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JANUARY/FEBRUARY 1991
Supplement

Journal of ANDROLOGY

16th Annual Meeting

Program and Abstracts

Published for THE AMERICAN SOCIETY OF ANDROLOGY
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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

SUMMARY OF THE 1991 ANNUAL MEETING OF THE AMERICAN SOCIETY OF ANDROLOGY

Saturday, April 27		Sunday, April 28		Monday, April 29		Tuesday, April 30	
Time	Event	Time	Event	Time	Event	Time	Event
8:00 AM–6:00 PM Exhibits*		**8:00 AM–6:00 PM Exhibits**		**8:00 AM–6:00 PM Exhibits**			
8:30 AM	POSTGRADUATE COURSE–I An Update on Fertilization in the Laboratory and in the Clinic (Ambassadeur Room) • Current Concepts in Mammalian Fertilization	8:00 AM	Opening Welcome (Ambassadeur Room)	6:45 AM	Past Presidents' Breakfast	8:00–9:00 AM	State-of-the-Art Lecture Dr. William Bremner (Ambassadeur Room)
		8:10 AM	SERONO LECTURE Dr. Tony Plant	8:00 AM	State-of-the-Art Lecture Dr. Deborah Segaloff (Ambassadeur Room)		
10:00–10:30 AM	BREAK	9:00–10:30 AM	Scientific Presentations	9:00–10:30 AM	Scientific Presentations	9:00–10:00 AM	Eulogy for Dr. Larry Ewing Award Ceremony & Business Meeting
		10:30–11:00 AM	BREAK	10:30–11:00 AM	BREAK	10:00–10:30 AM	BREAK
		11:00–12:30 PM	Scientific Presentations	11:00–12:30 PM	Scientific Presentations	10:30–12:30 PM	Symposium (Postgraduate Course-II—Joint Session) "Clinical Use of 5α-Reductase Inhibitors and Antiandrogens in Prostatic Disease"
12:00–1:30 PM	LUNCH	12:30–1:45 PM	LUNCH†	12:30–2:00 PM	LUNCH	12:30 PM	ADJOURN
1:30 PM	• Sperm Physiology in Clinical Medicine	1:45–3:45 PM	Scientific Presentations				
3:00–3:30 PM	BREAK	3:45–4:00 PM	BREAK	2:00–4:00 PM	Symposium "Recent Advances in Bio- and Immuno-Hormone Functions and Analyses"	1:30–6:30 PM	Postgraduate Course–II‡ Management Options for Benign Prostatic Hyperplasia
		4:00–5:30 PM	Symposium "Physiologic Mechanisms of Androgen Transport into Tissue"	4:00–6:00 PM	Poster Session II (Gouverneur Salons)		
5:00–6:00 PM	Student Soiree	5:30–7:30 PM	Poster Session I and Reception (Gouverneur Salons)	7:00 PM	Banquet		
6:00–7:30 PM	STUDENT COLLOQUIUM Dr. Rupert Amann "Andrology: What is Unobtainable from a Computerized Data Base"	8:30–10:30 PM	Clinical Laboratory Scientists Meeting (Ambassadeur Room)				

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-to-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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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 Fertilization 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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1984–1985	Rudi Ansbacher
1985–1986	Anna Steinberger
1986–1987	William D. Odell
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1988–1989	C. Wayne Bardin
1989–1990	Rupert Amann

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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–I: 4/27/91	
Member or non-member	$110
Student	30
Half-Day	55
CME credit	20
1991 Annual Meeting: 4/27–30/91	
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.

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McGill Center for Study of Reproduction, McGill University
Department of Pharmacology and Therapeutics, McGill University
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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 Miracar 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 Doval 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 Maison-neuve 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 three 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 Quest." 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.

what do you do with a giraff with 3 balls?
walk him + pitch to the elephant.

SERONO AWARD LECTURESHIP

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 Endcocrinology Study Section.

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1991 ... Philip Troen, M.D.

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 LECTURESHIP

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 Endcocrinology Study Section.

DISTINGUISHED ANDROLOGISTS

1976 ... Roy O. Greep, Ph.D., Sc.D.
M.C. Chang, M.D., Ph.D.
1977 ... Roberto E. Mancini, M.D.
1978 ... Robert J. Hotchkiss, M.D.
1979 ... Thaddeus Mann, M.D., Sc.D., Ph.D.
1980 ... John MacLeod, Ph.D.
1981 ... Alexander Albert, Ph.D., M.D.
1982 ... Eugenia Rosemberg, M.D.
1983 ... Kristen B.D. Eik-Nes, M.D.
1984 ... Mortimer B. Lipsett, M.D.
1985 ... Robert H. Foote, Ph.D.
1986 ... Alfred D. Jost, D.Sc.
1987 ... Emil Steinberger, M.D.
1988 ... Yves W. Clermont, Ph.D.
1989 ... C. Alvin Paulsen, M.D.
1990 ... Marie-Claire Orgebin-Crist, Ph.D.
1991 ... Philip Troen, M.D.

YOUNG ANDROLOGIST AWARD

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.

YOUNG ANDROLOGISTS

1982 ... L. J. D. Zaneveld, D.V.M., Ph.D.
1983 ... William B. Neaves, Ph.D.
1984 ... Lonnie D. Russell, Ph.D.
1985 ... Bruce D. Schanbacher, Ph.D.
1986 ... Stephen J. Winters, M.D.
1987 ... Ilpo T. Huhtaniemi, M.D., Ph.D.
1988 ... Larry Johnson, Ph.D.
1989 ... Barry T. Hinton, Ph.D.
1990 ... Luis Rodriguez-Rigau, M.D.
1991 ... Patricia M. Saling, Ph.D.

Sponsored by the Texas Institute for Reproductive Medicine and Endocrinology, P.A.

NEW INVESTIGATOR AWARD

1991 ... Recipient will be announced at the Awards Ceremony

Previous Recipients

1983 ... Thomas T. Tarter
1984 ... Peter S. Albertson
Randall S. Zane
1986 ... Mark A. Hadley
1987 ... Peter Grosser
1988 ... Stuart E. Ravnik
1989 ... Tracy L. Rankin
1990 ... Donna Bunch

Sponsored by the West Michigan Reproductive Institute

STUDENT INFORMATION

Student Soiree	Saturday, April 27, 1991, 5:00–6:00 PM
Guest of Honor	Donna Bunch, 1990 New Investigator Award Winner
Sponsor	Student Affairs Committee
Student Colloquium ...	Saturday, April 27, 1991, 6:00–7:30 PM (refreshments)
Speaker	Rupert P. Amann, Ph.D. Past President, American Society of Andrology
Topic	"Andrology: What is unobtainable from a computerized data base?"
Sponsor	Student Affairs Committee
Placement Service	A placement service for candidates and employers is sponsored by the Student Affairs Committee. The Placement Service Board will be near the Registration Desk. Contact: Dr. William Baird at 614/442-0122.
Student Awards	A New Investigator Award of $500 and five Student Merit Awards of $100 will be presented to students on the basis of their presentations at the Annual Meeting. The winners will be selected by the Awards Committee and announced at the Awards Ceremony, 9:00 AM, Tuesday, April 30.

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—"Physiologic 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 Plymate (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.

AMERICAN SOCIETY OF ANDROLOGY POSTGRADUATE COURSE—II

Tuesday, April 30, 1991
Montreal, Quebec

MANAGEMENT OPTIONS FOR BENIGN PROSTATIC HYPERPLASIA

Course Co-Directors: Joseph E. Oesterling, M.D. and Ronald W. Lewis, M.D.

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 1 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 1 of the Physician's Recognition Award of the American Medical Association.

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 epididymidis.** G. A. Cornwall, M.-C. Orgebin-Crist, and S. R. Hann.*

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.

10:15 A.M. 6 **Differential regulation of testicular growth factor receptor gene expression during 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, K. R. Robertson,* C. R. Day,* and J. W. Overstreet.

11:30 A.M. 9 **Monoclonal IgM antibodies against rabbit sperm for vaginal contraception.** P. E. Castle,* K. J. Whaley, T. R. Moench,* J. E. Hildreth,* W. M. Saltzman,* M. L. Radomsky,* T. E. Hoen,* and R. A. Cone.*

11:45 A.M. 10 **Stimulation of human sperm capacitation in protein-free medium by lipid transfer protein.** S. E. Ravnik and C. H. Muller.

12:00 P.M. 11 **Ultrastructural features of bull sperm cocultured with bovine uterine tube epithelial cells.** J. E. Ellington,* D. H. Schlafer,* and R. H. Foote.

* Not a member of the American Society of Andrology.

SUNDAY, April 28, 1991

12:15 P.M.	12 **Protamine is removed from hamster sperm nuclei during the decondensation step of fertilization.** S. Perreault and R. Barbee.*
12:30–1:45 P.M.	LUNCH†
	Scientific Presentations: CLINICAL ANDROLOGY
	Chairpersons: Luis J. Rodriguez-Rigau and Gilbert G. Haas, Jr.
1:45 P.M.	13 **A method for experimentally increasing testicular size and spermatogenesis in the rat.** P. Cooke,* R. Hess, E. Meisami,* and J. Porcelli.*
2:00 P.M.	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,* N. S. Padian,* and T. R. O'Brien.*
2:15 P.M.	15 **Is routine endocrine screening of impotent men necessary?** J. P. Jarow, A. Johnson,* and R. Sweat.*
2:30 P.M.	16 **Hypogonadism in men with type II diabetes and organic impotence: Response to testosterone therapy.** F. T. Murray, R. Johnson,* M. Geisser,* and J. Rountree.*
2:45 P.M.	17 **Standardization and comparability of computer-aided sperm analysis (CASA) instruments.** R. O. Davis and D. F. Katz.
3:00 P.M.	18 **The mediator of human corpus cavernosum relaxation is nitric oxide.** W. J. Aronson,* P. A. Bush,* G. M. Buga,* L. J. Ignarro,* and J. Rajfer.
3:15 P.M.	19 **Comparison of assessment of sperm morphology by computer assisted versus manual methods.** C. Wang, A. Leung,* W. L. Tsoi,* J. Leung,* V. Ng,* K. F. Lee,* and S. Y. W. Chan.*
3:30 P.M.	20 **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.*
3:45–4:00 P.M.	BREAK
4:00–5:30 P.M.	**Symposium on Physiologic Mechanisms of Androgen Transport into Tissue**
	Chairpersons: Gerard Cooke and Stephen Plymate
	Geoffrey Hammond: **Molecular Biology of Androgen Transport Proteins** — ABP cDNA
	William Rosner: **Biologic Effects of Androgen Transport Proteins**
	Carl Mendel: **The ~~Free Hormone Hypothesis~~: A Physiologically Based Mathematical Model**
5:30–7:30 P.M.	**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).
8:30–10:30 P.M.	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
9:00 A.M.	21 **Regulation of the truncation of LH receptors at the plasma membrane in Leydig cells.** A. P. West and B. A. Cooke.
9:15 A.M.	22 **Adult rat Leydig cell peroxisomes contain 3-hydroxy-3-methyl glutaryl-coenzyme A reductase (HMG-CoAR).** S. M. L. C. Mendis-Handagama, S. K. Krisans, and L. L. Ewing.

* Not a member of the American Society of Andrology.

† Joint meeting of the Editorial Board and Publication Committee Luncheon (Salon Senateur).

MONDAY, April 29, 1991

9:30 A.M.	23	**Diverse secretory patterns of rat clusterin and α_2-macroglobulin by Sertoli cells in bicameral culture chambers.** J. Grima,* R. A. Lockshin,* and C. Y. Cheng.
9:45 A.M.	24	**Dynamics of interactions of clusterin (SGP-2) with maturing spermatozoa.** D. R. Mattmueller and B. T. Hinton.
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. Schulman* and R. Sherins.
11:30 A.M.	29	**Human cervical mucus properties and sperm mucus interaction during the proliferative phase of the menstrual cycle.** D. F. Katz, A. I. Yudin,* J. B. Andrew,* B. L. Lasley,* and M. J. Zinaman.
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.
12:15 P.M.	32	**Gamete micromanipulation for the treatment of male infertility in the mouse.** D. J. Lamb, J. D. Stockton, L. I. Lipshultz, and M. L. Meistrich.
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.

TUESDAY, April 30, 1991

10:30–12:30 P.M. **Symposium on Clinical Use of 5α-Reductase Inhibitors and Antiandrogens in Prostatic Diseases (Postgraduate Course-II: Joint Session)**
Chairpersons: Jack Geller and Mostafa M. Elhilali

Julianne Imperato-McGinley: **Hormonal and Clinical Comparisons: Observations in Patients Treated with 5α-Reductase Inhibitors and 5α-Reductase Deficient Patients**
Elizabeth Stoner: **Use of 5α -Reductase Inhibitors as Treatment for Benign Prostatic Hyperplasia**
Nelson Stone: **Use of Antiandrogens as Treatment of Benign Prostatic Hyperplasia**
Jack Geller: **Overview of Enzyme Inhibitors and Antiandrogens in Prostatic Cancer**

12:30 P.M. ADJOURN

1:30–6:30 P.M. **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

33 **Use of a Y-chromosome-specific DNA probe to monitor the albumin column method of X–Y sperm separation.** A. R. Glass.

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

35 **Testicular injury induced by hypothermia and ischemic hypothermia.** W. K. Aulitzky, P. N. Schlegel, P. S. Li, M. Goldstein, D. M. Phillips, and C. W. Bardin.

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. Wientzeck.*

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

39 **Suppression of spermatogenesis by a GnRH antagonist plus testosterone in normal men does not affect sperm motion parameters (SMP).** M. C. Bastias, H. Kamijo,* and S. N. Pavlou.

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. Shanis, 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.

45 **Specific alterations in the dynamics of luteinizing hormone secretion in healthy aging men.** J. D. Veldhuis, R. J. Urban, G. Lizarralde, and A. Iranmanesh.

46 **Quantitative testicular biopsy in spinal cord injured men: Comparison of quantitative micrometric and cytometric analysis.** I. H. Hirsch, P. A. McCue,* R. B. Conn,* M. T. Flanigan,* and W. E. Staas.*

47 **HIV-I in the reproductive tract of a man with epididymal obstruction: A case report.** J. Pudney and D. Anderson.

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.

50 **Spermatic vein ligation versus transcutaneous embolization: Contrasting approaches to varicocele management.** R. M. Falk, C. Rosenblatt, A. J. Thomas, M. A. Geisinger, and G. K. Lammert.

51 **Paratesticular myosarcoma—Report of 2 cases.** S. Siew, S. B. Katlein,* and M. Fatteh.*

52 **Immunolocalization of human sperm antigens by light and electron microscopy.** M. Homyk and J. C. Herr.

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

54 **Some functional features of human Sertoli cells *in vitro*.** M. A. Drosdowsky, P. Foucault,* and S. Carreau.*

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

56 **Membrane lipid peroxidation in cavernosal smooth muscle cells from impotent men.** S. C. Sikka and W. J. G. Hellstrom.

SPERMATOGENESIS AND FERTILITY

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1 PRIOR EXPOSURE TO CALCIUM REDUCES MOUSE SPERM TRANSPORT TO THE OVIDUCT. P. Olds-Clarke, W. Wivell* and R. Sego*. Dept. of Anatomy & Cell Biology, Temple University School of Medicine, Philadelphia, PA 19140.

To be capable of fertilization in vitro, cauda epididymal sperm must first 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-P). For fertilization in vivo to occur, sufficient sperm must be transported through the female genital tract to the site of fertilization. To determine whether the reduction in fertilization 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 ½ to 4½ hrs. after AI of $3x10^6$ 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 11-287, 10 females), but 366 ± 98 (range of 131-983, 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 uterotubal junction (Gaddum-Rosse, 1981, Am. J. Anst. 160:333), preincubstion 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.** D.G. Cyr[a], B.Robaire[a], and O.W. Blaschuk[b]. [a]Dept. of Pharmacology and Therapeutics, McGill Univ., Montréal. [b]Dept. of Urology, Royal Victoria Hospital, and Dept. of Anatomy, Centre for the Study of Reproduction, McGill Univ., Montréal, Québec.

Testicular development involves complex cellular interactions. These interactions are important for germ cell maturation as well as for the maintenence of the blood-testis barrier as suggested by the presence of adherence junctions between germ cells and Sertoli cells as well as tight junctions be ween adjacent Sertoli cells. Previous studies have linked tight junctions in the kidney to the presence of cadherins. Cadherins are 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 orignally isolated: Epithelial-Cadherin (E-Cad), Neural-Cadherin (N-Cad), and Placental-Cadherin (P-Cad). We have previously reported the presence of N-Cad (4.7 and 3.7kb) and P-Cad (3.2kb) transcripts in testis. No E-Cad message was detected. For the presen t study, testes 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 spermatozoa. 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 63, and 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 spermatogenic wave. Supported by the Medical Research Council of Canada.

3 AN ANDROGEN-REGULATED PROVIRAL GENE IS PREFERENTIALLY EXPRESSED IN THE MOUSE CAPUT EPIDIDYMIDIS Gail A. Cornwall, M-C. Orgebin-Crist and Stephen R. Hann[1]*, Center for Reproductive Biology Research and [1]Department of Cell Biology, Vanderbilt University, Nashville, TN.

The recent focus of our studies has been to identify genes that are specifically expressed in the caput epididymidis and which ultimately may be important for sperm maturation. To identify these genes a mouse epididymal cDNA library was screened with a caput-specific subtractive probe. One of the caput-specific cDNAs identified was shown to be 96% homologous to sequences present in endogenous murine retroviral genes. Based on preliminary sequence analysis and transcript size, the proviral cDNA appears to represent a defective provirus that has deleted sequences essential for producing a functional virus. The defective proviral mRNA is surprisingly restricted in its expression. It is expressed preferentially in the caput epididymidis with minimal expression in the vas deferens and kidney. Moreover, the mRNA is androgen-regulated since 4 weeks postcastration revealed a dramatic reduction in message which recovered partially to precastrate levels following T-replacement. Although nondefective proviral sequences have been found to be expressed in mouse epididymis and several other tissues, our observations raise the question of why a defective provirus is expressed predominantly in the caput and not the corpus-cauda epididymis. It suggests that these proviral sequences have integrated into a region which has caput-specific sequence elements. Also possible is that the proviral LTR/enhancer may be influencing the expression of caput epididymal genes which may be important for sperm maturation. Recently, Southern blot analyses have been performed and a genomic library has been screened with the proviral sequence to begin to test these possibilities.

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. WJB Dorn Veterans Hospital, University of South Carolina School of Medicine, Columbia, SC 29201 and The Population Council, New York, NY 10021.

Insulin-like growth factor-I (IGF-I) is a 70 amino acid peptide which mediates effects of growth 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 and 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 while bands 1 and 2 cells 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 elutriation 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 testis 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.

5

EXPRESSION OF THE ANDROGEN RECEPTOR GENE IN THE RAT PENIS DURING SEXUAL MATURATION

Gonzalez-Cadavid, N., Swerdloff, R.S., Lammi, C., and Rajfer, J., Departs. of Surgery and Medicine, Harbor-UCLA Medical Center, Torrance, CA

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 maturation 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 blots. 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 developmental regulation of the expression of the AR gene. This may be extrapolable to human 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 CYNOMOLGUS MONKEY. 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-1 (IGF-1), TGF-α, 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 testes from the following stages of cynomolgus development; early prepubertal (1.5-2.5 yr), late prepubertal (2.5-3.5 yr), pubertal (3.5-4.0 yr), and adult (6-8 yr). A summary of the relative concentrations of the Rc mRNA levels are as follows:

receptor	early prepubertal	late	developmental stage pubertal	adult
Tf Rc	+	+ +	+ + +	+ +
IGF-1 Rc	+	+ + + +	+ +	+
EGF Rc	+	+ +	+	+
Ret Rc-α	+ +	+ +	+ +	+ +

The relatively high concentrations of testicular Tf Rc 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-1 and EGF Rc mRNA levels in the late prepubertal stage suggest that IGF-1 and TGF-α are important during early stages of sexual development.

(Human receptor cDNA's were obtained from American Type Culture Collection; supported in part by NIH grant HD25272)

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

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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 25% of the specific activity recovered from PC-12 and rat pituitary cells respectively. Thus PKC inhibitors such as sphingosine, staurosporin and H-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 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 towards fertilization.

8 COLD SHOCK INDUCES HYPERACTIVATED MOTILITY IN MOUSE SPERMATOZOA. E.Z. Drobnis, K.R. Robertson*, C.R. Day*, J.W. Overstreet. 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, $[Ca^{++}]_i$, may be involved in diminished functional competence. Since increasing the membrane permeability to $[Ca^{++}]_i$ with A23187 causes hyperactivated motility in mouse sperm, analysis of motility pattern may be capable of detecting this aspect of CS cryodamage. To test this hypothesis, sperm were collected from CD-1 mice into Tyrodes buffer (2 mg/ml BSA). The control sample 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 motile 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; 13.5 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 ($p > .05$). 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. = 300 ± 61 μm/sec) and ALH (24 ± 4.0 μm) and the lowest LIN (0.255 ± 0.062). Representation in this subpopulation increased from 6% of control sperm to 38% of CS sperm. The decrease in percent motile (20%) did not support a selection mechanism for this change. We conclude that CS induces a hyperactivated pattern of motility in a subpopulation of mouse sperm.

9 MONOCLONAL IgM ANTIBODIES AGAINST RABBIT SPERM FOR VAGINAL CONTRACEPTION. Philip E. Castle*, Kevin J. Whaley, Thomas R. Moench*, James E. Hildreth*, W. Mark Saltzman*, Michael L. Radomsky*, Timothy E. Hoen*, and Richard A. Cone*, Departments of Biophysics, Medicine*, Pharmacology*, and Chemical Engineering* The Johns Hopkins University, Baltimore, MD

We are investigating the use of monoclonal antibodies to create new prophylactic contraceptives such as a vaginal ring that releases a combination of monoclonals against sperm and STD pathogens. Immuno-infertility correlates best with antisperm antibodies in semen or cervical mucus, not in serum: in mucus, the amibodies agglutinate sperm or immobilize them (the 'shaking' effect). However, most monoclonals 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 find 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 polyvalent IgA 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 polyvalent antisperm monoclonals to pursue contraceptive tests in the rabbit. A BALB/c mouse was immunized with ejaculated rabbit sperm, separated from semen by 'swim-up', and injected intraperitoneally and subcutaneously. Spleen 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 monoclonals 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. We are currently evaluating MARS-M3 (Mouse Anti-Rabbit Sperm 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 LIPID TRANSFER PROTEIN. *Stuart E. Ravnik and Charles H. Muller*, Depts. 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 (Ravnik 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 subsequent acrosome reactions to take place.. Our previous studies have used BWW with BSA to incubate sperm. Because some albumin preparations are contaminated with LTP-I, we asked if LTP-I could stimulate human sperm capacitation in protein-free medium. Human sperm capacitation and acrosome reaction was measured using both sperm penetration assays and PSA lectin staining. 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 to 10 X 10^6 sperm /ml in BWW-PVA, treated 15min with LTP-I, washed, and incubated for 1hr further before adding to eggs or fixing for lectin staining. LTP-I was purified from human plasma by chromatography; the final LTP-I preparation had 11% Transfer/3hr 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: Penetration Index- 0.42 ± 0.11, %AR- 15.7 ± 2.0; Control: PI- 0.05 ± 002, %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 every 15 minutes for 2 hours. Sperm treated with LTP-I showed no stimulation of AR up to 45 minutes after treatment (30 min: LTP-I %AR=4.8 ± 0.5, Conuol %AR=4.5 ± 1.7), but after 45 minutes had significantly ($p<0.01$) more AR than control, increasing linearly up to 2 hours (1 hr: LTP-I 17 ± 2.2; Control 4.5 ± 2.4; 2 hr: LTP-I 24.5 ± 4.9, 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 HD 12629.

11 ULTRASTRUCTURAL FEATURES OF BULL SPERM COCULTURED WITH BOVINE UTERINE TUBE EPITHELIAL CELLS. J.E. Ellington,[1] D.H. Schlafer[1] and R.H. Foote.[2] [1]New York State College of Vet. Med., [2]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 or 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 fertilize cow 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 in TALP at 39°C in 5% CO_2 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-reaction. Another 10-20% of the cells showed non-specific changes 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 HD00884/HD21939.

12 PROTAMINE IS REMOVED FROM HAMSTER SPERM NUCLEI DURING THE DECONDENSATION STEP OF FERTILIZATION. S. Perreault and R. Barbee*, 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 pronucleus formation. Isolated epididymal sperm nuclei were treated sequentially with 5 mM DTT (10 min, to reduce protamine disulfide bonds), & 0.5mM monobromobimane (10 min, to fluorescently label free sulfhydryls). These nuclei were microinjected into hamster oocytes. Sperm nuclear fluorescence was scored at various times post injection (PI) on a brightness scale of 4 (maximum) to 0 (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 decondensed in DTT/SDS in vitro (i.e. reduction of disulfide bonds without protamine removal), they remained fluorescent. We conclude that protamine removal occurs rapidly during chromatin dispersion (decondensation) in the oocyte, is complete before pronucleus 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 Meisami*+ and John Porcelli*. Depts. 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 testes 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/g testis (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 more than doubled and the efficiency of sperm production (DSP/g testis) 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.

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*, N.S. Padian,[2]* T.R. O'Brien.[3]*Harvard Medical School, Boston, MA 02115; [2]UCSF, San Francisco, CA 94110; and [3]CDC, Atlanta, GA 30333.

Among persons infected with Human Immunodeficiency Virus Type 1 (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. Three of the men, two asymptomatic (CDC Stage II) and one with AIDS-related complex (ARC; CDC Stage IV), had moderate to low seminal WBC counts and undetectable levels of infectious HIV-1 or proviral DNA in their ejaculates. However in a series of three ejaculates from a fourth HIV-1 seropositive asymptomatic vasectomized man, high numbers of WBC, predominantly macrophages, were detected. Furthermore, two of the samples were positive for infectious HIV-1 by coculture on activated peripheral blood leukocytes and one also tested positive for HIV-1 proviral DNA by polymerase chain reaction. Our finding 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.

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

Most studies have shown that endocrinologic disorders are an infrequent 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 which 7 had documented hypogonadism with repeatedly low T and an elevated serum LH. One 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 (volume <19ml by orchiometer). However, 2 of 7 patients with an endocrinopathy had normal sized testes and 1 of 7 stated he had normal libido. Conversely, almost one-half of the impotent patients without an endocrinopathy had decreased libido and 10 had atrophic testes. 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.

16 HYPOGONADISM IN MEN WITH TYPE II DIABETES AND ORGANIC IMPOTENCE: RESPONSE TO TESTOSTERONE THERAPY F.T. Murray, R. Johnson*, M. Geisser* and J. Rountree*. University of Florida, Gainesville, FL 32610.

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±4.4(SD) and men with type II diabetes and organic impotence (n=7, age=48.9±1.35y) showed that serum bioavailable testosterone (7.7 ± 0.69 vs 5.78 ± 0.58 nm/l; $p^*0.05$) but not total testosterone (21.1 ± 2.4 vs 18.4 ± 2.3 nm/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 Depression 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.

17 **STANDARDIZATION AND COMPARABILITY OF COMPUTER-AIDED SPERM ANALYSIS (CASA) INSTRUMENTS.** RO Davis & DF Katz. Reprod. Biol. & Med., Univ. California, Davis, CA 95616.

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).

19 COMPARISON OF ASSESSMENT OF SPERM MORPHOLOGY BY COMPUTER ASSISTED VERSUS MANUAL METHODS

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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, N.Y.). The mean percent normal, small and amorphous head forms were not significantly different when assessed by the two methods. Whereas, the percent big and taper forms were significantly higher when analyzed by the Morphologizer. Significant positive correlations were obtained between the two methods for morphological classification of % normal ($r=0.52$, $p<0.0001$), % small ($r=0.50$, $p<0.0001$), % taper ($r=0.52$, $p<0.0001$) and % amorphous spermatozoa ($r=0.33$, $p<0.01$). Examination of the individual data showed that the differences between paired values obtained 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 (intra-technician CV less than 10% with either method). Overall, there was no apparent advantage of the commerically available Morphologizer over the manual method with regards to 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.

18 THE MEDIATOR OF HUMAN CORPUS CAVERNOSUM RELAXATION IS NITRIC OXIDE. W.J. Arousou*, P.A. Bush*, O.M. Buga*, L.J. Ignarro* and J. Rajfer. UCLA Medical Center, Los Angeles, CA 90024.

In an attempt to determine whether nitric oxide (NO) could be the specific mediator of human corporal smooth muscle relaxation, a series of experiments were performed in vitro using strips of human corporal tissue from 7 patients. The strips were hung in organ chambers, bathed 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 nonadrenergic-noncholinergic (NANC) neurons. The addition of a specific inhibitor of NO synthesis (N^G-nitro-L-arginine) prevented ACh from relaxing corporal tissue ($p<0.001$). Similarly, inhibitors of NO production N^G-nitro-L-arginine and N^G-amino-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 concomitant 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.

20 *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*, F. DeStefano*. Centers for Disease Control, Atlanta, GA 30333 and University of California, Davis, CA 95616.*

With recent advances in the technology for semen analysis, several automated standardized parameters of 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 sperm 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 measurements were made with a Cellsoft automated semen analyzer (Ast et al., 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 (straightline velocity $\geq$ 25 um/sec) and 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 covariates, 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.

21 REGULATION OF THE TRUNCATION OF LH RECEPTORS AT THE PLASMA MEMBRANE IN LEYDIG CELLS.

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The truncation of lutelnizing 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 3.3nM LH for 2h at 34°C had no detectable effect on [^{125}I]-hCG binding sites 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 NaN_3. Dibutyryl-cyclic AMP (0.01,0.1,1 mM) also caused a 30-50% loss of binding in MA10 and MLTC-1 cells, by inhibiting internalization. Phorbol 12-myrlstate-13-acetate (PMA) had no effect on binding or internalization of [^{125}I]-hCG. The loss in binding sites under all conditions was prevented by the addition of protease inhibitors (leupeptin, PMSF, aprotinin). 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 [^{125}I]-hCG, a radioactive-protein complex of approx. M_r 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.

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22 ADULT RAT LEYDIG CELL PEROXISOMES CONTAIN 3-HYDROXY-3-METHYL GLUTARYL-COENZYME A REDUCTASE (HMG-CoAR). [1]S.M.L.C. Mendis-Handagama, [2]S.K. Krisans, [1]L.L. Ewing. [1]Department of Population Dynamics, The Johns Hopkins School of Hygiene and Public Health, Maryland 21218. [2]Department of Biology, San Diego State University, California 92182.

Leydig cell peroxisome volume is positively and linearly correlated with testosterone secretion in hamter, 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; J.Androl.1990,11:270-8);latter transports cholesterol to mitochondria (J.Biol.Chem.1982,257:8928-34). Intra-peroxisomal SCP_2 in Leydig cells increases 5 fold above controls 30 minutes after a single SC injection of luteinizing hormone (Endocrinol.1990, 127:2947-54); latter occurs prior to the plasma testosterone peak. These observations suggest that peroxisomes 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. 1990,11:270-8). HMG-CoAR was immunolocalized in Leydig cell organelles via AuroProbe EM Protein A gold G10. 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 /cytoplasmic 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 HD07204.

23 DIVERSE SECRETORY PATTERNS OF RAT CLUSTERIN AND α_2-MACROGLOBULIN BY SERTOLI CELLS IN BICAMERAL CULTURE CHAMBERS. Josephine Grima,[1,2,*] Richard A. Lockshin,[2,*] and C. Yan Cheng.[1,2,3] [1]The Population Council, 1230 York Avenue, New York, New York 10021; [2]Department of Biological Sciences, St. John's University, Jamaica, New York 11439; and [3]The 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_2-macroglobulin (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 RIAs and bicameral culture chambers to study the polarized secretion of these proteins by Sertoli cells *in vitro*. Briefly, Sertoli cells (2-5 x10^6 cells/cm^2) were plated on Millicell[TM]-HA culture inserts and maintained in a humidified atmosphere of 95% O_2/5% CO_2 at 35C for a period of 4-13 days during which serum-free media]F12/DME, 1:1, v/v, supplemented with insulin,10 µg/ml; human transferrin, 5 µg/ml; epidermal growth factor, 2.5 ng/ml; and bacitracin, 5 µg/ml] were changed every 24-48 hr. The amounts of clusterin, AMG, and transferrin in the media obtained from the apical and basal chambers were quantified by RIAs. It was noted that clusterin was secreted exclusively to the apical chamber (A) over a 13 day culture period, since immunoreactive clusterin was not detectable in the basal (B) compartment. In contrast, AMG, a tetramer with an apparent Mr 8-fold greater than that of clusterin and transferrin (Mr 680,000 vs Mr 85,000 and 75,000, respectively), is secreted into the apical and basal compartments with an A/B ratio of 2.5±1.8:1 making it distinctively different from clusterin (A:B, 1:<0.0001) but similar to transferrin (A:B, 1.6±0.2:1). The secretions of clusterin and AMG into the apical and basal chambers were not regulated by either testosterone (T, 0-1 x10^{-5} M) or ovine FSH (0-1000 ng/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.

24 DYNAMICS OF INTERACTIONS OF CLUSTERIN (SGP-2) WITH MATURING SPERMATOZOA. Danette R. Mattmueller and Barry T. Hinton. Dept. of Anatomy and Cell Biology, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

Clusterin is a heterodimeric glycoprotein synthesized and secreted by rat Sertoli cells. An antigenically similar form is secreted in the epididymis. The specific role of clusterin is unknown, but it may act to protect spermatozoa from complement-mediated lysis. The goal of this study was to determine the presence of clusterin in the luminal fluid and its association with the membranes of developing spermatozoa in the presence and absence of androgen. We have previously demonstrated by 2-D Western blot analysis that in epididymal fluid the amounts of clusterin were: caput > corpus > cauda. Luminal fluid from cauda epididymides was collected from control and orchiectomized rats (6 and 12 days) and equal volumes of fluid were analyzed by 2-D Western blot probing for clusterin. Following orchiectomy, there was an increase in clusterin in the luminal fluid after 6 days and maximal amount after 12 days when compared with control cauda fluid. Amido black protein stain of Western blots before probing with the clusterin antibody revealed a decrease in known androgen dependent proteins in the fluids of 6 day and 12 day orchiectomized animals. Membrane proteins were extracted from equal numbers of caput and cauda sperm by detergents and analyzed by Western blot probing for clusterin. Blots of caput sperm membrane extracts revealed the presence of the epididymal form of clusterin (MW 40 kd and 32 Kd). Cauda sperm membrane extracts had a very low levels of epididymal clusterin. Cauda sperm was collected from animals 6 days after orchiectomy and sperm membrane proteins were extracted. Extracts from these sperm revealed levels of clusterin similar to that seen in caput sperm. Our findings suggest that in the normal animal, clusterin is secreted into the lumen of the proximal epididymis, where it binds to the sperm membrane. Distally, clusterin dissociates from the spermatozoa and is endocytosed by the epithelial cells. We hypothesize that androgen is required for (1) dissociation of clusterin from spermatozoa and (2) endocytosis.

We thank Drs. M. Griswold and S. Sylvester for providing clusterin antibody. Supported by NIH grant HD 18257.

25 **REGULATION OF STEADY-STATE 4-ENE STEROID 5α-REDUCTASE mRNA LEVELS IN THE RAT EPIDIDYMIS AFTER ORCHIDECTOMY AND TESTOSTERONE REPLACEMENT.** Robert S. Viger and Bernard Robaire. Dept. of Pharmacology & Therapeutics, Centre for the Study of Reproduction, McGill University, Montreal, Quebec, H3G 1Y6.

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 epididymal function. Previous work from our laboratory has characterized the endocrine regulation of the epididymal enzymatic 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, 300-350g, were either sham-operated to serve as controls or orchidectomized and simultaneously implanted with T-filled polydimethylsiloxane capsules of either 0 cm, 2.5 cm or 18.6 cm (3 x 6.2 cm) in length. Implants of 2.5 cm mimic serum T in control adult rats, while implants measuring 18.5 cm result 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 liver 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 initial segment of the caput epididymidis but only a 40-50% decrease in the caput, corpus, and cauda. With the 2.5 cm implants, mRNA levels were as low in the initial segment and the caput as in the orchidectomized rat, but completely maintained, relative to control, in the corpus and cauda epididymides. With the 18.6 cm dose, it was possible to maintain only 50% of the steady-state mRNA level in the initial segment, while complete maintenance was found for the rest of the tissue. These results demonstrate that epididymal 5α-reductase is regulated at the mRNA level and that the regulation by testosterone is different in the various segments of the epididymis. Supported by MRC of Canada.

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THE SIGNIFICANCE OF FLAGELLAR PATHOLOGY IN THE EVALUATION OF ASTHENOZOOSPERMIA H.E.Chemes*, 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 asthenozoospermia. 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 3.6% 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.This percentage progresively increased from groups 1(G1) to 4 (G1:33.9±6.9, G2: 52.8±6.2, G3:72.1±5.7, G4:89.6±4.8), while the values of fast forward motility were uniformly low throughout the groups and showed only minor diminutions with no statistical significance(G1:4.8±5.5, G2:4.3±5.6, G3:3.6±4.5, G4:2.4 ±3.6). Group 1 had values of axonemal anomalies overlapping those reported for normal men. Therefore, their astheno zoospermia was not due to an increase in abnormal flagella. Groups 2, 3 and 4 displayed slight (40-60%), moderate (61-80%) and great (81-100%) increases in sperm anomalies. In 30% of the patients (G1:n=27+ G2:n=32) axonemes were either normal or with slightly increased abnormality values,while in the remaining 70% (G3:n=76+G4:n=66) asthenozoospermia was related to an increase of pathological axonemes. These findings indicate a high incidence of sperm anomalies in patients with severe asthenozoospermia and stress the need of ultrastructural examination for their proper evaluation.

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, A. Bartke. Michael Reese Hospital, hicago, IL 60616 and Southern Illinois U iversity, Carbondale, IL 62901.

Previous work has shown that expression of human (h) GH in transgenic mice is associated with enlargment of seminal vesicles (SV) in young mice which is greatly accentuated as the animals age (Cecim,et al, 1990). 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 transgenic mice which possess elevated GH levels with no prolactin effects had SV with weights similar to aged-matched control mice. In order to further characterize this SV enlargement, we examined the cellularity and androgen receptor (AR) content of SV from six groups of animals: young (3-5 months) control mice, old (>12 months) control mice, young hGH transgenic mice, old hGH transgenics, young bGH transgenics, and old bGH transgenic mice. Results revealed that young transgenic mice (hGH and bGH) possessed larger SV than their nontransgenic littermates (control: 45 ± 5, hGH: 63 ± 5, bGH: 71 ± 1 mg weight after fluid expression). However, this enlargement was not associated with an increased cell number (DNA content) nor a difference in AR content (control: 1814 ± 342, young hGH: 1714 ± 510, young bGH: 2334 ± 390 fmmoles/mg DNA). With aging, the bGH transgenic SV exhibited massive hyperplasia whereas the bGH transgenic SV did not show this response (control: 50 ± 6, hGH: 172 ± 32, bGH: 81 ± 8 mg weight). The old hGH SV showed a marked increase in cell number (DNA content) and a marked decrease in cell size and/or glandular secretions (protein:DNA ratio) when compared to old controls and to young hGH transgenic SV. Tissue AR content (fmmoles/mg DNA) was markedly reduced in old hyperplastic hGH SV (236 ± 49) when compared to young hGH SV (1714 ± 510), old control SV (1550 ± 410) and old bGH SV (3229 ± 284). Histological assessment revealed a massive increase in stromal tissue in the hyperplastic SV from old hGH transgenic mice. Immunohistochemistry indicated the absence of AR in the hyperplastic stromal areas where as the acinar epithelial cells showed similar AR staining intensity to the control SV. The present results suggest that the aging-associated SV hyperplasia in hGH transgenic mice is a result of a massive increase in stromal tissue which is low or devoid of AR suggesting a loss of androgen dependence for growth. (Supported by NIH Grants DK40890 and HD20033)

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.Schulman* and R.Sherins. Genetics & IVF Institute, Fairfax, VA.

The aim of this study was to assess the value of sperm morphology and ability of capacitated sperm to undergo acrosome reaction (AR) as predictors of fertilization. Semen specimens were obtained from 250 infertile men participating in our IVF program. Sperm morphology was determined with the Dif-Quik stain in 215 specimens used at IVF. To assess AR an additional semen was obtained from 76 patients; sperm were washed free of seminal plasma using a modified Tyrode's medium (HSM) supplemented with 0.3% BSA, filtered through glasswool, capacitated for 22 hr in HSM with 2.6% BSA, then exposed to 10% human follicular fluid. AR was assessed by FITC-labeled Pisum Sativum lectin. Age of wife and number of eggs inseminated were not correlated with male assessment brackets. Data were analyzed using Spearmen correlation and median response cumulative curves (see table below). AR response (% AR above control) in normal fertile men was 5% or more; normal morphology among donors was greater than 14%.

	% normal morphology				% AR above control			
test response	<4	4-6	10	22	1	4-5	6-10	>10
% eggs fert.	5	19	50	60	0	0	33	42
	r=0.31, p<0.0001				r=0.41, p<0.0002			

As % normal morphology or AR response increased, there was greater success in IVF. If normal morphology was <4%, half the patients fertilized <5% of eggs while 50% of eggs fertilized with 10% normal morphology. When AR response was less than 5% there was no fertilization for half the patients; when AR response was 6-10% the median response for patients was 33%. AR and morphology were correlated (r=0.396), yet both had importance in determining IVF performance. Data suggest that sperm morphology in semen and AR inducibility are useful in predicting IVF success.

29 HUMAN CERVICAL MUCUS PROPERTIES AND SPERM MUCUS INTERACTION DURING THE PROLIFERATIVE PHASE OF THE MENSTRUAL CYCLE D.F. Katz[1], A.I. Yudin[1*], J.B. Andrew[1*], B.L. Lasley[2*] and M.J. Zinaman[3]. Depts. of OBGYN[1] and Vet. Reprod.[2], University of California, Davis, CA and Dept. of OBGYN[3], Georgetown University, Washington, D.C.

Cervical mucus properties are optimal for sperm penetration and survival at the time of ovulation, and mucus is thought to be a barrier to sperm during the remainder of the menstrual cycle. We investigated this phenomenon, collecting daily mucus from normal women, beginning in the early proliferative phase. Cycle phase was monitored via measurement of urinary and serum hormones. Mucus properties were assessed by transmission electron microscopy after preparation by a freeze substitution technique. Fresh human sperm-mucus interaction was analyzed in vitro using a new plane slide protocol, which included videorecording. Sperm movement was analyzed using computer aided sperm analysis (CASA). We found good correlations among the architecture of the mucus microstructure, sperm penetration, and serum estradiol (E) levels. The early, cellular mucus, associated with low estradiol (E≤20 ng/ml), contains a compact microstructure, with interstices of the order of 0.08 μm in diameter. This size is an order of magnitude less than that for ovulatory mucus (E≥200 ng/ml), where the mucin molecules are less electron dense and there is more fine structure. Sperm velocity in periovulatory mucus correlates with estradiol level. Nonetheless, sperm can penetrate the low-E, early proliferative phase mucus: flagellar movement is more constrained, and velocities are lower, than at ovulation. However, such early mucus penetrability must be considered in understanding the interval during which insemination can lead to successful sperm transport in vivo. (Supported by IISNFP BR-US006).

30 SPERM MOTILITY, CREATINE KINASE PARAMETERS AND HAMSTER OOCYTE SPA IN PERCOLL FRACTIONS OF HUMAN SEMEN. G. Huszar, M. Corrales*, L. Vigue*, #P. Quinn*, The Sperm Physiology Laboratory, Dept. of Ob/Gyn, Yale Univ. Sch. of Med., New Haven, CT 06510 and #Inst. for Repr. Res., Los Angeles, CA.

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 (conc: 64±15 million sperm/ml, mot: 45±2%, vel: 39±2 μm/sec, CKACT: 0.19±0.05 CK IU/100 million sperm and CKM: 35±3%, all values mean ± SEM) on a discontinuous gradient of 40% and 80% Percoll (400 x 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%, 33±3% and 89±2% with a motile sperm 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, 0.06±0.01 and 0.03±0.005 CK IU/100 million sperm, the CKM values were 27±3%, 63±4% and 84±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 ova we have performed SPA and found 10-fold higher penetration (both rate and index) when assaying sperm fractions arising from the pellet vs the top or intermediate phase of the gradient. These data demonstrate that the 40-80% discontinuous Percoll gradients provide a one-step preparation method that concentrates in the pellet more than 50% of the motile sperm. The sperm in the pellet is also the best in motility (89±2%), CKACT (0.03±0.005 CK IU/ 100 million sperm), CKM (84±4%), and SPA properties. The improved fertilizing potential of sperm fractions with such parameters 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 (Huszar et al, J. Androl., 1990; Molec. Repr. and Dev., 1990) (Supp. HD-19505).

31 EVIDENCE FOR PEROXIDATIVE DAMAGE TO HUMAN SPERM DURING CRYOPRESERVATION

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The present need to cryopreserve human sperm used in artificial insemination donor (AID) programs has focussed attention on the cell damage to the cells from the process. We have examined the possible role of phospholipid peroxidation in this damage. Comparison of phospholipids of pooled human sperm samples from AID donors before and after cryopreservation in egg yolk medium showed 84% loss of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) plasmalogens, which contain peroxidatively labile polyunsaturated acyl and vinyl ether moieties. Loss of ^{3}H-arachidonic acid in both diacyl and plasmalogen PC and PE due to cryopreservation was 61% and 41%, respectively, while loss of palmitic acid was nil, consistent with peroxidative degradation. We had previously shown that human sperm contain the peroxidative protection enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPX) (J. Androl.8 (1987) 338-348). SOD activity was found to be lost during cryopreservation, while GPX activity remained unaffected. Time to complete loss of motility under aerobic incubation and initial percent motility decreased in proportion to this SOD loss. Isolation of a highly motile subpopulation from a given sample prior to and after cryopreservation by glass wool filtration revealed that these cells had SOD activities that were nearly equal and higher than that of the fresh sample, regardless of the wide variation in the initial SOD activities between fresh samples. We conclude that a given sperm sample has a distribution of SOD activities among the cells, that the population of cells with low 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.

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32 GAMETE MICROMANIPULATION FOR THE TREATMENT OF MALE INFERTILITY IN THE MOUSE. [1,2]D.J. Lamb, [1]J.D. Stockton, [1]L.I. Lipshultz, [3]M.L. Meistrich. [1]Scott Dept. Urol. & [2]Dept. Cell Biology, Baylor College of Medicine, [3]Dept. Exper. Radiotherapy, U.Tex. MD Anderson Cancer Center, Houston, TX 77030

In vitro fertilization (IVF) has been used to treat certain types of male infertility, but is often unsuccessful due to severe defects in sperm number, motility, morphology or 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 function. The bouncy mutant (bc/bc) was found to be infertile *in vivo* (0% fertilizations, 5 trials, 79 eggs). However, IVF resulted in a fertilization rate indistinguishable from controls (92%±7, 3 trials, 50 eggs). In contrast, another mutant mouse strain, blind sterile (bs), produce homozygous (bs/bs) males who lack mature sperm. Hence these animals are incapable of fertilization either *in vivo* or *in vitro*. Sperm from bs/bs mice were not present and therefore were not micromanipulated. Interestingly, the heterozygous +/bs males were found to produce sperm which were fertile *in vivo*, but not *in vitro* (0% fertilizations, 8 trials, 263 eggs), although normal fertilization rates were observed for our standard IVF controls (71%±15, 3 trials, 80 eggs). PZD+IVF did not result in fertilization with the +/bs sperm (0%, 2 trials, 90 eggs). However, PVS overcame the block to fertilization *in vitro* and resulted in a fertilization rate of 57%±15 (4 trials, 65 eggs). Sperm from azh/azh males which have abnormal head morphology as their only defect did not fertilize eggs *in vitro* (4 trials, 65 eggs). While PZD was ineffective (0%, 29 eggs), PVS resulted in a fertilization rate of 22%±11 for azh/azh 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.

Posters Sunday

33 USE OF A Y-CHROMOSOME-SPECIFIC DNA PROBE TO MONITOR THE ALBUMIN COLUMN METHOD OF X-Y SPERM SEPARATION.

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The popular Ericsson method of X-Y sperm separation for pre-conception gender selection, in which sperm are passed through albumin columns 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 1ml 10% 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 42C in 2X SSC, followed by washing twice in 6X SSC/45% formamide at 42C, 2X SSC at 22C, and 0.2X SSC at 50C. Anti-digoxigenin antibody coupled to alkaline phosphatase was added, and the labeled probe was detected with NBT/X-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 ±0.6[SE] %) and the corresponding aliquots processed for X-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 spermatogenesis (Proc. Natl. Acad. Sci., 1990; 87:1431). PYR is known to exhibit an anti-malarial effect by inhibition of dihydrofolate reductase (DHFR) in the plasmodium. 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 folinic acid, the product of the DHFR reaction, at various dosages for 55 days. Breeding trials revealed that folinic 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 folinic acid and were normal or near normal ($P < 0.05$) with higher dosages of folinic 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 folinic 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 folinic acid. Studies are currently underway in our laboratory to characterize testicular DHFR.

35 TESTICULAR INJURY INDUCED BY HYPOTHERMIA AND ISCHEMIC HYPOTHERMIA. W.K. Aulitzky, P.N. Schlegel, P.S. Li, M. Goldstein, O.M. Phillips, C.W. Bardin. The Population Council and Division of Urology, NYH-Cornell, New York, NY 10021

Previous studies have shown that hypothermic testicular ischemia results in induction of Sertoli-cell only syndrome (SCO) in Lewis rats. However, the effects of HTI versus hypothermia alone (HA) have not been elucidated in a long term study. Isogenic Lewis rats were subjected to contralateral orchiectomy and ipsilateral hypothermia (4°C) or ischemic hypothermia for 3 hours. Ipsilateral vasectomy and removal of the involved testis from the scrotum was performed to prevent collateral testicular blood flow. Testicular function was evaluated by histology (general appearance and seminiferous tubular diameter(STD)), testis weight(TW), and serum FSH levels. Evaluation was perfo med at 2 days, 1,2,6, 12 and 18 weeks after che procedure (n=6 for each time point). Results demonstrated a decrease in mean TW after HA from 1.42 ±.11 to .64±.29 within one week, that returned to control values 18 weeks after HA. HTI also resulted in decreased mean TW from 1.42±.11 to .66±.11 after one week, but no return of TW was detected after 18 weeks. Similarly, STD decreased from 245±32 to 150±28 microns within one week after HA, and returned to 192±42 microns 18 weeks after the procedure. After HTI, tubular diameter decreased to 160±32 microns, and remained decreased at 18 weeks. Testicular histology was normal in 97.8% of control testes, but SCO histology was present in 96% of HA and 97% of HTI tubules within 2 weeks. 67% of tubules were normal 18 weeks after HA, whereas no normal tubules were seen 18 weeks after HTI. FSH levels increased significantly in HA animals at one week and returned to baseline after 6 weeks. FSH levels in the HTI group were not affected.

Taken together, these results suggest a permanent injury to germ cells with 3 hours of HTI, whereas HA induced only reversible injury to the seminiferous tubule.

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DILATION OF THE RIGHT TESTICULAR VEIN IN RATS WITH LEFT VERICOCELE

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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 a LV on the function of the right testicle we performed an experimental varicocele model in 40 Wistar rats by occluding the left renal vein partly and ligating the collateral vein between the left testicular vein (LTV) and the left common iliac vein. Five rats were sham operated (group A). Eight weeks after the operation, the varicocelized rats underwent laparotomy. 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 initial operation, the rats of group C showed significant reduction of : a) the fertility rate, b) the right testicular versus intraabdominal temperature differences, c) the right epididymal spermatozoal content, motility and glucose consumption, d) the weight of the right testicle, 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 LV improved the parameters representing the harmful consequences of the LV in the right testicle, 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 testicle.

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EVIDENCE THAT ABNORMAL STAINING BEHAVIOR OF HUMAN SPERM TAILS IS CAUSED BY EPIDIDYMAL 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 or at the transition from caput to corpus epididymidis. This observation is of great clinical relevance in 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-glocosidase was found. These parameters improved after antiphlogistic/antibiotic 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.

38 ADVERSE EFFECTS OF CYCLOPHOSPHAMIDE (CPA) ON PROGENY OUTCOME CAN BE MEDIATED THROUGH THE EPIDIDYMIS IN RATS. J. Qiu*, B. Hales* and B. Robaire. 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 paternal 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 undertaken. (1) Male rats were treated with saline (group A: control) or CPA (group B: 6.8 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 effect was observed on pre-implantation loss at any treatment time among any of the groups, but there was a time dependent and 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: 4.6±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 (10.0 mg/kg/day for 7 days) was not different from that produced by CPA treatment alone (Control: 3.6±3.2%; Ligation: 5.9±3.9%; CPA: 36.9±2.8%; CPA plus ligation: 35.9±5.3%). (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±2.2; 10 mg/kg: 4.6±1.9; 30 mg/kg: 21.4±5.0%; 70 mg/kg: 26.5±4.2%). 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 spermatozoal transit through the epididymis and is mediated in the caput and corpus epididymidis, but not in the cauda epididymidis. Together the three 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

39 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% at baseline to 34.0% on week 4 and to 21.1% on week 6. In contrast, VEL, LIN, ALH and BCF did not change in any of the subjects. Azoospermia 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.

40 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 end point, 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-, U- or chi-square tests to differentiate between two groups or, for more complex designs, a form of ANOVA. 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 LOGIT 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: $p(x) = 1/[1+e^{-l(x)}]$, where $0 \leq p \leq 1$ and $l(x) = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$, weighted as a function of the total fertilization events, is evaluated using a general linear models 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: $y_{(x)} = \gamma / 1 + e^{\beta(\tau - x)}$, which approximates a logistic function. The parameters γ and τ, representing the asymptote and point of half-maximal fertility, 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.

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THE EFFECT OF CLOMIPHENE CITRATE ON SPERM MORPHOLOGY. B.S. Shanis, J.H. Check, A. Bollendorf. 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.

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 1 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 (>6 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 % or total normal forms (volume x count x (% normal/100)) 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 8.7 ± 5.3% before treatment as compared with 4.9 ± 3.5% after treatment, group C 12.5 ± 6.9% vs 6.7 ± 3.6%). Three months of CC caused no change in the absolute number (expressed as 10^6) of morphologically normal sperm (8.0 ± 7.8 before and 8.7 ± 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 25.9 ± 36.5 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 VARICOCELECTOMY. C.B. Dhabuwala, Sayeed Hamid*, J.E. Pontes*, 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 varicocele were unilateral, 38 (79%) were diagnosed clinically (confirmed by doppler examination) group 1, in 10 (21%) patients the varicocele was diagnosed by doppler examination, group 2. All these patients underwent internal spermatic vein ligation. Repeated measures of multivariant analysis of variance show a significant improvement in sperm density and sperm morphology in both the groups, following varicocelectomy ($p = .011$ & $p = .001$), the same analysis does not show a significant improvement in forward motility ($p = .448$). 19 out of 38 (50%) men in group I became fertile, 3 out of 10 (30%) men in group 2 became fertile. There was no significant difference in fertility between the groups ($p = > .05$). Pre operative sperm density of > 20 mill/ml and spouse under 30 years of age were found to be good prognostic indicators of fertility.

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

We report our experience with 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 (PGE_1) 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 (500mcg/cc) and 1.2 cc of 0.9% normal saline mixed together to produce a vial 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 22-80 yrs. In 52 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, 46 patients (46%) 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 that also monitors penile rigidity (RigiScan™ by Dacomed) (NPTR). Previous techniques may not have noted abnormal rigidity despite normal tumescence.

To test the reproducibility of NPTR 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 B & 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.

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SPECIFIC ALTERATIONS IN THE DYNAMICS OF LUTEINIZING HORMONE SECRETION IN HEALTHY AGING MEN

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Concomitants of aging such as illness, medication use, and impaired activity status can influence function of the hypothalamo-pituitary-testicular axis adversely. However, the nature and extent of significant alterations that occur in the gonadal axis in healthy ambulant older males are not well defined. Moreover, since most studies have relied on RIA of occasionally sampled serum LH concentrations, age-associated changes in LH bioactivity and/or episodic LH secretion and in vivo LH clearance are poorly defined. Accordingly, here we have combined: (i) a new sensitive and specific 2-site immunoradiometric assay (IRMA) of LH in blood sampled every 10 min for 24 hr in each of 22 healthy active unmedicated men age 21-73; and (ii) a novel deconvolution algorithm (PNAS 84: 7686, 1987) to evaluate in vivo LH secretion and clearance. The LH IRMA correlated highly ($r = + 0.975$) with in vitro LH bioactivity in a Leydig cell bioassay. Mean and integrated 24-hr serum LH concentrations did not vary significantly with age over the 6 decades studied. Deconvolution analysis revealed unchanging daily LH secretion rates (nominally 132 ± 22 IU/24 hr), as well as an age-invariant in vivo LH half-life (mean 56 ± 5 min) and mass of LH secreted per burst (mean 2.8 ± 0.3 mIU/ml distribution volume). In contrast, deconvolution-calculated LH secretory burst amplitude decreased significantly with increasing age ($r = - 0.52$, $P < 0.01$), whereas secretory burst frequency ($r = + 0.43$, $P = 0.04$) and duration both increased significantly with age ($r = + 0.66$, $P < 0.001$). Tonic LH secretion was not observed at any age. We conclude that healthy aging of the male hypothalamo-pituita y-gonadal axis is marked by an accelerated frequency of discrete LH secretory bursts of prolonged duration and attenuated amplitude.

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HIV-1 IN THE REPRODUCTIVE TRACT OF A MAN WITH EPIDIDYMAL OBSTRUCTION: A CASE REPORT

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We have immunocytochemically analyzed reproductive organs obtained at autopsy from male AIDS patients, using a cocktail of monoclonal antibodies to HIV-1 antigens (p24, gp120, gp41). Reproductive tissues from 24 cases have been studied to date, and HIV-1+ cells identified in 9 patients. In all cases but one, HIV-1+ cells, all of lymphoid morphology, were present in low abundance in the interstitium and seminiferous tubules of the testis, epithelium of the epididymis, and stroma of the prostate. This exceptional case was a 39-year-old male homosexual with a previous history of gonorrhea and evidence of epididymal obstruction. The efferent ducts were highly distended with spermatozoa and immature germ cells whereas the epididymis, distal to this region, was devoid of germ cells. Present in the lumen of the efferent ducts were numerous large cells that were positive for HIV-1 antigens. These cells resembled macrophages, known HIV-1 host cells, and engulfed heads of spermatozoa could be observed in their cytoplasm. Vasectomy has been advocated to reduce the saxual transmission of HIV-1. As yet little data exist on where HIV-1 infiltrates the lumen of the reproductive tract to subsequently appear in semen to know if this would be effective. Furthermore, as reported here, blockage of the excurrent ducts causing accumulation of germ cells could attract HIV-1 infected macrophages which are not killed by the virus and could act as reservoirs for HIV-1. Activation of these cells could dramatically increase the viral burden in vasectomized AIDS patients and contribute to disease progression. ?

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QUANTITATIVE TESTICULAR BIOPSY IN SPINAL CORD INJURED MEN: COMPARISON OF QUANTITATIVE MICROMETRIC AND CYTOMETRIC ANALYSIS.

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Spermatogenic 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 micrometry (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 6 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 dissolution of the tissue specimen in Hank's balanced salt solution. The prepared cell suspension was adjusted to a cell count of 3×10^6/mL. Nuclear DNA staining was accomplished by the Vindelov method (Cystometry 3:323, 1983). A minimum of 10^5 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 eligibilty of spinal cord injured men for semen recovery techniques

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TESTOSTERONE (T) SUPPRESSES BIOACTIVE LH MORE COMPLETELY IN AZOOSPERMIC THAN OLIGOSPERMIC MEN

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In normal men, chronic administration of T (200 mg) markedly suppresses FSH and LH by RIA and reduces sperm production, azoospermia occurs in only 50-60% of the men. We wished to determine whether more complete suppression of gonadotropins by T could be an explanation for azoospermia in some men, while other men remained oligospermic. 19 men received T weekly by muscular injection. T injections continued until either azoospermia (3 consecutive azoospermic specimens) was achieved (n= 12), or 6 months elapsed without azoospermia being achieved (oligospermic, n=7). FSH and LH levels were measured by RIA and bioassay before and during the 6th month of T-treatment. Bio-LH and T data shown below:

	Before Treatment		After Treatment	
	Bio-LH	T	Bio-LH	T
Azoo	519±56*	3.7±0.2	42±2.3*	8.0±0.6
Oligo	272±49	4.2±0.7	65±8.5	6.4±1.1

Conclusions: 1) Greater suppression of bio-LH by T may increase the likelihood of producing azoospermia in experimental male contraceptive regimens, 2) Bio-FSH, immuno-FSH and immuno-LH levels (data not shown) decreased after T-treatment but were not significantly differen between groups, and 3) T values before and after treatment were not significantly differen between groups.

49 VENOGENIC 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 evaluable 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.

50 SPERMATIC VEIN LIGATION VERSUS TRANSCUTANEOUS EMBOLIZATION: CONTRASTING APPROACHES TO VARICOCELE MANAGEMENT.

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Sixty-eight men with varicoceles underwent standard inguinal or retroperitoneal ligation (36 patients) or radiographic transcutaneous embolization (32 patients). Comparison between the two groups was made regarding effectiveness, complications and the cost of varicocele obliteration. The failure rate, defined as nondisappearance of the varicocele by physical examination and doppler ultrasound was 11% in the surgical group and 6% in those who underwent embolization. Following surgery, six men had minor complications (2-wound infections, 1-urinary retention, 1-vomiting, 1-incisional pain requiring overnight hospitalization). One of the embolization patients had marked inguinal tenderness which resolved with anti-inflammatory drug treatment. The cost of both procedures is approximately the same if each is performed in an outpatient setting. Patients who undergo embolization are able to return to work without restriction 48 hours after the procedure. Men undergoing surgery returned to work after four days if they had a sedentary job and others having more physically demanding work may be off for a week or more depending on the policy of their employer as well as their level of comfort. It would appear therefore that embolization has some advantages over surgery. If there is a experienced interventional radiologist available to do the procedure both options should be offered to patients who are candidates for varicocele obliteration.

51 PARATESTICULAR MYOSARCOMA-REPORT OF 2 CASES

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We repon 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 presence of cystic change, foci 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, in some of them. Occasional sarcomeres 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 1.5 cm. It was moveable and somewhat tender. 10 weeks later a 2.5 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 predominant spindle cell pattern. Immunohistochemical studies were positive for desmin and vimentin. Spindle cells in a loose flocculent stroma were noted on electron microscopy. There was an external membrane and pinocytotic 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 orchiectomy.

Ultrastructural 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.

52 IMMUNOLOCALIZATION OF HUMAN SPERM ANTIGENS BY LIGHT AND ELECTRON MICROSCOPY.

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We describe immunofluorescent and EM immunogold localization of cognate antigens recognized by a panel of monoclonal antibodies (mAbs), S19, S69, S71, S72, S75, and S77 submitted to the WHO workshops on human sperm antigens (Anderson, D.J.,1990: "Prospects for Immunocontraception", Wiley-Liss, p.103-106). S19 was surface reactive while the other mAbs (S69, S71, S72, S75 and S77) were directed towards internal antigens. Permeabilization of the sperm with 95% ethanol or fixation with 2% paraformaldehyde 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 with paraformaldehyde and tannic acid was tested for mAbs S69 to S77 by immunofluorescence microscopy. Little or no staining was observed, indicating that the epitopes recognized by these mAbs were adversely affected by these fixatives. We therefore employed a pre-embedding immunogold staining technique prior to fixation with Karnovsky's buffer, osmication, dehydration and embedding 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 mAbs S71, S72, S75 and S77, which required sperm permeabilization to show their acrosomal locus by LM, did not immunoreact with the plasmalemma 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 mAbs S75 and S77 appeared to be associated with the inner acrosomal membrane. Supported by WHO Antifertility Vaccines Steering Committee, NIH T32 HD07382 and HD23789.

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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 be 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 charcoal treated serum until 7 more days. At confluency, cells were washed and incubated for 60 minutes at 37° with various concenrrations (0.1-2 nM) of [^{3}H] R-1881 in absence and presence of excess of cold R-1881 (5uM). 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, 0.4pM 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.

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SOME FUNCTIONAL FEATURES OF HUMAN SERTOLI CELLS IN VITRO.

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Testicular tissue was obtained from 8 healthy patients (mean age 28 years, range : 16 - 47 years) in agreement with the International Ethical Rules. They were washed several times with Ham F12/DME (1:1, v/v) medium containing antibiotics (Penicillin, Streptomycin, Fungizone), then mechanically dissociated before being submitted to a three steps enzymatic treatment (collagenase/dispase ; STI ; DNase at decreasing concentrations ranging from 0.05 to 0.0005 %) at 32° C during 10 to 40 min. The purified Sertoli cells (purity > 97 %, viability > 98 %) were then plated (2 x 10^6 cells/ml) in medium without insulin and transferrin but in presence of FCS (10 %) and antibiotics for 2 days. The medium was changed and replaced by a fresh medium without FCS ; on day 4 medium without pyruvate was added in the presence or absence of hFSH (5 ng/ml), dbcAMP (1 mM), testosterone (T 200 ng/ml), MIX (0.5 mM). The levels of lactate, pyruvate, total proteins and estradiol 17β (E2) were measured in medium 24 h later using specific kits.

An age-related increased of Sertoli cell lactate production was obvious (1.20 and 15.53 µmol/mg protein, respectively at 19 and 47 years). The addition of a saturating concentration of either FSH or dbcAMP (in the presence of MIX) was weakly effective (10-40 %) in improving the lactate output in young subject, and without effect in the oldest ones. It is to note that no pyruvate synthesis was detectable (< 2.5 nmol/mg prot).

By contrast, whatever the age, the basal E2 production did not change significantly (100-200 pg/mg protein), in the presence of T and MIX, a 6.7 fold increase of E2 was observed and the Sertoli cells from youngest patients seemed to be more sensitive to FSH (20-30 %). These data demonstrate that the human Sertoli cells in vitro are able to produce E2 under FSH control whereas the lactate synthesis is not modified.

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THE 5α-REDUCTASE INHIBITOR, 4-MAPC, INHIBITS DNA SYNTHESIS IN THE VENTRAL PROSTATE OF RATS.

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Treatment of young adult rats with the 5α-reductase inhibitor, 4-MAPC, (10 mg/kg/d) for 14 days caused a 37% reduction in ventral prostate weight, but only an 18% reduction in total DNA, and testosterone propionate (TP; 1 or 3 mg/kg/d) partially overcame this effect. (Ghusn et al, 1989) To further evaluate the effects of this agent on DNA synthesis, adult male rats were castrated and the prostates were allowed to involute for 7 days before daily injections for 3 days with sesame oil; TP (1 mg/kg), TP+4-MAPC (10 mg/kg), TP + cyproterone acetate (CA; 10 mg/kg). They were sacrificed 24 hours after the last injection and the ventral prostate was excised, minced, and incubated for 1 hour in medium containing 3H-thymidine. Results are expressed as Mean±SEM (cpm/ug DNA).

INTACT	SESAME OIL	TP	TP+4-MAPC	TP+CA
13,493	1883	106,744	30,248	17,571
±1,877	±220	±16,003	±6,780	±5,077

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% ($p<0.0001$).

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.

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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 cell damage leading to impotence, an in-vitro cell culture model was developed and characterized. Two to three pieces (4mm^3) of human corpora cavernosa from men with arteriogenic impotence were removed at the time of prosthesis 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% confluency after passage one/two) 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 axones, reticulum, mitochondria, thickened basement membrane, increased collagen and some degenerative changes in arteries and vacuolated appearance in smooth muscle cells. Lipid peroxidation (n moles malonyldialdehyde/mg protein) in non-BHT treated samples (0.4 ± 0.1 in cavernosal 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.

57 TESTICULAR STEROIDOGENESIS *IN VITRO* IN YOUNG ADULT NON-OBESE DIABETIC (NOD) MICE

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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. Hemitestes from each mouse were incubated with or without 12.5mIU hCG/ml, and media testosterone (T) was determined by RIA. Body and testes weights, but not seminal vesicle weight, were significantly reduced in NOD vs. ICR mice. Hemitestes 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.

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Over 40% of the phospholipids present in ejaculated spermatozoa are ether lipids. In nongerminal tissues, ether lipids have been demonstrated to be synthesized from PC or lyso-PC and long chain fatty alcohols. Alternatively, ether lipids can be produced by acylating lyso-ether lipids. This study was undertaken to determine whether ejaculated sperm could synthesize ether lipids from a variety of precursors such as 3[H]-1-O-alkyl-glyceryl-3-phosphorylcholine (lyso-PAF), 14[C]-1-hexadecanol [16:0-ol], phosphatidylcholine [PC], and either palmitic [16:0] or docosahexaenoic [22:6] acids. Ejaculates were obtained from fertile volunteers and were washed to yield approximately 10^7 sperm per assay. The incubation buffer consisted of 20mM ATP, 20mM MgCl2, 0.1mM coenzyme A, 5mM dithiothreitol, 380mM Tris [pH 8.4] and 50uM fatty acid or lyso-phosphetide. Phospholipids were extracted with chloroform and methanol and spotted on washed LK5 TLC plates which were run in a mobile phase consisting of chloroform:triethylamine:ethanol:water [30:34:30:8; v/v]. Spots corresponding to standards were scraped and counted. Blank incubations were performed in the absence of acyl substrates. When 3[H]-16:0-ol was included in the incubation with unlabeled lyso-PC or PC, a labelled PC product could not be isolated. Similarly, when 3[H]-lyso-PAF 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.

59 RAPID DISAPPEARANCE OF SPERMATOZOA AFTER VASOCCLUSION (VC) IN THE DOG.

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The reproductive system of the male dog is unique in that seminal vesicles and bulbo-urethral glands are absent. Thirty adult male Beagle dogs providing ejaculates containing at least 500 X 10^6 sperm with 90% motility were studied. The dogs' vasa were occluded percutaneously using a Vasocclude™ clip applying device through a small scrotal puncture hole. Dogs were ejaculated and semen analyses performed. The first 24 dogs were completely azoospermic 1 wk following VC. In view of these unexpected results, we studied six more dogs to determine the specific time 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.ml	Count ($x10^6$/ml)	Motility %
PRE-VC ± SEM	4.01±1.41	510.46±165.01	90±7
POST-VC 1 DAY	3.80±1.10	1±1.1	0
3 DAYS	3.00±0.71	0	0
5 DAYS	6.03±3.00	0	0
7 DAYS	5.81±2.00	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 take 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 germ cells from the reproductive tract following VC is consistent with this hypothesis. (Supported by USAID-DPE3050-A-00-8059-00).

60 FAILURE OF PLATELET ACTIVATING FACTOR (PAF) ANTAGONISTS (BN52021 AND WEB2086) TO BLOCK PAF INDUCED CHANGES IN SPERM FUNCTION

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PAF antagonists have been reported to effect gamete interaction and fertilization. We have examined 2 PAF antagonist, WEB2086 and BN52021, for their ability to block PAF induced changes in sperm function.

Motile sperm were separated from semen using a 97%/48.5% discontinous Percoll column. After separation, sperm were washed 2X in BWW containing 0.6% HSA (fraction V) and incubated for 18 hr to effect capacitation. The sperm were exposed to the antagonist (15 min) followed by addition of PAF (15 min), washed in PBS, fixed in ETOH for 45 min at 4C, and acrosome reaction (AR) assessed with FITC labeled PSA lectin (Table 1). Alternatively, sperm were treated with antagonist (ANT) or HSA (CON) prior to PAF treatment, then labeled with FITC prior to the SPA such that different sperm populations (ANT and CON) could be distinguished when the SPA was evaluated using the same group of zona free hamster oocytes (Table 2). ANT and CON samples were also evaluated for sperm attachment and fusion to zona free oocytes. Oocytes were preloaded with Hoechst dye 33342 and gametes were co-incubated for 30 min before evaluation (Table 2).

Table 1
Effect of antagonists on PAF induced AR N=3

	10^{-3}M	10^{-6}M	10^{-8}M	10^{-12}M	10^{-14}M	10^{-18}M
% AR (CON/BN/WEB)	15/20/18	15/14/15	15/16/15	15/14/16	15/15/17	15/17/13
% Motile	34/28/33	56/48/50	56/45/49	56/53/50	56/54/53	56/48/54

(PAF concentration 10^{-14}M)

Table 2
Sperm-oocyte interaction N=4

	CON[BN]	BN52021	WEB2086	CON[WEB]
% sperm penetrated	50	48	55	45
%motile	60	63	58	62
# fused/# attached	5/13	4/15	7/15	6/11
% AR	12	13	11	13

(antgonist concentration 10^{-12}M, PAF concentration 10^{-14}M)

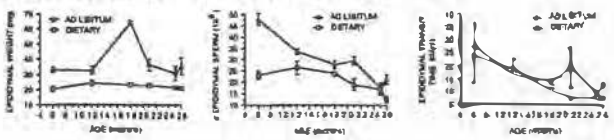
A reduction in sperm motility was observed for both antagonists at 10^{-3}M (Table 1), this was also noted for 10^{-3}M PAF. BN52021 and WEB2086 were unable to block PAF induced AR or effect sperm-oocyte fusion and penetration of zona free hamster oocytes in vitro under the conditions used in our mixed gamete interaction system.

61 **ERADICATION OF LEYDIG CELLS DURING PUBERTY I: DAILY SPERM PRODUCTION, TESTOSTERONE AND STRUCTURE OF THE SERTOLI/SPERMATID INTERACTION.** D.F. Cameron and S.J. Nazian, Departments of Anatomy and Physiology & Biophysics, College of Medicine, University of South Florida, Tampa, FL 3361

Sertoli cells form junctional specializations with step 8 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 completion of spermiogenesis, rats were injected with ethylene dimethylsulphonate (EDS) at 40 days of age to eradicate the Leydig cells just prior to the onset of the testosterone rise and the developmental presence of step 8 spermatids. At weekly intervals beginning with 26 day old rats and continuing through day 96, testis weights were obtained, serum and testicular interstitial fluid (TIF) testosterone was assayed, daily spermatid production/testis (DSP/T) was determined and testicular tissue was processed for light and electron microscopy. DSP/T was reduced in EDS-treated rats but returned to normal by three weeks post-treatment. This was concurrent with abnormal Sertoli-spermatid junctional associations which by the third week post-treatment appeared normal. A significant reduction in testis weight parcelled reduced serum testosterone both of which returned to normal by the second week post-treatment. Results suggest that a reduction of TIF testosterone, but not serum testosterone, corresponds closely to a spermiogenic lesion involving the Sertoli-spermatid junctional interaction. Supported, in part, by NIH-BRSG grant 2 S07 RR RR05749-18.

62 EFFECT OF AGE AND DIETARY RESTRICTION ON NUMBER AND TRANSIT TIME OF EPIDIDYMAL SPERM. L. Johnson, M.R. May and D.L. Busbee. Department of Veterinary Anatomy and Public Health, Texas A&M Univeristy, College Station, TX 77843.

Dietary restriction (DR) is known to delay age related changes and extend the life of mice. Age-related changes in number of epididymal sperm, transit time of sperm through the epididymis, and the effects of DR on these changes were evaluated. At weaning, C57BL/6N-NIA mice were placed on a diet (NIH-31) fed *ad libitum* (AD) or restricted to 60% of the AD caloric intake and sacrificed. The number of sperm in the epididymis was determined by phase contrast cytometry of epididymal homogenates. Transit time was calculated by dividing the number of epididymal sperm by the daily sperm production (DSP) of the attached testis. Age had no effect on epididymal weight, but there was an age-related reduction in DSP, number of epididymal sperm, and transit time of 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 10^6 vs 20.5 ± 0.9 x 10^6; $P < 0.01$) 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.



63 EFFECT OF AGE AND DIETARY RESTRICTION ON SPERMATOGENESIS IN MICE L. Johnson, M.R. May* and D.L. Busbee, Department of Veterinary Anatomy and Public Health, Texas A&M University, College Station, Texas 77843-4458.

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 spermatids per testis by the 4.84 day life span of these spermatids. DSP/G was calculated by dividing DSP/T by the testicular parenchymal weight. Over both treatments, testicular and parenchymal weights were significantly decreased with age. Both DSP/G and DSP/T were highest at 21 months of age, but were significantly decreased at 26 months of age. DSP/G was not uniform in adult mice, but increased to 18-21 months and decreased thereafter. Over all ages, DR had no significant effect on testicular (.082 ± .002 g vs .079 ± .002 g) and parenchymal weight (.067 ± .002 g vs .065 ± .001 g), DSP/G (32.69 ± 1.27 x 10^6 vs 34.08 ± 1.25 x 10^6), or DSP/T (2.16 ± .09 x 10^6 vs 2.18 ± .09 x 10^6). While two-way ANOVA revealed no significant interaction ($P<0.07$) between diet and age group, there was a trend for a delay in obtaining maximum DSP/T at 12 months and obtaining age-related, reduced DSP/T at 25 months in the DR group compared to *ad libitum*. Supported by Grants AG02260 and AG07734.

64 BLIND-ENDING TUBULES IN THE DUCTULI EFFERENTES OF THE RAT. Rex A. Hess, Robert Guttroff* and Paul Cooke*. Veterinary Biosciences, University of Illinois, Urbana, IL 61801.

Aberrant or blind-ending tubules (BET) are found in the head of the epididymis of most large mammals (Hemeida 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-8 efferent ductules were found in the rat (mean = 6). The ductules formed as individual tubules from channels of the rete, external to the testis. Most ductules began to merge in the coni vasculosi region where the tubules became narrow and tortuous. Merging 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 diverticuli 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 nonciliated 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 rat BET trap sperm and become involved 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.

65 UPTAKE OF OVIDUCT FLUID (ODF) PROTEINS BY BOVINE SPERM MEMBRANE DURING IN VITRO CAPACITATION. Tamara McNutt, Lee Rogowski* and Gary Killian, Dairy Breeding Research Center, Penn State University, University Park, PA 16802.

Sperm undergo capacitation and display unique head to head agglutination patterns when incubated in medium containing ODF. These observations suggest that an alteration of the sperm surface occurs during capacitation by components of ODF. To test this hypothesis, ^{125}I-labeled ODF proteins and antiserum to ODF were used to determine whether ODF proteins associate with the sperm membrane during in vitro capacitation. ODF was collected from indwelling oviduct cannulae (Kavanaugh and Killian, 1988, J Invest Surg 1:201). Washed sperm (50 x 10^6 sperm/ml) were incubated at 39°C in 5% CO_2 up to 4 h in a protein-free modified Tyrodes medium (Parrish, et al., 1988, BOR 38:1171) containing 1 mg PVA/ml (MTM), MTM containing heparin (MTM-H; 10 µg/ml), or MTM supplemented with 40% ODF or 0.5 ng ^{125}I-labeled ODF proteins. Daily samples of ODF collected during the estrous cycle were combined according to serum progesterone concentrations into luteal (> 1.5 ng/ml) and non-luteal (< 1.5 ng/ml) pools. Sperm incubated in MTM-H, MTM or 40% ODF were assessed at 0, 2 and 4 h, and sperm incubated in radiolabeled ODF were analyzed at 2 h. At each time point, sperm were pelleted, washed, recentrifuged and the resulting pellet suspended in membrane solubilization buffer (Jones and Brown, 1987, Biochem J 241:353). Solubilized sperm membrane proteins, incubation and wash media and ODF proteins were separated using 1D SDS-PAGE. All samples were run in duplicate; gels from one set were stained, the other western blotted and reacted with antiserum to ODF. Gels containing separated membranes from sperm incubated in radiolabeled ODF were stained, dried and autoradiogtaphs prepared. Bovine sperm incubated with non-luteal ODF adsorbed four proteins, a doublet at 80 to 90 kDa, and three others at 66, 53 and 24 kDa, whereas sperm incubated with luteal ODF only adsorbed three proteins at 66, 53 and 24 kDa. The amount of the 66 kDa protein decreased during the incubation, whereas the 80 to 90 kDa protein bound by the sperm remained unchanged. Sperm also transformed ODF proteins in the 53 kDa region, from 2 to 3 separate proteins. Proteins from ODF which are enriched on the sperm surface may be factors influencing sperm capacitation, the acrosome reaction or preparation for interaction with the oocyte.

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66 THE EFFECTS OF PROSTAGLANDINS ON SPERM MOTILITY FROM THE MOUSE CAUDAL EPIDIDYMIS J.R. Mabee* and B. Peitz, Department of Biology, California State University, Los Angeles, CA 90032

Semen is the richest natural source of prostaglandins (PG), however, PG function in fertility remains elusive. While sperm motility is a requisite for successful in vivo fertilization, data regarding PG effects on motility is conflicting. Some studies show PGE1, PGE2, and PGF2@ to increase sperm velocity (Aitken, et al, 1985 JRF 73:139-46), while others show these same PGs to have no effect (Gottlieb et al, 1988 Fertil Steril 49:322-7). The purpose of this study was to test the effects of PGE1, PGE2, PGF1@ and PGF2@ on CD-1 mouse caudal epididymal sperm. Sperm were allowed to swim out of minced caudal epididymides into a modified Kreb's Ringer bicarbonate (KRB) media in a humidified, gas controlled incubator at 37°C for 15 min. Samples were then incubated for 30 and 90 min in either PGE1 or PGE2 (10^{-8}-10^{-13}M) or PGF1@ or PGF2@ (10^{-9}-10^{-13}M), using KRB and KRB with EtOH as controls. Treatment samples were then videotaped, and analyzed to determine curvilinear velocity (Vc), and linearity (Ln) using the Experc Vision system (Motion Analysis, Santa Rosa, CA). Two subpopulations of sperm were noted in this strain; those with and without cytoplasmic droplets. Data analysis was performed among and between the subpopulations. Mean control Vc values were 235 ± 46 u/sec for sperm with cytoplasmic droplets, and 190 ± 39 u/sec for sperm without cytoplasmic droplets ($p < .05$). Mean control Ln values were .464 ± .127, and .371 ± .146 respectively ($p < .05$). However, no consistent statistically significant differences were noted between all treatment groups and both controls for Vc and Ln at either 30 or 90 min (3 repeat expts with an average of 21 sperm per sample). PGE1 appears to have no effect on Vc and Ln in mouse sperm.

67 PRELIMINARY CHARACTERIZATION OF BIOACTIVE FACTORS IN HUMAN FOLLICULAR FLUID THAT CAUSE SPERM HYPERACTIVATION

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Previous research in our laboratories has shown that human follicular fluid causes capacitated human sperm to display behavior that resembles hyperactivation (Fetterolf and Rogers, Biol. Reprod. 40, Suppl. 1:146, 1989). Here we report preliminary characterization and purification of the bioactive substance(s) in human follicular fluid that stimulates sperm motion. Sperm from known fertile donors was prepared for bioassay using the swimup procedure in Ham's F-10 medium + 10% (v/v) early cycle maternal serum (ECMS) and allowed to capacitate for 3-6 hours at 37°C in 5% CO_2. The bioassay quantifies sperm motion using computerized digital image analysis after a 15 minute coincubation of swimup sperm with 10% ECMS (v/v;control) or follicular fluid. Fractionation of follicular fluid using centrifugation (Centricon-10, Amicon) or filtration (PD-10, Amicon) indicates that the sperm motion activating factor(s) is recovered in the fraction above 10,000 molecular weight. Protease digestion (Pronase, Sigma) of follicular fluid and subsequent heat inactivation at 60°C for 1 hour do not destroy the activity. A total lipid fraction of follicular fluid extracted using chloroform:methanol stimulates sperm motion. The ED50 for this total lipid extract is between 1:2,000 and 1:20,000 dilution. These preliminary findings are consistent with the hypothesis that the sperm motion activating factor is a lipid or hydrophobic peptide.

68 SEQUENCE AND EXPRESSION OF A NOVEL TESTIS ANTIGEN. A.B. Diekman* and E. Goldberg, Dept. of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208 and T.K. Roberts*, S.C. Shih*, D. Kay* and B. Boettcher*, Dept. of Biological Sciences, University of Newcastle, NSW 2308, Australia.

Antibodies that bind to spermatozoa have been implicated in some instances of human infertility. In an attempt to discover potential antigens, sera from male and female infertile patients were used to screen a λgt11 human testis cDNA expression library. An isolated clone with a 600 bp insert was used to prepare a fusion protein which reacted in an immunoblot assay with sera from nine of eleven patients diagnosed as immunologically infertile. Using the insert as a probe to rescreen the library, three longer clones were obtained. The longest clone (2.2 kb) was isolated and sequenced. This cDNA begins with 222 bp of 5' untranslated region before the start codon and ends with 476 bp of 3' untranslated region followed by a poly A tail. The open reading frame (1503 bp) encodes a peptide of 501 amino acids. Neither the cDNA nor the derived amino acid sequences are represented in the GenEMBL data library. The peptide chain's secondary structure was predicted by computer analysis to have an α-helical, β-sheet structure characteristic of globular proteins and to contain three putative glycosylation sites indicative of membrane localization. Structural motifs which could indicate a possible function are not readily apparent. A signal peptide is not present at the amino terminus nor is there an apparent transmembrane region in the amino acid sequence of the peptide. mRNA encoding this peptide is expressed in human testes as demonstrated by polymerase chain reaction amplification of a 590 bp product from both the cloned cDNA and cDNA prepared from human testis RNA using reverse transcriptase. Expression of this message in human somatic tissue is under investigation. This peptide may be useful as an antigen for contraceptive vaccine development as well as a reagent for use in diagnosing a class of immunological infertility.

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69 DNA UPTAKE BY MAMMALIAN SPERMATOZOA

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Mouse epididymal and washed ejaculated rabbit spermatozoa were preincubated (5% CO_2, 37°C) in capacitation medium (30 mg/ml BSA) before incubation (30-60 min; 5% CO_2; 37°C) with ^{32}P-labeled pcDM1 mouse met cDNA (9.8 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 ^{35}S-labeled pcDM1 met cDNA, revealed that DNA binding occurred primarily in the postnuclear 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 dioxigenin-labeled pcDM1 met cDNA, after the membranes were permeabilized with digitonin, suggesting the intracellular localization of the DNA. Little or no uptake of ^{32}P-labeled pcDM1 met cDNA occurred when added to immotile/dead spermatozoa obtained by long term incubation in the absence of substrate or to paraformaldehyde-killed spermatozoa. Addition of ^{32}P-labeled pcDM1 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. Boughton Trust.

70 COLUMN SEPARATION OF SPERMATOZOA FROM SUBFERTILE STALLIONS.

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A study was performed to investigate the ability of column separation techniques to isolate motile sperm from stallion ejaculates. Semen from 5 stallions was extended 1:1 with Texas A&M, clear extender. Original motility ranged 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 lengths: 2 cm, 3 cm, 4 cm; and centrifugation times: 30, 60, 90, and 140 sec. Columns were pretreated 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 (%R) and percentage change in motility (%MC). There were significant differences in %R for column type ($p < 0.0001$) and length ($p < 0.0001$); and in %M improvement ($p < 0.0001$) and %MC ($p < 0.0001$) for column type.

Column Type	%R	%MC	Length	%R
Sephadex	$45^{b} \pm 3$	$35^{b} \pm 5$	2 cm	$63^{a} \pm 3$
Glass Wool	$29^{c} \pm 2$	$63^{a} \pm 5$	3 cm	$47^{b} \pm 3$
Nylon Wool	$51^{b} \pm 3$	$54^{ab} \pm 5$	4 cm	$41^{c} \pm 3$
Glass Beads	$75^{a} \pm 3$	$44^{ab} \pm 5$		

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, %MC 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.

71 EFFECT OF OSMOLALITY ON DENSITY OF BULL SPERM SEPARATED ON NYCODENZ^{R} GRADIENTS.

R.H. Foote, S.R. Hough* and B.E. Carril*. Department of Animal Science, Cornell University, Ithaca NY 14853-4801.

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^{R}, 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++ TALP to 250 x 10^{6} sperm/ml. Three fractions, 100% fresh (75-80% motile), 100% killed and a 50:50 mixture of sperm were prepared and 1 ml of these suspensions each were layered on discontinuous gradients of 23, 24, 25 and 44% Nycodenz prepared with distilled water (220, 231, 237 and 407 mOsmols, respectively), and 27, 28, 29 (prepared with a Tyrodes solution to give 342, 345, and 343 mOsmols, respectively) and 44% Nycodenz (as above). Sperm were centrifuged at 100,000 x g, 20°C, for 60 minutes. In the 23-25% hypotonic 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 89% motile. In the killed sperm treatment 100% were in the 44% layer (density=1.233 g/ml). The 50:50 treatment was intermediate. In the hypertonic 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.

72 CLINICAL FEATURES OF PERSISTENT MULLERIAN DUCT SYNDROME (PMDS) IN A HORSE

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A grey, 8-yr-old, Thoroughbred horse was donated to the NYSCVM 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 glans 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 LxW), oval, 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 (T4) 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 2 h and 272 ng/ml 4 h post hCG. Laparoscopy was used to visualize the genital tract. A small (15 cm x 1.5 cm LxW) symmetric uterus suspended by a broad ligament was visualized. Near the tip of each horn a testes-like gonad, with an epididymis, was present. A sac-like structure was identified ventral to the body of the uterus. A cervix was not present. The karyotype of this horse was 64,XY. We believe this to be the first report of PMDS in horses.

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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 subpopulations 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 8.0 uIU acrosin/million sperm which is clearly in the abnormal range (<14 uIU/10^6 sperm). Three patients had a mean acrosin value of 20.1 uIU/10^6 sperm which is in the indeterminate range (14-25 uIU/10^6 sperm). Two other patients and four proven fertile donors had mean acrosin values in the normal range (>25 uIU/10^6 sperm). The normal fertile controls had a mean acrosin value of 32.5 uIU/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.

74 EVIDENCE FOR MODULATION OF THE HUMAN ACROSOME REACTION BY G-PROTEINS R.A. Anderson, K.A. Feathergill and L.J.D. Zaneveld. Rush Medical Center, Chicago, IL 60612

Studies have implicated cAMP 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. Cholera toxin (CTX), pertussis toxin (PTX) and 5 mM NaF/10 μM $AlCl_3$ (fluoroaluminate) were used to evaluate G-protein involvement in the human AR. The AR of washed (no albumin added) uncapacitated 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 nM A23187 and fluoroaluminate combined gave an AR of 21.6 (19.1-24.2)%, higher than the maximal AR (AR_{max}) induced by either A23187 (17.3 (14.8-19.9)%) or fluoroaluminate (15.1 (12.7-17.7)%). Neither NaF nor $AlCl_3$ affected the AR. AR induction by CTX, but not PTX, required activation with dithiothreitol. PTX-induced AR_{max} was 22.2% (from linear regression of transformed data); [PTX] at 0.5 AR_{max} (ED_{50}) = 5.3 pM. 1 nM A23187 increased the ED_{50} for PTX (11.0 pM); the curve changed from rectangular hyperboloid to sigmoidal; AR_{max} was 52% greater than the A23187-induced AR_{max}. CTX-induced AR_{max} was 15.2%; ED_{50} = 6.9 nM. A small response, 5.4 (3.5-7.3)%, was produced by the CTX B subunit (0.35 μM). The A subunit had no effect. PTX (0.95 pM) decreased the ED_{50} for CTX (2.3 nM) with no change in AR_{max} (15.9%). Unlike the A23187-induced AR, neither PTX- nor CTX-induced AR was Ca^{2+}-dependent. The above findings suggest that the human AR may be modulated by multiple G-proteins and may occur via separate, though interacting, Ca^{2+} dependent and -independent pathways. Supported by NIH grant HD 19555.

75 PURIFICATION AND PARTIAL AMINO ACID SEQUENCE OF TWO FORMS OF PROACROSIN FROM BOVINE SPERMATOZOA.
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Acrosin, an acrosomal trypsin-like protease is postulated to enable the spermatozoa to penetrate the zona pellucida of the ovum during fertilization. In all mammalian spermatozoa studied, the majority of acrosin exists as enzymatically inactive precursor forms, proacrosin. Recent evidence also suggests that proacrosin is involved in secondary binding of sperm to the zona pellucida (ZP2). We now report the purification and partial N-terminal amino acid sequences of proacrosins from bovine cauda epididymal sperm. Two forms were purified from acid extracts of spermatozoa by ammonium sulfate fractionation, gel filtration on Sephadex G-150, and affinity chromatography on Concanavalin A Sepharose 4B; their molecular weights determined by SDS-PAGE were 44,000 and 38,000. The overall purification was 13-fold with respect to crude acid acrosomal extract. Both can be converted to active proteinases after being resolved on gelatin-SDS-PAGE. The M_r 38,000 component was isolated by reverse phase HPLC. Thirty nine amino acid residues at the N terminus have about 67% and 74% sequence similarity with boar and human proacrosin, respectively. The amino acid sequence of 14 amino acids at the N-terminus of the high molecular weight component (M_r 44,000) is identical with that of the low molecular weight component. Proacrosin autoactivation followed the classical S-shaped activation curve and can be blocked by highly specific acrosin inhibitors. Calcium (10mM) stimulated the proteolytic activity of bovine acrosin ten fold, and it enhanced acrosin hydrolysis of N-benzoyl-L-arginine ethyl ester (BAEE) three fold. Michaelis-constant (K_m) for BAEE was 0.13 mM.

76 **CRYOPRESERVATIVE DILUENTS CONTAINING BOVINE SERUM ALBUMIN (BSA) STABILIZE THE ACROSOMES OF HUMAN SPERM DURING CRYOPRESERVATION.** E.Z. Drobnis, K.R. Robertson*, S.J. Samuels* and J.W. Overstreet. Division of Reproductive Biology, Department of Obstetrics & Gynecology, School of Medicine, University of California, Davis, CA 95616

Some sperm which remain motile following cryopreservation (CP) have sustained sublethal damage and have reduced functional competence. In human sperm, a consequence of this cellular injury is destabilization of the acrosome and premature acrosome reaction (AR). We have used a five factor, two-level experimental design, to compare cryopreservative diluents for their ability to reduce this damage. In eight experiments, donor semen (donors not selected for sperm cryosurvival) was divided into four samples. Two samples were washed to remove seminal plasma (SP), then all samples were diluted to twice their original volume with assigned diluents and frozen (1°C/min to 4°C, 10°C/min to -100°C) in cryostraws. Sperm were analyzed after thawing (t = 0 h) and after 3 h incubation postthaw (t = 3 h) for: 1. motility (CASA), 2. viability (%V = exclusion of supravital dye), and 3. AR (%AR = absence of FITC-PSA staining in viable sperm). Washing, diluent (seminal plasma, citrate, TEST, HSPM), and egg yolk (0% *vs.* 20%) affected motility but had no effect ($p > 0.05$) on %AR. In contrast, BSA (0% *vs.* 0.2%) had no effect on motility but reduced %AR ($p = 0.004$).

	t = 0	t = 3
With BSA	11 ± 2	13 ± 3
Without BSA	16 ± 2	21 ± 4

There were no significant first-order interactions of BSA with the other factors. We conclude that BSA stabilizes the acrosome of human sperm during cryopreservation, reducing premature acrosome reactions.

77 PARTIAL CHARACTERIZATION OF SP-10 INTERACTIONS WITH HUMAN ACROSOMAL MEMBRANES
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A WHO taskforce on contraceptive vaccines identified the human intra-acrosomal protein SP-10 as a candidate vaccine immunogen (Anderson, et al. 1987 J Reprod Imm 10:231-57). EM studies of mature, ejaculated sperm localized SP-10 to the acrosomal membranes and the acrosomal matrix. After acrosome reaction (AR), immunofluorescent (IF) studies revealed that some SP-10 is retained on the sperm head, apparently associated with the inner acrosomal membrane (IAM) and equatorial segment (ES) (Herr et al. 1990 Biol Reprod 42:181-183). In the present study sperm were treated with 1% Triton X-114 (TX) to fractionate Triton-extractable and Triton-resistant pools of SP-10. Western blots show that in TX phase partitioning studies, both pools of SP-10 were detected in the hydrophilic aqueous phase suggesting that SP-10 is not an amphipathic integral acrosomal membrane protein. The IF staining patterns of TX-resistant SP-10 resemble the SP-10 IF patterns of AR sperm suggesting that this pool of SP-10 is associated with the IAM and ES which are exposed by Triton treatment. Incubation of TX-treated sperm with three washes of 1.5M NaCl partially extracted TX-resistant SP-10 from the sperm head while complete extraction of this SP-10 pool was achieved by treatment with 150mM NaSCN (a chaotropic salt) and pH extremes of pH 2 & 10. We propose that TX-resistant SP-10 may be peripherally associated with the IAM and ES, or with a TX-resistant acrosomal "scaffolding". [Support:NIH HD16767 & 23789]

78 ROLE OF ACROSINE IN THE HUMAN SPERM ACROSOME REACTION. P. Morales, P. Vigil*, M. LLANOS*. Unit of Reproduction and Development, Catholic University of Chile and INTA, Universidad de Chile. Santiago, Chile.

One of the crucial steps in the process of fertilization in mammals is the sperm-zona pellucida (ZP) interaction. In this interaction it is posible to distinguish two events, sperm-ZP binding and sperm-ZP penetration. It has been suggested that a serine protease located in the acrosome, acrosine, 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. Motile 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, 5 % CO_2. After 4.5 h, the sperm were treated with 1mM of p-aminobenzamidine (pAB) or with DPBS (control) for 30 min. Then, 2-3 non-viable human oocytes were added to each sperm suspension. After 3 h, the number of sperm penetrated and bound to the ZP were determined. The results indicated that pAB inhibited sperm-ZP penetration significantly (56±8 % control vs 0 % treated, x±sem, n=8), without decreasing sperm-ZP binding. In addition, there was a marked decrease in the percentage of acrosome reactions induced by the ZP in the pAB treated group (39±5 % control vs 6±2 % pAB). Additional experiments, in which the sperm were treated with 1 mM of the protease inhibitors pAB, TLCK (Na-p-Tosyl-L-Lysine Methyl Ester), or NPGB (p-Nitrophenyl p'-Guanidino-Benzoate), showed a marked inhibition of the acrosome reaction induced by human follicular fluid (50 to 73 % inhibition) in comparison to the control group. These results suggest that acrosine could be participating in the process of acrosome reaction in the human sperm. Supported by RF 89033/42 and FONDECYT 0823/90.

79 PROGESTERONE (P) CONTENT IN HUMAN FOLLICULAR FLUID (hFF) IS RELATED TO ITS ACROSOME REACTION-INDUCING ABILITY. D. Vantman, G. Gutierrez*, P. Kohen*, P. Vigil*, P. Morales. Paula Jaraquemada Hospital and Unit of Reproduction and Development, Catholic University of Chile. Santiago, Chile.

The acrosome reaction (AR) is a prerequisite for the success of fertilization in mammals. It has been suggested that P could be one of the molecules able to induce the AR in the female genital tract. In the present study we have evaluated the relationship between the ability of hFF to stimulate the AR and its steroid levels. 18 hFF samples were obtained during egg retrieval in 6 patients undergoing assisted fertilization. Fluids were centrifuged at 10,000 xg to remove cellular debris and then kept at -80°C until use. Motile sperm were selected by a two-step Percoll gradient and then resuspended in modified Tyrode's medium (26 mg/ml BSA). After the sperm concentration was adjusted to $1x10^7$ cells/ml, the cells were incubated for 18 h at 37 °C, 5 % CO_2. To test the ability of hFF to induce the AR, the sperm were treated with 20 % hFF for 15 min. In each experiment, all hFF samples were simultaneously tested using a single semen sample. The percentage of AR sperm was determined using the PSA-FITC lectin. Each hFF sample was also analyzed by RIA to determine the levels of P, 17-hydroxy progesterone (17-OHP), testosterone (T), and estradiol (E). In addition, seven of these hFF were treated with dextran charcoal. Then, an aliquot of each sample received 10 µg/ml of P and the other aliquot received 0.5 % DMSO. Their ability to induce the AR and their steroid level were tested as described above. The results have indicated that there was a positive and highly significant correlation between the AR-inducing activity and the P level of hFF (R^2 = 0.72, P<0.005). Treatment of hFF with dextran charcoal caused both a decrease in P concentration (10 µg/ml charcoal-treated vs 10,000 µg/ml untreated hFF) and a decrease in 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. Supported by RF 89033/42 and FONDECYT 0823/90.

80 ELECTRON MICROSCOPICAL EVALUATION OF THE ACROSOMAL STATUS OF SPERMATOZOA TIGHTLY BOUND TO AND PENETRATING THROUGH HUMAN HEMIZONAE PELLUCIDAE STORED IN A BUFFERED SALT SOLUTION
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The hemizona assay (HZA) has been developed as a homologous bioassay to evaluate sperm binding potential to the zona pellucida. In this study we used immature human oocytes harvested from surgically removed ovaries and stored in a buffered salt solution for up to 60 days. Oocytes were bisected into matching hemispheres (hemizonae) and coincubated with the spermatozoa from a fertile man at a concentration of 500,000 motile/ml for 4 hours. The hemizonae with bound spermatozoa were prepared and fixed for transmission electron microscopy (TEM) using standard techniques. All evaluated spermatozoa were tightly bound to the outer surface or had begun penetration into the zona pellucida. A total of 25 out of 108 zona-bound sperm cells were evaluated by transmission electron microscopy for acrosomal status and location within the zona pellucida. Twenty (80%) of the zona-bound spermatozoa were partially or completely acrosome reacted, while 6 (20%) of the zona-bound sperm had intact acrosomes. Penetration pathways or digestive tunnels were seen within the zona matrix. The results illustrate that typically, spermatozoa that are tightly bound to the human zona pellucida show induction of the acrosome reaction under HZA conditions. Importantly, following storage of human oocytes in salt solution (buffered at pH 7.4), the zonae pellucidae retain their biological functional properties for up to 60 days.

81 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 fertilization status. Acrosin activity did not correlate with parameters of standard semen analysis. The mean ($\pm$ SD) acrosin activity index was higher when 1 or more mature oocytes fertilized (10.5 $\pm$ 6.0), compared to 5.5 $\pm$ 4.7 when no mature oocytes fertilized (P=0.002). Acrosin activity before sperm processing also correlated with the proportion of mature oocytes that were fertilized (r^2=.55, P=0.001). Sensitivity of the assay was 62%, specificity was 82%. The positive likelihood ratio was 3.7, suggesting that acrosin activity is a suitable parameter to measure for the biochemical evaluation of one aspect of sperm function.

83 SPONTANEOUS AND INDUCED ACROSOMIC REACTION IN CAUDA EPIDIDYMAL EQUINE SPERMATOZOA.

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The male mammalian gamete undergoes changes known as capacitation (of which, the acrosomic 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 became of interest to elicit AR in stallion sperm taken from the cauda and characterize it by the triple staining technique (modified from Talbot and Chacón, 1981).

Samples were taken from 5 healthy stallions after castration and used if progressive motility was over 60%. One aliquot was kept in culture medium and another had BSA added to a final concentration of 3 mg/ml. Sperm samples were incubated for 0, 2, 4 and 6 hs at 37°C (with 100% humidity, 5% CO2 and 95% air. Half an hour before and at 4 and 6 hs of incubation 50 µg/ml of LPC (lysophosphatidyl choline) was added to the BSA containing samples. Spontaneous (0 min of incubation) AR was found in 11.7% of living sperm. When BSA was added, 18.2%; 20.5%; and 26.2% of AR were found after 2, 4 and 6 hs. LPC induced 33.5% of AR in alive sperm by 4 hs.

In conclusion, cauda epididymal sperm have spontaneous AR. It can be induced by addition of BSA to the incubation medium and synchronized if LPC is added. Thus, in vitro reacted, capacitated living spermatozoa are obtained. They could eventually be used for interaction with oocytes and represent a new and promising tool for zootechnical advance in equine reproduction.

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82 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 capacitation 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 supportive 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 motion 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 #1R43HD26682-01.

84 HUMAN SPERM FUNCTION INHIBITION BY ANTIBODIES AS ASSESSED BY BOTH THE SPA AND HZA

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The sperm penetration assay (SPA) and the hemi-zona assay (HZA) assess different functional properties of spermatozoa. The SPA assesses capacitation, acrosome reaction, membrane fusion and sperm nuclear decondensation while the HZA assesses the ability of sperm to bind to the zona pellucida. Some monoclonal antibodies may be directed to sperm antigens that are involved in functions detected by the SPA and others may recognize antigens that are involved in sperm-zona interaction. To assess the ability of a series of 19 monoclonal antibodies to inhibit sperm function each was assayed in both the SPA and HZA. The monoclonals were prepared by immunization of mice with an NP40 extract of human sperm plasma membranes. The SPA methodology involved an overnight incubation of washed human sperm in BWW in the presence or absence of antibody at a 1:10 dilution. Prior to addition of zona-free eggs the sperm samples were washed once by centrifugation at 500 g for 5 minutes. The HZA methodology included a swim-up in Hams F-10 supplemented with 0.3% HSA prior to addition of antibody. Sperm and hemi-zonae were incubated at a concentration of 2 million/ml in the presence or absence of antibody for 4 hours prior to evaluation of tight sperm binding. Of the 19 monoclonals tested 7 showed inhibition (< 50% of control) in the SPA and 5 in the HZA. The % penetration for inhibitory antibodies was: DH3, 34%; DH13, 33%; DH14, 35%; S73, 39%; S74, 36%; S75, 35%; and S79, 23%. The HZA index for inhibitory antibodies was: DH3, 13; DH4, 21; DH13, 20; DH14, 31; and DH205, 23. Three antibodies showed inhibition in both assays suggesting interference of multiple sperm functions and potential usefulness as contraceptive agents.

85 THE INCIDENCE OF ANTISPERM ANTIBODIES IN SEMEN, SERUM AND CERVICAL MUCUS. G.M. Centola, L.B.V.Emilson*, Andrology Laboratory, Dept. of Obstetrics-Gynecology, University of Rochester Medical Center, Rochester, NY.

Antisperm antibodies have 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. Sperm (n=71), serum (female, n=103; male, n=24) and midcycle cervical mucus (CM; n=22) specimens were assessed for IgG and IgA binding using the direct (semen) or indirect (serum, CM) Immunobead Binding Test. Greater than 20% binding was considered e pooitive test. The % positive for IgG and IgA in each group was as follows:

	Semen	Serum		CM
		Female	Male	
IgG	8.5	9.7	25.0	9.1
IgA	1.4	3.9	8.3	18.2

The female and male serum 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 serum, while IgA was directed at the tail in the semen and the head and tail in the serum. CM IgG and IgA were primarily directed at the tail. Of the females positive for serum IgG (10/103), 2 were positive for CM IgG. Of those positive for IgA (4/103), 2 were positive for CM IgA. There has been only 1 pregnancy in a female positive for serum and CM IgG and IgA following menotropin induction and IUI. There have been fertilizations in vitro by antibody positive semen. Although the incidence of antisperm antibodies in this patient group appears low, it is considered a significant etiologic factor for the infertility. Antisperm antibody testing should remain as a diagnostic test for couples presenting with factors suggestive of an immunologic basis for the subfertility.

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

Patient anti-sperm antibody (AsAb) status in immunofluorescence AsAb assays is generally determined aginst reference AsAb negative end known positive controls. Positive controls for this assay system however,are frequently unavailable, and often unreliable. Our laboratory has thus begun utilizing an artificially engineered positive control. Aggregated IgG was prepared by heating a 1.0 mg/ml solution to 63°c for 30 minutes and will nonspecifically bind to human sperm. Twelve assays were performed studying 5 and 10 million sperm. All specimens were from previously studied AsAb negative controls and washed twice with excess phosphate buffered saline / .3% bovine serum albumin (PBS/BSA). Fifty microliters of aggregated IgG was added to each specimen and incubated 2 hours. The mean percent positive sperm was estimated using a 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 (+/-8.8) when studied the same as above. 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 appropriate assignment of AsAb status of infertility patients. Studies to determine the long term stability of aggregated IgG at -40°c are being pursued.

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COMPARISON OF A FEW TECHNIQUES FOR SPERM ANTIBODY SCREENING

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Immunologic infertility has been evaluated by various assay systems to detect the presence of antisperm antibodies (ASA) in serum and seminal plasma (SP). We have tested SP for ASA and compared the following techniques: indirect Immunobead test (iIBT), enzyme-linked immunosorbent assay (ELISA), and a new latex agglutination test (LA). ELISA has been used in our laboratory as a screening test for ASA in SP. Specimens can be frozen, precluding the need for testing fresh semen while permitting batch analysis. While iIBT is the current standard for detection of ASA, it is not suitable for high volume testing. In an attempt to combine the advantages of both tests, LA was evaluated. Seminal plasma from 39 patients was separated via centrifugation and frozen at -20 C until tested. For ELISA, SP was serially diluted, applied to sperm-coated microtiter plates and incubated at 37 C for 1 hour, followed by subsequent incubations with enzyme conjugated antihuman immunoglobin and substrate. Absorbances were read on a spectrophotometer and values ≥0.150 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 10ul of antigen-coated bead suspension with 20ul of SP and observing for visible agglutination after 2 minutes. All 39 specimens tested negative by iIBT. Three specimens tested positive by ELISA and 8 specimens tested positive by LA. Using iIBT as a reference method, ELISA and LA had specificities of 92.3% and 79.5% respectively. In our laboratory, ELISA proved to be a more suitable screening test for ASA in SP than LA. We recommend that a positive result by either test be followed up and confirmed by iIBT and direct IBT for definitive diagnosis of immunologic infertility.

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 prefreeze, and inversely correlated with serum FSH and LH. Semen was cryopreserved using 6-7% glycerol (usually in egg-yolk buffer) and staged freezing, then was stored under liquid nitrogen. Accumulated motile sperm post-thaw were used to project fertility potential and anticipated reproductive technology. Seventeen men had over 150 million total sperm post-thaw (55% recovery), suitable for at least four IUI cycles. Thirty-three men had 15-150 million total 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 multiagent chemotherapy (usually MOPP or 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.

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

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In 1989 we reported (J. Androl. 10:139, 1989) significant correlations between objectively determined quality of sperm motility, occurrence of pregnancy and incidence of early pregnancy wastage in couples undergoing gamete intra-fallopian tube transfer (GIFT). The incidence of early pregnancy loss was significantly increased in couples with disturbed linearity 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 process of exclusion of "inadequate" spermatozoa might not have occurred leading to increased incidence of oocyte fertilization by abnormal spermatozoa, in turn resulting in higher incidence of pregnancy wastage.

The study presented here was designed to investigate whether this relationship between quality of sperm motility and early pregnancy loss is unique to GIFT or IVF. For this purpose we studied 44 randomly selected couples who conceived following treatment for infertility (no GIFT or IVF). The incidence of first trimester spontaneous abortion was correlated to sperm count, morphology, percent motility, velocity and linearity. Sperm motility and its quality were determined objectively utilizing a computer-assisted multiple-exposure photographic technique. Linearity of sperm motility correlated inversely with early pregnancy wastage (30% with mean linearity >8, 17% <8). The total number of spermatozoa with "effective motility" (linearity >8 and velocity >20um/sec) correlated best with pregnancy outcome (37.5% miscarriage with <20 million/ejaculate, 10% miscarriage with >20 million/ejaculate).

These data confirm our prior study dealing with GIFT and other studies with IVF, which suggested that quality of sperm motility correlates not only with occurrence of pregnancy but also with pregnancy outcome. This indicates that disturbance of normal sperm motility may be an index of the functional quality of the spermatozoon, which relates not only to its capacity to fertilize but also to other aspects of its physiology necessary for healthy embryonic development.

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90 THE EFFECT OF REFRIGERATION IN TEST-YOLK BUFFER ON CAPACITATION, THE ACROSOME REACTION, AND FERTILIZING ABILITY OF SPERM IN SELECTED IVF PATIENTS.

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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 as to the effect that the procedure has on stimulating capacitation, the acrosome reaction, and fertilization. In this study, we have analyzed sperm penetration capacity on 476 prospective IVF patients and identified 143 patients with decreased penetration ability as measured by the SPA. These patients were divided into two IVF treatment groups in which a fresh semen sample prepared by serum swim-up was either used alone, or mixed with a sample previously incubated in TEST-yolk buffer. Previous semen quality, age, and number of motile sperm added to the eggs were similar for both groups. The eggs were analyzed for evidence of fertilization, and the percentage of viable, acrosome-reacted sperm was determined before and after addition of calcium ionophore A23187. The mean percent fertilization was 66.2 ± 2.9 for the group using refrigerated and fresh samples, and 47.1 ± 3.9 for the control group. The pregnancy rate was 26.0% compared to 11.3% for the control group. The percentage of viable, acrosome reacted sperm was 20.0 ± 1.4 (mean ± SE) for the control group and 32.3 ± 2.0 for the treatment group. The percentage increased significantly in both groups after addition of ionophore A23187. 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 during IVF.

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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.

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This presentation describes the application of Assisted Conception to a group of men with motile sperm concentrations (MSC) of less than 5 million spermatozoa per ml. A total of 43 couples underwent 54 treatment cycles. Of these, 13 couples underwent 16 treatment cycles by pronuclear stage tubal transfer ("PROST")[1], a further 21 couples were treated by tubal embryo stage transfer("TEST")[2]. Of these 29 couples, 5 underwent treatments by both TEST and PROST. The remaining 14 couples, due to the presence of tubal disease, were treated by means of 14 cycles of In Vitro Fertilisation and Embryo Transfer ("IVF-ET"). Of the 43 men in this study, 12 had a MSC of less that 1 million per milliliter and in the 12 cycles of treatment carried out in these patients the sperm were treated, prior to insemination, with the phosphodiesterase inhibitor pentoxylilline[1].

Failure of fertilisation occurred in 17 of the 54 cycles of treatment (32%) and in 6 of the 12 cycles of treatment carried out in the men with a MSC of less than 1 million. A total of 13 pregnancies resulted from the 48 treatment cycles, 6 from the 16 cycles of PROST, 5 from the 24 cycles of TEST and a further 2 from the 14 cycles of treatment by IVF-ET. From the 12 cycles of treatment in the 12 men with a MSC of less than 1 million per millilliter, 1 pregnancy was achieved. Of the 13 pregnancies, 9 are on-going or have already delivered while the remaining 4 gestations were all anembryonic pregnancies.

This series has produced in a pregnancy rate of 30% per patient, or 24% per attempt if the failed fertilisation cycles are excluded. there results a pregnancy rate per transfer of 42%, and an on-going pregnancy rate per transfer of 29%. Thus while the fertilisation failure rate remains a problem, Assisted Conception clearly has an important part to play in the treatment of male infertility.

1. Yovich et al. (1986) Fertil. Steril. 50:179.
2. Yovich et al. (1988) Lancet I; 468.

92 EFFECT OF LUTEINIZING HORMONE (BOVINE AND EQUINE) ORIGIN IN IN VITRO MATURATION OF EQUINE OOCYTES

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Current research has demonstrated that the optimal protocol for in vitro equine oocyte maturation at 15 hours is Tissue Culture Medium (TCM 199) and mare serum from the day of ovulation (MSO; Willis et al, unpublished data). The aim of the continuing research was to improve the culture conditions and determine the effect of different sources of luteinizing hormone (LH) on the in vitro maturation of equine oocytes. The hormones evaluated were bovine luteinizing hormone (bLH; 1, 10, 100 µg/ml; fresh or frozen stock solution) and equine luteinizing hormone (eLH; 100 µg/ml, frozen stock solution). The effects of bLH and eLH on in vitro maturation were compared to a control of TCM 199 with 15% MSO. For storage the hormones were frozen in PBS. For the experiments the bLH was either added fresh at culture , or from frozen stock solution to TCM 199 with 15% MSO. Nuclear maturation was then evaluated using Nomarski optics at 1000x (oil) magnification.

Hormone	Time (hrs)	Germinal Vesicle (%)	Resumption of Meiosis (%) *	Metaphase II (MII) (%)	Degenerate (%)
None (control)	0	27/36[a] (75%)	5/36 (14%)	3/36[a] (8%)	0/36 (0%)
None (control)	15	4/84[b] (5%)	20/84 (24%)	40/84[b] (48%)	20/84 (24%)
1 bLH	15	9/58[bc] (16%)	15/58 (26%)	17/58[c] (29%)	17/58 (29%)
10 bLH	15	21/65[c] (32%)	11/65 (17%)	17/65[c] (26%)	16/65 (25%)
100 bLH	15	19/52[c] (17%)	11/52 (21%)	8/52[c] (15%)	14/52 (27%)
100 eLH (fr)	15	1/37[b] (3%)	11/37 (30%)	19/37[b] (51%)	6/37 (16%)
1 bLH (fr)	15	0/31[b] (0%)	11/31 (35%)	10/31[b] (32%)	10/31 (32%)
10 bLH (fr)	15	0/76[b] (0%)	25/76 (33%)	37/76[b] (49%)	14/76 (18%)
100 bLH (fr)	15	1/40[b] (2.5%)	14/40 (35%)	17/40[b] (43%)	8/40 (20%)

* Resumption of meiosis = germinal vesicle breakdown plus metaphase I. Unlike superscripts in the same column indicate a significant statistical difference ($p<0.05$)

In vitro culture for 15 hr of equine oocytes significantly ($p<0.001$) decreased the percentage of germinal vesicles from 0 hr. There was no significant increase in the percent of MII oocytes with fresh or frozen LH (either bovine or equine). The fresh bLH, however, significantly ($p<0.05$) increased the number of oocytes retaining their germinal vesicle. The lack of apparent effect of eLH was surprising, but the MSO did have an initial 13.4 ng/ml eLH. The inhibitory effect of the fresh bLH may be explained by competing for the eLH site without any stimulatory effect. (This research was supported by the University of Georgia VMES grant 29-26-GR207-002. The authors thank Dr. Harold Papkoff Hormone Research Institute, CA for eLH and Dr. Bolt USDA Animal HormoneProgram, MD for bLH)

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PREDICTION OF HUMAN IN-VITRO FERTILIZATION OUTCOME USING A MODIFIED SPERM PENETRATION ASSAY

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Numerous methodologies have been proposed over the past 10 years to improve the ability of the sperm penetration assay (SPA) to predict male fertilizing 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 Yolk 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 59 patients (88%) using SPA-SW protocol and in 61 patients (91%) using SPA-RW protocol. Six patients (9%) 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.

Poster II Monday

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SOURCES OF VARIATION OF THE SPERM PENETRATION ASSAY UNDER FIELD STUDY CONDITIONS
S.M. Schrader, T.W. Turner, and S.D. Simon. National Institute for Occupational Safety and Health, Cincinnati, OH 45226

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 in 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 (SD_B) and within worker (SD_W), interclass correlation (R_I) and the coefficient of variation for both total (CV_T) and within worker (CV_W) were determined. The R_I for the variables were ranged from 43% to 63% indicating good repeatability, but the CV_T ranged from 46 to 98 and the CV_W ranged from 25 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.

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SPERM HEAD MORPHOMETRY: FITTING POPULATIONS INTO TOLERANCE INTERVALS. T.W. Turner, S.M. Schrader, and S.D. Simon. National Institute for Occupational Safety and Health, Cincinnati, Ohio 45226

Tolerance intervals having a 95% confidence that 75% of the observations will be included have been reported previously for sperm head morphometric parameters (J 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=19; 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 75% 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 then 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.

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

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Little is known about the differences between normal and various clases of abnormally shaped sperm to respond in assays which assess the functional ability of sperm. We have analyzed semen samples from 48 semen donors and 31 infertility 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-deactivated serum, and analyzed at 0, 3, 6 and 24 hours for each of the above assays. Each assay was recounted for each individual class of morphology. Morphologically normal sperm were significantly ($p<0.05$) 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 ($p<0.01$) in all assays compared to tapered sperm. The HOS assay showed the greatest disparity between morphology classes. The percentage reacted was 69.2 ± 5.1 (mean ± SE) for normal morphology, 26.3 ± 4.0 for tapered, 12.2 ± 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.

97 **COMPARISON OF FLOW CYTOMETRIC ANALYSES WITH CLASSICAL VIABILITY AND MORPHOLOGIC PARAMETERS OF BOVINE SPERMATOZOA.** Scott A. Ericsson[1], Duane L. Garner[1,2], Troy W. Downing[2]* and Clifton E. Marshall[3]. Departments of [2]Animal Science and [1]Biology, University of Nevada, Reno, NV 89557. [3]Select 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/craters. The flow cytometric analyses, which utilized two different fluorescent staining protocols (1 and 2), were used to estimate spermatozoal viability, morbidity and morphology. These staining combinations assessed the functional capabilities of spermatozoal plasma membranes, mitochondria, and acrosomes. Plasma membrane integrity was assessed with dihydroethidium (HED), carboxydimethylfluorescein diacetate (CDMFDA) or propidium iodide (PI). Acrosomal membrane integrity was examined using fluorescein isothiocyanate-labeled *Pisum sativum* agglutinin (PSA). Mitochondrial function was estimated by the level of rhodamine 123 (R123) staining. Protocol 1 consisted of PI, R123 and CDMFDA; 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 spermatozoal quality assessments. Specific fluorescent staining patterns indicated that the percentage of viable spermatozoa was negatively correlated with the percentage of abnormal heads ($r=-.80$; $P<0.05$). The percentage of dead spermatozoa was positively correlated with the percentage of abnormal heads ($r=.73$; $P<0.05$) and negatively correlated with intact acrosomes at 0 hr ($r=-.81$; $P<0.05$). Tri-functional staining systems 1 and 2 provided quantifiable information reflective the quality of cryopreserved bovine spermatozoa. Supported, in part, by grants USDA 89-37240-4735, NSF-DMB 8518021.

98 **TRI-FUNCTIONAL CYTOMETRIC ANALYSES OF FERTILITY-TESTED CRYOPRESERVED BOVINE SPERMATOZOA.** Scott A. Ericsson[1]*, Duane L. Garner[1,2], Troy W. Downing[2] and Clifton E. Marshall[3]. Departments of [2]Animal Science and [1]Biology, University of Nevada, Reno, NV 89557. [3]Select 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), carboxy dimethyl fluorescein diacetate (CDMFDA) or propidium iodide (PI). Also, acrosomal membrane integrity was assessed with fluorescein isothiocyanate-labeled *Pisum sativum* agglutinin (PSA) and mitochondrial function was determined using rhodamine 123 (R123). Protocol 1 consisted of PI, R123 and CDMFDA; 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, moribund and dead spermatozoa and the cellular debris. Multiple regression analyses were used to measure the relationship between these phenotypes 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 ($R^2=0.99$, $P<0.05$) and the CNR by the percentage of dead cells at 1.5 hr and moribund spermatozoa at 4 hr ($R^2=0.75$, $P<0.05$). Analysis of protocol 2 stained spermatozoa showed that ENR could be predicted by the percentage of viable and moribund spermatozoa at both incubation times ($R^2=0.99$, $P<0.05$) and the CNR by the percentage of viable and moribund spermatozoa at 1.5 hr and viable spermatozoa at 4 hr ($R^2=0.99$, $P<0.05$). 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.

99 COMPARISON OF CELL TRAK TO MANUAL METHODS. C.L. Gnatuk*, MC Larrison*, JM Engberg*, and G. D. Ball. Mayo Clinic, 200 1st St. S.W., Rochester, MN 55905.

This study compared the results for sperm density from Cell Trak (Motion Analysis Corp.) automated system 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: Semen from Fertility Clinic and donors, collected into sterile containers via masturbation after 48-hrs abstinence, was allowed to liquify 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 set-ups: (1) 15 frames (F) at 15 frames per second (F/S); (2) 60 F at 60 F/S; (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 that at least 95% of differences between 2 measurements on the same sample fall within ± 2SD 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 annlysis for densities between the 2 methods found that automated setting 1 produces results from 88% above to 57% 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.

100 RABBIT SEMEN QUALITY. NUMBER OF SPERM INSEMINATED AND FERTILITY. R.H. Foote, P.B. Farrell* and M.E. Simkin*. Department of Animal Science, Cornell University, Ithaca, NY 14853-4801.

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 1000×10^3, 500×10^3 and 100×10^3 sperm. Fertilization rate was 96, 81 and 64%, respectively ($n=987$, $P < 0.05$). Another trial, with 100×10^3, 50×10^3 and 25×10^3 sperm, resulted in 63, 68 and 41% fertilization ($n=1119$, $P < .05$). For the automated semen analyses a Hamilton-Thorn 2030 unit was used to analyze sperm recorded on 3/4" video tape, using a 10X negative phase objective and a 6.7X 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 (54 fields) in analyzing eight ejaculates used in fertility trial 2. The range in progressively motile sperm was 42 to 85%. Progressively motile sperm and fertility were correlated ($r=.33$). Further refinements in instrumentation and inseminations with low numbers of sperm are planned. Supported by EPA contract CR815428-01.

101 **A HETEROLOGOUS SALT-STORED ZONAE PELLUCIDAE ASSAY FOR ASSESSING SPERM CAPACITATION AND THE IMPACT OF TERATOSPERMIA IN THE CHEETAH (*Acinonyx jubatus*).** JoGayle Howard, MarkBarone, MitchellBush* and David Wildt*. National Zoological Park, Smithsonian Institution, Washington, DC 20008.

Our studies reveal that: 1) teratospermia 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 felids, including the cheetah, ejaculate high proportions of morphologically abnormal sperm. This study examined the utility of ZP-intact, salt-stored domestic cat oocytes for: 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. Domestic cat oocytes were matured *in vitro* for 48 h and, following removal of cumulus cells, stored at 4°C in a hypertonic salt solution: 0.5 M $(NH_4)_2SO_4$, 0.75 M $MgCl_2$, 0.2 mM $ZnCl_2$, 0.1 mg/ml polyvinylalcohol (PVA) and 40 mM HEPES buffer (pH 7.4). Electroejaculates from 10 cheetahs were evaluated for sperm motility and morphology and diluted in Hams F10 medium containing: 1) no protein (PVA); 2) 0.4% bovine serum albumin (BSA); or 3) 5% fetal calf serum (FCS). Sperm were washed, pre-incubated ($4x10^6$ motile sperm/ml) for 1 h, then co-incubated ($2x10^5$ motile sperm/ml) 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 inner ZP-penetrated sperm and the morphology of all bound and penetrated forms. Cheetah ejaculates 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 flagellar (39.8%) defects. Cheetah sperm penetrated cat ZP in the presence of BSA (34.9±5.8%) and FCS (49.1± 9.4%), however, penetration was lower ($P<0.05$) when sperm were incubated in protein-free (PVA) medium (7.4±3.4%). The number of ZP-penetrated sperm/ ovum was 8 to 11-fold higher ($P<0.05$) in BSA (0.6±1.1) and FCS (0.8±0.2) compared to PVA (0.07±0.03). The morphology of bound sperm included normal forms (41.5%) and sperm with midpiece (43.6%) and flagellar (14.9%) defects, but every sperm within or through the inner ZP was morphologically normal. These results indicate that: 1) albumin or serum facilitates capacition of cheetah sperm; 2) although pleiomorphic sperm bind to ZP, only structurally-normal sperm are capable of ZP penetration; and 3) this heterologous zona penetration assay appears useful for assessing factors influencing fertilization in nondomestic felids. (Supported by NIH Grants K01RR00045 and HD 23853)

102 SPERM SELECTIVE CAPACITY OF THE HUMAN ZONA PELLUCIDA UNDER HEMIZONA ASSAY (HZA) CONDITIONS OR Franken, R Menkveld, TF Kruger, SC Oehninger, GD Hodgen
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We have previously reported a positive and significant correlation between the percentage of morphologically normal spermatozoa in semen and the number of sperm tightly bound to the zona pellucida. Here, we compare the % of morphologically normal spermatozoa present in semen with the % normal sperm forms (1) following a standard swim-up procedure and (2) tightly bound to the human zone pellucida after 4 hours of gametes coincubation under HZA conditions. Sperm morphology was recorded for semen, swim-up and zona bound sperm using strict criteria. The mean (±SEM) percentage normal forms for normozoospermic men (n=4) in semen, after swim-up and zona bound spermatozoa were 21.5±1.6%, 27.5±2.9% and 44.8±3.4%, respectively ($p=0.02$, HZA vs. semen and swim-up sperm). The mean (±SEM) % of normal forms present in the semen, after swim-up and zona pellucida bound for teratozoospermic men (n=11), were 3.7±0.9%, 5.8±1.6% and 15.6±4.9%, respectively. Significant differences existed between the % normal forms found in the swim-up and zona bound spermatozoa ($p=0.01$ and $p=0.0007$, respectively) compared to the spermatozoa present in the semen. These results suggest that (1) the zona pellucida has a selective binding capacity for normal sperm forms, both for normo- and teratospermic semen samples ; and (2) this property may help in the selection of sperm for assisted fertilization techniques in cases of severe male-factor infertility.

103 SYNCHRONOUS ASSESSMENT OF UTERINE SPERM MOTILITY AND FERTILIZING ABILITY IN THE HAMSTER. S. Jeffay*, V. Slott*, J. Suarez*, R. Barbee* and S, Perreault. NSI Technology Services, and Reprod. Toxicol. Br., US EPA, Res. Triangle Park, NC 27711.

In order to characterize the relationship between sperm motility and fertilizing ability in toxicologic studies, it is important to assess both endpoints in the same animal. To do this, female hamsters are bred at 0630 on the morning of estrus (lights-on, 0700). Uteri are surgically exposed 1.5-2.5 h later, and a sample of sperm is removed (using a 16g IV catheter), diluted, video-taped, and analyzed with computer aided semen analysis (HTM-2000, software version 6.5R, Hamilton-Thorn Research, Danvers, MA). Six hr after breeding, females are killed to recover eggs and evaluate fertilization. Unlike epididymal sperm which have fairly straight paths, the majority (>80%) of uterine sperm swim in large circles. When characterized for 3 sec intervals (at 7 frames/sec), circular path diameters averaged 144 μm (range 100-200 μm) with curvilinear velocity, Vcl = 149 ± 44 μm/sec (mean ± SD) for 49 randomly selected paths. Vcl was similar for sperm with straight paths (164 ± 58 μm/sec, n = 38). Eggs were fertilized at a consistently high rate (98.3 ± 3%, n=11 females). In contrast, in females bred shortly after lights-on (0715), samples were difficult to collect and analyze due to a paucity of uterine sperm (despite sperm-positive vaginal smears). Also fertilization rates were slightly depressed (94.8 ± 8.1%, n=11) and a low incidence of polyspermy was seen. Thus this strategy, with breeding at 0630, may be useful for quantifying effects of toxicants on uterine sperm motility in relation to sperm function.
This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

104 DETERMINATION OF VIABILITY AND ACROSOMAL STATUS OF HUMAN SPERM BY FLOW CYTOMETRY M.E. Kiel*, B.F. Burke*, E. Clegg, M. J. Zinaman, Department of Obstetrics and Gynecology, Georgetown University Medical Center, Washington, DC 20007 and U.S. Environmental Protection Agency, Office of Reseach and Development, Washington, DC 20460

The ability to determine the acrosomal status (AS) of viable human sperm has become a common research tool which has led to an increased understanding of sperm physiology. The most common available method utilizes a supra vital stain and an FITC-lectin with fluorescence microscopy (FM). Determination of AS in living sperm is performed by manually counting up to 200 cells. We attempted to facilitate this process by utilizing flow cytometry (FC) which is more rapid and allows the determination of AS in millions of cells. Sperm cells capacitated in BWW 3% BSA were directly stained or challenged with follicular fluid (20% vol/vol) to induce the acrosome reaction. To assess the viability of sperm, samples were stained with ethidium monoazide (EMA), which labels nucleic acids, after exposure to a fluorescent light source, by producing covalent bonds with dead cells. After washing, viabilities were checked and found to correspond to EMA binding levels. Samples were then fixed with ethanol and stained with FITC - conjugated *P. sativum* (FITC-PSA) to determine acrosomal status. The samples were evaluated by flow cytometry and by inspection with a fluorescence microscope. The flow cytometer identified 3 subgroups: I-viable, acrosome-reacted; II-viable, non-acrosome reacted, III-nonviable. Slides made from the samples and read by microscope verified the results obtained by flow cytometry. The distribution of sperm (mean) for the groups is as follows:

	I		II		III	
	FM	FC	FM	FC	FM	FC
FF Challenge	31	28	26	24	43	48
No Challenge	10	8	52	49	38	43

Sperm challenged with follicular fluid demonstrated increased percentages of viable, acrosome-reacted cells compared to unchallenged cells to a similar degree by both methods. Flow cytometric assessment of AS using EMA in conjunction with FITC-PSA is an attractive alternative to more conventional methods.

105 COMPARISON OF GLYCEROL AND 1,2-PROPANEDIOL AS CRYOPROTECTANT AGENTS FOR HUMAN SPERM

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To achieve acceptable post-thaw motility of human sperm, cells must be frozen in the presence of cryoprotectant compounds. In part due to historical protocols, and in part due to its proven efficacy, the standard cryoprotectant for human sperm has been glycerol; in spite of its apparent adverse effects on motility. Although 1,2-propanediol (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 (v:v) 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 N_2. Cells were thawed and examined for (1) motility, using CASA, and (2) plasma and acrosomal membrane integrity, using indirect immunoflurescence (T6 monoclonal antibody). Results indicate that cells frozen in glycerol had greater ($P<.01$) post-thaw motility than those in PrOH [motility: 43.8 ± 3.8 ($\bar{X}$% $\pm$SEM) vs 1.9 ± 1.2; 18.2 ± 4.7; 15.5 ± 4.0; 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 ($P>0.05$) among control (28.1 ± 4.6) or 1M (34.8 ± 2.7) and 0.5M (39.2 ± 5.0) 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(#1RO1HD25949-01). T6 monoclonal antibody was graciously supplied by D.P. Wolf, Oregon Regional Primate Research Center, Beaverton, OR.*

106 METHODS USED TO PREPARE RAT EPIDIDYMAL SPERM FOR CASA INFLUENCE MOTILITY ENDPOINTS.

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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 medium used, and sample chamber depth. Sperm samples were obtained from the distal cauda epididymidis and analyzed with an HTM-2000 motility analyzer (Hamilton Thorn 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 axpressed 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 (≥77%) 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%, 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.

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THE USE OF THE KRUGER STRICT TEST FOR SPERM MORPHOLOGY IN DISTINGUISHING THE FERTILE FROM SUBFERTILE MALE.

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There are reports suggesting that the Kruger strict test for sperm morphology might 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 conceptions. 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 $\geq 10x10^6$/ml. When the Kruger test was ≤4% 14 of 28 (50%) conceived versus 46 of 77 (57%) when the level was >14%. 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 2%. 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 >14%. There were 14 of 34 (41%) pregnancies in 6 months with Kruger tests ≤4%, compared to 10 of 34 (29.4%) 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 $<10x10^6$ or greater.

108 **ACCURACY AND PRECISION OF COMPUTER-AIDED SPERM ANALYSIS (CASA) IN MULTI-CENTER STUDIES.**

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Results from traditional semen analysis are not highly correlated with fertility. Reasons for this include subjective assessment of semen parameters, and/or inadequate definition and control of study populations. The introduction of CASA, and new interest in multi-center studies, have begun to address these problems. In this study, methods were developed to standardize CASA at two centers. Four CTS instruments (Motion Analysis Corp.), two each at two sites, were used to repeatedly analyze four semen specimens from the Video Tape Reference Standard for Human Semen Analysis (College of American Pathologists). Machine accuracy was verified by manual analysis of the videotape. The coefficient of variation (CV) for each instrument was between 1-4% for each CASA variable. Mean values varied slightly as a function of digitization threshold. A minimum CV was obtained at a particular threshold setting, implying the existence of an optimum. Due to high within-machine precision, slight differences in means were statistically significant for some variables and specimens, although these differences are unlikely to be biologically significant. These results show that multi-center standardization of CASA instrumentation is possible, but that threshold settings are one source of variation which needs to be controlled using CVs, or another means of objective calibration. (HD15149 & ES03614)

109 IMPAIRMENT OF SPERM MOTILITY BY WATER-SOLUBLE LUBRICANTS AS ASSESSED BY COMPUTER-ASSISTED SPERM ANALYSIS

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Men participating in diagnostic or population-based fertility studies and sperm donor programs, as well as couples attempting to acheive pregnancy often desire a lubricant for semen collection or intercourse. Lubricants are usually not recommended due to spermicidal activity. Several new water soluble lubricants have recently been introduced with unknown effects on sperm motion and viability. Sperm from normal men were incubated for 20 minutes at 37° with 10% KY (Johnson and Johnson), saliva or the newer lubricants, Aetroglide (Aetro-Lube) and Today (VLI). Aliquots of each mixture were analyzed for sperm motility, and curvilinear velocity (VCL) using computer-assisted sperm analysis (CASA, CTS, Motion Analysis Corp). Smears were prepared and stained with eosin-nigrosin for viability assessment. The average sperm motilities after exposure to saliva, Today, Astroglide and KY were 91%, 62%, 45%, and 34% percent of control respectively. Average VCL of the motile sperm with saliva, Today, Astroglide and KY were 90%, 79%, 80%, and 59% of the control value. Viability staining supported a concentration dependent spermicidal activity of the tested lubricants. Although the newer lubricants have less spermatotoxicity than KY, they induce demonstrable reduction in motility and viability. Men collecting semen or couples attempting pregnancy should be cautioned to avoid the lubricants tested.

110 PROGRESSIVE MOTILITY OF PROCESSED SPERM PERMITS IMPROVED PREDICTION OF SPERM PENETRATION ASSAY OUTCOME.

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We examined semen samples from 52 infertile couples to determine whether computer assisted semen analysis parameters from unprocessed ejaculate (RAW) or from sperm who had undergone washing and swim-up procedure (WASH) would predict the outcome of the sperm penetration (SP) assay. 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).

	RAW:			WASH:		
	abnormal	normal	P	abnormal	normal	P
T	31.3±2.5	42.0±2.9	.008	59.8±2.8	74.6±2.6	.0005
U	10.1±1.1	16.1±2.5	.020	24.6±2.3	41.1±3.9	.0003
I	59.0±2.9	48.0±3.2	.016	28.4±2.6	16.5±2.1	.0014

The following correlations were found: 1) SP vs T: (RAW) r=.405, p=.0029; (WASH) r=.423, p=.0018. 2) SP vs U: (RAW) r=.468, p=.0005; (WASH) r=.623, p=.0001. 3) SP vs I: (RAW) r=-.390, p=.0042; (WASH) r=-.428, p=.0015. Proportions of T, U and I showed significant differences in men with 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.

111 FLOW CYTOMETRIC ANALYSIS OF WHITE BLOOD CELLS IN HUMAN SEMEN

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Methods for accurate identification of white blood cells (WBC) in human semen has been limited to phase contrast microscopy, and histochemical staining techniques. The presence of nucleated cells other than sperm (NCOS) in semen, potentiates the false positive and false negative identification of WBC. Our laboratory has initiated an investigation to phenotype the WBC present in control semen specimens using flow cytometry. Two fold excess of RPMI-1640 / 5% human AB serum (RPMI/AB) was added twice to wash the semen, resuspending it in a final concentration of 4 million sperm/ml. Five ml of semen suspension was layered on 10 ml of 49.5% continuous gradient percoll, and centrifuged 30 minutes at 800 g utilizing the entire specimen. The interface was aspirated, washed, resuspended in excess RPMI/AB, and refrigerated over night. The isolated NCOS cells were split in thirds and incubated with fluorescein (FITC) conjugated and phycoerythrin (PE) conjugated combinations of monoclonal antibodies. Combinations included CD-45 FITC / CD-3 PE , CD-3 PE / CD-4 FITC, or CD-3 PE / CD-8 FITC. Criteria for analysis of WBC on the Coulter model 753 flow cytometer consisted of a positive light scatter gate established by ficoll-paque isolated CD-45$^+$ peripheral blood cells. A second gate set on CD-45$^+$ cells from the percoll continuous gradient prep assured collection of NCOS cell data only. Analysis of CD-45$^+$ and subsequent cell sorting confirmed a CD-45$^+$ "bright" (WBC) population and "dim" (other nucleated cells) population. Two color analysis of both the CD-45$^+$ bright and dim demonstrated CD-45$^+$ / CD-3$^+$ / CD-8$^+$ / CD-4$^+$ and CD-45$^+$ / CD-3$^-$ / CD-8$^-$ / CD-4$^-$ cells respectively as compared to appropriately conjugated IgG1 negative controls. Phenotyping of WBC in semen provides insight into potential cytokines liberated by WBC. Monoclonal antibodies to CD-45 distinguish WBC from other nucleated cells in semen. Studies are under way to evaluate the activation status of CD-45$^+$ cells in semen.

112 IMPROVEMENT IN SEMEN QUALITY AFTER FILTRATION THROUGH L-4 MEMBRANE. COMPARISON OF RESULTS WITH SWIM-UP TECHNIQUE.

Ann Manglona, *Ashok Agarwal, Kevin R. Loughlin, Boston, MA (Presentation to be made by Dr. Agarwal).

L4 leucocyte adsorption medium (Pall 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 on 15 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 on a Cell-Soft semen analyzer. Semen 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. Semen specimens were mixed with 5.0 ml of Human Tubal Fluid medium by vortex and passed through mounted discs 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 spermatozoa from its surface. Filtered specimens were centrifuged at 600g 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 recovered was 25% (swim-up) and 27% (L4); total motile sperm count 34% (swim-up) and 40% (L4). Sperm motility on the other hand showed an increase from 61% (swim-up) to 82% (L4) ($P<0.0002$) and velocity increased from 47 u/second (swim-up) to 52 u/sec. (L4) ($P<0.048$). Our results demonstrate that sperm filtration through L4 membrane gives superior results as compared to traditional swim-up technique.

113 SEMEN SAMPLE PREPARATION FOR INTRAUTERINE INSEMINATION COMPARING THE WASH, COLUMN FILTRATION AND SWIM-UP METHOD M. Beck, R. Shelden*. Robert Wood Johnson Medical School, New Brunswick, N.J. 08903.

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 (v*c*m)	%RECOVERY	VELOCITY (u/sec)	%NORMAL
WASH	15.1(2.1)	71(3.8)	43(1.6)	30(2.1)
COL	7.1(1.3)	37(3.3)	46(0.7)	20(1.8)
SU	4.6(1.0)	27(2.5)	68(1.8)	67(1.8)

Total motile, percent recovery and normal morphology were significantly higher in the washed samples than after column filtration ($p<0.001$), but no difference in velocity. When the column and the swim-up method was compared, there was no significant difference in the total motile sperm. Percent recovery with the column was significant ($p<0.02$), but velocity and percent normal morphology was significantly better ($p<0.001$) in the swim-up preparation.

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MOTILE DENSITY LEVELS DISTINGUISHING FERTILE FROM SUBFERTILE MALES J.H. Check, A. Bollendorf, B. Shanis. 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.

The World Health Organization establishes a normal motile density as $\geq 10x10^6$/ml. Thus if a couple presents with infertility all men with a motile density below the minimal level will be considered as having a male factor problem and he would thus be considered at least a contributing factor to the infertility problem. If a repediable etiology was present eg a varicocele, surgical correction might be suggested. It is well known, however that some men have achieved pregnancies with extremely low motile densities. A study was thus initiated to determine in couples where a female infertility factor was identified and corrected what critical motile density level would define the subfertile male. We performed baseline semen analysis on the male partners of 281 couples with a minimum of 1-2 years of infertility. All female fertility factors were deemed normal or corrected. The men were grouped into 5 groups based on the average of 2 specimens. Group 1 ($<2.5x10^6$/ml), Group 2 (≥ 2.5 to <5), Group 3 (≥ 5 to < 10), Group 4 (≥ 10 to < 15), and Group 5 ($\geq 15x10^6$/ml). The 6 month pregnancy rates were as follows: Group 1 - 7 of 32 (22%), Group 2- 9 of 13 (69%), Group 3 - 25 of 31 (81%), Group 4 - 27 of 34 (74%), and Group 5 - 139 of 171 (81%). No statistical differences were found comparing Group 2 to Groups 3, 4, or 5 but statistical differences were found comparing Group 1 to the other 4 groups. The data suggest that the $10x10^6$/ml motile density level used by the WHO to distinguish fertile from subfertile males is too high and could lead to unnecessary treatment of the male. Instead our data suggest that the critical level be reduced to $2.5x10^6$/ml and even this is relative rather than absolute since 22% still achieved pregnancies during a 6 month interval. Perhaps more extensive data might find Group 2 men as slightly less fertile.

115 THE USE OF A SPERM "SWIM-DOWN" PROCEDURE FOR PROCESSING FROZEN-THAWED HUMAN SEMEN

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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 assessed. Frozen semen from 9 donors was thawed at 22°C for 20 min. Thawed semen (0.4 ml) was mixed with Ham's F-10 medium/1% HSA (1 ml) in a 15 ml graduated polypropylene 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 (#1) to the top fraction (#7). Aliquots (5 ul) were evaluated for % motility using an automated semen analyzer and sperm concentration determined with a hemocytometer. The % motile sperm recovery $\pm$ s.e.m. evaluated in fractions 1-7 after a 0.5 hr swim-down was: 42.9 $\pm$ 4.0, 15.3 $\pm$ 3.0, 7.4 $\pm$ 0.7, 7.5 $\pm$ 0.6, 6.4 $\pm$ 0.6, 5.7 $\pm$ 0.8 and 10.7 $\pm$ 2.0, respectively. After 1.5 hr the recoveries were: 68.6 $\pm$ 5.2, 7.6 $\pm$ 1.9, 4.8 $\pm$ 1.0, 2.6 $\pm$ 0.9, 2.7 $\pm$ 0.5, 3.9 $\pm$ 1.1, 4.9 $\pm$ 1.2. 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 (0.5hr = 61.9 $\pm$ 6.2%; 1.5 hr = 71.3 $\pm$ 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 with respect to post-thaw motile sperm was 31.9 $\pm$ 7.9% (0.5 hr) and 33.9 $\pm$ 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 IUI and yields a motile sperm recovery 4-5 times greater than reported with swim-ups.

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DETERMINATION OF THE ORIGIN OF THE REACTIVE OXYGEN SPECIES IN SEMEN AND ASSOCIATION WITH QUANTITATIVE SEMEN ANALYSIS.

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Lipid peroxidation has been suggested as one of the major causes for defective sperm function (1). The susceptibility of spermatozoa to peroxidation is increased by their capacity to generate reactive oxygen species (ROS) (2). Every semen sample contains leucocytes principally neutrophils and macrophages (3) both known to be copious 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 discontinuous percoll gradients (2). In order to clarify the origin of ROS, leucocytes were extracted from the 40-80% percoll fraction with immunomagnetic beads coated with a common leucocyte antibody. Quantitative semen analysis was performed on a Hamilton-Thorn motility analyser. Leucocyte derived ROS accounted for almost 70% of the response with a significant correlation between leucocyte numbers and levels of ROS ($R=0.92$).

Using multiple regression analysis only best cross frequency was significantly affected by ROS originating either from sperm or leucocytes. This might not be surprising considering the high levels of ROS scavenging enzymes such as superoxide dismutase in seminal plasma and the short half life of ROS. The effect of the "extracellular" ROS on membrane fusion events such as acrosome reaction and oocyte penetration are yet to be determined(3).

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117 **CRYOPRESERVED OVA DIMINISH THE SENSITIVITY OF THE OPTIMIZED SPERM PENETRATION ASSAY.**

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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 ass y, resulting in a 40-fold increase in sensitivity as compared with routine methods. The optimized SPA uses only fresh hamster ova. However, some clinical laboratories are using commercially available cryopreserved ova to avoid the expense of maintaining and injection live animals. To determine whether the cryopreserved ova are suitable for use in our optimized SPA, we compared the penetrability of cryopreserved ova obtained from 2 commercial sources (A & B) with our fresh ova. Sperm from 3 fertile donors were each tested at 3 different sperm concentrations (1,5,10 X 10^6 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 ova were uniformly decreased to only 39%,r=0.99 (A) and 32%,r=0.89 (B) of the rate for fresh ova. In another experiment 4 patient samples were tested in duplicate at our routine concentration of 5 X 10^6/ml using two batches of frozen ova from source A. The fresh ova results were 4,14,21,31 SCI. In contrast the frozen ova scores were 1,3,3,9 SCI reflecting only 25% (r=0.91) of the fresh values. These results clearly demonstrate that frozen ova do not have the same penetrability as fresh ova even though their appearance at the time of incubation are indistinguishable from fresh ova. They should not be used interchangeably with fresh ova in the optimized SPA.

	FRESH Ova			FROZEN Ova A			FROZEN Ova B			
	[1]	[5]	[10]	[1]	[5]	[10]	[1]	[5]	[10]	←Sperm
Donor #1	10	25	36	5	10	14	1	9	14	Conc.
Donor #2	10	25	32	3	9	12	3	6	11	(x10^6/ml)
Donor #3	3	18	34	2	6	14	1	1	7	

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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Cervical mucus testing has been utilized to evaluate male infertility; however, little has been done to compare in vitro testing with in vivo. Our purpose was to study sperm penetration in patients with a well-timed mucus specimen, as well as a bovine mucus control and compare these to in vivo performance after cervical insemination. Cervical mucus was obtained from 11 patient using BBT and urine LH for cycle timing and bovine mucus was used as a control (Humegen, Charlottesville, VA). Semen was provided by husbands after 2 days abstinence and evaluated to confirm normal parameters, then inseminated into the cervix. Previous semen parametars were normal according to the WHO criteria. Patient cervical mucus was graded (score >8) by WHO criteria and placed in one penetration track. The bovine mucus was inserted in the other parallel track and the liquified semen was placed with the mucus. A penetration of vangard sperm less than 10mm was considered poor after 30 min. Evaluation in vivo was performed by obtaining an endocervical sample from the patient 1 1/2 h after insemination and a poor test consisted of less than 5 motile sperm/hpf. For 6 patients with poor patient mucus penetration, the post-cervical insemination was equally poor in all cases. In the 5 other cases there was excellent penetration in vitro but only 1 had an excellent in vivo evaluation. It appears there is a sperm dysfunction despite otherwise normal semen and cervical parameters and the mucus penetration has a better negative correlation. This may allow a more focused therapy.

119 TIGHT BINDING OF SPERMATOZOA TO THE ZONA PELLUCIDA: COMPARISON OF RESULTS USING FRESH VERSUS SALT-STORED OOCYTES AND INFLUENCE OF OVARIAN GONADOTROPIN STIMULATION UNDER HEMI-ZONA ASSAY (H2A) CONDITIONS

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The H2A is a homologous, reproducible, internally-controlled bioassay that evaluates tight binding of human sperm to the human zona pellucida. Previous studies have validated H2A results using salt-stored and frozen-stored immature oocytes obtained from surgically-removed ovarian tissue. This study aimed to evaluate the sperm binding capacity of (1) fresh vs. salt-stored oocytes (metaphase I) and (2) immature-prophase I or PI - (salt-stored) eggs recovered from ovarian tissue vs. PI oocytes (salt-stored) retrieved after superovulation with gonadotropins. Oocytes were microbissected before the assay and coincubated for 4 hours with swim-up processed semen. Results: (1) the mean number (x±SE) of tightly bound sperm for frash and salt-stored metaphase I oocytes (n=11) were 118.6 ± 12.9 and 122.7 ± 14.0, respectively (normal semen) and 37.1 ± 7.0 and 33.4 ± 7.3 (infertile semen), raspectively (not significant). (2) PI oocytes recovered in tha natural cycle (n=5) had significantly higher number of tightly bound sperm than PI eggs (n=3) obtained after superovulation (31.1 ± 5.5 vs. 15.3 ± 4.5, respectively; p<0.05). These results show that (1) salt-stored human eggs (pH 7.0, stored up to 60 days) have equal binding ability than fresh, living human oocytes under H2A conditions; (2) zona-binding properties of PI oocytes seem to be negatively affected by gonadotropin ovarian stimulation.

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

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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 anaesthesia and aspiration was performed with a 22 gauge Wescott needle attached to a Gilbaugh aspirator (Bard Urological, Marietta, Ga.). Flow cytometry was conducted on a Coulter EPICS 451 flow cytometer (Hialeah, FL), using the 488 argon ion laser line at 15mW to induce flourescense. 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 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 sperm 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 % motility (43.2 ± 28.2) were within 2 S.D.'s. 96% values of the flow cytometry were within 2 S.D.'s (1C: 78.2 ± 5.6; 2C: 10.6 ± 4.0; and 4C: 7.05 ± 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.

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IMPROVEMENT IN SPERM MORPHOLOGY AND REMOVAL OF ROUND CELLS IN SEMEN AFTER FILTRATION OF EJACULATES THROUGH L4 MEMBRANE. Ann Manglona, *Ashok Agarwal and Kevin R. Loughlin, Boston, MA (Presentation to be made by Dr. Agarwal)

Human semen specimens contain various "round cells" (immature germ cells, white blood cells) as well as mature spermatozoa. Previous reports suggest that removal of these round cells can enhance the fertilizing capacity of semen specimens. We report our experience adapting the L4 leucocyte adsorption medium (Pall Biosupport Corporation, 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 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 in 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 using a Makler chamber under a 20 x phase objective lens. Each specimen with a round cell count > or = 5×10^6/ml was tested for the presence of white blood cells (granulocytes) by the Endtz test. Peroxidase positive white blood cells staining dark brown were counted under the 20 x objective using a Makler chamber. The results were recorded as million/ml.

Our results demonstrate a significant increase in normal sperm forms and a decrease in abnormal forms after filtration of specimens through the L4 membrane. Normal sperm forms increased from 48% to 84% ($P<0.0001$); abnormal forms decreased from 26% (head defects) to 10% ($P<0.0001$); neck defects from 5% to 2% ($P<0.002$); tail defects from 11% to 4% ($P< 0.03$) and undifferentiated round cells from 10% to <1% ($P<0.008$) after filtration. Round cell count decreased from 8×10^6/ml to 0.5×10^6/ml ($P<0.002$) after filtration. Similarly white blood cell counts measured through the Endtz test also decreased from 3.4×10^6/ml to 0.1×10^6/ml. These results demonstrate that the filtration of semen specimens through the L4 membrane results in significant improvement in sperm morphology and should be considered as an adjunct in sperm processing.

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SPERM MOTILITY BEFORE AND AFTER PREPARATION FOR INTRAUTERINE INSEMINATION C.R. Sanborn*, L.E. Meuli, and F.W. Byrn*. Department of Ob/Gyn, University of New Mexico, Albuquerque, NM 87131.

Two methods of separating sperm from the seminal plasma prior to intrauterine insemination (IUI) were compared using a computer assisted semen analysis system (CellSoft ™, Cryo Resources, Inc). Male patients from the Reproductive Endocrinology/Infertility Clinic collected samples by masturbation. Samples from each individual were prepared using two methods: either triple wash (TW), (Wong et al, 1986) or glasswool separation (GW), (Rhemrev et al, 1989). Pre-preparation and post-preparation motility parameters on samples obtained from the same patient were subject to statistical analyses. Percent motility, velocity, and amplitude of lateral head motion (ALH) were analyzed using Student's t-test. We noted a significant increase ($p<0.005$) in the percent motile fraction post-GW when compared to GW pre-treatment values. Furthermore, we also found that percent motility post-GW treatment was significantly higher ($p<0.05$) when compared to percent motility post-TW. There was no significant difference observed in percent motility following TW treatment when compared to TW pre-treatment parameters. Lastly, no significant differences were observed in the velocity or ALH parameters among the four compartments. These findings suggest that glasswool separation, in contrast to triple wash preparation, produces a sample with a higher post-preparation fraction of motile sperm for use in IUI, while comparably separating sperm from seminal plasma.

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EFFECT OF SP-cAMP ON SPERM MOTILITY IN PATIENTS WITH UNEXPLAINED INFERTILITY. *Ashok Agarwal and Kevin R. Loughlin, Boston, MA (Presentation to be made by Dr. Agarwal)

Sperm motility is one of the most important semen parameters affecting the fertility of an individual. Sp-cAMP is a thiophosphate 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 (HTF) (Irvine Scientific, CA) in a IEC 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 polypropylene tubes (5 ml capacity). 100 ul of washed sperm were added into tubes containing equal volumes of cAMP at concentrations of 0, 2.5, 5.0 and 10.0 uM, suspended in HTF medium. The tubes were incubated at 37°C for 30 minutes. A computerized semen analysis was done on each aliquot at the end of incubation period on a Cell-Soft automated semen analyzer.

There was an increase in motility between sperm incubated with Sp-cAMP (0 uM) and all other drug concentrations. The greatest improvement of sperm motility was seen with 5.0 uM Sp-cAMP which was statistically significant ($P<0.001$). Our results appear in the table below.

Concentration Sp-cAMP (uM)	Motility (%)
0	29
2.5	39
5.0	48
10.0	38

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

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DETRIMENTAL EFFECT OF CRYOPRESERVATION ON HUMAN SPERM VIABILITY. J.H. Check, M.L. Check, R. Long. 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.

Most programs performing cryopreservation of spermatozoa are predominantly interested in the post-thaw motile density. Though adverse effects on the sperm membrane have been previously reported, the failure to measure viability most likely reflects the assumption that if the motile density is good then the viability would probably follow suit. We evaluated 13 cryopreserved-thawed specimens from known fertile donors (when used fresh) which yielded after thawing a motile density of $\geq 10\times10^6$/ml. Not one specimen had a viability $\geq$ 50%. The highest viability was 48% with a hypo-osmotic swelling test (HOS) of 45%. Five subsequent specimens were next evaluated from commercial sources; one source claimed that they prechecked viability tests (center 1) and the other did not (center 2). All 3 thawed specimens from center 1 had viability levels over 50% (72, 62, and 51%) and the HOS specimens were 64, 52, and 47% respectively. The evaluation of 2 specimens from center 2 showed only 17% and 24% viability respectively. The cryopreservation technique used by our group employed test-yolk buffer as the cryoprotectant but our buffer had 12% glycerol and center 1 had 7% and center 2 used a "flash freezing" method not employing a cryoprotectant. We washed out the cryoprotectant in 5 additional specimens with motile densities $>10\times10^6$/ml but still the viability was <50%. Officials from center 1 stated that they reject 11 of 12 donors in order to achieve their criteria for selection. There are data suggesting that poor fertility outcome is seen despite normal motile densities when the viability is below 50%. Perhaps this is why one center reported only a 1% success rate with cryopreserved-thawed sperm following intracervical insemination but 11% with intrauterine insemination, ie perhaps poor viability manifests with poor-longevity. More data is needed to determine if cryopreserved specimens with viability $\geq$ 50% are more fertile than those <50% despite normal motile densities similar to the results seen with fresh semen.

125 PLATELET ACTIVATING FACTOR (PAF) STIMULATES MOTION PARAMETERS OF CRYOPRESERVED HUMAN SPERM. W.J.G. Hellstrom, 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 because of 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. Semen 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 10^6 sperm) were incubated at 25°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, and amplitude of 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 not significant at lower (0.03 and 0.1μM) PAF doses. V_{SL} (μ/sec) was significantly ($p<0.05$) stimulated with higher PAF doses (13 ± 1 in control; 18 ± 2 with 1μM and 20 ± 3 with 3μM after 1 hour). A significant ($p<0.05$) stimulatory 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 V_C and ALH were not significant ($p>0.05$) because of a larger variation. Our data demonstrates that PAF can stimulate the motion parameters significantly (% motility, V_{SL}, and LIN) at 0.3 μM and at higher doses. This may improve the chances of conception when employing cryopreserved sperm in assisted reproductive procedures.

127 SUCCESSFUL ARTIFICIAL INSEMINATION OF CRYO-PRESERVED PHEASANT SEMEN. B. Durrant and C. Burch*. CRES, Zoological Society of San Diego, San Diego, CA 92112.

Avian 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 Impeyan pheasants (Lophophorus impeyanus) and diluted in 21°C Beltsville Poultry Semen Extender for transport (10-15 min) to the laboratory for analysis. Maintanence of motility during transport was enhanced at 5°C vs. 21°C ($p<.001$). Post-thaw motility was greater ($p<.05$) 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) or 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 5°C bath. All samples were frozen at 1°C/min to -20°C then 50°C/min to -80°C before plunging into liquid nitrogen. Semen evaluation revealed the following means:

Volume (ml)	Concentration (10^6/ml)	Motility index (motility X speed^2)	
0.019	111.85	fresh	851
		frozen	367

In fresh samples, motility was as accurate a measurement 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.

126 CRYOPRESERVATION OF HUMAN SPERM IS DETRIMENTAL TO HYPERACTIVATED MOTILITY POTENTIAL. G.M. Centola, L.J. Burkman, K. White*, and L.B.V. Emilson*, Andrology Laboratory, Ob/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 pellucida. Hyperactivated motility (HA) is another significant factor in fertilization. This study has examined whether cryo damage extends to HA potential as well. Sperm were acquired from donors in an artificial insemination program, within 0.5 h of ejaculation (20 samples). Each specimen was divided in half for fresh versus frozen studies. The fresh sperm were washed with Ham's F10 medium plus 3.0% human serum albumin, resuspended in F10 and assessed for motility at 0 hours, as well as after 3h and 6h of a swim-up incubation (37°C, 5% CO_2). Automated motion analysis was performed with the Hamilton-Thorn 2030 system (= HTM) at low sperm density (2 x 10^6/ml) and in a deep chamber. Computerized HA identification relied on 3 criteria (curvilinear velocity ≥ 100 um/sec, linearity ≤ 65, and ALH ≥ 7.5 um). The remaining seminal portion was processed for cryo: dilution with Human Sperm Preservation Medium + 7.5% glycerol and nitrogen vapor freezing. Sperm were thawed at room temperature after ≥ 48 hours. The thawed sample was diluted with F10 medium and a portion was assessed at 0 hours. The residual thawed sperm were washed twice, layered with F10 and examined after 3h and 6h of incubation. As previously reported, the fertile men were heterogenous 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 [fresh/thawed] were [81% ±3; 57% ±4], [69% ±3; 46 ±4], and [59 ±1; 47 ±3 um/sec]. Whereas all 20 fresh cases exhibited some HA (range for peak value: 1-14%), 9 of the 20 had 0% HA post-thaw. The hyperactivation assessment revealed a mean of 6 ±0.8% for the maximal values in fresh samples. After cryo, HA fell significantly by 50% to 3± 0.8% ($p< 0.05$). 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 *late* HA peaks (6h, fresh), 5 of 7 exhibited no HA post-thaw. This significant loss in HA potential is consistent with the decline in other fertilization functions after cryo. A 50% reduction in hyperactivated capacity is another factor requiring increased numbers of thawed sperm for inseminations involving zona-intact eggs.

128 HUMAN SPERM CRYOPRESERVATION- TEST YOLK BUFFER OR HSPM? G.L.A. Horbay, R. Cooblal*, and F. Choma*. Division of Reproductive Medicine, Toronto General Hospital, Univ. of Toronto, Toronto, Ont. Canada.

Normal semen samples with a minimum of 60 X 10^6 motile sperm/ml were selected from 13 patients undergoing evaluation for IVF. Liquefied semen was diluted 1:1 with TEST yolk buffer (TYB) freezing medium or human sperm preservation medium (HSPM). Aliquots of EJACulate and of each semen-cryoprotectant mixture (CPM) were left at room temperature(RT) as CONTrols. The remainder of each sample was packaged in cryostraws and frozen in liquid nitrogen (LN_2) vapours with a manually controlled 3 stage freezing device, and stored in LN_2. Frozen samples were thawed at RT and evaluated at 20 min. EJAC, 2h EJAC CONT, 2h TYB CONT and HSPM CONT, and post thaws (TYB PT, HSPM PT) were all evaluated by CASA. Manual counts were performed on triplicate samples. Motility factors evaluated for each sample were %MOTility, VELocity, LINearity, ALHmean, and BCF. Survival (SF) and cryosurvival (CSF) factors were calculated as were motility indices (MI) [%MOT X VEL/100] and return of MI (RMI). Mean results were compared between treatments for each parameter using t-test; only differences at the 95% confidence level are reported. EJAC CONT had increased LIN and BCF over EJAC- all other parameters were unchanged. Compared to EJAC, TYB CONT had decreased VEL and ALH, and increased LIN and BCF; they were not different from EJAC CONT in any way. HSPM CONT showed a marked decrease in %MOT from the EJAC, and marked decreases in %MOT, LIN, SF and RMI compared to EJAC CONT. HSPM CONT showed marked decreases in %MOT and SF, decreased LIN and increased ALH compared to TYB CONT. Both TYB PT and HSPM PT showed decreased %MOT, CSF and RMI from their respective CONT, and marked decrease in %MOT, LIN, CSF AND RMI from EJAC CONT. TYB PT differed from HSPM PT having increased LIN and decreased ALH. Although not significantly so, TYB PT showed higher %MOT, BCF, CSF and RMI than HSPM CONT. Where CSF is >50%, RMI is higher in TYB PT ($p< 0.001$) with no difference in CSF between CPMs. TYB appears less cytotoxic to sperm than HSPM, and overall gives a better freeze-thaw sample.

129 PREVALENCE OF LEUKOCYTOSPERMIA IN MALE INFERTILITY PATIENTS IN BEIJING, CHINA A-W. Wang 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. Semen samples from 102 male infertility patients and 10 normal fertile men were stained for peroxidase positive cells (granulocytes) by the Endtz technique. Samples containing $>1 \times 10^6$ 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 2%, $p<0.09$). All but one of the pacients 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.

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UPPER EPIDIDYMAL ASPIRATION OF SPERMATOZOA AND ASSISTED CONCEPTION IN THE TREATMENT OF OBSTRUCTIVE AZOOSPERMIA.

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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 'blow outs', 3 had idiopathic upper epididymal obstruction, 2 more had bilateral congenital absence of the vasa deferentia, and the remaining 3 men had upper epididymal obstruction due to Young's Syndrome, previous surgery and to epididymitis. 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 vasa deferens. A total of 4 attempts were made among the 3 men with idiopathic obstruction and 1 attempt each was made in the 1 man with Youngs syndrome and in the 2 men with obstruction after surgery and after epididymitis. 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 at sperm aspiration, no motile sperm could be obtained and in 1 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 among 5 patients. The fertilisation rate ranged between 7% to 86% among the 7 attempts at which fertilisation occurred, producing a mean fertilisation rate of 40%. Transfers of between 1 and 3 embryos were carried out in the consorts of all 5 of these men producing 2 pregnancies. One pregnancy occurred from a patient with idiopathic obstruction resulting in a live birth but the other pregnancy, from a man with post vasectomy upper epididymal "blow outs', ended in a first trimester miscarriage. These results produced a pregnancy rate of 10 % per attempt, 17% per patient and 29% per transfer.

131 RECOVERY OF SPERM PRODUCTION FOLLOWING A NEW CHEMOTHERAPY REGIMEN FOR HODGKIN'S DISEASE

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Chemotherapy for Hodgkin's Disease (HD) generally employs alkylating agents (e.g. procarbazine) and can result in permanent azoospermia in most of the men treated. We are studying the gonadal effects of NOVP, a new chemotherapy combination consisting of 3 courses of mitoxantrone, vincristine, vinblastine and prednisone over 2 months, followed by radiotherapy for treatment of Stages I-III HD. In 13 of 14 men analyzed, pretreatment sperm counts were in the normospermic range. However, at 2-3 months from the start of chemotherapy, only 1 of 13 men analyzed was normospermic; and 4 of these men were azoospermic. Recovery begins rapidly; at 2-3 months from the end of chemotherapy, 7 of 10 men analyzed were normospermic and the other 3 were oligospermic. This result indicates that A spermatogonia survive the treatment despite the killing of later stage cells. A secondary decline (median of 20-fold) in sperm counts following abdominal radiotherapy occurred in all 8 men, despite the low measured median gonadal dose of 24 cGy. However, this was transient and all 5 patients who have been followed for at least 10 months from the end of therapy, are normospermic. Although sperm count is reduced by NOVP, as with most chemotherapy combinations, in contrast to treatment with MOPP there is a rapid recovery of sperm production, eventually reaching normal levels. Thus, proper choice of chemotherapy drugs used and minimizing the gonadal dose of radiation can preserve the fertility of young men undergoing curative treatment for HD. (Supported by Grant CA-17364 from NIH).

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

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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, no adverse effect on spermatozoan quality has been reported. This study was therefore undertaken to determine the in vitro effect of these pollutants on sperm quality. The pollutants studied were hexachlorbenzol (HCB), 22'66' tetrachlorbinphenol (PCB), Clophen (a mixture of polychlorinated biphenylsCl), chlorpyritos (a cholinesterase inhibitorCH) or DDE (a metabolite of DDT).

Donor ejaculates were washed in BWW medium and 10^6 spermatozoa were resuspended in 1 ml BWW containing 0.1, 1.0, 10, 100, or 1000 ng (solubilized in 1% DMSO) of these compounds. Compared to spermatozoa in BWW + 1% DMSO, all compounds studied yielded a dose and time-dependent effect on sperm motility (SM), hypoosmotic swelling (HOS) test and dye exclusion (vital) test. After 8 hour incubation, even at a concentration of 1 ng/ml, a significant ($P<0.05$) reduction in % SM for Cl and PCB, % HOS +ve sperm for HCB and DDE and % vital sperm for DDE was observed as compared to the control.

133 A NEW SIMPLE METHOD FOR PREPARING SPERMATOZOA FOR INSEMINATION USING THE NEW SPERMPREP™ FILTRATION METHOD

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Motile, morphologically normal human spermatozoa can be separated from semen with various spermatogenic deficiencies or characteristics via the SpermPrep™ filtration method (Zavos, Infertility 13:25, 1990). Such selection could yield higher rates of conception in procedures such as intrauterine 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 normospermic specimens 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 liquified 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) 218.6 ± 30.7 ($x10^6$), 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, hypoosmotically swollen sperm (HOS) 72.3 ± 7.4. The specimens were filtered for 4 min recovering a filtrate volume of 4.1 ml. Both the filtered and non-filtered aliquots (remained in SpermPrep™) were centrifuged and resuspended in 0.4 ml and 0.6 ml modified Ham's F-10, assessed and used for IUI and intracervical inseminations (ICI) respectively. The spermatozoal parameters in the two specimens are shown below (mean ± SE).

Sample	C	MO	GR	MOR	HOS	SR*
IUI	36.7 ± 6.9	78.7 ± 5.1	3.6 ± 0.2	85.1 ± 6.0	86.7 ± 6.9	16.8
ICI	139.6 ± 28.3	63.7 ± 7.9	3.4 ± 0.3	63.1 ± 8.2	68.6 ± 7.5	63.9

The results indicate that the new SpermPrep™ provides additional features such as speed and efficiency (*SR: % 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.

134 SELECTION OF VIABLE SPERMATOZOA VIA SPERMPREP™ FILTRATION FOLLOWING TWENTY-FOUR HOUR CRYOSTORAGE AT 5°C IN TEST-YOLK BUFFER

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In vitro fertilization (IVF) rates and sperm penetration into zona-free hamster oocytes is significantly enhanced when spermatozoa are diluted with Test-Yolk buffer (TYB; Fertil Steril 52:1077, 1989; JIVET 6:207, 1989). Motile, morphologically normal sperm can be separated from semen via the SpermPrep™ filtration method (Infertility 13:25, 1990). The objective of this study was to determine if spermatozoa following a short term storage at 5°C in TYB could be recovered and improved via SpermPrep™ filtration for use in various forms of noncoital reproduction (IUI, IVF, GIFT, etc.). Twenty ejaculates were assessed, liquified and an aliquot containing 100.0 x 10^6 sperm were diluted 1:1 (v/v) in TYB and cooled to 5°C for24 hrs (Zavos, et al., Fertil. Steril. 34:607). After storage the supernatant was removed. The remaining pellet was rewarmed and resuspended in 1.0 ml (37°C) modified Ham's F-10. Following 15 min of incubation the rewarmed spermatozoa were filtered via the SpermPrep™ according to the manufacturers specifications (Fertility Technologies, Inc. Natick, MA 01760). The mean (± SE) ejaculate characteristics and final semen parameters after rewarming and filtration are shown below (sperm counts are in millions). Treatments (TRTS): 1: fresh, 2: rewarmed, 3: filtered.

TRTS	Total Count	Motility	Grade	Morphology	HOS	TFSF
1	100.0 ± 0.0	59.7 ± 7.6	3.3 ± 0.4	57.9 ± 9.6	66.2 ± 5.3	34.6 ± 5.1
2	100.0 ± 0.0	48.2 ± 9.3	2.9 ± 0.5	53.3 ± 9.9	51.3 ± 6.7	25.7 ± 5.7
3	38.7 ± 5.0	78.9 ± 6.1	34.0 ± 0.2	69.7 ± 6.2	86.1 ± 4.2	21.3 ± 3.2

Viability over a 3 hr incubation revealed that filtered spermatozoa had the highest survival. The results showed very clearly that TYB cryostored spermatozoa survived well and improved significantly ($P<0.05$) when filtered via the SpermPrep.™ Of notable clinical importance, 82% of the TFSF (total functional sperm fraction = total sperm x % motility x % normal) originally applied (rewarmed) to the SpermPrep™ were recovered. Thus, SpermPrep™ filtration may be beneficial in recovering TYB cryostored spermatozoa for use in various forms of noncoital reproduction.

135 MODIFIED PERCOLL GRADIENT CENTRIFUGATION FOR PREPARING MOTILE SPERM FROM INFERTILE MEN. N Tanphaichitr, C Lawrence, M Leveille, D Caldwell and J Carnegie. Human IVF Labs, Reproductive Biology Unit, Dept Obs/Gyn, Loeb Inst, Ottawa Civic Hospital, Univ Ottawa, Ontario, Canada.

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 68.5 ± 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 intrauterine insemination.

136 PARACRINE REGULATION OF HUMAN PROSTATIC GROWTH BETWEEN NEOPLASTIC EPITHELIAL CELLS AND STROMAL CELLS D Djakiew, B. Pflug*, R. Delsite* and M Onoda.* Department of Anatomy and Cell Biology, Georgetown University School of Medicine, Washington, D.C. 20007.

The interactions between a neoplastic prostatic 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 ^{3}H-thymidine incorporation. The TSU-prl cells and stromal cells were cultured separately in F-12/DME with testosterone (10^{-7} M). Conditioned media were dialysed and lyophilized to obtain the concentrated 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) repectively, 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 cell 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 reduced the relative growth 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 and 50 ug/ml stromal cell secretory protein exhibited 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.

137 ENDOCYTOTIC PATTERNS OF TESTICULAR PROTEINS IN THE EXCURRENT DUCTS OF THE RAM. D.N. Rao Veeramachaneni and Rupert P. Amann. Colorado State University, Fort Collins, CO 80523

Although about 85% of the protein present in rete testis fluid (RTF) is absorbed in the proximal excurrent ducts of the ram, the amount of androgen-binding protein (ABP) in the lumen of the central caput epididimidis remains unchanged (*J Androl 11:140, 1990*). To determine if only some testicular proteins are selectively absorbed at specific sites in the excurrent ducts, proteins in four HPLC fractions of ovine RTF viz., ABP, 27/34 kD dimer, clusterin, and transferrin were colloidal gold-labeled and microinjected into the lumen of the ductuli efferentes (DE), and the initial (IS), proximal (PC), central (CC), and distal (DC) segments of the caput, and the proximal corpus (CP) epididymidis (4 rams/site/protein). Ductal tissue at each injection site was sampled 30 min later and processed for electron microscopy. Gold particles present in 6400 µm2 area of epithelial cells cut longitudinally were counted. Data were analyzed using ANOVA and Duncan's multiple range test.

	DE	IS	PC	CC	DC	CP
ABP	1825 ^{A}	78 ^{Aa}	73 ^{Aa}	51 ^{Ab}	27 ^{Ac}	24 ^{Ac}
27/34 Dimer	1673 ^{A}	32 ^{Ba}	23 ^{Ba}	15 ^{Ba}	7 ^{Bb}	10 ^{Ab}
Clusterin	1595 ^{A}	35 ^{Ba}	6 ^{Ba}	62 ^{Ab}	30 ^{Aa}	72 ^{Bb}
Transferrin	1288 ^{A}	12 ^{Ba}	4 ^{Ba}	6 ^{Ba}	7 ^{Ba}	30 ^{Ab}

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

Irrespective of protein injected, substantially more gold was endocytosed in the DE than in any segment of the epididymis. In the epididymis, whereas more ABP was endocytosed in proximal sites, endocytosis of clusterin and transferrin was more in distal sites than in proximal sites. Thus, maintenance of a similar amount of ABP through the DC as that entering in RTF probably is by recycling of endocytosed ABP or secretion, rather than by selective "sparing" from endocytosis, in the more proximal regions of excurrent ducts.

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

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Experiments were conducted to evaluate Win 49,596 ((5alpha,17alpha)-1'(methylsulfonyl)1'H-pregn-20-yno-[3,2c]- pyrazol-17-ol), a novel steroidal androgen receptor antagonist, in animal models of prostate cancer. Oral administration of Win 49,596 for 28 days inhibited ($p<0.05$) growth of the androgen-sensitive PAP variant of the Dunning R-3327 prostatic carcinoma in intact male rats to 61, 44 and 41% that of intact controls (100%) at 30, 100 and 300 mg/kg/day, respectively. Tumor inhibition at 100 and 300 mg/kg/day Win 49,596 was similar ($p>0.10$) to that observed in castrate controls (50%) and in intact rats administered the nonsteroidal androgen receptor antagonist flutamide (45%) orally at 15 mg/kg/day. Castration (24%), Win 49,596 (43, 38 and 34% at 30, 100 and 300 mg/kg/day, respectively) or flutamide (33%) 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-82 human prostatic carcinoma xenografted into athymic nude male mice. Oral Win 49,596 for 35 days inhibited ($p<0.05$) tumor growth to 58, 67 and 45% that of intact controls (100%) at 30, 100 and 300 mg/kg/day, respectively. Tumor inhibition was similar to that observed in intact male mice administered flutamide (48%) orally at 30 mg/kg/day but less than that following castration (15%). Ventral prostate weights were also reduced ($p<0.05$) in castrate mice (21%) as well as in intact mice administered Win 49,596 (89, 58 and 46% at 30, 100 and 300 mg/kg/day, respectively) or flutamide (52%) relative to intact controls (100%). Finally, at equivalent total daily dosages of either 150 or 300 mg/kg/day Win 49,596, twice a day (BID) dosing was more effective than once a day (SID) dosing in inhibiting tumor growth (63% at 150 mg/kg SID; 37% at 75 mg/kg BID; 33% at 300 mg/kg SID; 24% at 150 mg/kg BID) relative to intact controls (100%). The inhibitory effects of Win 49,596 at 150 mg/kg BID on tumor growth were similar to that observed following castration (18%). Although Win 49,596 treatment reduced ($p<0.05$) ventral prostate weights relative (58% at 150 mg/kg SID; 48% at 75 mg/kg BID; 50% at 300 mg/kg SID; 45% at 150 mg/kg BID) to intact controls (100%), there was no difference ($p>0.10$) between SID versus BID dosing. These results suggest that Win 49,596 may have utility in the treatment of hormonally dependent metastatic prostate cancer in humans.

139 ERADICATION OF LEYDIG CELLS DURING PUBERTY II: REPRODUCTIVE HORMONES AND ACCESSORY ORGANS. S.J. Nazian and D.F. Cameron, Departments of Physiology & Biophysics and Anatomy, College of Medicine, University of South Florida, Tampa, FL 33612

The peripubertal FSH rise is believed to play an important role in the onset of sexual maturation in the male rat, but the factors controlling it are not clear. In order to determine if the pubertal testosterone rise plays a role in the termination of the FSH increase, male rats were injected with ethylene dimethylsulphonate (EDS) at 40 days of age to eradicate the Leydig cells just prior to the onset of the testosterone rise. Groups of rats were decapitated at weekly intervals beginning at age 26 days through age 96. Organ weights, serum and testicular interstitial fluid (TIF) were obtained. Serum was analyzed for testosterone and FSH by radioimmunoassay. TIF testosterone was also determined. Compared to vehicle injected controls, EDS treatment resulted in a delay in the peripubertal increase in the relative weights of prostates and seminal vesicles of about 2 weeks. Serum testosterone remained at pretreatment levels for 1 week post-injection. TIF testosterone remained at pretreatment concentrations for considerably longer and was significantly lower than control values for 2 weeks post-injection. EDS treatment resulted in serum FSH levels that were elevated by 1 week post-injection. They remained significantly higher than vehicle-injected controls until 96 days of age. These results suggest that the pubertal testosterone increase plays an important role in terminating the peripubertal FSH rise and that serum testosterone and TIF testosterone may vary independently. Sponsored by BRSG S07 RR05749.

140 GLUCURONYL TRANSFERASE ACTIVITY FOR ANDROGENS IN RAT TISSUES IN VITRO. K. Pirog,* R.V. Clark and O.C. Collins. VA Medical Center and Department of Medicine, Emory University School of Medicine, Atlanta, GA 30322.

Horton et al. demonstrated direct formation 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 determined the activity of microsomal UDP-glucuronyltransferase (UDPGT), EC 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 extrahepatic tissues (kidney, intestine, preputial gland, prostate) of the male Sprague Dawley rat.

We demonstrated small but significant UDPGT activity for androstane-3α,17β-diol (more than 80% conjugated in position 17β) by skin microsomes: (pmol/mg protein/2 hr) penile skin: 0.087 ± 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: 0.532 ± 0.125. No UDPGT activity for testosterone or androsterone was detected in these tissues. Microsomes from preputial glands did not show any significant UDPGT activity toward either of the androgen steroids tested. The UDPGT activity for androstanediol was 10^6-fold higher in liver microsomes: 6.94 ± 1.18 nmol/mg protein/min. The liver microsomes also showed significant UDPGT activity for testosterone: 6.56 ± 0.73 nmol/mg/min and androsterone: 4.32 ± 0.19 nmol/mg/min. These results suggest that the extrahepatic contribution is low and the liver is the major source of androgen glucuronides is low in rat. UDPGT activity was distributed evenly in sexual and non-sexual skin. Furthermore, UDPGT activity in the extrahepatic organs was limited to androstanediol, while the liver UDPGT isoenzymes also conjugated androsterone and testosterone.

141 CHARACTERIZATION OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) IN TESTIS, EPIDIDYMIS AND VAS DEFERENS OF MONKEY B. Radhakrishnan*, W. Niklinski*, C.A. Suárez-Quian. Georgetown University, Depr. of Anatomy and Cell Biology, Washington, DC 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 immunocytoohemistry using a polyclonal antibody (RK2) 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 RK2 revealed a specific band at approximately 170 kDa, 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 3.7% 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 RK2 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, immunostnining of the EGFR was observed on both the basolateral and the luminal borders of the epididymal epithelium. Similar staining was noticed in vas deferens. There was no positive staining in the interstitium 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 EGF in the primate testis may act at 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 at the basal or lumina) sides of the cells, or that these tissues serve as sites of EGF transcytosis across the epithelium.

142 EPIDERMAL GROWTH FACTOR: PRODUCTION AND RECEPTOR BINDING TO THE SEX ACCESSORY ORGAN OF MALE MICE. Seethalakshmi, L, Liu, A., Flores, C*, Kinkead, T*, Menon, M.* & Davis, R. University of Massachusetts Medical Center, Worcester, Ma 01655

Sexually mature male mice (8 wks old) were divided into three groups a) sham control (SC) b) sialoadenectomized (S) and c) S+EGF (2.5 ug/d/m) treated. EGF was administered subcutaneously for 28 days. Sialoadenectomy reduced significantly the weight of seminal vesicle (SV). Treatment of S animals with EGF maintained the normal weight of SV. Determination of EGF by RIA indicated that the SV ranked second to salivery glands in the levels of EGF. After sailadenectomy, the levels of EGF could not be detected in the circulation or in the SV. In contrast, prostate still contained EGF, although the levels were very low. These two organs also demonstrated receptor binding to ^{125}I EGF. The binding was maximum after 1-2 hours of incubation at room temperature. Both the organs demonstrated two binding sites, one high affinity and low capacity (K_d = 0.87 nM, n=28 p M for SV; K_d = 0.58 nM, n=34 pM for PR) and the other one low affinity and high capacity (K_d=39.5 nM, n=390 pM for SV; K_d=6nM, n=150 pM for PR). These data suggest that EGF may have an important role to play in the sex accessory organs. EGF is important for the maintenance of SV weight and SV accumulates EGF from the circulation while the prostate may produce EGF.

143 EPIDERMAL GROWTH FACTOR(EGF): AN IMPORTANT ROLE IN TESTICULAR FUNCTION

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In order to understand the role of EGF in testicular function, preliminary experiments were carried out using sexually mature C3H mice (8 weeks old) which were divided initially into three groups a) sham control (SC); b) sialoadenectomized(S); c) S+EGF (SE: 2.5 ug/d/m) treated. Subsequently, another group of animals were subjected to either flutamide(F) or S+F or SE+F 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 preleptotene and pachytene spermatocytes, and round spermatids. In contrast, testosterone(T) levels increased while FSH remained unaffected and LH levels decreased. Treatment of S animals with EGF maintained spermatogenesis and T levels at the control values. F alone reduced sperm counts and motility by 50% and elevated 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 the values in the F group. The testis also demonstrated two binding sites for EGF, one with higher affinity (k_d =0.70nM) and lower capacity (n=27pM), and the other with lower affinity (K_d =13.2nM) but higher capacity (n=156 pM). The binding of EGF to testis was maximum after 1-2 hours of incubation at room temprature. These results suggest that EGF may have an important role in the regulation of testicular function and that may be independent of T action.

144 EFFECTS OF HORMONES ON THE DISTRIBUTION OF JUNCTION ASSOCIATED SERTOLI 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, associated with the Sertoli-spermatid junctional specialization. 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+, retinoic acid, lactic acid and EGF. In Sertoli monocultures with no hormone or testosterone, actin and vinculin were diffuse 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 maximizes spermatid binding to Sertoli cells by insuring structural "competency" of the Sertoli cell. Supported, in part, by NIH-BRSG grant 2 S07 RR RR05749-18.

145 ACID AND ALKALINE PHOSPHATASE ACTIVITIES IN TESTES AND ACCESSORY REPRODUCTIVE GLANDS OF TRANSGENIC MICE EXPRESSING BOVINE GH GENE.

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Previous studies from our laboratory indicated that expression of human (h)GH 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 hGH is lactogenic and somatotropic and bovine (b)GH is only somatotropic in rodents, the purpose of the present study was to examine the consequences of expression of bGH on histological structure and activity of hydrolytic enzymes in testes and accessory reproductive glands. Adult male MMT/bGH transgenic and nontransgenic mice (15-16 weeks old) were used. The testes, SV and ventral prostate (VP) were dissected out and weighed using 6 animals per group. Significant differences were recorded in body wt. (Control: 30.2 ± 1.0, bGH: 45.6 ± 1.9), SV wt. (Control: 65.8 ± 3.9, bGH: 84.7 ± 2.5 mg) and VP wt. (Control: 9.6 ± 0.8) bGH: 22.0 ± 2.7 mg) while no differences were observed in testes wt. Acid phosphatase activity in SV was significantly increased in transgenic animals compared to their normal littermates (Control: 374.4 ± 31.2, bGH: 480.5 ± 24.2 ug p-nitrophenol produced /mg protein/30 min) while no differences were observed in alkaline phosphatase activity in the same organ (Control: 1500.5 ± 182.8, bGH: 1324.5 ± 128.7). The activities of both acid and alkaline phosphatase were increased in the VP of bGH transgenic mice (Acid- Control: 252.9 ± 19.5, bGH: 360.1 ± 10.0; Alkaline- Control: 490.6 ± 52.4, bGH: 717.9 ± 54.9) 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 bGH transgenic and nontransgenic mice. The alveolar epithelial cells in SV were more elongated while the follicular epithelial cells in VP of bGH 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 increased size of the glandular secretory cells and that these changes are mediated by the somatotropic effect of bGH.

147 BINDING OF ACTINOMYCIN D TO SPERM

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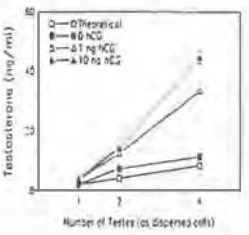
Toxic chemicals and drugs have been detected in semen but little is known of their interaction with spermatozoa. If such agents can bind to spermatozoa, they may be transported to, and possibly into, the oocyte and effect embryo/fetal development. In order to determine if spermatozoa can take up toxic chemicals, the binding of actinomycin D to spermatozoa was evaluated. Actinomycin D is an antitumor, immunosuppressant drug which intercalates with DNA and has been shown previously to effect 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 two times by resuspending in mBWW and centrifugation. The washed spermatozoa were resuspended in mBWW and several concentrations of spermatozoa were incubated at ambient temperature with tritated actinomycin D for up to 6 hours. At selected time intervals the spermatozoa were collected on an ultrafiltration 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 INCREASING CONCENTRATIONS OF FETAL LEYDIG CELLS IN CULTURE AMPLIFY THE STEROIDOGENIC RESPONSE. T. Sokka*, I. Huhtaniemi 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.



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CHARACTERIZATION OF GELATIN-DEGRADING METALLOPROTEINASES OF THE DUNNING RAT PROSTATE TUMOR

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A common property of many tumors is an increased activity of metalloproteinases that can cleave proteins of the extracellular matrix. We have examined the enzymic characteristics and effects of castration on metalloproteinase activities in the Dunning rat prostate tumor using gelatin-containing SDS-PAGE zymography. Nine distinctive bands of Ca^{2+}-dependent proteinase (C-DP) activities were detected in zymograms of the tumor; activities of 64, 71, and 76 Kd being prominently expressed and those of 33, 42, 85, 98, 115, and 120 Kd showing weaker activities. A 91 Kd activity was detected in the absence of Ca^{2+}. The C-DP activities of different prostatic lobes of the Copenhagen rat were distinctive from the tumor and from one another: i.e., these were a 62 Kd proteinase in the anterior; 59, 62, and 67 Kd forms in the ventral; and 52, 62, and 72 Kd activities in the lateral lobes. The C-DP of the Dunning tumor were active over a broad pH range and were inhibited by EDTA and EGTA. Sr^{2+} and Ba^{2+} were the only divalent cations able to substitute for Ca^{2+} with some efficacy. There was little change in the predominant molecular forms of proteinase following castration; however, the 98 Kd C-DP activity decreased and the 91 Kd Ca^{2+}-independent activity increased as a result of this treatment. The identification of individual proteinase 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 Dept. of Veterans Affairs)

149 RESPONSIVENESS OF MALE ACESSORY REPRODUCTIVE GLANDS TO ANDROGEN DEPRIVATION IN MICE TRANSGENIC FOR HUMAN OR HUMAN PLACENTAL VARIANT GROWTH HORMONE. M. Ceolin and A. Bartke, Dept. of Physiology, School of Medicine, Southern Illinois University, Carbondale, IL 62901.

Previous work from our laboratory has shown a dramatic age related enlargement of seminal vesicles (SV) and coagulating glands (CG) in mice transgenic for human (h) and human placental variant (V), with the mMT-1 promoter, but not for bovine growth hormone (GH) with the mMT-1 or PEPCK promoters. The present study intended to investigate the sensitivity of SV and CG to androgen deprivation. Old (6-12 mo) transgenic mMT-1/hGH mice, young (3-6 mo) transgenic mMT-1/hGH.V and their normal littermates were castreted (C) or sham operated (S), using 6 animals per group, and examined 15 days later. The SV and CG in hGH.V and normal animals showed similar pattern of regression after castration. In contrast, hGH males showed no CG regression (399±108 vs. 408±63mg), thus differing from the response in normal controls (145±48 vs 24±8, P<.001). Blotted SV wt was reduced in normals (53±3 vs. 21±3, P<.0001) but not in transgenics (182±45 vs. 110±14). In addition, after castration SV contents were reduced 4 fold in transgenic males and 24 fold in controls. Compared to normal animals, intact hGH.V transgenic males had a decrease in DNA/mg of tissue and an increase in prot/DNA ratio suggesting increased cell size, but the response to castration was similar in both SV and CG: ↓ DNA/mg tissue and ↓ prot/DNA ratio indicating cell shrinkage and inactivity. Intact old hGH animals also showed ↓ DNA/mg tissue but no differences in prot/DNA ratio when compared to intact normals. Upon castration, SV changes were similar to those in normals, however, prot/DNA ratio in transgenics did not show the expected decrease. Histological observations showed an abnormally enlarged stromal tissue in both SV and CG of transgenic animals with no stromal atrophy after castration. The results suggest that secretory epithelia does not make a major contribution to the wt of the enlarged SV and CG of old hGH males and that the hypertrophied stromal tissue of these transgenic animals is not responsive to castration.

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151 REPRODUCTIVE TOXICITY OF BORIC ACID (BORA) IN MALE DEER MICE. P.A. Fail, H.R. Sauls*, and S.W. Dennis*
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BORA is a reproductive toxicant in Swiss CD-1 male mice (Fail et al, J. Androl. 10:45, 1990). Swiss mice treated with BORA for 9 weeks had lower body and liver weights (wts), decreased basal testosterone concentrations and diminished response to HCG [Grizzle et al., Biol. Reprod. 40 (Suppl 1):153, 1989]. To assess the toxicity of BORA in wild rodents, adult male deer mice (Peromyscus maniculatus) were exposed to 0, 4500, or 9000 ppm BORA in feed for 8 weeks (n=60/group). On week 9, 30 males per group were mated, challenged with HCG (25 IU) or saline and necropsied. Pregnancies did not occur at 9000 ppm (0%) and were reduced at 4500 ppm (50%) compared to controls (67%). Although basal testosterone did not differ among groups, response to HCG was decreased by BORA. Testosterone 60 min after HCG was ($\bar{X}$+SEM) 21.5+3.0, 26.0+4.8 and 15.9+3.2 ng/ml, at 0, 4500, and 9000 ppm, respectively. Testicular wts were 182+7, 144+12, and 53+7 mg. Total accessory sex organ (TASO) wts were 329+17, 307±21, and 221±16 mg. The germinal epithelium at 9000 ppm was degenerated and consisted of Sertoli cells, spermatogonia and occasional spermatocytes. The 4500 ppm group had fewer elongated spermatids than controls. Body and liver wts were unchanged. The other 30 males per group were allowed to recover from BORA for 9 weeks, mated, given HCG or saline and necropsied. Pregnancy rates were 71, 75 and 69% at 0, 4500, and 9000 ppm. Testes were normal in size, histological appearance and response to HCG across all groups. TASO were also normal. Thus, BORA is a reproductive toxicant in deer mice, but recovery is complete. Deer mice appear to be less sensitive to BORA than Swiss mice, because deer mice did not lose body weight or have depressed basal testosterone.

150 INHIBIN α-SUBUNIT IN MALE RATS Seiichi Saito and Robert J. Ryan*, Department of Urology, Sapporo Medical College, Sapporo 060, Japan and Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN 55905

Several reports have demonstrated that immunoreactive inhibin is localized in the Sertoli cells in the testis, and that endogenous inhibin plays a physiological role in suppressing FSH secretion in infantile male rats at an age when Sertoli cells contain the largest amount of inhibin. We developed and characterized antibodies against the synthetic fragments of each subunit of inhibin as reported previously (Endocrinol., 125: 898, 1989). In this reports, we studied the role of inhibin in modulating FSH secretion as a function of sexual maturation in male rats using antibodies against inhibin α-subunit. Immunohistochemical study was performed by immunogold-silver staining in male rats. Modulating FSH secretion in sexual maturation was studied by immunoneutralization. Male rats were injected s.c. with antibody every day from birth to adulthood. 5 out of original group 25 rats were sacrificed at 10 day intervals and had their testes and blood removes at these times. The testes were weighed and stained with H-E staining, also serum FSH was measued. The results show that immunoreactive inhibin α-subunit is localized in the Sertoli cells, especially in young cases. There were no significant differences in the testes weights and histology between the antibody-administered group and the control group. Marked FSH increase was observed in the antibody-administered group from 5 to 30 days. No significant difference was detected in the over 50 day group. From these results, it is suggested that inhibin plays a role in suppressing FSH secretion 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 HISTOGRAM OF BILATERAL TESTES AFTER VASECTOMY

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Studies in animals have revealed a wide variety of testicular changes after vasectomy, ranging from no change, disordered spermatogenesis, ultrastructual 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. Transscrotal 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 380 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.

153 SYNERGISM OF FSH AND TESTOSTERONE ON SPERMIOGENESIS IN HYPOPHYSECTOMIZED RATS. H.F.S. Huang, L. Pogach, E. Nathan*, W. Giglio*, J.J. Seebode* UMD New Jersey-New Jersey Medical School , Newark, N. J. 07103

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 spermiogenesis was noted in all TC implanted HPX rate. Although elongated epermatids 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 testicular ABP contents in rats with 2 or 5 cm TC implants, and increased the epididymal ABP levels significantly in all TC implanted rats. The ABP status in testis and its transport to epididymis were closely correlated with the final maturation of spermatids. These results suggest that FSH enhanced the accumulation of T in testis, and that T and FSH synergistically stimulated Sertoli cell ABP production which may have some role in the control of the final steps of spermiogenesis.

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THE EFFECTS OF CONCOMITANT CISPLATIN AND TESTOSTERONE ADMINISTRATION ON RAT TESTICULAR GERM CELLS

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We have previously reported that cisplatin reduces the number of preleptotene primary spermatocytes and inhibits epididymal sperm motility after a single dose of the drug, and have correlated this impairment with drug disposition and circulating testosterone levels. The present study undertook to ascertain whether concomitant testosterone therapy with cisplatin administration overcame the cellular damage associated with this anticancer agent.

Three groups of 6 Wistar rats each were used for the study. Group 1 received one dose of 8 mg cisplatin / kg body weight, group 2 received cisplatin plus 100 ug testosterone s.c. daily and group 3 received the vehicle only and served as the control group. The animals were sampled after 5 days and testicular germ cell counts were made at stage VII of the seminiferous epithelial cycle.

Preleptotene primary spermatocytes and step 7 and step 19 spermatids were reduced in number in the cisplatin group by 21%, 20% and 28% respectively. No significant differences in cell counts were noted between groups 2 and 3. Epididymal sperm number was reduced in group 1 by 50%, whilst sperm motility and abnormality were adversely affected in both groups 1 and 2.

These results indirectly confirm that cisplatin adversely affects the germ cells by inhibiting testosterone synthesis and or secretion, since concomitant testosterone therapy prevents damage to germ cells and epididymal sperm number. Since sperm motility and abnormality are affected in both groups 1 and 2, it appears as though this adversity is mediated directly by the drug on epididymal sperm and not via lowered testosterone levels.

155 TESTICULAR ISCHEMIA AND REPERFUSION INJURY: ITS PREVENTION, IMMUNE RESPONSE AND EFFECTS ON CONTRALATERAL TESTES

S. Akhter, S. Sridhar*, N.M. Katlowitz*, Y. Lamy*, K.M. Butt*, J.N. Cunningham* and G.J. Wise*. Maimonides Medical Center, 4802 10th Avenue, Brooklyn, NY 11219.

Testicular Ischemia and reperfusion injury and its possible prevention by Heparin, Oxypurinol and super Oxide dismutase, subsequent immune response and effects on contralateral testes were studied in sprague daweley rat model. Ipsilateral testes were studied using light microscopy. Immune response was measured 8 weeks later by capillary tube agglutination test (CTAT) and enzyme linked immunosorbent assay (ELISA) measuring antitesticular antibodies. Contralateral testes were studied 8 weeks later using light microscopy. Significant histological protective effects were found in all three treatment groups undergoing one hour ischemia followed by reperfusion of ipsilateral testes. Significant immune response was noticed in untreated (ischemia-no drugs) as well as in treated animals with antibody titres > 1:1024. No histological damage was found in any of untreated or treated contralateral testes. Thus it is concluded that Heparin, Oxypurinol and PEG-SOD offer significant protection from reperfusion injury in short term ipsilateral testicular ischemia. A significant immune response follows ischemia and reperfusion injury which is not prevented by therapeutic agents. Despite significant immune response no evidence of histological injury was noticed in contralateral testes upto eight weeks.

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