infertility. Since leukocytospermia may be attributable in many cases to asymptomatic genital tract infections, its prevalence could vary widely in different populations depending on sexual practices and the prevalence of sexually transmitted pathogens. In this study we determined the incidence of leukocytospermia in male infertility patients from the People's Republic of China. Seven samples from 102 male infertility patients and 10 normal fertile men were stained for peroxidase positive cells (granulocytes) by the indirect technique. Samples containing >1 x 10⁶ granulocytes/ml were considered leukocytospermic (WHO, 1987). Seven out of 102 (6.9%) samples from infertility patients and 0/10 samples from fertile donors were leukocytospermic. Fifty-seven of the infertility patients had varicoceles; the incidence of leukocytospermia was slightly higher in this group than in men without varicoceles (11% vs 7%, p<0.09). All but one of the patients with leukocytospermia had a poor sperm count and/or poor sperm motility.

This study indicates that the prevalence of leukocytospermia in infertility patients may be lower in China than in the West, but that it is still an important clinical problem associated with infertility.

130 UPPER EPIDIDYMAL ASPIRATION OF SPERMATOZOA AND ASSISTED CONCEPTION IN THE TREATMENT OF OBSTRUCTIVE AZOOSPERMA

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Obstructive lesions of the epididymis commonly cause male infertility. This presentation describes upper epididymal aspiration of sperm and Assisted Conception in the treatment of this disorder. A total of 14 patients underwent 20 attempts to achieve pregnancy using this technique. Of the 14 patients, 6 had obstruction due to upper epididymal post-vasectomy blowouts, 3 had idiopathic upper epididymal obstruction, 2 more had bilateral obstruction, 3 had vasectomy and vas deferens, and the remaining 3 men had upper epididymal obstruction due to Young's Syndrome, previous surgery and to epidydymitis. Of the 20 attempts at treatment, 7 were carried out among the post-vasectomy patients, and 6 in the 2 men with congenital absence of the vas deferens. A total of 14 attempts were made among the 3 men with idiopathic obstruction and 1 attempt each was made in the 1 man with Young's syndrome and in the 2 men with obstruction after surgery and after epidydymitis. In all 14 patients, the sperm were aspirated from high in the caput epididymis.

Of the 20 attempts, 9 (45%) resulted in failed fertilisation, in 3 (15%) of the attempts the semen analysis was normal. In one patient fertilisation was achieved but the embryos failed to divide. However, between 1 and 3 embryos were generated in each of the remaining 7 attempts. The fertilisation rate ranged between 7% to 90% among the 7 attempts at which fertilisation occurred, producing a mean fertilisation rate of 40%. Transfer of between 1 and 3 embryos were carried out in the consorts of all 5 of these men producing 2 pregnancies. One pregnancy occurred following a patient with idiopathic obstruction resulting in a live birth but the other pregnancy, from a man with post vasectomy upper epididymal obstruction, ended in a missed miscarriage. These results produced a pregnancy rate of 10% per attempt, 17% per patient and 29% per transfer.

132 THE IN VITRO EFFECTS OF CHLORINATED HYDROCARBONS ON HUMAN SPERM QUALITY.


Environmental pollutants such as the chlorinated pesticide and polychlorinated aromatic hydrocarbons has been detected in human tissues and body fluids including the semen. Although these pollutants are toxic in vitro, adverse effect on spermatogonial quality has not been reported. This study was therefore undertaken to determine the in vitro effect of these pollutants on sperm quality. The pollutants studied were hexachlorobenzol (HCB), 2,2'6' tetrachlorobiphenol (PCB), Clophen (a mixture of polychlorinated biphenyls), chlopyrifos (a cholinesterase inhibitor), and DDE (a metabolite of DDT).

Spermatozoa were washed in BMW medium and 10² spermatozoa were resuspended in 1 ml BMW containing 0.1, 1.0, 10.0, or 1000 ng (solubilized in 1% DMSO) of these compounds. Compared to spermatozoa in BMW + 1% DMSO, all compounds studied yielded a dose and time-dependent effect on sperm motility (SM), thermotactic swimming (TST) and viability (vital) test. After 8 hour incubation, even at a concentration of 1 ng/ml, a significant (P<0.05) reduction in SM for PCB, HCB, and DDE as well as viable sperm for HCB and DDE was observed as compared to the control.
136: "A NEW SIMPLE METHOD FOR PREPARING SPERMATOZOA FOR INSEMINATION USING THE NEW SPERRMPREP™ FILTRATION METHOD"

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Spermatozoa can be separated from semen with various spermaticographic deficiencies or characteristics via the SpermPrep™ filtration method (Zavos, Infertility 15:25, 1990). Such selection could yield higher rates of conception in procedures such as intratubal insemination (IUI) or other forms of assisted reproductive techniques. The present study examined the qualitative improvements of spermatozoa recovered from a new improved SpermPrep™ designed to filter spermatozoa from nonviable sperm for IUI. The current method is used routinely in preparing specimens for IUI at the Andrology Institute of Lexington, KY USA. The ejaculates (N=32) were assessed and diluted 1:1 with a modified Ham's F-10, properly liquidified and filtered through the new SpermPrep™ according to the manufacturer's specifications (Fertility Technologies, Inc. Natick, MA 01760). The semen parameters assessed prior to filtration were: count (C) 2106 ± 307 x 10^6/mL, volume (V) 3.1 ± 0.5, % motility (MO) 61.0 ± 5.6, grade (GR) 3.3 ± 0.3 (0-4), % normal morphology (MOR) 68.7 ± 7.1, hypoosmotic swelling sperm (HOS) 72.3 ± 7.4. The specimens were filtered for 5 min recovering a filtrate volume of 1.1 ml. Both the filtered and non-filtered aliquots (recovered in SpermPrep™) were centrifuged and resuspended in Mardel Spermatozoa for use with IUI and intravaginal inseminations (ICI) respectively. The spermatid parameters in the two specimens are shown below (mean ± SE).

<table>
<thead>
<tr>
<th>Sample</th>
<th>C</th>
<th>MO</th>
<th>GR</th>
<th>MOR</th>
<th>HOS</th>
<th>5+</th>
</tr>
</thead>
<tbody>
<tr>
<td>UFI</td>
<td>367.6 ± 6.9</td>
<td>78.7 ± 3.1</td>
<td>3.6 ± 0.2</td>
<td>83.1 ± 6.0</td>
<td>86.7 ± 6.9</td>
<td>16.8</td>
</tr>
<tr>
<td>PCI</td>
<td>139.6 ± 28.3</td>
<td>63.7 ± 3.9</td>
<td>3.4 ± 0.3</td>
<td>63.1 ± 8.2</td>
<td>68.6 ± 7.5</td>
<td>63.9</td>
</tr>
</tbody>
</table>

The results indicate that the new SpermPrep™ provides additional features such as speed and efficiency (e.g., % sperm recovered) that enables the recovery of high quality spermatozoa for IUI with the remaining non-filtered spermatozoa being introduced ICI to saturate the cervix. This mode of filtration and performance of IUI-ICI could have synergistic effects and be advantageous for infertile couples with moderate to no male factor and female with or without cervical hostility and limitations.

137: "MODIFIED PERCOLL GRADIENT CENTRIFUGATION FOR PREPARING MOTILE SPERM FROM INFERTILE MEN"

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Centrifugation of human semen through a Percoll-gradient (2 ml each of 45% and 90%) has been used to prepare motile sperm from normal semen samples (sperm concentration-SC ≥ 20 million/ml, % sperm motility-MOT > 40%, normal morphology-NM > 50% and volume-V > 1.5 ml). The percoll pelletted sperm (PPS) were mainly motile (Tanphaichitr et al., Gamete Res. 20:67-81, 1988). In this study, we used this technique to prepare motile sperm from infertile men. A modified gradient (1 ml each of 45% and 90% Percoll) was used instead. A study was performed in 10 semen samples having subnormal % MOT + one or more subnormal sperm parameters (NM, SC and V). Samples were grouped into those having the % MOT <10, 10-20 and >20. Three samples were in the first group with % MOT of 5.8 ± 3.2 (S.D.); three in the second and four in the third group with % MOT of 16 ± 2.6 and 34 ± 4.5, respectively. < 1 ml of these samples was used in each gradient. The recovered PPS had % MOT of 13.7 ± 7.8, 60.7 ± 8.3 and 58.5 ± 12.8 for the first, second and third group, respectively. The increase of % MOT in the PPS was 194 ± 121, 282 ± 37, and 108 ± 67.8 respectively for these three groups. Although the % sperm recovered was only 8.2 ± 4.5 for the 10 samples, the total number of sperm recovered was sufficient for the in vitro fertilization or intracutaneous insemination.


The interactions between a neoplastic epithelial cell line (TSU-prl) and a human prostatic stromal cell line was investigated in regard to their paracrine regulation of growth, as indicated by 3H-thymidine incorporation. The TSU-prl cells and stromal cells were cultured separately in F12/DME with testosterone (10^-7 M). Conditioned media were dialysed and lyophilized to obtain the conditioned secretory proteins. The TSU-prl secretory proteins enhanced the relative growth of stromal cells by 1.7-fold in a dose dependent manner up to a concentration of 100 ug/ml. Treatment of the TSU-prl proteins with antibodies against acidic or basic fibroblast growth factor (aFGF or bFGF) respectively, did not significantly modify the stimulatory effect of the TSU-prl proteins, whereas treatment with nerve growth factor (NGF) antibody resulted in further stimulation of stromal cell proliferation by 4-fold. In the corresponding opposite interaction, stromal cell secretory proteins enhanced the relative growth of TSU-prl cells by 2.6-fold in a dose dependent manner up to a concentration of 20 ug/ml. Treatment of the stromal cells proteins with antibodies against aFGF and bFGF did not significantly modify the ability of the stromal cell protein to maximally stimulate TSU-prl relative growth, whereas NGF antibody inhibited the stimulation of TSU-prl cells to 20% of maximum levels. Immunofluorescence studies localized NGF-like protein in vesicular structures of both the TSU-prl cells and the stromal cells. Furthermore, PC-12 cells cultured in the presence of 100 ug/ml of TSU-prl conditioned media showed neurite outgrowth. These results suggest that a NGF-like protein present in the prostate mediates paracrine interactions between stromal cells and a neoplastic epithelial cell line.
ENDOCYTIC PATTERNS OF TESTICULAR PROTEINS IN THE EXCERCUTANT DUCTS OF THE RAM. D.N. Rao Vemaramachandi a D Rupert P. Amann. Colo ado State University, Fon Collins, CO 80253

Although about 85% of the protein present in testis tissue fluid (TTF) was absorbed in the proximal excurrent ducts of the ram, the amount of androgen-binding proteins (ABP) in the luminal fluid of the cauda epididymis remains unchanged [Androl 11:140, 1990]. To determine if only some testis specific proteins are selectively absorbed at specific sites in the excercunt ducts, proteins in four HPLC fractions of ovine TTF vs. ABP, 27/74 kD dimer, cys, and transferrin were separated by gel filtration and proteinase K digestion (CNE) and their respective (IS) proximal duct (CP), central duct (CD), and distal duct (DC) sites were counted. Data were analyzed using ANOVA and Dunnet's multiple range test.

Means in a column with different capital superscripts or in a row for epididymal sites with different small superscript are different (P<0.05).

Irrespective of protein injected, substantially more protein was endocytosed in the DE as compared to any segment of the epididymis. In the epididymis, whereas more ABP was endocytosed in proximal site, endocytosis of cys and transferrin was more in distal sites than in proximal sites. Thus, maintenance of a similar amount of ABP through the DC suggests that endocytosed ABP is not sequestration, rather, is by selective "sparking" from endocytosis, in the more proximal regions of excercunt ducts.

Grant HD-14501

138 EVALUATION OF WIN 49,596, A NOVEL STEROIDAL ANDROGEN RECEPTOR ANTAGONIST, IN ANIMAL MODELS OF PROSTATE CANCER


Experiments were conducted to evaluate Win 49,596 (Ciba-Geigy) for its effects on sexual androgen receptor antagonism, in animal models of prostate cancer. Oral administration of Win 49,596 for 28 days inhibited (p<0.05) growth of the andromone-sensitive R-24 variant of the Bummer hard tumor in SCID nude mice to 61, 44, and 47% of that observed following treatment with 10, 50, and 100 mg/kg/day, respectively. In similar experiments, doubling of tumor volume at 20 and 200 mg/kg/day was associated with similar (p>0.10) to that observed in scrotal skin controls (50%). In intact rats administered the steroidandrogen receptor antagonists flutamide (50mg/kg/day) in an oil vehicle or in a vehicle containing testosterone (1mg/kg/day). Caudal (24h), Win 49,596 (5, 30, and 50X, or 30, 100, and 300 mg/kg/day, respectively) or flutamide (250) treatment also decreased (p<0.05) prostate weight in tumor-bearing animals relative to intact controls (100%). Studies were also conducted to determine the effect of Win 49,596 on growth of the androgen-dependent PC-3 human prostate carcinoma transplanted into athymic male mice. Oral administration of Win 49,596 for 35 days inhibited (p<0.05) tumor growth to 58, 67, and 45% of intact controls (100%) at 30, 100 and 300 mg/kg/day, respectively. Tumor inhibition was similar to that observed in intact mice treated either with flutamide (50mg/kg/day) or with testosterone (1mg/kg/day) but less than that following castration (103).

140 GLUCURONYL TRANSFERASE ACTIVITY AND ANDROGENS IN RAT TISSUES IN VITRO.

K. Pirog, R.W. Clark, and O.C. Collins, VA Medical Center and Department of Medicine, Emory University School of Medicine, Atlanta, GA 30322.

Horizon et al. demonstrated direct excitation of C-19 androgen glucuronides by human and rat sexual skin and concluded that sexual skin was the major source of androgen glucuronides in plasma (J. Androl., 11:161, 1990). In this study, we have determined the activity of microsomal UDP-glucuronyltransferase (UGPT), CE 2.4.1.17, in skin from various areas of the body (abdominal, scrotal, penile) and compared it to the enzyme activity in liver and some extrahypothalamic tissues (kidney, intestine, preputial gland, prostate) of the male Sprague Dawley rat.

We determined small but significant UGPT activity for androstan-3-ol, androsterone, androstanediol in position 17;B by skin microsomes: (pmol/mg protein/h), penile skin: 0.082 ± 0.023; scrotal skin: 0.062 ± 0.011; abdominal skin: 0.097 ± 0.039; prostate: 0.075 ± 0.015; intestine: 0.055 ± 0.027; kidney: 0.537 ± 0.125. No UGPT activity for testosterone or androsterone was detected in these tissues. Microsomes from preputial glands did not show any significant UGPT activity toward either of the androgen steroids tested. The UGPT activity for androstanediol was 10-fold higher in liver microsomes: 6.94 ± 1.18 nmoI/mg protein/min. The liver microsomes also showed significant UGPT activity for testosterone: 6.56 ± 0.73 nmoI/mg/min and androsterone: 4.32 ± 0.15 nmoI/min. These results suggest that the extrahepatic contribution is low in the liver and the major source of androgen glucuronides is in liver. UGPT activity was distributed evenly in sexual and non-sexual skin. Furthermore, UGPT activity in the extrahypothalamic organs was limited to androstanediol, while the liver UGPT isozymes also conjugated androsterone and testosterone.
Journal of Andrology - January/February 1991

141 CHARACTERIZATION OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) IN TESTIS, EPIDIDYMIS AND VAS DEFERENS OF MONKEY B. Radhakrishnan*, W. Niklinski*, C. A. Sudrez-Quian, Georgetown University, Dep. of Anatomy and Cell Biology, Washington, D.C. 20007.

Recent studies on epidermal growth factor have elicited interest regarding its potential effects on spermatogenesis. This study was undertaken to demonstrate the presence of EGFR in testis, epididymis and vas deferens in the monkey by immunohistochemistry using a polyclonal antibody (RRK2) raised against a peptide specific sequence of the intracellular domain of the human EGFR. Immunoblotting of membrane preparations of liver (as a control), testis and epididymis with RRK2 revealed a specific band of approximately 150KD, corresponding to the known molecular mass of the EGFR. Reproductive tissues viz. the testis, epididymis and vas deferens, and also liver, were obtained from Baboons approximately 3 years old. Frozen sections, 5-7 um. were prepared, fixed in 3% paraformaldehyde (10 min) and permeabilized with 0.1% Triton X-100 (45 sec). Next, sections were blocked with 10% non-immune goat serum, incubated with either RRK2 or pre-immune serum, and immunostained using biotinylated goat anti-rabbit antibody, streptavidin-peroxidase and substrate-chromogen mixture. The results in liver showed positive staining along the basolateral membranes of the hepatocytes. The testis showed positive staining indicating the presence of EGFR in Leydig and Sertoli cells. In epididymis, immunostaining of the EGFR was observed on both the basolateral and luminal borders of the epididympal epithelium. Similar staining was noticed in vas deferens. There was no positive staining in the interstitial tissue of epididymis or in the smooth muscle cell layers of vas deferens. The sections of all tissues treated with pre-immune serum were negative. These results suggest that EGFR in the primates may act as the level of somatic cells. In addition, the basolateral and luminal EGFR staining in epididymis and vas deferens may suggest these cells respond to an EGF source both on the basal or luminal sides of the cells, or these tissues serve as sites of EGF transcytosis across the epithelium.

In order to understand the role of EGFR in testicular function, preliminary experiments were carried out using sexually mature C57Bl/6J mice (8 weeks old) which were divided initially into three groups a) sham control (SC); b) sialoadenectomized (SA); c) SA+EGF (100 μg/kg, i.p.) treated. Subsequently, another group of animals were subjected to either (tumicidine(F) or S+EGF treatments. The animals were sialoadenectomized and for 28 days EGF and F were injected subcutaneously. The removal of salivary glands did not affect the body weight and the weights of testis and epididymis, but reduced sperm counts and motility. Quantitative determination of spermatogenesis indicated that S reduced the number of pre-pachytene and pachytene spermatocytes, and round spermatids. In contrast, testosteron(T) levels increased while FSH remained unaffected and LH levels decreased. Treatment of S animals with EGF maintained spermatogenesis and T levels at control values. F alone reduced sperm counts and motility by 50% and reduced T levels by 2 fold. SF treatment elevated T levels by 5 fold as compared to the F group but did not decrease the sperm count any further. However sperm motility was further reduced. Administration of EGF to the SF group maintained the sperm counts, motility and T levels comparable to the values in the F group. The tests also demonstrated two binding sites for EGF, one with higher affinity (Kd = 0.70nM) and lower capacity (n = 0.27pm), and the other with lower affinity (Kd = 1.26nM) but higher capacity (n = 156pm). The binding of EGF to testis was maximum after 1-2 hours of incubation at room temperature. These results suggest that EGF may have an important role in the regulation of testicular function and that may be independent of T action.


Sexually mature male mice (8 weeks old) were divided into three groups a) sham control (SC); b) sialoadenectomized (SA); c) SA+EGF (100 μg/kg, i.p.) treated. EGF was administered subcutaneously for 28 days. Sialoadenectomy reduced significantly the weight of sialic acid (SA), Treatment of S animals with EGF maintained the normal weight of SA, Determination of EGF by ELISA indicated that the EGF remained second to salivary glands in the levels of SA. After sialoadenectomy, the levels of EGF could not be detected in the circulation or in the SA. In contrast, prostate still contained EGF, although the levels were very low. There were two organs demonstrated two binding sites, one high affinity and low capacity (Kd = 0.67 nM, 10% at 5 nM of EGFR for SA; Kd = 0.36 nM, 20% at 5 nM of EGFR for P and the other one low affinity and high capacity (Kd = 30, 15% at 50 nM of EGFR for SA; Kd = 4.9, 85% at 150 nM of EGFR). These data suggest that EGF may have an important role in the sex accessory organs. EGF is important for the maintenance of SA weight and SA secretions and EGFR from the circulation while the prostate may produce EGF.

144 EFFECTS OF HORMONES ON THE DISTRIBUTION OF JUNCTION ASSOCIATED SEROTOLI CYTOSKELETAL PROTEINS IN VITRO. K.E. Muffly and D.F. Cameron, Department of Anatomy, College of Medicine, University of South Florida, Tampa, FL 33612.

Sertoli cells form junctional specializations with germ cells. Although Sertoli junctional structure has been well-described, control of junctional formation and function is poorly understood. It appears, that in vitro, FSH+testosterone are necessary to achieve maximal spermatid binding to Sertoli cells. Actin and vinculin are cytoskeletal proteins known to be involved with junctional complexes and are present in Sertoli cells. Actin and vinculin distribution. Results suggest that FSH and EGF are involved with a greater score of the Sertoli cell junctional formation and function. The effects of FSH, testosterone and FSH+testosterone on the distribution of these two junction-related proteins were determined by immunostaining in Sertoli cells isolated from 18-20 day-old rats and cultured on Matrigel for 48h either alone or with round spermatids. The DMEM-F12 medium was supplemented with ITS+, retinoid acid, lactose acid and EGF. In Sertoli monolayers with no hormone or testosterone, actin and vinculin were diffused throughout the cell. FSH and FSH+testosterone, however, stimulated the peripheral distribution of these two proteins. The addition of spermatids did not appear to alter the effects of FSH or themselves effect actin and vinculin distribution. Results suggest that FSH and EGF maximize spermatid binding to Sertoli cells by insuring structural "competency" of the Sertoli cell. Supported, in part, by NIH BRSG grant 2 RO1 RR05749-19.
ACID AND ALKALINE PHOSPHATASE ACTIVITIES IN TESTES AND ACCESSORY GLANDS OF FEMALE WISTAR RATS EXPRESSED IN VITRO ON CAE. P.K. Choudhury A. Bartke. Department of Physiology, School of Medicine, Southern Illinois University, Carbondale, IL 62901.

Previous studies from our laboratory indicated that expression of human (hCH) in transgenic mice is associated with increase in testicular weight and massive enlargement of the seminal vesicles (SV) along with abnormalities in neuroendocrine functions. Since hCH is luteinizing hormone receptors and luteinizing hormone is known to stimulate testosterone production via hCG receptor, therefore, the purpose of the present study was to examine the consequences of expression of hCH in histological structure and activity of acidic enzymes in testes and accessory reproductive glands. Adult male hCH/hCG transgenic and nontransgenic mice (6-12 weeks old) were used. The testes, SV and ventral prostate (VP) were dissected out and weighed using a balance per group. Significant differences were recorded in body wt. (Control: 150 ± 2 g, hCH: 120 ± 3.2 g) and VP wt. (Control: 9.6 ± 0.8 g, hCH: 32.0 ± 2.7 mg) while no differences were observed in testes wt. Acid phosphatase activity to SV was significantly increased in transgenic animals compared to their nontransgenic mates (Control: 374.6 ± 21.7, hCH: 450.5 ± 24.2 U per enzymatic product/mg protein/20 min) while no differences were observed in alkaline phosphatase activity in the same region (Control: 160.0 ± 18.2, hCH: 124.5 ± 128.7). The activities of both acid and alkaline phosphatases were increased in the VP of hCH transgenic mice (Control: 252.9 ± 19.5, hCH: 360.1 ± 10.0; Alkaline-Controle: 80.6 ± 32.4, hCH: 717.9 ± 53.9) while no differences in both the enzyme activities were observed in testes. Histological evaluation of testes indicated that the cellular associations in the seminiferous epithelium are similar in hCH transgenic and nontransgenic mice. The acinar epithelial cells in SV were more elongated while the follicular epithelial cells in VP of hCH transgenic animals had increased nuclear diameter when compared to the corresponding tissue from nontransgenic animals. These studies indicate that hypertrophy of SV and VP and increased activity of hydrolytic enzymes in these organs are possibly due to the glanular secretory cells and that these changes are mediated by the somatotropic effect of hCG.}

SIXTEENTH ANNUAL MEETING

145 ACID AND ALKALINE PHOSPHATASE ACTIVITIES IN TESTES AND ACCESSORY GLANDS OF FEMALE WISTAR RATS EXPRESSED IN VITRO ON CAE. P.K. Choudhury A. Bartke. Department of Physiology, School of Medicine, Southern Illinois University, Carbondale, IL 62901.

BINDING OF ACTINOMYCIN D TO SPERM. D.P. Walker, L.J. Zanelle, and K.L. Polakowski, Dept of Pharmacodynamics, Univ of Ill College of Medicine, Chicago, IL 60612, Dept of Obstetrics, Rush Univ, Rush Medical Center, Chicago, IL 60612 and Dept of Ob/Gyn, Washington Univ, St. Louis, MO 63110

I. Toxic chemicals and drugs have been detected in semen but little is known of their interaction with spermatozoa. I. Such agents can bind to spermatozoa, they may be transported to, and possibly into, the acrosome and affect embryo/fetal development. In order to determine if spermatozoa can take up toxic chemicals, the binding of actinomycin D to spermatozoa was evaluated. Actinomycin D is an antimicrobial, immunosuppressive drug which intercalates with DNA and has been shown previously to affect reproduction. Freshly ejaculated human spermatozoa were washed once by centrifugation (10 min, 500 x g) through 10% Ficoll in albumin free modified BWW(mBWW) and then washed twice by resuspending in mBWW and centrifugation. The washed spermatozoa were resuspended in mBWW and several concentrations of spermatozoa were incubated at ambient temperature with radioactive actinomycin D for up to 6 hours. At selected time intervals the spermatozoa were collected on an ultracentrifuge manifold using gentle vacuum and repeatedly washed with mBWW. The filtration membrane was then saturated with a tissue solubilizer and left for one hour before the addition of scintillation cocktail and counting in a scintillation counter. Maximum binding occurred prior to 2 hours and the counts were directly proportional to the number of sperm present in the incubation mixture. The results demonstrate that spermatozoa tightly bind actinomycin D in a direct proportion to the number of spermatozoa. The nature of this binding and the potential for this type of interaction leading to oocyte exposure of a chemical needs further evaluation.

146 INCREASE CONCENTRATIONS OF FETAL LEYDIG CELLS IN CULTURE AMPLIFY THE SOMATOMEDIN RESPONSE. T. Sokka1, T. Hietanen2, and D. Warren. Department of Physiology, University of Turku, Finland and Department of Physiology and Biophysics, University of Southern California, Los Angeles, CA 90033.

Leydig cells and mixed testicular cells in culture have been widely used as models to study Leydig cell function. In previous studies of fetal testis cells in culture, we have noticed that testosterone production varied widely with the number of cells used in the culture. Thus, we felt that it was important to carefully quantify the amount of testosterone produced by increasing numbers of fetal testis cells and correlate this with the response to increasing doses of hCG. Fetal testes, 20.5 days of gestation, were incubated with 0.4% collagenase until all cell to cell connections were disrupted. The cells were then washed with medium 199 and the cells from 1, 2 or 4 testis equivalents were placed in 0.4 ml of medium 199 in a 24 well culture plate. After 24 hours to allow the cells to attach to the plate, the medium was removed and replaced with fresh medium containing 0.1 or 10 ng/ml hCG. After 48 hours, testosterone in the medium was measured. The figure shows the effect of varying concentrations of cells and hCG on testosterone production by the fetal testis cells. We have concluded that when culturing fetal Leydig cells, cell density is critical and that cell to cell contact or a paracrine mechanism amplifies the hCG effect on testosterone production.

147 CHARACTERIZATION OF GELATIN-DEGRADING METALLOPROTEINASES OF THE DUNNING RAT PROSTATE TUMOR. M.J. Wilson1, S. Kapoor2, M.M. Vogel1 and A.A. Sinha2. Research Service, VA Medical Center and Dept of Medicine and Pharmacology1 and Genetics and Cell Biology2, Univ of Minnesota, Minneapolis, MN 55417

A common property of many tumors is an increased activity of metalloproteinases that can cleave proteins of the extracellular matrix. We have examined the enzymatic characteristics and effects of caspase on metalloprotease activities in the Dunning rat prostate tumor using gelatin-containing SDS-PAGE zymography. Nine distinctive bands of Ca2+-dependent protease (CDP) activities were detected in zymograms of the tumors; activities of 64, 71, and 76 Kd being prominently expressed and those of 33, 42, 65, 98, 115, and 120 Kd showing weaker activities. A 91 Kd activity was detected in the absence of Ca2+. The CDP activities of different paratotic lobes of the Copenhagen rat were distinct from the tumor and from another i.e., these were a 62 Kd protease in the anterior; 59, 62, and 67 Kd forms in the ventral; and 59, 62, and 72 Kd activities in the lateral lobes. The CDP of the Dunning tumor were active over a broad pH range and were inhibited by EDTA and EGTA, S2+ and Ba2+ were the only divalent cations able to substitute for Ca2+ with some efficacy. There was little change in the predominant molecular forms of protease following castration; however, the 98 Kd CDP activity decreased and the 59 Kd Ca2+-independent activity increased as a result of this treatment. The identification of individual protease activities and whether the castration-induced changes in activity are related to tumor progression to hormone Insensitive status remains to be determined. (Supported by Research Funds of the Dep't of Veterans Affairs)

148 METALLOPROTEINASES OF THE DUNNING RAT PROSTATE TUMOR. M.J. Wilson1, S. Kapoor2, M.M. Vogel1 and A.A. Sinha2. Research Service, VA Medical Center and Dept of Medicine and Pharmacology1 and Genetics and Cell Biology2, Univ of Minnesota, Minneapolis, MN 55417

A common property of many tumors is an increased activity of metalloproteinases that can cleave proteins of the extracellular matrix. We have examined the enzymatic characteristics and effects of caspase on metalloprotease activities in the Dunning rat prostate tumor using gelatin-containing SDS-PAGE zymography. Nine distinctive bands of Ca2+-dependent protease (CDP) activities were detected in zymograms of the tumors; activities of 64, 71, and 76 Kd being prominently expressed and those of 33, 42, 65, 98, 115, and 120 Kd showing weaker activities. A 91 Kd activity was detected in the absence of Ca2+. The CDP activities of different paratotic lobes of the Copenhagen rat were distinct from the tumor and from another i.e., these were a 62 Kd protease in the anterior; 59, 62, and 67 Kd forms in the ventral; and 59, 62, and 72 Kd activities in the lateral lobes. The CDP of the Dunning tumor were active over a broad pH range and were inhibited by EDTA and EGTA, S2+ and Ba2+ were the only divalent cations able to substitute for Ca2+ with some efficacy. There was little change in the predominant molecular forms of protease following castration; however, the 98 Kd CDP activity decreased and the 59 Kd Ca2+-independent activity increased as a result of this treatment. The identification of individual protease activities and whether the castration-induced changes in activity are related to tumor progression to hormone Insensitive status remains to be determined. (Supported by Research Funds of the Dep't of Veterans Affairs)
We developed and characterized antibodies against the syn­
when Sertoli cells contain the largest amount of inhibin. A
plays a role

days.

bin Oβi-subunit is localized
and that endogenous inhibin plays a physi­
ological role in

antibody every day from birth to adulthood.

Modulating FSH secretion in sexual maturat­
tion was studied

and that inhibin's role

changes were still observed in the over 50
day group. From these results, it is suggested that inhibin plays a role in spermatogenesis in infantile male rats, especially before puberty. However, inhibin's role might not be particularly important in those ages, as was shown by the lack of significant changes in the gonads of the antibody-administered group.

152 DNA HISTOCGRAM OF BILATERAL TESTES AFTER
VASECTOMY

L. M. Lee, A. W. Chiu*, M. T. Chen*, L. S. Chang*,
Division of Urology, Department of Surgery, National Yang­
Ming Medical College, and Veterans General Hospital -
Taipei, Taiwan 11217, R. O. C.

Studies in animals have revealed a wide variety of testicular changes after vasectomy, ranging from no change, disordered spermatogenesis, interstitial change of spermatid, to interstitial fibrosis. We conduct an animal experiment in which DNA histogram change of bilateral testes was used to evaluate the spermatogenesis function after unilateral vasectomy. Transgenic unilateral vasectomy was performed in 30 Sprague Dawley rats. Serial testis
aspirations were analyzed by flow cytometer before and after vasectomy. Mean body weight of these rats increased from 350 to 450 grams, 6 rats expired during this study.

There was no change of haploid cell ratio in the sham operation group. In the ipsilateral side of vasectomized testis, there was a decrement of haploid cells after one week, from 74% to 68%. Nevertheless, this change showed no statistical significance. P value > 0.05, and this decrement recovered one week later. No change was found in the contralateral testis till one month postoperatively.

The pathology examination of bilateral testes also revealed normal spermatogenesis. We considered there was no significant change of spermatogenesis in bilateral testes of rats after unilateral vasectomy.
The present study examined the relationship between functional status of Sertoli cells and the maintenance of spermatogenesis in hypophysectomized (HPX) rats. Implantation of 2-10 cm testosterone capsules (TC) for 90 days restored serum T of HPX rats 2-10 times above the normal controls but did not increase T levels in testis significantly. Daily injection of FSH enhanced the accumulation of T in testis significantly. Maintenance of early spermatogenesis in all TC implanted rats. Testicular ABP content was stimulated by TC implants alone without concomitant increase in epididymal ABP levels. FSH injection doubled the HPX rate. Although elongated spermatids were present, step 18/19 spermatids were only noted in rats bearing 10 cm TC implants. FSH injection resulted in the completion of spermiogenesis in all TC implanted HPX rats. Testicular ABP content was stimulated by TC implants alone without concomitant increase in epididymal ABP levels. FSH injection doubled the HPX rate. Although elongated spermatids were present, step 18/19 spermatids were only noted in rats bearing 10 cm TC implants. FSH injection resulted in the completion of spermiogenesis in all TC implanted HPX rats. Testicular ABP content was stimulated by TC implants alone without concomitant increase in epididymal ABP levels. FSH injection doubled the HPX rate. Although elongated spermatids were present, step 18/19 spermatids were only noted in rats bearing 10 cm TC implants. FSH injection resulted in the completion of spermiogenesis in all TC implanted HPX rats.
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