JANUARY/FEBRUARY 1996
Supplement

Journal of ANDROLOGY

American Society of Andrology

21st Annual Meeting

April 25–April 29, 1996
Minneapolis, Minnesota

Program and Abstracts

Published by THE AMERICAN SOCIETY OF ANDROLOGY

Thursday, April 25

8:00–5:00 PM	Andrology Laboratory Workshop (Nolte)
12:00–1:00 PM	Lunch served at Workshop site
1:00–9:00 PM	Executive Council Meeting (Regents)

Friday, April 26

8:00–12:00 NOON	Postgraduate Course (Ballroom B, C, D)
12:00–1:00 PM	Lunch at the course site
1:00–5:00 PM	Postgraduate Course (Ballroom B, C, D)
7:00–9:00 PM	ASA Welcoming Reception (Ballroom B, C, D)

Saturday, April 27

7:45–8:00 AM	Welcome and Opening Remarks (Ballroom B, C, D)
8:00–9:00 AM	Serono Lecture: "The Making of a Spermatozoon: Regulation of Gene Expression during Male Germ Cell Differentiation" (Ballroom B, C, D)
9:00–10:00 AM	AUA Lecture: "Germ Cell Cancer of the Testis: Biologic, Anatomic, and Management Factors Impacting Fertility" (Ballroom B, C, D)
10:00–10:30 AM	Refreshment Break/Exhibits (Ballroom A/Prefunction/Faculty)
12:00–1:30 PM	Lunch (on your own) Women in Andrology Luncheon (Nolte) Business meeting 12:00–12:30 Speaker and Lunch 12:30–1:30
1:30–3:00 PM	Symposium I: "Development of the Cell Types in the Testis" (Ballroom B, C, D)
3:00–3:30 PM	Refreshment Break/Exhibits (Ballroom A/Prefunction/Faculty)
3:30–4:30 PM	Oral Session II (Ballroom B, C, D)
4:30–6:30 PM	Poster Session I (H.H.H./Northrop/Coffman/Campus)
7:00–9:00 PM	Banquet (Prefunction/Ballroom B, C, D)

Sunday, April 28

7:00–8:00 AM	Past Presidents' Breakfast (Presidents)
8:00–9:30 AM	Oral Session III (Ballroom B, C, D)
9:30–10:00 AM	Refreshment Break/Exhibits (Ballroom A/Prefunction/Faculty)
10:00–11:00 AM	ASA/NME State-of-the-Art Lecture: "Genetics of Sperm Structure and Function" (Ballroom B, C, D)
11:00–12:00 NOON	Pharmacia & Upjohn Clinical Debate: "Resolved: With Recent Advances in Micromanipulation, There is no Longer a Need for the Male Infertility Specialist" (Ballroom B, C, D)
12:00–1:30 PM	Lunch (on your own)
12:00–1:30 PM	Simultaneous Events 1. Editorial Board Luncheon (Presidents) 2. Laboratory Science Forum "The ART Technologist's Role in Lab Accreditation: Quality Control, Quality Assurance, Competency Measurements" (Alumni) 3. "Using Andrology and the ASA Home Page" (Nolte)
1:30–2:30 PM	Symposium II: "Techniques, Failure Mechanisms and Adverse Health Consequences of Vasectomy" (Ballroom B, C, D)
2:30–3:30 PM	ASA Business Meeting and Award Ceremony (Ballroom B, C, D)
3:30–4:00 PM	Refreshment Break/Exhibits (Ballroom A/Prefunction/Faculty)
4:00–6:00 PM	Poster Session II (H.H.H./Northrop/Coffman/Campus)
7:00–9:00 PM	Student Colloquium: "Publishing a Paper—Tricks of the Trade" (Ballroom B, C, D)
9:00–10:00 PM	Student Soiree (Presidents)

Monday, April 29

8:00–9:30 AM	Oral Session IV (Ballroom B, C, D)
9:30–10:00 AM	Refreshment Break/Exhibits (Ballroom A/Prefunction/Faculty)
10:00–11:00 AM	Buckeye State-of-the-Art Lecture: "HIV in the Male Reproductive Tract and Semen" (Ballroom B, C, D)
11:00–12:00 NOON	Special "Hot Topic" Lecture: "Spermatogonial Transplants in Mouse Testes" (Ballroom B, C, D)

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1996 Presidential Message

Welcome to the 21st Annual Meeting of the American Society of Andrology.

The scientific program, postgraduate course, and social events are the result of the year-long dedicated efforts of Michael Griswold, Jon Pryor, and David Hamilton who, respectively, chaired the Program, the Postgraduate Course, and the Local Arrangements Committees. They should be commended for presenting us with this outstanding and exciting program.

I am looking forward to greeting all the members of the American Society of Andrology at this meeting, and hope that, as in the past, it will be a forum for scientific exchange and an occasion for the renewal of friendships.

See you in Minneapolis April 25–29, 1996.

Marie-Claire Orgebin-Crist
President

Past Presidents

1975–1977	Emil Steinberger
1977–1978	Don W. Fawcett
1978–1979	C. Alvin Paulsen
1979–1980	Nancy J. Alexander
1980–1981	Philip Troen
1981–1982	Richard M. Harrison
1982–1983	Richard J. Sherins
1983–1984	Andrzej Bartke
1984–1985	Rudi Ansbacher
1985–1986	Anna Steinberger
1986–1987	William D. Odell
1987–1988	Larry L. Ewing
1988–1989	C. Wayne Bardin
1989–1990	Rupert Amann
1990–1991	Howard Nankin
1991–1992	David W. Hamilton
1992–1993	Ronald S. Swerdloff
1993–1994	Bernard Robaire
1994–1995	Glenn R. Cunningham

General Information

Headquarters

Radisson Hotel Metrodome
615 Washington Avenue, S.E.
Minneapolis, MN 55414
TEL: (612) 379-8888
FAX: (612) 379-8436

Mail-In Registration

Chris Gosch
Business Manager - ASA
P.O. Box 15171
Lenexa, KS 66285-5171
TEL: (913) 541-9077, Ext. 474
FAX: (913) 541-0156

On-Site Registration

Big Ten Room and Foyer Booth
Friday, April 26 and Saturday, April 27:
7:30 AM to 7:00 PM
Sunday, April 28:
7:30 AM to 5:00 PM
Monday, April 29:
7:30 AM to Noon

Exhibits

Exhibits will be in the foyer of University Ballrooms B, C and D, in University Ballroom A and in the Faculty Room. They will be open 9:00 AM to 5:00 PM Friday, April 26 to Sunday, April 28.

Slide Preview and Press Room

The ***Collegiate Room*** will be open during the meeting hours (7:30 AM to 6:00 PM) from Friday, April 26 to Sunday, April 28, and from 7:30 AM to Noon on Monday, April 29.

Miscellaneous Meetings

The ***Rotary Room*** can be reserved for committee meetings or small group meetings (please contact Debbie Elder in the ASA Business Office).

Smoking Regulations

Smoking is not allowed in any of the meeting rooms, or in the foyer exhibit space.

Transportation and Travel Arrangements

By Car: From Interstate 35W, Coming from the South: Go north on 35W, and follow it (keep to the right) as it circles to the east of Downtown. You will see an exit sign (Exit 17C) to U of M. Follow signs to the east bank, which will put you on Washington Avenue. The hotel is on the left at the third set of traffic lights.

From Interstate 35W, Coming from the North: Go south on 35W to the Washington Avenue exit. Turn left and follow Washington Avenue over two bridges. At the end of the second bridge, turn left, which will put you on Washington Avenue (again). The hotel is on the left at the third set of traffic lights.

From Interstate 94, Coming from the East: Exit at the sign for U of M and continue to Washington Avenue. Turn left and follow Washington Avenue to the third set of traffic lights (or to the street just before the third set of traffic lights). The hotel is on your right.

From Interstate 94, Coming from the West: Just south of Downtown there are two short tunnels on Interstate 94. You should be in the left lane when entering the first tunnel (you will be warned because there will be signs warning trucks carrying hazardous material not to go through the tunnel) and immediately at the end of the second tunnel (which is about one mile from the first) bear left onto Interstate 35W. Do not change lanes!! Continue on, following signs to U of M east bank, which will put you on Washington Avenue. The hotel is on the left at the third set of traffic lights.

By Airplane and Car: For those wanting to have personal transportation in the Twin Cities, all major (and some minor) car rental firms are represented at the Twin Cities' International Airport.

When leaving the airport, bear left at the exit, which enters Highway 55 (west) from the left. You will need to move from the left lane to the right lane fairly quickly to exit to Minneapolis (this can be tricky at certain times of day). Then, follow signs to Highway 62 (Crosstown) and take 62 to Interstate 35W. Go north on 35W, and follow it as it circles to the east of Downtown. You will see an exit to U of M. Follow signs to the east bank, which will put you on Washington Avenue. The hotel is on the left at the third set of traffic lights.

By Taxi or Airport Shuttle Service: Taxi fare from the airport to the meeting hotel is $25.00. There are frequent Airport Express vans. Fares are $10.00 one way or $15.50 round trip.

Travel Arrangements and Hotel Reservations: Arrangements for special airfares can be made by contacting Ann Adams at Buck Rogers Travel (TEL: 1 (800) 580-2472; (915) 581-6063. FAX: (915) 581-8172). Mention that you are with the American Society of Andrology.

Hotel Reservations

Hotel reservations should be made by using the Hotel Reservation form in the registration package, or by calling hotel directly (TEL: 1 (800) 333-3333; (612) 379-8888). Make your reservations by March 25, 1996 to be assured of receiving meeting rates.

Student Information

Colloquium	Sunday. April 28, 7:00 - 9:00 PM, in the ***University Ballroom B, C, D.*** **"Publishing a Paper - Tricks of the Trade"**
Speakers	Susan Suarez, Don Tindall, Don Cameron, Ken Ginsburg
Colloquium Sponsor	California Cryobank
Mixer	Immediately following the colloquium in the ***Presidents Room.***

Placement Service: A Placement Service for candidates and employers is available. The Placement Service Board will be near the Registration Desk. To register prior to the meeting, contact: Dr. Christopher De Jonge, Department of OB/Gyn, University of Nebraska Medical Center, 600 S. 42nd Street, Omaha, NE (TEL: (402) 559-8834; FAX: (402) 559-5015)

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.

Message from the Local Arrangements Committee

Welcome to the great city of Minneapolis!! This is the 21st Annual Meeting of the ASA and it promises to be an exciting event. Jon Pryor and his Postgraduate Committee have put together a very interesting program, as has Mike Griswold and the Program Committee. We have been able to engage the Dudley Riggs' Brave New Workshop improvisation group, which should provide a lively atmosphere at the banquet.

I realize that most of your time will be spent at the meeting, but Minneapolis has many attractions if you can find a few hours. Theaters, museums, music, sports and recreation are unsurpassed and all of them are within a short distance from the hotel by bus, taxi or car. We will have a list of events at the meeting. In addition, there are many opportunities for shopping, both in the Downtown and in Mall of America (about 20 minutes south of the hotel). Leading department stores such as Saks 5th Avenue, Nieman Marcus, Nordstroms and many others, as well as many boutiques are waiting for you.

The Radisson Metrodome Hotel is located in the middle of the east bank campus of the University of Minnesota and is just across the street from the Academic Health Center. The hotel has undergone a major renovation in the past year or so, so everything should be in tiptop shape.

We hope you have a marvelous time in Minneapolis. We look forward to seeing you!

David W. Hamilton, Ph.D.
Chair, Local Arrangements Committee

Local Arrangements Committee: Kevin Billups, Bo Crabo, Kathy Ensrud, David Hamilton (Chair), Jon Pryor, Linda Raab, Ken Roberts, Jon Siiteri, Donald Tindall and Michael Wilson.

Message from the Program Chairperson

The annual meeting is the glue that holds a society like ours together. While it may seem to some people that "Andrology" is a tightly focused theme for a society, the fact is, this society has a membership that ranges from the private practitioner to the basic scientist, and member's interests run from clinical situations that are unique to urologists to questions of basic science that can be applied to any area of biology. It is a tough assignment for the program committee to put together an agenda that is interesting and exciting to the majority of our members. The program committee has tried to focus on some themes that will be of general interest and may impact both basic science research and the clinical practice.

For the first time we will have two "keynote" addresses. The Serono Lecturer for this year is Dr. Norman Hecht of Tufts University who will discuss "The Making of a Spermatozoon: Regulation of Gene Expression during Male Germ Cell Differentiation". Dr. Hecht has been a recognized leader in the field of gene expression in haploid cells. In addition, the American Urological Association has offered to sponsor an annual lecture honoring a prominent urologist. We are fortunate that the first AUA Lecturer will be Dr. John Donohue of Indiana University who will discuss "Germ Cell Cancer of the Testis: Biologic, Anatomic, and Management Factors Impacting Fertility". Dr. Donohue's pioneering work in the treatment of testicular cancer is known throughout the world. In addition to developing a modified method of retroperitoneal lymph node dissection that preserves ejaculation, his group has recently accumulated long term significant data regarding fertility potential following previous chemotherapy for treatment of testicular cancer.

The symposia are meant to convey in-depth information on focused subject areas. The first of two symposia will be concerned with the development and origin of the cells in the testis and will include discussions by Dr. Joanne Orth on Sertoli cells, Dr. Matt Hardy on Leydig cells, and Dr. Janet Heasman on germ cells. The advisability, efficacy and safety of vasectomies has been a controversial topic among andrologists and the general public. The second symposium is entitled, "Techniques, Failure Mechanisms and Adverse Health Consequences of Vasectomy" will directly address elements of this controversy.

Two areas of increasing importance to Andrology have been selected for State-of-the-Art lectures. First, the influence of the vast amount of available DNA sequence information and the powerful new techniques developed for molecular genetics are making the genetics of complex systems and disease processes more understandable. In this regard, Dr. Mary Ann Handel will present the ASA/NME State-of-the-Art Lecture entitled "Genetics of Sperm Structure and Function". Second, the Buckeye State-of-the-Art Lecture will be entitled, "HIV in the Male Reproductive Tract and Semen" and will be given by Dr. Deborah Anderson. This is a very important area of interest to clinicians and basic scientists and an area where andrology interfaces with a serious disease problem that threatens millions of people world-wide.

One of the continuing features of the annual meeting is the clinical debate. In 1996 the subject of the clinical debate is "Resolved: With Recent Advances in Micromanipulation, There is no Longer a Need for the Male Infertility Specialist". The scheduled participants have promised us a lively discussion.

A new feature of this meeting will be a "Hot Topic" lecture. The idea of this lecture is to have a late-breaking and important new research development presented to the members. Successful germ cell transplantation was recently reported by Dr. Ralph Brinster of the University of Pennsylvania. Dr. Lonnie Russell has been collaborating with Dr. Brinster in the characterization of the transplants and Dr. Russell will present the "Hot Topic" lecture entitled, "Spermatogonial Transplants in Mouse Testes". This subject is of immediate interest to basic scientists who are interested in the propagation of stem cells and the role of supporting cells in the testis and will be intriguing to clinicians if the practice of germ cell transplantation can be moved from the laboratory to the clinic.

Michael D. Griswold, Ph.D.
Program Chairperson

Continuing Medical Education Credit

The University of Minnesota is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians.

Annual Meeting:
The University designates this continuing education activity as Category 1 of the Physician's recognition Award of the American Medical Association. One credit hour may be claimed for each hour of participation up to a maximum of 17.0 hours.

Post Graduate Course:
The University of Minnesota designates this continuing medical education activity for 7.0 hours in Category 1 of the Physician's Recognition Award of the American Medical Association.

Educational Objectives for CME Credit

ASA Annual Meeting

By attending the 1996 Annual Meeting program, the participant will learn about:
* The importance of gene expression in haploid cells;
* The treatment and diagnosis of testicular cancer;
* The development of germ cells, Sertoli cells and Leydig cells in the testis;
* The advances in understanding the genetics of sperm formation;
* The techniques and potential problems associated with vasectomy;
* The association of HIV with the male reproductive tract;
* The latest experiments on spermatogonial transplants.

ASA Post Graduate Course

Following this course entitled "Endocrinology of the Male: From Head to Testis", the participants will be able to:
* Describe the possible role of the estrogen receptor in male reproduction;
* Describe the physiology and clinical utility of 5α-reductase inhibitors in benign prostatic hyperplasia;
* Outline the steroid and hypothalamic feedback mechanisms which regulate pituitary gonadotropins, and potential therapies to regulate LH and FSH levels;
* Discuss the progression of physical and hormonal events which accompany sexual maturation in the male;
* Describe the mechanism by which prostate growth is influenced by both androgens and growth factors;
* Know the current applications of GnRH analogs to contraception in men and prostatic disease;
* Demonstrate how to measure Inhibin and Activin in human serum plasma and body fluids;
* Understand the indications, benefits and risks of androgen replacement therapy, and be able to discuss available androgen preparations and those which are under investigation;
* Describe the potential effects of stress on reproduction.

1996 American Society of Andrology
Postgraduate Course
April 26, 1996, Radisson Hotel Metrodome
Minneapolis, Minnesota

"Endocrinology of the Male: From Head to Testis"

Course Director:	**Jon L. Pryor**
8:00–8:10 AM	Welcome and Introduction **Jon L. Pryor**
8:10–8:55 AM	Androgen Receptors **Donald Tindall**
8:55–9:40 AM	Transcriptional Regulation of the Gonadotropin Genes **Margaret Shupnik**
9:40–10:00 AM	**Refreshment Break**
10:00–10:45 AM	Disruption of the Estrogen Receptor Gene **Kenneth S. Korach**
10:45–11:30 AM	Activin and Inhibin **Teresa R. Woodruff**
11:30–12:00 NOON	Questions and Answers **All Morning Speakers**
12:00 NOON	**Lunch (On your own)**
1:00–1:40 PM	Physiology of GnRH: Antagonists and Agonists **William Bremner**
1:40–2:20 PM	Endocrinology of Puberty **Howard Kulin**
2:20–2:40 PM	**Refreshment Break/Exhibits**
2:40–3:20 PM	Testosterone Replacement Therapy **Glenn Cunningham**

3:20–4:00 PM	5 alpha Reductase Inhibitors in the Prostate **Roger Rittmaster**
4:00–4:15 PM	Questions and Answers
4:15–5:00 PM	Panel on Stress and Reproduction **Ron Weber** **Richard Clark** **Richard Sherins (Moderator)**

Serono Lecture

Saturday, April 27, 8:00 AM

Norman Hecht, Tufts University, Medford, Massachusetts "The Making of a Spermatozoon: Regulation of Gene Expression during Male Germ Cell Differentiation"

American Urological Association (AUA) Lecture

Saturday, April 27, 9:00 AM

John Donohue, Indiana University Medical Center, Indianapolis, Indiana "Germ Cell Cancer of the Testis: Biologic, Anatomic, and Management Factors Impacting Fertility"

ASA/NME and Buckeye State-of-the-Art Lectures

Sunday, April 28, 10:00 AM

Mary Ann Handel, University of Tennessee, Knoxville, Tennessee "Genetics of Sperm Structure and Function"

Monday, April 29, 10:00 AM

Deborah Anderson, Harvard Medical School, Boston, Massachusetts "HIV in the Male Reproductive Tract and Semen"

Symposium I— "Development of the Cell Types in the Testis"

Saturday, April 27, 1:30 PM

Janet Heasman, University of Minnesota, Minneapolis, Minnesota "Development and Migration of Germ Cells"

Joanne Orth, Temple University, Philadelphia, Pennsylvania "Development of Sertoli Cells and Gonocytes in Perinatal Testis"

Matthew Hardy, The Population Council, New York, New York "Origin and Development of Leydig Cells"

Symposium II— Techniques, Failure Mechanisms and Adverse Health Consequences of Vasectomy

Sunday, April 28, 1:30 PM

Arnold Belker, University of Louisville, Louisville, Kentucky "Vasectomy Techniques, Rates of Sperm Clearance, Recanalization and Paternity Considerations for Sperm Negative Patients After Vasectomy"

Stuart Howards, University of Virginia, Charlottesville, Virginia "Is Vasectomy Safe?"

Pharmacia & Upjohn Clinical Debate

Sunday, April 28, 11:00 AM

"Resolved: With Recent Advances in Micromanipulation, There is No Longer a Need for the Male Infertility Specialist"

Moderator: Richard Sherins, Genetics and IVF Institute, Fairfax, Virginia

Debater, Pro: Alan DeCherney, New England Medical Center, Boston, Massachusetts

Debater, Con: Rebecca Sokol, University of Southern California School of Medicine, Los Angeles, California

Special "Hot Topic" Lecture

Monday, April 29, 11:00 AM

"Spermatogonial Transplants in Mouse Testes"

Ralph Brinster, University of Pennsylvania, Philadelphia, Pennsylvania

Presentor: Lonnie Russell, Southern Illinois University, Carbondale, Illinois

Program Committee

A. Belker, W. Bremner, T. Brown, E. Goldberg, M. Griswold (Chair), M. Hardy, P. Olds-Clarke, J. Pryor, L. Russell, B. Sanborn, T. Turner

Abstract Review Committee

A. Belker, W. Bremner, T. Brown, E. Goldberg, M. Griswold (Chair), M. Hardy, N. Hecht, K. Kim, P. Olds-Clarke, J. Pryor, L. Russell, B. Sanborn, T. Turner

Serono Award Lectureship

Norman B. Hecht, Ph.D., is a Professor in the Department of Biology at Tufts University. He received his B.S. degree from Rensselaer Polytechnic Institute and his Ph.D. in microbiology from the University of Illinois in 1967. After three years of postdoctoral research studying the regulation of meiosis in plants at the University of California, San Diego, he joined the faculty at Tufts in 1970. His research interests have centered on mechanisms that regulate mammalian germ cell differentiation. Dr. Hecht's laboratory was one of the first to apply the techniques of modern molecular biology to male germ cells. His early work demonstrated that haploid gene expression plays a prominent role in the differentiation of germ cells. These pioneering studies have led to the identification and characterization of a large number of post-meiotically expressed genes and an understanding of how transcriptional and translational regulation controls the spatial and temporal expression of male specific proteins. Recently, his research interests have focused on post-transcriptional gene regulation with an emphasis on DNA- and RNA-binding proteins in the testis. The significance of these studies goes far beyond spermatogenesis, since one testicular RNA-binding protein has been shown to repress translation of stored "paternal" mRNAs in germ cells and also to be essential for transporting mRNAs along microtubules in brain cells. He is also applying his laboratory's genetic engineering expertise to the development of novel male contraceptives by targeting essential germ cell specific proteins for suppression. Dr. Hecht has authored more than 130 manuscripts and has served on the editorial boards of the Journal of Andrology and Journal of Experimental Zoology and as a guest editor of Developmental Genetics for a special issue on male gametogenesis. Presently, he is an Associate Editor of Molecular Reproduction and Development and the Journal of Cellular Biochemistry. Dr. Hecht's contributions to the field of male reproductive biology extend far beyond laboratory research. He has been a consultant on the use of molecular biomarkers in toxicology to a Reproductive and Developmental Toxicology Panel of the National Research Council, the vice chairman and chairman of Gordon Conferences on Mammalian Gametogenesis and Embryogenesis, a member of the Clinical Sciences Study Section of NIH, and a member of the Steering Committee of the Task Force on Methods for the Regulation of Male Fertility of the World Health Organization. He is currently a member of the Reproductive Biology Study Section of NIH, a council member of the American Society of Andrology, and the leader of a research group of the International Consortium on Male Contraception.

Serono Lectureship Recipients

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

Distinguished Service Award

Philip Troen is the recipient of the 1996 Distinguished Service Award. Dr. Troen received his A.B. from Harvard College in 1944 and his M.D. from Harvard Medical School in 1948. After internship at Boston City Hospital and residency in Medicine at the Beth Israel Hospital in Boston, he was an Endocrinology and Metabolism fellow at the Mayo Clinic. He then returned to the Beth Israel Hospital as a postdoctoral fellow and research fellow. Dr. Troen was appointed Assistant Professorof Medicine at Harvard Medical School in 1960 and moved to the University of Pittsburgh in 1964 as Professor of Medicine and Physician-in-Chief at the Montefiore Hospital. He has served as Associate and Vice-Chair of the Department of Medicine at the University of Pittsburgh School of Medicine and is currently Interim Chair, Division of Endocrinology and Metabolism and Physician-in-Chief Emeritus. Dr. Troen has had a distinguished research career. He received the Distinguished Andrologist Award from the American Society of Andrology in 1991. He has studied steroid production and metabolism in the placenta and testis, pituitary/gonadal interactions, testicular function and the bases for testicular disease, and control of gonadotropin hormone release and secretion. Dr. Troen has been an active member of the American Society of Andrology. He was actively involved in the founding of the Society. He has served on the Program and Publications Committee (1975–76), Executive Council (1977–80), as Vice President (1979–80) and President (1980–81), and as Chair of the International Liaison Committee (1981–93) and the Publications Committee (1990–93). He has been active in the International Society of

Distinguished Service Award Recipients

1994 C. Alvin Paulsen 1995 Andrzej Bartke

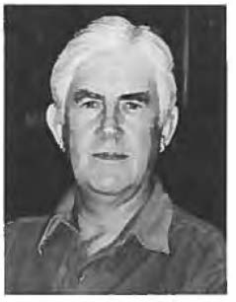
Sponsored by the Genetics & IVF Institute

Andrology, serving as Secretary (1981–89), President (1989–93), and currently as Past President. He was on the program organizing committee for three International Congresses of Andrology. He has also served on the Program Committee and Publications Committee for the Endocrine Society. Dr. Troen has served on numerous occasions as a grant and contract reviewer for NIH, FDA and WHO. He has also served on the editorial boards of Andrologia, the International Journal of Andrology, the Journal of Clinical Endocrinology and Metabolism, and the Journal of Andrology. Dr. Troen has been an effective mentor. He has trained a number of persons who have gone on to be active contributors to the American Society of Andrology and to reproductive endocrinology in general. For his enthusiastic support of the discipline and his numerous efforts on behalf of andrology, the Society is pleased to award Dr. Philip Troen the Distinguished Service Award.

Young Andrologist Award

Paul S. Cooke is the recipient of the 1996 Young Andrologist Award. Dr. Cooke received his B.A. from Westminster College and Ph.D. in Endocrinology from the University of California at Berkeley in 1983. He was an NIH NRSA fellow at the University of California San Francisco. Dr. Cooke was appointed assistant professor in the Department of Veterinary Biosciences at the University of Illinois at Urbana-Champaign in 1987 and associate professor in 1993. In 1991, Dr. Cooke and colleagues at Illinois made the novel observation that early postnatal hypothyroidism induced the adult testis to double its size and sperm production, as well as to increase prostate size, without increasing serum testosterone. Over the past few years, Dr. Cooke has developed this model further and shown that this treatment increases both Leydig and Sertoli cell number. These are particularly important observations, since adult testicular Sertoli cell number is a limiting factor in sperm production. Dr. Cooke has embarked on mechanistic studies to understand this phenomenon, as well as applied studies aimed at increasing adult testicular size and sperm production in domestic animals. Dr. Cooke is equally as productive in other areas, including reproductive toxicology, mechanism of action of steroid hormones and stromal/epithelial interactions in reproductive tract development. Dr. Cooke is well funded from NIH and USDA. He has been the recipient of the Levine Award for research excellence at the University of Illinois. Dr. Cooke serves on the editorial board of the Journal of Andrology and has been a consultant for the EPA and NSF. He has been an invited speaker at national and international meetings. Dr. Cooke is an excellent teacher and is actively training a number of enthusiastic students. For his research accomplishments and promise, the Society is pleased to award Dr. Paul Cooke the Young Andrologist Award.

Distinguished Andrologist Award



J. Michael Bedford is the recipient of the 1996 Distinguished Andrologist Award. Dr. Bedford received a B.A., M.A., and Vet. M.B. from Cambridge University and a Ph.D. in physiology from the University of London. Dr. Bedford was a Scientist at the Worcester Foundation from 1959 to 1961. He served as lecturer and teacher in Physiology at the Royal Veterinary College and the University of London and then returned to the Worcester Foundation (1966–67). He joined the Department of Anatomy at Columbia University in 1967 as Assistant Professor, was appointed Associate Professor in 1970, and was appointed Professor of Reproductive Biology and Cell Biology and Anatomy at Cornell University Medical College in 1972. He became the Percy and Harold Uris Professor of Reproductive Biology at Cornell in 1981. Dr. Bedford has been honored in many ways, including the Serono Award for Distinguished Contributions to Andrology (1982) by our Society, the Research Award from the American Philosophical

Young Andrologists

1982 L.J.D. Zaneveld	1989 Barry T. Hinton
1983 William B. Neaves	1990 Luis Rodriguez-Rigau
1984 Lonnie D. Russell	1991 Patricia M. Saling
1985 Bruce D. Schanbacher	1992 Gary R. Klinefelter
1986 Stephen J. Winters	1993 Robert Chapin
1987 Ilpo T. Huhtaniemi	1994 Wayne J.G. Hellstrom
1988 Larry Johnson	1995 Christopher J. De Jonge

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Distinguished Andrologists

1976 Roy O. Greep	1986 Alfred D. Jost
M. C. Chang	1987 Emil Steinberger
1977 Roberto E. Mancini	1988 Yves W. Clermont
1978 Robert J. Hotchkiss	1989 C. Alvin Paulsen
1979 Thaddeus Mann	1990 Marie-Claire Orgebin-Crist
1980 John MacLeod	1991 Philip Troen
1981 Alexander Albert	1992 C. Wayne Bardin
1982 Eugenia Rosemberg	1993 Anna Steinberger
1983 Kristen B. D. Eik-Nes	1994 Richard J. Sherins
1984 Mortimer B. Lipsett	1995 Rupert P. Amann
1985 Robert H. Foote	

Society (1993), and the Bruce Stewart Memorial Lecturer for the American Fertility Society (1995). Dr. Bedford's research interests can be characterized as ground-breaking, for he pioneered many of the observations upon which current understanding of male reproductive biology is based. His work has focused on spermatogenesis, epididymal physiology, sperm transport and fertilization, using a wide variety of species. He demonstrated the development of sperm fertilizing capability in the epididymis and showed changes in sperm surface charge and the development of disulfide bonds in sperm nuclear proteins during maturation. He investigated in detail the role of the male genital tract in the maintenance of sperm fertility, factors affecting decapacitation, and the ultrastructure of the acrosome reaction. He demonstrated that the sperm plasma membrane overlying the equatorial segment is the site of fusion with the oocyte membrane. He undertook comparative studies of gametes and fertilization to dissect fundamental principles in gamete function. This study has led Dr. Bedford to advance provocative ideas on the evolution of mammalian gametes. Dr. Bedford has contributed a number of seminal reviews of lasting impact. He is known by many for his originality, high degree of scholarship, critical analysis of reproductive biology, particularly at the *in vivo* level, and his willingness to take on controversial topics and propose new mechanisms of action. Dr. Bedford has served on the editorial boards of a wide range of journals and on advisory boards for NIH, WHO, AID, EPA, and the International Planned Parenthood Foundation. For his ground-breaking work, his thoughtful perspective on reproductive strategies throughout the animal kingdom, his enthusiasm for science and encouragement of young investigators, the Society is pleased to present Dr. Michael Bedford with the Distinguished Andrologist Award.

New Investigator Award

Recipient will be announced at the Awards Ceremony on Sunday, April 28, 1996.

New Investigator Award Recipients

1983 Thomas T. Tarter	1990 Donna O. Bunch
1984 Peter S. Albertson	1991 Robert Viger
Randall S. Zane	1992 John Kirby
1986 Mark A. Hadley	1993 Michael A. Palladino
1987 Peter Grosser	1994 Linda R. Johnson
1988 Stuart E. Ravnik	1995 Mehdi A. Akhondi
1989 Tracy L. Rankin	

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Future Meetings

1997—Baltimore, Maryland, February 21-25: Contact Thomas Chang, Department of Urology, Johns Hopkins Medical School, 611 North Wolfe Street, Baltimore, MD 21205. Tel: (410) 955-1042; Fax: (410) 955-0833.

1998—Long Beach, California, March 27-31: Contact Dr. Shalender Bhasin, Division of Endocrinology, Walter P. Martin Research Center, Harbor–UCLA Medical Center, Torrance, CA 90509. Tel: (310) 222-1855; Fax: (310) 533-0627.

Laboratory Science Forum Program

"The ART Technologist's Role in Lab Accreditation: Quality Control, Quality Assurance, Competency Measurements"

Chair: Susan Tarchala, B.S.

Speaker: Gail Compton, M.A.S., B.L.M.

Date: Sunday, April 28, 1996

Time: 12:00–1:30 PM

Place: Alumni Room

(Box lunch available for $15—See Registration Form)

Student Colloquium (sponsored by California Cryobank, Los Angeles, CA): "Publishing a Paper—Tricks of the Trade"

Chair: Grace M. Centola, Ph.D.

***Speakers:** Susan S. Suarez, Ph.D.—"When is Your Data Ready to be Published?"
Donald J. Tindall, Ph.D.—"The *Journal* and the Editorial Board—Behind the Scenes
Donald F. Cameron, Ph.D.—"Reviewing a Paper"
Kenneth A. Ginsburg, M.D.—"How to Give a Talk About Your Data"

Date: Sunday, April 28, 1996

Time: 7:00–9:00 PM

Place: Ballroom, B, C, D

*All speakers will be involved in a joint panel discussion/question and answer session following their individual talks.

(Student Soiree immediately following Colloquium)

Women in Andrology Lunch

"Never Meet a Banker on an Empty Stomach—Lessons on Becoming an Entrepreneur"

Chair: Donna Vogel, M.D., Ph.D.

Speaker: Susan A. Rothmann, Ph.D.

Date: Saturday, April 27, 1996

Time: 12:00–12:30 PM (Business Meeting)
12:30–1:30 PM (Speaker and Lunch)

Place: Nolte Room

Andrology Laboratory Workshop (Hosted by: Andrology Laboratories Committee (ALC) of the American Society of Andrology)

"Winning Ways of Laboratory Data Presentation: Analyzing and Presenting Your Laboratory Data"

Directors: Christopher J. De Jonge, Ph.D. and Stephen Simon, Ph.D.

Topics: "It's Greek to Me!"—Statistical Data Analysis
"Where's that Form?—Documenting Data for Regulatory Agencies and Inspection"
"What Does This Result Mean?—Reporting Clinical Data in Understandable Ways"
"The Art of ART—Alternative Slide and Poster Presentations, Manuscripts, and Electronic Presentations"

Date: Thursday, April 25, 1996

Time: 8:00 AM–5:00 PM

Place: Nolte Room

The course is designed for Laboratory Directors and Supervisors. New material will be presented using an innovative small group format. Registration fee is $225 with a late fee of $25 after March 1, 1996.

SOCIAL/ORGANIZATIONAL EVENTS AT A GLANCE

Thursday, April 25, 1996

9:00 AM–11:00 AM	Publications Committee Meeting (Rotary Room)
8:00 AM–5:00 PM	Andrology Laboratory Workshop/Lunch (Nolte Room)
1:00 PM–9:00 PM	Executive Council Meeting/Dinner (Regents Room)

Friday, April 26, 1996

8:00 AM–5:00 PM	Postgraduate Course/Lunch (University Ballroom B, C, D)
7:00 PM–9:00 PM	Welcoming Reception (University Ballroom B, C, D)

Saturday, April 27, 1996

7:45 AM–5:00 PM	ASA Meeting (University Ballroom B, C, D)
12:00 PM–1:30 PM	Women in Andrology Lunch (Nolte Room)
7:00 PM–9:00 PM	Banquet (Prefunction/University Ballroom B, C, D)

Sunday, April 28, 1996

7:00 AM–8:00 AM	Past-Presidents Breakfast (Presidents Room)
12:00 PM–1:30 PM	Laboratory Science Forum/Box Lunch (Alumni Room)
12:00 PM–1:30 PM	Editorial Board Meeting/Lunch (Presidents Room)
12:00 PM–1:30 PM	Using Androlog and the ASA Home Page (Nolte Room)
2:30 PM–3:30 PM	Awards Ceremony/Business Meeting (University Ballroom B, C, D)
3:30 PM–4:00 PM	Prize Drawing Sponsored by Exhibitors (Ballroom A/Prefunction/Faculty)
7:00 PM–9:00 PM	Student Colloquium (University Ballroom B, C, D)
9:00 PM–10:00 PM	Student Soiree (Presidents Room)

21st Annual Meeting

Thursday, April 25

8:00–12:00 NOON	**Andrology Laboratory Workshop** (Nolte Room)
12:00–1:00 PM	**Lunch served at Workshop** (Nolte Room)
1:00–5:00 PM	**Andrology Laboratory Workshop** (Nolte Room)
1:00–9:00 PM	**Executive Council Meeting** (Regents Room)

Friday, April 26

8:00–12:00 NOON	**Postgraduate Course: Endocrinology of the Male: From Head to Testis** (University Ballroom B, C, D)
8:00–8:10 AM	**Introduction** Jon L. Pryor
8:10–8:55 AM	**Androgen Receptors** Donald Tindall
8:55–9:40 AM	**Transcriptional Regulation of the Gonadotropin Genes** Margaret Shupnik
9:40–10:00 AM	Refreshment Break/Exhibits (Ballroom A/Prefunction/Faculty)
10:00–10:45 AM	**Disruption of the Estrogen Receptor Gene** Kenneth S. Korach
10:45–11:30 AM	**Activin and Inhibin** Teresa R. Woodruff
11:30–12:00 NOON	**Questions and Answers**
12:00–1:00 PM	Lunch at the course site (University Ballroom B, C, D)
1:00–1:40 PM	**Physiology of GnRH: Antagonists and Agonists** William Bremner
1:40–2:20 PM	**Endocrinology of Puberty** Howard Kulin
2:20–2:40 PM	Refreshment Break/Exhibits (Ballroom A/Prefunction/Faculty)
2:40–3:20 PM	**Testosterone Replacement Therapy** Glenn Cunningham
3:20–4:00 PM	**5 alpha Reductase Inhibitors in the Prostate** Roger Rittmaster
4:00–4:15 PM	**Questions and Answers**
4:15–5:00 PM	**Panel on Stress and Reproduction** Ron Weber, Richard Clark, Richard Sherins (Moderator)
7:00–9:00 PM	**ASA Welcoming Reception** (University Ballroom B, C, D)

Saturday, April 27

7:45–8:00 AM	**Welcome and Opening Remarks** (University Ballroom B, C, D) Marie-Claire Ørgebin-Crist, President David Hamilton, Local Arrangements Chairperson

8:00–9:00 AM — **Serono Lecture** (University Ballroom B, C, D)
Norman Hecht **"The Making of a Spermatozoon: Regulation of Gene Expression during Male Germ Cell Differentiation"**
Chairperson: Marie-Claire Orgebin-Crist

9:00–10:00 AM — **AUA Lecture** (University Ballroom B, C, D)
John Donohue **"Germ Cell Cancer of the Testis, Biologic, Anatomic, and Management Factors Impacting Fertility"**
Chairperson: Arnold Belker

10:00–10:30 AM — Refreshment Break/Exhibits (Ballroom A/Prefunction/Faculty)

10:30–12:00 NOON — **Oral Session I**
Somatic Cells of the Testis (Ballroom B, C, D)
Chairpersons: James Hutson, Paul Cooke

10:30 AM 1 **Expression and regulation of steroidogenic acute regulatory protein (StAR) messenger ribonucleic acid levels in rat Leydig cells by human chorionic gonadotropin and cytokines** / T. Lin, D. Wang
10:45 AM 2 **Testicular microvascular blood flow after loss of Leydig cells** / T.T. Turner, L.A. Caplis
11:00 AM 3 **Partial purification of a lipid factor from testicular macrophages that stimulates testosterone secretion by Leydig cells** / J.C. Hutson, C. Garner, P.A. Doris
11:15 AM 4 **Developmental pattern of Leydig cells in intact or transplanted testes in Fischer rats** / L. Johnson, L.C. Suggs, G.U. Falk
11:30 AM 5 **Neonatal estrogen treatment: effects on Sertoli cell proliferation and testis development in the rat** / K.L. Joyce, R.A. Hess, P.S. Cooke
11:45 AM 6 **Expression of the c-kit receptor in spermatogenic cells and normal sperm** / J. Sandlow, H.-L. Feng

12:00–1:30 PM — Lunch (on your own)
12:00–1:30 PM — **Women in Andrology Luncheon** (Nolte Room)
Susan A. Rothman **"Never Meet a Banker on an Empty Stomach—Lessons on Becoming an Entrepreneur"**
Chairperson: Donna Vogel

1:30–3:00 PM — **Symposium I: Development of the Cell Types in the Testis** (Ballroom B, C, D)
Chairperson: Barry Zirkin

Janet Heasman "Development and Migration of Germ Cells"
Joanne Orth "Development of Sertoli cells and Gonocytes in Perinatal Testes"
Matthew Hardy "Origin and Development of Leydig Cells"

3:00–3:30 PM — Refreshment Break/Exhibits (Ballroom A/Prefunction/Faculty)

3:30–4:30 PM — **Oral Session II**
Epididymis (Ballroom B, C, D)
Chairpersons: Daulat Tulsiani, David Bunick

3:30 PM (7) **Partial characterization and localization of galactose-containing glycoproteins on rat testicular spermatozoa: evidence for their degalactosylation by a galactosidase purified from rat epididymal fluid** / M.D. Skudlarek, M- C. Orgebin-Crist, D.R.P. Tulsiani
3:45 PM 8 **Comparison of epididymal epithelial localization of proteins D and E using dual labeling by two monoclonal antibodies and confocal imaging** / K.M. Ensrud, D.W. Hamilton
4:00 PM (9) **Immunocytochemical localization of estrogen receptor in efferent ductules and epididymis of the adult male rat** / D.H. Gist, R.A. Hess, J. Bahr, D. Bunick
4:15 PM 10 **Endothelin-1 is present and biologically active in human epididymis** /M. Maggi, A. Peri, S. Amerini, T. Barni, S. Granchi, G. Barbagli, A. Natali, L. Gloria, G.B. Vanelli, G. Forti

4:30–6:30 PM — **Poster Session I includes Student Award Candidates** (H.H.H./Northrop/Coffman/Campus)

Fertilization, IVF—I

11 **Effects of chronic renal failure (CRF) on sperm fertilizing capacity** / Y. Yamamoto, I. Miyagawa, P. Incze, S. Andrighetti, A. Zini, N. Sofikiti

12 **Acrosomally intact or acrosomally reacted sperm injections into rabbit oocytes** / K. Shimamoto, I. Miyagawa, Y. Yamamoto, K. Ono, H. Okada, N. Sugiyama, K. Taniguchi, F. Sasaka, R. Kotoku, N. Sofikitis

13 **The correlation of sperm morphology with sperm function tests after swim-up and Percoll separation** / J.W. Graczykowski, H. Okuda, R.Z. Sokol

14 **Abstract Withdrawn**

15 **Validation of hypoosmotic swelling as a viability test by Hoechst-33258 and eosin-nigrosin in fresh and cryopreserved human spermatozoa** / S.C. Esteves, R.K. Sharma, A.J. Thomas, Jr., A. Agarwal

16 **Initial sperm viability assessment predicts the survival of viable sperm after separation** / E.E. Gottenger, H.M. Nagler, N. Virji

17 **Processing of human spermatozoa does not reduce membrane damage or spontaneous acrosome reaction following cryopreservation** / R.K. Sharma, S.C. Esteves, A.J. Thomas, Jr., A. Agarwal

Sperm Biology

18 **A bilateral effect of left varicocele (LV) on epididymal sperm acrosin profiles** / H. Okada, I. Miyagawa, Y. Yamamoto, K. Ono, P. Incze, S. Andrighetti, S. Girardi, A. Zini

19 **Identification, localization and activation of P42-mitogen-activated protein kinase ($p42^{MAPK}$,ERK2) in human spermatozoa** / M. Luconi, T. Barni, B. Vannelli, C.Krausz, G. Forti, E. Baldi

20 **Purification of a human sperm maturation-related glycoprotein, GP-83** / G-H. Sun, Y-C. Lin, H-Y. Tseng, Y-W. Guo, G-H. Sun

21 **Sperm mediated effect on mouse embryo development** / Y. Kuribayashi, C. Gagnon

22 **Molecular cloning of the gene encoding GP-83, a human sperm maturation-related glycoprotein** / H-W. Liu, Y- Y. Tseng, Y-C. Lin, Y-W. Guo, S-H. Sun

23 **Coculture of human sperm with oviduct cells** / J. Ellington, A.Benson, G. Hiss, S. Brisbois, G.Rice, R. Wright

24 **Protein kinase A (PKA) may be involved in the platelet activating factor (PAF) induced human sperm acrosome reaction (AR)** / R.A. Tom, M.J. Angle, P.J. Turek

25 **Comparative study of screening test for antisperm antibody** / S. Saito

26 **Binding of vasoactive intestinal peptide to human sperm surface** / Y. Siow, M.E. Fallat, E.A. Klar, S.C. Yoffe, A.M. Belker

27 **A 18kDA nuclease is associated with rat spermatocyte apoptosis induced by 2-methoxyethanol (ME)** / R.N. Wine, W.W. Ku, R.E. Chapin

28 **Kinetics of mouse sperm cell lysis in hypotonic medium: implications for determination of the water permeability (L_p) of the murine sperm plasma membrane** / E.E. Noiles, K.A. Thompson, B.T. Storey

29 **Isolated defect of acrosomal function in men with otherwise normal sperm maturity and fertilizing potential** / G. Huszar, L. Vigue

Fertility, Infertility

30 **Single intramuscular injection of 1000 mg testosterone undecanoate to hypogonadal men maintains physiological serum testosterone levels for 7 weeks** / H.M. Behre, S. Kliesch, B. Lemcke, E. Nieschlag

31 **Pharmacokinetics and efficacy of transbuccal testosterone in the treatment of hypogonadal men** / A. Dobs, K. Les, D. Hoover, R. Allen

32 **Decreased bone mineral density at the hip of adult hypogonadal men as compared with normal pubertal boys** / M.R. Mascarenhas, J. Garcia-e-Costa, A. Galvao-Teles

33 **The role of cadmium in the etiology of varicocele associated infertility** / S. Benoff, I.R. Hurley, M. Barcia, F.S. Mandel, D. Kostouros, G.W. Cooper, A. Hershlag

34 **A challenge to the concept that the use of calcium channel blockers cause reversible male infertility** / D. Katsoff, J.H. Check

35 **Percutaneous testicular biopsy in men with azoospermia or severe oligospermia: results and intra-operative findings** / B.C. Mellinger

36 **Ethnic differences in spermatogenic potential in humans** / L. Johnson, L. Rodriguez, R.S. Swerdloff, X.H. Wang, C. Wang

37 **Reproductive assessment of military personnel associated with military duty assignments** / S.M. Schrader, R.E. Langford, T.W. Turner, D.O. Lundy, S.D. Simon

Semen

38 **Polymerase chain reaction (PCR)-based quantitation of sexually-transmitted pathogens in semen of infertility patients with leukocytospermia** / G. Bezold, E. Yanushpolsky, D.J. Anderson

39 **Alterations of the parameters of semen on patients with varicocele pre and post variococelectomy** / C. Bravo, A. Arredondo, R. Tapia-Serrano
40 **Test kit to determine sperm count in semen** / J.G. Alvarez
41 **Evaluation of subnormal semen parameters and subsequent zona pellucida (AP) thickening following in vitro fertilization (IVF)** / J. Locuniak, J.H. Check, D. Lurie, H.G. Adelson, L. Hoover
42 **Use of semen as biopsy material for assessment of health status of the male reproductive tract** / D.N.R. Veeramachaneni, C.L. Moeller, B.W. Pickett, H.R. Sawyer
43 **The use of iodixanol as a density gradient material for the isolation of motile, morphologically normal human sperm from semen** / T.T. Smith, D. Turner, W. Whitford
44 **Quality control of the measurement of the alpha-glucosidase activity in seminal plasma** / C. Spiessens, E. Woutes, D. Vandershueren
45 **Development and validation of a procedure to determine the concentration of testosterone dihydrotestosterone and estradiol in the human ejaculate** / A.J. Peters, R.S. Jeyendran, R.T. Chatterton, Jr.
46 **Nitric oxide synthase (NOS) activity in human seminal plasma** / A. Zini, M.K. O'Bryan, P.N. Schlegel
47 **The biological basis of the resazurin reduction test** / N. Lammertijn, A. Zalata, F. Comhaire, A. Christophe

Testis and Epididymis

48 **Presence of isoforms of LH and FSH in serum of man with testicular damage** / A. Arredondo, M. Masson, M.E. Fanseca, R. Tapia-Serrano
49 **Decreased spermatogenesis as the result of an induced autoimmune reaction directed against the gonadotropin receptors in male rats** / K.M. Graf, M.D. Griswold
50 **Cyclophosphamide (CPA) induces apoptosis in the germ cells of rat testes in a cell and stage specific manner** / L. Cai, B.F. Hales, B. Robaire
51 **Androgen-induced growth factor (AIGF/FGF-8) is expressed in canine prostate and testis: cloning of cDNA encoding canine AIGF/FGF-8** / H. Canatan, S.K. Kulp, W.Y. Chang, F. Shidaifat, Y. Sugimoto, R.W. Brueggemeier, Y.C. Lin
52 **Alterations in catalase and glutathione peroxidase mRNA expression in the rat testis after surgical cryptorchidism and efferent duct ligation** / A. Zini, M. Goldstein, P.N. Schlegel
53 **The proliferative capacity of rat Leydig cells declines during pubertal development** / R.S. Ge, M.P. Hardy
54 **Physiological concentrations of glucocorticoid regulate serum testosterone and 11beta-hydroxysteroid dehydrogenase in purified Leydig cells** / H.-B. Gao, R-S. Ge, M.P. Hardy
55 **Developmental expression and differential regulation of CU/ZN superoxide dismutase, Mn superoxide dismutase, glutathione peroxidase and catalase mRNAs during spermatogenesis in the mouse** / W. Gu, N. Hecht
56 ***In situ* evaluation of gamma-glutamyl transpeptidase mRNA expression in the rat epididymis following loss of testicular factors** / D.B. Rudolph, C.M. Barber, M.H. Stoler, B.T. Hinton
57 **Orchidectomy induces a wave of apoptotic cell death in the epididymis** / X.P. Fan, B. Robaire
58 **Localization of testins in Sertoli cells and germ cells in vitro** / K.E. Muffly, D.F. Cameron
59 **Evidence for toxicant-induced acceleration in epididymal transit time** / J.D. Suarez, G.R. Klinefelter
60 **Expression and functions of cyclin A and cyclin A-dependent kinases in the mouse testis** / S.E. Ravnik, D.J. Wolgemuth
61 **Human testis and rat testis are equally sensitive to 2-methoxyethanol: a direct *in vitro* comparison** / L.-H. Li, R.N. Wine, R.E. Chapin
62 **Characterization of the protein osteopontin in human male reproductive tissues and cells** / J.E. Siiteri, D.J. Swanlund, L.A. Pinke, J.L. Pryor, K.P. Roberts, D.W. Hamilton
63 **Adult estrogen receptor knock-out mice have abnormal seminiferous tubules, rete testes and efferent ductules** / R.A. Hess, D. Bunick, K. Seo, D.B. Lubahn
64 **Ultrastructure of a Sertoli-Leydig cell tumor of the ovary** / S. Siew, S. Katlein
65 **Effect of increased scrotal temperature on sperm production in normal men** / C. Wang, V. McDonald, A. Leung, L. Superlano, L. Hull, R.S. Swerdloff
66 **Cryptorchidism and male intersex—clinical analysis and histological and endocrinological investigations** / S. Saito

Erectile Function and Ejaculation

67 **Adrenomedullin induces penile erection in the cat** / H.C. Champion, J.A. Santiago, R. Wang, D.H. Coy, W.A. Murphy, P.J. Kadowitz, W.J.G. Hellstrom
68 **Cessation of smoking produces rapid improvement in erectile function** / A. Guay, G. Heatley
69 **Comparison of transurethral and intracavernosal administration of PGE_1** / R. Wang, H.C. Champion, N. Sofikitis, W.J.G. Hellstrom

70 **Abstract Withdrawn**
71 **Long-term passive smoking in the rat reduces penile nitric oxide synthase without impairing the erectile response to electrical stimulation of the cavernosal nerve** / Y. Xie, H. Garban, Ch. Ng, J. Rajfer, N.F. Cadavid
72 **Clinical and laboratory treatment of retrograde ejaculation** / R.D. Eward
117 **IGFBP-5 expression is associated with involution of the ventral prostrate in castrated and finasteride-treated rats** / R.S. Rittmaster, L. Thomas, P. Cohen

7:00–9:00 PM **Banquet** (Prefunction/Ballroom B, C, D)

Sunday, April 28

7:00–8:00 AM **Past Presidents Breakfast** (Presidents Room)

8:00–9:30 AM **Oral Session III** (Ballroom B, C, D)
Fertility/infertility/contraception
Chairpersons: Erwin Goldberg, Larry Johnson

8:00 AM 73 **Decline in semen quality: global or selective?** / C.A. Paulsen, N. Berman, C. Wang
8:15 AM 74 **Submicroscopic deletions of Y chromosome outside the DAZ region in a subset of infertile men implicate additional Y-specific genes in the pathogenesis of male infertility** / H. Najmabadi, V. Huang, M. Subarao, P. Yen, D. Bhasin, S. Naseeruddin, W. Taylor, D.M. DeKretser, K. Loveland, H.W.G. Baker, R.I. McLachlan, S. Bhasin
8:30 AM 75 **Contraceptive vaccine development: the B-cell epitope of a novel testis antigen** / E. Goldberg, Z-G. Liang, G.-Y. Wang, P.A. O'Hern
8:45 AM 76 **Sperm chromatin structure assay (SCSA) predicts human fertility potential** / D. Evenson, M. Zinaman, E. Clegg, L. Jost
9:00 AM 77 **Effects of a nonscrotal testosterone transdermal system on sexual function over 6–12 months of treatment in hypogonadal males** / S. Arver, A.S. Dobs, A.W. Meikle, S. Sanders, N.A. Mazer
9:15 AM 78 **No decline in daily sperm production in a group of North American men over a seven-year period** / L. Johnson, R. Levine, J.J. Barnard, W.B. Neaves

9:30–10:00 AM Refreshment Break/Exhibits (Ballroom A/Prefunction/Faculty)

10:00–11:00 AM **ASA/NME State-of-the-Art Lecture** (Ballroom B, C, D)
Mary Ann Handel **"Genetics of Sperm Structure and Function"**
Chairperson: Dolores Lamb

11:00–12:00 NOON **Pharmacia & Upjohn Clinical Debate** (Ballroom B, C, D)
"Resolved: With Recent Advances in Micromanipulation, There is no Longer a Need for the Male Infertility Specialist"
Moderator: Richard Sherins
Discussants: Alan DeCherney, Rebecca Sokol

12:00–1:30 PM Lunch (on your own)
12:00–1:30 PM **Simultaneous Events**
1. **Editorial Board Luncheon** (Presidents Room)
2. **Laboratory Science Forum** (Alumni Room) Gail Compton **"The ART Technologist's Role in Lab Accreditation: Quality Control, Quality Assurance, Competency Measurements"**
3. Craig Niederberger **"Using Androlog and the ASA Home Page"** (Nolte Room)

1:30–2:30 PM **Symposium II** (University Ballroom B, C, D)
"Techniques, Failure Mechanisms and Adverse Health Consequences of Vasectomy"
Chairperson: Mark Sigman
Arnold Belker **"Vasectomy Techniques, Rates of Sperm Clearance, Recanalization and Paternity Considerations for Sperm Negative Patients After Vasectomy"**
Stuart Howards **"Is Vasectomy Safe"**

2:30–3:30 PM **ASA Business Meeting and Award Ceremony** (University Ballroom B, C, D)

3:30–4:00 PM Refreshment Break/Exhibits (Ballroom A/Prefunction/Faculty)

4:00–6:00 PM **Poster Session II (H.H.H./Northrop/Coffman/Campus)**

Sperm Quality and Motility

Media and dilution procedures for processing human, rabbit and bull sperm for computer-assisted sperm analysis / R.H. Foote, P.B. Farrell

80 **Human sperm motility and other CASA variables measured directly on specimens treated with Hoechst 33342 dye** / R.H. Foote, P.B. Farrell, M.J. Zinaman

81 **Energy and sperm motility in seminal hyperviscosity** / G.R. Mendeluk, M.J. Munuce, C. Carizza, M. Sardi, C. Bregni

82 **Effect of Lipopolysaccharide (LPS) and interferon-gamma (IFN-gamma) on sperm motion in vitro** / R. Wang, L.S. Estrada, H.C. Champion, M. Rajasekaran, S.C. Sikka, W.J.G. Hellstrom

83 **Optimizing motility following cryopreservation of epididymis and testicular sperm** / A.H. Amin, B.T. Storey, L. Blasco, J.L. Marmar, S. Heyner

84 **Morphological and morphometric comparisons of rabbit spermatozoa exposed to lead** / T. Turner, T. Zimmerman, W. Moorman, S. Schrader, S. Simon

85 **Does elevated blood lead increase sperm velocity?** / S.R. Skaggs, M.A. Hart, T.W. Turner, W.J. Moorman, J.C. Clark, S.M. Schrader, S.D. Simon

86 **Pro-inflammatory chemokine (IL-8) mediated effects on human sperm motility** / S.C. Sikka, L.S. Estrada, H.C. Champion, R. Wang, M. Rajasekanan, W.J.G. Hellstrom

87 **Sperm parameters in men with varicoceles (Vx) prior to and after surgery (Sx) compared to men without varicoceles** / G. Centola, D. Dever

88 **Flow cytometric techniques to identify and enumerate viable sperm cells** / D. Redelman, D. Garner

89 **Changes in sperm motion parameters following Percoll sperm wash for motile sperm cell separation** / J. Rafferty, R. Filer, M. Cornman

90 **Effect of pre-freeze Percoll wash and artificial motility stimulation on cryopreserved human spermatozoa** / R.K. Sharma, S. Kohn, A.J. Thomas, Jr., A. Agarwal

91 **Effect of pentoxifylline on the intrinsic force of human sperm** / P. Patrizio, Y. Liu, G.J. Sonek, M.W. Berns, Y. Tadir

92 **Sperm quality in the various stages and histologic types of testicular cancer** / M. Tolentino, Jr., I. Ayzman, A.J. Thomas, Jr., A. Agarwal

93 **Time course of in vitro sperm acrosome reaction** / G. Centola, V. Lewis, E. Andolina, S. Weisensahl, R. Herko

94 **Capacitation of clouded leopard sperm: II. effect of calcium and cyclic amp on acrosome reaction and zona penetration** / B.S. Pukazhenthi, J.A. Long, D.E. Wildt, M. Bush, J.G. Howard

95 **Capacitation of clouded leopard sperm: I. effect of protein on acrosome reaction and zona penetration** / J.A. Long, B.S. Pukazhenthi, D.E. Wildt, S. Murray, S. Barrett, J.G. Howard

Fertilization, IVF—II

96 **Fertilizing potential of frozen/thawed epididymal spermatozoa from fresh cadavers** / P.S. Li, S. Girardi, A. Zini, I. Miyagawa, T. Toda, D. Mourtzinis, A. Koutselinis, N. Sofikitis

97 **Profile of donor rejection in a sperm bank program** / I. Ayzman, S. Kachoria, C. Curtis, R.K. Sharma, A.J. Thomas, Jr., A. Agarwal

98 **A study of failed fertilization—relative frequency of male vs. female factors and therapeutic options in next cycles** / J.H. Check, C. Hourani, A. Baker, A. Nazari

99 **Viable pregnancies with intracytoplasmic sperm injection for poor hypo-osmotic scores** / D. Katsoff, L. Hoover, J.H. Check

100 **Antioxidants improve turkey sperm viability, membrane integrity and motility during liquid storage** / A.M. Donoghue

101 **Intracytoplasmic sperm injection (ICSI) is treatment of choice for subnormal total sperm acrosin activity** / B.M. Bani, Q. Winger, A. Deutsch, I.S. Tummon

102 **The use of the hemizona assay (HZA) as an advanced diagnostic tool in assisted reproduction** / M.C. Mahony, S. Oehninger

103 **The predictive value of motile sperm with normal morphology in donor insemination** / C.L. Gnatuk, R.S. Legro, J. Moessner, J. Miller, W.C. Dodson

104 **Impact of human sperm morphologic abnormalities on intrauterine insemination (IUI) pregnancy rates** / D.S. Karabinus, T.J. Gelety

105 **ICSI with percutaneous artificial spermatocele sperm aspiration (ASSA-ICSI) in failure cases of mesa-ICSI** / J.T. Seo, Y.S. Lee, J.P. Hong, Y.S. Park, J.H. Jun, H.J. Lee, I.P. Son

106 **Modified guanidinium thiocyanate method is ideal for human sperm DNA preparation to be used for evaluation of the X and Y composition of the sperm** / A.M. Hossain, B. Rizk, R. Yeoman, I.H. Thorneycroft

107 **In vitro laboratory treatments have impact on hypoosmotic swelling pattern when applied to human sperm** / A.M. Hossain, R. Selukar, C. Huff, B. Rizk, S.C. Lynn, Jr.

108 **One of the fractions of Percoll eliminated sperm can be added to Percoll recovered sperm to increase sperm yield in Percoll wash procedure** / A.M. Hossain, B. Rizk, C. Huff, I.H. Thorneycroft

109 **Acquisition of testicular sperm from men with non-obstructive azoospermia** / J.L. Marmar, M.Gibbs, S. Heyner

110 **Double fluorescent stain for accurate assessment of human-hamster ICSI outcome** / R. Dolgina, P. Studney, L. Ross, G.S. Prins, C. Neiderberger

111 **Mannose binding assay in dutch belted rabbits** / M.J. Breitenstein, S.M. Schrader

112 **Discontinuous Percoll optimizes sperm populations used for assisted reproductive technologies (ART)** / K.E. Tucker, B.S. Hurst, C. Dymecki, B. Mendelsberg, S. Guadagnoli, C.A. Awoniyi, W.D. Schlaff

113 **Microsurgical epididymal sperm aspiration and IVF outcome** / S.M. Tarchala, R.G. Rawlins, S.R. Mack, E. Radwanska, Z. Binor, M. Wood Molo, B.A. Soltes, L. Levine, E. Lenting

114 **Fertilization after intracytoplasmic injection of round spermatid from cryptorchid mouse** / I. Sasagawa, R. Yanagimachi

115 **Pregnancies in black-footed ferrets and siberian polecats after laparoscopic artificial insemination with fresh and frozen-thawed semen** / J.G. Howard, D.R. Kwiatkowski, E.S. Williams, R.W. Atherton, R.M. Kiethin, E.T. Thorne, M. Bush, D.E. Wildt

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116 **Nitric oxide depresses vas deferens contractility in the rat in vivo** / P.J. Turek, K. Aslam

118 **Neonatal estrogen exposure leads to prostate lobe-specific dysplasia and adenomas in the aging rat** / G.S. Prins, L. Birch, S.H. Ye, V. Ray

119 **Quantitation of 5alpha-reductase type 1 and 2 mRNA in nonhuman primate male reproductive tissues** / M.C. Mahony, K. Gordon

120 **Seminal fluid findings in men with non-bacterial prostatitis or prostatodynia** / C.H. Muller, R.E.Berger, S.O. Ross, I. Rothman, J.N. Krieger

121 **Abstract Withdrawn**

122 **Characterization of two serine protease inhibitors in the secretions of the rat ventral prostate** / C. Casey, M. Woodson, A.S. Sinha, M.J. Wilson

123 **Seminal plasma peptidase activities as indicators of prostate function in men** / R. Bellrichard, C. Ercole, J. Pryor, H. Hensleigh, K. Ahmed, P. Reddy, M.J. Wilson

124 **Central hypogonadism: differentiating idiopathic hypogonadism (IH) from pituitary tumors (PT)** / M. Wiederkehr, G. Wand, A.S. Dobs

Cryopreservation

125 **Artificial stimulation of cryopreserved human spermatozoa in cancer patients** / R.K. Sharma, O.F. Padron, S. Kohn, A.J. Thomas, Jr., A. Agarwal

126 **Antioxidants improve the quality and survival of cryopreserved bovine sperm** / J.L. Bailey, S. Tardif, C. Petitclerc

127 **Response of frozen-thawed bovine spermatozoa to the hypoosmotic swelling test at various temperatures** / J.R. Correa, P.M. Zavos

128 **Preparation of frozen-thawed bovine spermatozoa via various sperm selection techniques employed in assisted reproductive technologies** / J.R. Correa, P.M. Zavos

129 **Dilution associated changes in osmotic pressure of the cryopreservation medium during washing of frozen thawed bovine spermatozoa** / J.R. Correa, P.M. Zavos

130 **A simplified approach to the swim-up method; use of a new one step swim-up/swim-down standardized technique** / P.M. Zavos, P.N. Zarmakoupis-Zavos

131 **Effect of sperm capacitation before freezing and its influence on the extent of spontaneous acrosome reaction** / S.C. Esteves, R.K. Sharma, A.J. Thomas, Jr., A. Agarwal

132 **The relationship between quality of cryopreserved donor semen and pregnancy in artificial insemination** / A. Agarwal, I. Ayzman, C.Curtis, S. Kachoria, A.J. Thomas, Jr.

133 **Effect of centrifugation speed or intermediate buffer on improvement in post-wash frozen spermatozoa** / S. Kohn, S. Vemulapalli, R.K. Sharma, O.P. Padron, A.J. Thomas, Jr., A. Agarwal

134 **Clinical application and limitation of monoclonal acrobead test in fresh and cryopreserved semen samples** / R.K. Sharma, O.F. Padron, A.J. thomas, Jr., A. Agarwal

135 **Comparison of post-cryopreservation sperm quality in patients with testicular cancer, Hodgkin's disease, and leukemia** / O.F. Padron, R.K. Sharma, A.J. Thomas, Jr., A. Agarwal

136 **Effect of cryopreservation of activable proacrosin levels in bovine sperm** / D.L. Garner, C.A. Burner, D. Redelman, J.C. Powers

137 **Morphologic variation and megacephaly in pre- and post-cryopreserved human sperm** / R.J. Swanson, M.S. Morshedi, S.L. Taylor, C.C. Coddington, S.C. Oehninger

138 **Improved results of thawed sperm cryopreserved with stage cooling with a cellevator** / M.L. Check, J.H. Check, D. Katsoff

139 **Comparison of thawing temperature on semen parameters of cryopreserved sperm** / D.J. Check, D. Katsoff, J.H. Check

7:00–9:00 PM **Student Colloquium** (University Ballroom B, C, D)
Publishing a Paper—Tricks of the Trade
Chairperson: Grace Centola
Panel: Susan Suarez, Don Tindall, Don Cameron, Kenneth A. Ginsburg

9:00–10:00 PM **Student Soiree** (Presidents Room)

Monday, April 29

8:00–9:30 AM **Oral Session IV** (University Ballroom B, C, D)
Sperm Biology
Chairpersons: Bayard Story, Susan Suarez

8:00 AM 140 **Intra-acrosomal events and destruction of single-stranded DNA in sperm during human epididymal sperm maturation process** / S. Girardi, P.S. Li, A. Zini, I. Miyagawa, N. Sofikitis

8:15 AM 141 **Hydroperoxide reducing capacity of glutathione** / K.A. Thompson, J.G. Lavarez, B.T. Story

8:30 AM 142 **Effect of interaction with oviductal epithelium on intracellular calcium levels of hamster sperm** / S.S.Suarez, M.C. Lo

8:45 AM 143 **New chemical markers of sperm function** / F. Comhaire, A. Zalata, C. Depuydt, A. Christophe

9:00 AM 144 **The immunohistochemical localization of urokinase-type plasminogen activator (uPA) and urokinase-type plasminogen activator receptor (uPAR) in human spermatozoa** / C.L. Xiong, W.J. Xia, X.B. Huang, J.Y. Shen

9:15 AM 145 **$[Ca^{2+}]_i$ increase and acrosome reaction in response to progesterone predict fertilization success in in vitro fertilization (IVF)** / C. Krausz, L. Bonaccorsi, P. Maggio, M. Luconi, B. Fuzzi, S. Pellegrini, L. Criscuoli, G.G. Forti, E. Baldi

9:30–10:00 AM **Refreshment Break/Exhibits** (Ballroom A/Prefunction/Faculty)

10:00–11:00 AM **Buckeye State-of-the-Art Lecture** (University Ballroom B, C, D)
Deborah Anderson "HIV in the Male Reproductive Tract and Semen"
Chairperson: Donna Vogel

11:00–12:00 NOON **Special "Hot Topic" Lecture** (University Ballroom B, C, D)
Ralph Brinster, Lonnie Russell*, "Spermatogonial Transplants in Mouse Testes"
Chairperson: Martin Dym

*Presentor

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001 EXPRESSION AND REGULATION OF STEROIDOGENIC ACUTE REGULATORY PROTEIN (StAR) MESSENGER RIBONUCLEIC ACID LEVELS IN RAT LEYDIG CELLS BY HUMAN CHORIONIC GONADOTROPIN AND CYTOKINES.

T. Lin and D. Wang*. WJB Dorn Veterans Hospital and the Department of Medicine, University of South Carolina School of Medicine, Columbia, SC 29208

The rate-limiting step in steroidogenesis is the delivery of the substrate cholesterol from cellular stores and the outer mitochondrial membrane to the inner mitochondrial membrane involving a cycloheximide-sensitive transport protein. Recently, this transport protein was cloned by Clark et al. (JBC 269: 28314, 1994) and was named as steroidogenic acute regulatory protein (StAR). In the present study, we evaluated the expression and regulation of StAR mRNA in rat Leydig cells. Leydig cells were obtained from 55-65 day-old Sprague-Dawley rats by collagenase digestion of decapsulated testes, followed by centrifugal elutriation and Percoll density gradient centrifugation. StAR mRNA was expressed in Leydig cells with two major transcripts, 3.4 and 1.6 kb. Freshly isolated Leydig cells expressed high levels StAR mRNA which decreased markedly after 24 h in culture. HCG (0.1 - 10 ng/ml) induced StAR mRNA levels in a dose-dependent manner. StAR mRNA levels increased as early as 30 min and peaked at 2 h after the addition of hCG (10 ng/ml). Previously we have reported that interleukin-1 (IL-1) and tumor necrosis factor-α (TNFα) inhibit hCG-induced cyclic AMP and testosterone formation and P450scc mRNA expression. In the present study, we found that the addition of IL-1β (0.1 - 10 ng/ml) or TNFα (0.1 - 10 ng/ml) markedly reduced StAR mRNA levels in a dose-dependent manner. In conclusion, expression of StAR mRNA in rat Leydig cells is up-regulated by hCG and down-regulated by IL-1β and TNFα. Multiple steps of steroidogenic pathway are affected by cytokines including the rate-limiting step, StAR.

003 PARTIAL PURIFICATION OF A LIPID FACTOR FROM TESTICULAR MACROPHAGES THAT STIMULATES TESTOSTERONE SECRETION BY LEYDIG CELLS J.C. Hutson, C. Garner* and P.A. Doris*. Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Testicular macrophages from adult rats have been shown to secrete an unidentified factor that stimulates testosterone secretion by Leydig cells. The purpose of the present studies was to purify this factor from medium of testicular macrophages following 24 hrs of culture. Leydig cell-stimulating activity was assessed by measuring the amount of testosterone secreted by Leydig cells in culture by RIA. It was found that the factor responsible for stimulating Leydig cells was extractable in organic solvents leaving no activity in the aqueous phase. Treatment with dextran-coated charcoal completely removed Leydig cell-stimulating activity. The active factor was concentrated from an ether extract and subsequently chromatographed on a C_{18} reversed phase HPLC column using a gradient of 70-100% methanol developed over 2 min followed by a 14 min elution with 100% methanol (all containing 0.1% acetic acid). Activity was found in a fraction with a retention time of approximately 11 min. This retention time was significantly longer than that observed for PGE_2 or testosterone (approximately 6 min), as well as other metabolites in the steroidogenic pathway leading to testosterone. In conclusion, we have been successful in partially purifying a lipophilic factor from testicular macrophages that stimulates testosterone secretion by Leydig cells. Studies are underway to determine the identity of this factor using tandem mass spectroscopy.

002 TESTICULAR MICROVASCULAR BLOOD FLOW AFTER LOSS OF LEYDIG CELLS. T. T. Turner and L.A. Caplis. Department of Urology, University of Virginia, Charlottesville, VA

It has previously been shown that testicular microvascular blood flow (MBF) exhibits vasomotion, or pulsatile flow not associated with heart beat or respiration. This vasomotion is apparently dependent on testosterone (Damber et al., Biol. Reprod. 37:1291). We have undertaken several qualification trials to establish the use of laser-Doppler flowmetry for the study of MBF in our lab. Further, we have tested the hypothesis that the loss of vasomotion after Leydig cell and testosterone depletion is due to a loss of responsivity of the microvasculature to adrenergic stimulation. Testicular MBF was studied with testes in the scrotum and in a terperature-regulated testicle recepticle. Mean ± s.e. MBF in control animals was 13.3 ± 1.7 perfusion units (PU)., the mean vasomotion amplitude was 3.4 ± 0.6 PU, and the cycle frequency was 10.3 ± 0.8 cycles per min. These MBF parameters did not differ between left and right testes, between scrotal testes and testes in the testis recepticle, or among testicular regions. In vivo perifusion of seminiferous tubules and their vasculature with a maximally stimulating concentration of epinephrine (0.1 ug/ul perifusion fluid) reduced MBF by 89%. Animals receiving 75 mg ethane dimethane sulfonate (EDS) five days prior to study showed elimination of Leydig cells and testicular venous testosterone. Mean MBF was unaffected by treatment, but vasomotor activity was eliminated. Nevertheless, EDS treated testes perifused with epinephrine as above still vasoconstricted to reduce MBF by 92%. We conclude that the loss of vasomotor activity after loss of Leydig cells and testosterone is due to regulatory effects on microvasculature and is not due to intrinsic changes in the vascular smooth muscle subsequent to the loss of testosterone or other Leydig cell products. This work supported by NIH grant DK 45179.

004 DEVELOPMENTAL PATTERN OF LEYDIG CELLS IN INTACT OR TRANSPLANTED TESTES IN FISCHER RATS.

L. Johnson, L.C. Suggs, and G.U. Falk. Department of Veterinary Anatomy and Public Health, Texas A&M University, College Station, TX 77843-4458.

Castrated host rats with transplanted testes have elevated serum LH concentrations; however, the transplants maintain accessory sex gland weights and serum testosterone concentrations at a level in hosts similar to intact rats. The objective was to characterize the developmental pattern of Leydig cell size, volume density, volume per testis, and number in intact and transplanted testes. Testes from 4-6 rats per group (10, 20, 30, 40, 60 days of developmental age or time after transplantation) were fixed with glutaraldehyde, further fixed in osmium, and embedded in Epon. Toluidine blue stained 0.5 μm sections were evaluated by stereology to determine the volume density of Leydig cells or Leydig cell nuclei. Unstained 20 μm sections were observed by Nomarski optics to determine the height and width of individual Leydig cell nuclei to calculate their volumes. Testicular parenchymal weight was greater (240 ± 64 vs 59 ± 12 mg; $p<0.01$) for intact than transplanted testes. Except for day 10, the volume density of Leydig cells or Leydig cell nuclei was greater in transplanted testes. Volume densities of Leydig cells increased early after transplantation and then stabilized, but tended to decrease with age in intact testes. The number of Leydig cells increased ($p<0.05$) with age or time after transplantation. With growth of both transplanted or intact testes, the volume/testis or number/testis of Leydig cells increased ($p<0.05$). The volume/testis of Leydig cells was similar in intact and transplanted testes. Individual Leydig cells were larger in transplanted testes (724 ± 99 vs 1,293 ± 94 fl; $p<0.01$), but their number was less (12.9 ± 3.6 vs $4.5 \pm 1.3 \times 10^9$; $p<0.05$). In spite of reduced testicular size of transplanted testes, the endocrine status of host rats was maintained by transplanted testes which had an increased volume density of and increased individual size of Leydig cells. This transplantation model may be useful in identifying factors that stimulate Leydig cells. NIH AG11093-10

005 NEONATAL ESTROGEN TREATMENT: EFFECTS ON SERTOLI CELL PROLIFERATION AND TESTIS DEVELOPMENT IN THE RAT. Kimberly L. Joyce*, Rex A. Hess and Paul S. Cooke. Dept. of Veterinary Biosciences, Univ. of Illinois, Urbana, IL 61801.

Neonatal estrogen treatment decreases adult rat testicular weight and sperm production and causes histological abnormalities in the seminiferous epithelium. However, the mechanism of this effect is unknown. Recent reports have suggested that estrogens could decrease adult testis size and function by decreasing proliferation of Sertoli cells, which express estrogen receptors and are the major regulator of the magnitude of sperm production. The goal of the present study was to determine if neonatal estrogen treatment altered Sertoli cell proliferation and/or Sertoli cell death in the rat. Male Sprague-Dawley rats were untreated (control) or injected on d at birth with 500 µg of estradiol benzoate subcutaneously. Sertoli cell proliferation was examined using thymidine autoradiography in control and treated rat testes at 5, 10, 15, and 20 days. Apoptosis was also examined using immunocytochemistry in control and treated rat testes at 5, 20, and 45 days. Remaining rats were euthanized at 90 days to determine a variety of reproductive parameters. Developmentally, at 5, 10, 15, 20, and 45 days, testis weights were decreased compared to controls. Sertoli cell proliferation was similar at 5, 10, 15, and 20 days in control and treated animals, with proliferation being very high at the first time point examined (5 days=12%). This value rapidly declined in both control and treated groups; Sertoli cell mitogenesis was completely absent by 20 days of age. In addition, in both groups apoptosis appeared to be confined almost exclusively to germ cells with no significant Sertoli cell apoptosis at any age. Histologically, germ cell development was similar in both groups initially, but by 45 days there was a marked decrease in tubule diameter and late-step elongated spermatids were diminished in the treated rats. In adult estrogen-treated rats, testis weights and daily sperm production were decreased 59% and 78%, respectively. Histologically, many tubules were devoid of germ cells and consisted only of Sertoli cells; however, some adjacent tubules were undergoing apparently normal spermatogenesis in adult estrogen-treated rats. In conclusion, the magnitude and duration of Sertoli cell proliferation and apoptosis do not appear to be affected by estrogen treatment. Thus, histological and functional abnormalities in seminiferous epithelium following early estrogen treatment may result from *functional* changes in Sertoli cells which impair their ability to support germ cell development and/or other effects induced by neonatal estrogen treatment (e.g., excurrent ductal occlusions) rather than decreased Sertoli cell mitogenesis or increased apoptosis.

006 EXPRESSION OF THE C-KIT RECEPTOR IN SPERMATOGENIC CELLS AND NORMAL SPERM. J.I. Sandlow, Huai-Liang Feng*. Department of Urology, University of Iowa, Iowa City, IA 52242-1089.

The c-kit proto-oncogene encodes for a transmembrane receptor and is associated with the maturation of several cell types including germ cells. Loss or alteration of its expression leads to anemia, albinism and sterility in mice. Therefore, it is important to study the expression of c-kit during spermatogenesis, spermiogenesis and sperm function.

We examined the expression of the c-kit receptor in the testis and sperm of the mouse, the rat and the human. Utilizing an immunostaining technique with a monoclonal antibody to the c-kit receptor, we stained the testes of normal mice and rats as well as biopsy specimens from normal men undergoing infertility evaluation. All human specimens showed normal spermatogenesis. We also compared the staining for the c-kit receptor in fixed and unfixed normal sperm from mice, rats and humans.

The results demonstrated specific staining for the c-kit receptor in the acrosomal granules (AG) of the spermatids and in the acrosomal region of spermatozoa in the testis. We noted weak staining in the cytoplasm of the early spermatogenic cells. Staining of the fixed and unfixed normal human sperm was demonstrated in the acrosomal region and the entire tail. Staining was noted in the acrosome and the principal piece of fixed normal mouse sperm, whereas unfixed mouse sperm showed staining in the acrosome and in the entire tail. In the normal rat, staining was noted only in the acrosome of both fixed and unfixed sperm. Following the acrosome reaction, staining for the c-kit receptor in normal sperm is absent.

These studies suggest that the c-kit proto-oncogene is expressed in the acrosomal granules of spermatids and the acrosome and tail of normal sperm in the mouse, rat and human. It appears that the pattern of expression is different for each species; however, this may be related to the type of antibody used. Following the acrosome reaction, the c-kit receptor is no longer expressed, suggesting a role in the acrosome reaction or fertilization. It is also possible that c-kit may play a role in the formation of the acrosome.

007 Partial Characterization and Localization of Galactose-Containing Glycoproteins on Rat Testicular Spermatozoa: Evidence for Their Degalactosylation by a Galactosidase Purified from Rat Epididymal Fluid. M.D. Skudlarek, M.-C. Orgebin-Crist, and D.R.P. Tulsiani. Center for Reproductive Biology Research, Vanderbilt University, Nashville, TN 37232.

Previous studies from this laboratory have identified a rat epididymal luminal fluid acid ß-D-galactosidase activity which also optimally hydrolyses a glycoprotein substrate at neutral pH (Skudlarek, Tulsiani, Orgebin-Crist, Biochem. J. 286:907-914, 1992). The luminal fluid ß-D-galactosidase has been separated into two molecular forms by ion-exchange chromatography on a column of DE-52 and the two forms have been purified to apparent homogeneity (Tulsiani, Skudlarek, Orgebin-Crist, Biochem. J. 305:41-50, 1995). The potential role of the enzyme in modification of rat sperm plasma membrane glycoproteins was examined by labeling the surface glycoproteins on intact spermatozoa from the rete testis and cauda epididymidis by oxidation with galactose oxidase followed by reduction with $NaB[^3H]_4$. The [^{3}H]labeled spermatozoa were detergent solubilized, resolved on SDS/PAGE under reducing conditions, and the radiolabeled glycoproteins were visualized by autoradiography. Data from these studies show the presence of three glycoproteins (apparent molecular weight 125 kDa (major), 145 kDa and 170 kDa (minor)) on testicular spermatozoa which were not present on cauda spermatozoa. The chemical nature of the [^{3}H]labeled oligosaccharide(s) was assessed by quantifying radioactivity released after treatment of the radiolabeled sperm membrane glycoproteins with various exo-and endo-enzymes. Most of the tritium was released after treatment with purified luminal fluid ß-D-galactosidase and O-glycanase. Little or no radioactivity was released by the treatment with N-glycanase, endo ß-D-galactosidase or N-acetyl ß-D-glucosaminidase. Taken together, the results suggest that the [^{3}H]galactosyl residue(s) is present on O-linked disaccharide(s) and that epididymal luminal fluid ß-D-galactosidase is capable of releasing the [^{3}H]galactosyl residue(s) from testicular sperm plasma membrane. O-galactosylated glycoproteins were localized on the sperm plasma membrane by cytochemistry using peanut agglutinin (PNA)-FITC, a lectin which preferentially binds to Gal ß1,3GalNAc-linkage found in O-linked glycoproteins. The lectin stained the acrosomal region of testicular (but not caudal) spermatozoa. Combined, these results suggest that O-linked glycoproteins present on the plasma membrane of the acrosomal region of the sperm head are degalactosylated during sperm maturation. Supported in part by Grants HD03820, HD25869 and HD05797 from NIH.

008

COMPARISON OF EPIDIDYMAL EPITHELIAL LOCALIZATION OF PROTEINS D AND E USING DUAL LABELING BY TWO MONOCLONAL ANTIBODIES AND CONFOCAL IMAGING.

Kathy M. Ensrud and David W. Hamilton, Department of Cell Biology & Neuroanatomy, University of Minnesota Medical School, Minneapolis, MN 55455.

Proteins D and E (AEG) are highly homologous proteins, secreted by the epididymal epithelium, that become associated with the sperm plasma membrane as sperm course through the epididymis. Using monoclonal antibodies specific for each of the proteins, Protein D has been shown to localize to the sperm head, while protein E resides on the tail. By employing a technique whereby two mouse monoclonal antibodies can be visualized with different fluorescent indicators on the same tissue section, and by using confocal imaging to detect two fluorochromes simultaneously, we have been able to show different patterns of epithelial expression for the two proteins along the length of the epididymis. Protein D expression occurs in the mid-caput region, continues into the distal caput and corpus regions at a lower level than in the caput, and then reappears in the epithelium of the cauda region. Staining is diffuse in the cytoplasm, and in many cells it is intense in the Golgi and along the microvillous border. Protein E is expressed in the distal caput epididymidis and continues into the corpus region. The antigen is first visualized as intense staining in the Golgi apparatus and on the microvillous border of the cells, with little diffuse staining apparent. No Protein E expression is observed in the cauda epithelium. These results suggest differential regulation of the production of these two highly similar proteins along the length of the epididymal duct. (Research support by USPHS grant HD-11962 to DWH)

009 IMMUNOCYTOCHEMICAL LOCALIZATION OF ESTROGEN RECEPTOR IN EFFERENT DUCTULES AND EPIDIDYMIS OF THE ADULT MALE RAT

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It has been known for many years that estrogenic compounds, such as DES, induce abnormal development and function of the male reproductive system when exposures occur perinatally. However, the mechanisms by which estrogens cause these abnormalities are not understood. Contributing to this lack of understanding is the fact that few data are available regarding the function of estrogen in the developing male and the sources of estrogen and its function in the adult male reproductive tract. Therefore, the present study immunocytochemically examines the reproductive tract of the adult male rat for the presence of estrogen receptors (ER). Tissues from the efferent ductules, epididymis and vas deferens were dissected at 4°C from anesthetized 100 day old Sprague-Dawley rats, cut into small pieces in 10% sucrose, treated with microwave irradiation (600 watts; 6 sec), and frozen in liquid propane (according to Slayden et al., Endocrinol. 136:4012, 1995). Frozen tissue sections were also treated with microwave irradiation to stabilize the ER and post-fixed in paraformaldehyde-picric acid prior to immunostaining using an ER21 antibody (anti-rat ER; supplied by Dr. Geoffrey Greene) and visualized using the avidin-biotin peroxidase staining kit. The strongest immunostaining was observed in efferent ductules and initial segment epididymidis regions of the male tract, with the nonciliated cells of the efferent ductules exhibiting a clearly greater intensity for ER than any other cell type among the epithelia lining the male tract. Principal epithelial cells showed a progressive decrease in ER immunostaining from the initial segment through the cauda epididymidis. Very weak to no staining was observed in the cauda principal cells and no staining was found in epithelial cells of the vas deferens. Ciliated cells of the efferent ductules were ER-positive, basal cells throughout the tract were moderately positive, but the clear cells showed no reactivity. Connective tissue cells were also ER-positive throughout the reproductive tract, but the smooth muscle cells beneath the epithelia exhibited the strongest staining. These observations suggest that the proximal regions of the male reproductive tract in the rat are primary targets for estrogen and that estrogen may play a role in the regulation of epididymal function, particularly the efferent ductules.

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010 ENDOTHELIN-1 IS PRESENT AND BIOLOGICALLY ACTIVE IN HUMAN EPIDIDYMIS.

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We previously reported that specific transcripts and proteins for endothelin-1 (ET-1) and related receptors are present in human testis (J. Androl., 16:213, 1995). In addition we reported the presence of authentic ET-1 in human seminal plasma. However, in the same study we did not detect significant differences among the seminal ET-1 levels found in normospermic, oligospermic, azospermic and vasectomized subjects. Hence, alternative sources other than Sertoli cells of the testis has been suggested for ET-1 in seminal fluid. We therefore initiated studies to verify if other portion of the male genital tract express ET-1 gene and protein. We found specific transcripts for ET-1 and its converting enzyme ECE-1 in human epididymis. Immunocytochemical studies indicated that the positive signal was localized in the endothelial cells of the vessels and in the abluminal tubular cells in direct contact with smooth muscle fibers. [^{125}I]ET-1 *in situ* autoradiography indicated that binding sites are present in smooth muscle cells of the tubules and blood vessels. To characterize these receptors we preformed binding studies on epididymal membranes obtained from patient undergoing surgery for different urological diseases. Mathematical analysis of families of competition curves among ([^{125}I]ET-1, [^{125}I]ET-3), the corresponding unlabeled peptides, the ETA antagonist BQ123, the ETB agonist IRL 1620 and the non-peptide antagonist SB209670 indicated the presence of two distinct subtypes of ET-1 receptotrs in epididymis. We essentially found elevated concentration of ETA receptors (5400 fmoles/mg. prot) and a lower density of ETB receptors (200 fmoles/mg prot.). Accordingly, smooth muscle cells express the mRNA for both the subtype of ET receptors. To investigate the functions of these receptors we performed isometric contractility studies on epididymal strips *in vitro*. We found that ET-1 was as potent as noradrenaline in stimulating epididymal contractility with EC_{50}=3 nM. In conclusion our study suggest that ET-1 and its receptor are not only present in human testis but also in epididymis and involved in the regulation of tubular contractility.

011 EFFECTS OF CHRONIC RENAL FAILURE (CRF) ON SPERM FERTILIZING CAPACITY

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We have previously shown that CRF has a detrimental effect on spermatogenesis and epididymal sperm maturation process in rabbits and rats (J Androl 1992, P45; Fertil Steril 1991, S104). In the present study we investigated the effect of CRF on sperm fertilizing potential. CRF was induced in 20 male four-week-old Wistar rats (group A) by performing five-sixths nephrectomies in two stages (see above references). An additional ten rats underwent a two stage sham-operation and served as a control group (group B).

Eight weeks later, peripheral serum creatinine and urea level were significantly higher ($P<0.05$; Wilcoxon's test) in group A than in group B. Caudal epididymal spermatozoa were collected from each animal and were processed for insemination of ten mature rat oocytes. Sperm suspensions containg 0.5×10^7 spermatozoa/ml were used. Caudal epididymal spermatozoa from CRF-animals but not from control animals had been filtered via SpermPrep ™ tubes to isolate sperm fractions showing strong forward progression. By doing so, there was no significant difference ($P>0.05$) between groups A and B in the % sperm motility in the final suspensions processed for inseminations. A volume of 0.05 ml of sperm suspension was introduced to 0.45 ml of Toyoda and Chang medium (J Reprod Fertil 1974, 36:9) containing the oocytes. The % oocytes with two pronuclei was significantly lower ($P<0.05$) in group A than in group B 18 hours after inseminations (23 vs 82, respectively). In addition, the % cleaved oocytes was significantly lower ($P<0.05$) in group A than in group B 36 hours after inseminations (13 vs 65, respectively).

The present findings suggest that CRF has an adverse effect on the overall sperm fertilizing capacity.

012 ACROSOMALLY INTACT OR ACROSOMALLY REACTED SPERM INJECTIONS INTO RABBIT OOCYTES.

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We compared the outcome of intracytoplasmic injections (ICSI) of acrosomally intact spermatozoa vs acrosomally reacted spermatozoa. Caudal epididymal spermatozoa were recovered from mature White New Zealand rabbits. Spermatozoa were washed in RD medium (J Reprod Fertil 1991;91:113) and resuspended at the same medium supplemented with 3 mg/ml rabbit serum albumin. Then, sperm samples were incubated at 37 °C under 5% CO_2 for 12 hours. At the end of the incubation period, 50 mature rabbit oocytes were injected with acrosomally intact spermatozoa (group A) and an additional 50 oocytes were injected with acrosomally reacted spermatozoa (group B). One spermatozoon was injected into an oocyte. A confocal scanning laser microscope providing high magnification (X 4,500) attached to microsanipulator was used for observation of the sperm acrosomal status. Injected oocytes were cultured in RD medium supplemented with taurine (10 mM) for 72 hours.

Group	Cleaved oocytes at 24 hours	Morulae plus blastocysts at 72 hours
A	13 a	2 a
B	42 b	18 b

Within each colusn: a vs b:$P<0.05$(Chi square test-Yates's correction).

The significantly lower fertilization and embryo development rates after ICSI procedures with acrosomally intact spermatozoa suggest that entrance of the acrosomic cap with its enzymes within the rabbit oocyte has detrimental effects on the fertilization process. Furthermore, oocytes fertilized with acrosomally intact spermatozoa have lower potential for development.

013 THE CORRELATION OF SPERM MORPHOLOGY WITH SPERM FUNCTION TESTS AFTER SWIM-UP AND PERCOLL SEPARATION

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Semen from patients who undergo intrauterine insemination (IUI) must be processed in order to remove the seminal plasma and debris and to enhance the fertilizing ability of the specimen. The most commonly used methods of sperm processing are Percoll gradient separation (PGS) and swim-up (SU). Both methods yield an acceptable motile sperm recovery. However, the impact of POS and SU on sperm morphology is variable. In order to determine if the method of sperm separation affects sperm function, the semen samples from 21 men were initially analyzed using the WHO and strict morphology criteria. Specimens were then divided and processed either by PGS or SU. The morphology assessment was repeated on processed sperm and specimens were incubated overnight in TEST-yolk buffer. The hamster zona-free ova penetration assay (SPA) and the sperm mannose receptor binding (ManRec) were then performed. The WHO morphology results were grouped into 5 categories according to the head size (small=tapered + small + flat & deficient acrosome + acrosomeless + pinhead; large=immature + large; round; coiled tails) and compared between the neat and processed samples. The %penetration in SPA (%SPA) and %mannose-bound sperm were correlated between different treatments. The results in the table are expressed as the mean±SD values (^{a}p<0.05 difference between neat and the treatment, ^{b}p<0.05difference between the treatments). The %SPA for SU sperm was significantly higher than for PGS sperm. There was a significant (p<0.05) negative correlation between WHO large head category and %SPA. Both, SU and PGS significantly reduced the number of coiled tail sperm. In conclusion, both sperm separation methods significantly increased the %normal forms by WHO criteria but not by strict criteria. The higher %SPA in sperm processed by SU as compared to PGS are probably due to other molecular changes not reflected by morphology. These functional alterations didn't include the ability to bind mannose which was not different between the treatments.

	Neat	PGS	SU
WHO normal	37.6±11.3	44.5±10.8^a	46.5±16.1^a
WHO small	29.5±10.5	29.4±9.5	30.3±12.1
WHO large	21.4±6.0	21.7±7.0	16.9±7.9^a
WHO round	3.9±2.6	3.3±2.8	3.2±2.9
WHO coiled	5.2±9.0	0^a	0.1±0.1^a
Strict/normal	22.1±10.5	23.5±13.3	25.9±13.3
Strict/amorph.head	19.4±8.2	22.0±8.0	19.9±7.8
Strict/amorph.neck	0.7±1.7	1.0±1.5	0.4±1.1
Strict/small acros.	35.0±12.7	35.8±12.3	32.9±12.2
Strict/no acrosome	8.9±5.4	5.57±3.1^a	6.7±3.9
Strict/acros>70%	14.1±6.9	12.2±8.2	14.2±9.1
SPA: %penetration	N/A	23.5±21.65^a	37.9±24.7^a
ManRec: %Type II	N/A	1.7±1.8	1.0±1.3
ManRec: %TypeIII	N/A	4.9±5.9	5.1±5.9

Abstract withdrawn.

015 VALIDATION OF HYPOOSMOTIC SWELLING AS A VIABILITY TEST BY HOECHST-33258 AND EOSIN-NIGROSIN IN FRESH AND CRYOPRESERVED HUMAN SPERMATOZOA. S.C. Esteves*, R.K. Sharma, A.J. Thomas Jr. and A. Agarwal. Andrology Research & Clinical Laboratories, Department of Urology, Cleveland Clinic Foundation, Cleveland, OH 44195.

Assisted reproductive procedures such as intracytoplasmic sperm injection (ICSI) are possible even with a few non-motile but viable spermatozoa. Sperm viability rather than motility is critical in assisted reproduction. Motility excludes cells that are viable but nonmotile. Assessment of viability by vital stains is accurate but their effects on sperm integrity is unclear. The hypoosmotic swelling (HOS) test is advocated as an indicator of membrane integrity and normal functional ability in fresh human spermatozoa. However, reports on its use in cryopreserved samples are lacking. The purpose of this study was to determine the validity of HOS as a test of viability in fresh and frozen human spermatozoa. Fresh semen specimens from 11 normal donors were divided into two aliquots: the first aliquot received no treatment, and the other was processed by the swim-up technique. Both aliquots were then diluted with TEST-yolk buffer (1:1 v/v) and cryopreserved by the liquid nitrogen vapor method. To validate the results of HOS test each specimen was evaluated by eosin-nigrosin (EN) staining and Hoechst-33258 staining before and after freezing. Two hundred spermatozoa from each specimen were scored to assess the percentage viability. A highly positive correlation was seen in both raw and swim-up specimens (r=0.95, P=0.0001) between the viability scores of fresh sperm measured by the HOS test and by Hoechst-33258 staining. After cryopreservation, no correlation was seen between HOS test scores and Hoechst-33258 or EN scores. However, there was a high correlation between Hoechst-33258 and EN scores (r=0.72; P<0.004). HOS test is a simple and non-deleterious assay that can accurately evaluate viability in fresh human sperm. However, this test may not be clinically helpful in the selection of viable cells in cryopreserved samples for assisted reproduction.

016

INITIAL SPERM VIABILITY ASSESSMENT PREDICTS THE SURVIVAL OF VIABLE SPERM AFTER SEPARATION

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Semen analyses (SA) provide data that is utilized in the management of infertile patients. Most clinicians review sperm concentration, total sperm numbers, % motility and morphology data carefully. Although sperm viability is also routinely presented, the value of this data is not clear. This retrospective review of sperm viability data of 29 patients undergoing SA and swim-up separation for intrauterine insemination was designed to evaluate the predictive value of fresh sperm viability status.

SA were performed according to WHO standards. Motile sperm were separated by a swim-up method. Sperm concentration, viability and percent motility were assessed before and after swim-up and compared to values obtained after overnight incubation (O/I). The fresh sperm mean viability was 75%; increasing to 85% after swim-up (p<0.001). After O/I, viability decreased to 61% (p<0.001). Mean motility of the fresh sperm was 29%; after swim-up motility increased to 73% (p<0.001) and decreased to 29% (p<0.001) after overnight culture. Of the 26 patients with normal initial viability, 7 (27%) had abnormal viability after O/I. Of the 3 patients with abnormal initial viability, 2 had normal viability after separation, however only one remained normal after O/I. These 3 had abnormal initial motility and after O/I, all had abnormal motility.

Fresh sperm viability may have some predictive value of sperm survival after separation techniques. Although our preliminary conclusions require further analyses including a larger number of patients, SA with poor initial viability appear likely to have abnormal sperm survival even after separation of viable motile sperm. However, individuals with normal initial viability are not assured of maintaining normal viability and motility after sperm preparation and incubation. Initial viability should be assessed and utilized in the formulation of patient treatment plans.

017

PROCESSING OF HUMAN SPERMATOZOA DOES NOT REDUCE MEMBRANE DAMAGE OR SPONTANEOUS ACROSOME REACTION FOLLOWING CRYOPRESERVATION. R.K. Sharma, Sandro C Esteves*, A.J. Thomas Jr. and A. Agarwal, Andrology Research & Clinical Laboratories, Department of Urology, Cleveland Clinic Foundation, Cleveland, OH 44195.

In spite of advances in assisted reproduction, the survival and fertilizing ability of thawed spermatozoa is poor. Sperm processing is a fundamental step in assisted-reproduction. The purpose of this study was to evaluate if sperm processing by swim-up technique before freezing reduced post-thaw membrane damage and spontaneous acrosome reaction. Samples from 15 normal donors were divided into two aliquots. The first aliquot was frozen without any treatment (unprocessed), and the second after processing by the swim-up method. The samples were evaluated for sperm motion characteristics with a computer-assisted semen analyzer. Viability and membrane integrity were assessed with Hoechst-33258 staining and the hypoosmotic swelling (HOS) test. Acrosomal status was evaluated by fluorescent isothiocyanate-conjugated peanut agglutinin assay before freezing and after thawing. Compared with the unprocessed samples, samples processed by the swim-up method had better motion characteristics, viability, and membrane integrity before freezing ($P<0.005$). After thawing, the above measures decreased significantly ($P<0.005$) in both types of samples. Spontaneous acrosome reaction was significantly increased in viable spermatozoa in both types of samples ($P<0.005$) after thawing. Although the swim-up technique allows selection of a highly motile, viable, and acrosome-intact sperm population in fresh specimen, this improvement is not seen after cryopreservation. Acrosome damage in viable cells after cryopreservation is independent of prior treatment. The decrease in sperm motion and functional parameters after cryopreservation can explain the poor fertilizing ability of human spermatozoa especially in samples with poor pre-freeze semen characteristics as in oligozoospermic and cancer patients.

019

IDENTIFICATION, LOCALIZATION AND ACTIVATION OF P42-MITOGEN-ACTIVATED PROTEIN KINASE ($p42^{MAPK}$,ERK2) IN HUMAN SPERMATOZOA.

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Mitogen-activated protein kinases (also named extracellular-regulated kinase, ERK) are important intracellular enzymes that convert extracellular signals in intracellular responses, and are involved in mediating several cell functions such as growth and differentiation. However, recent evidence suggests the possibility that these enzymes are also involved in the activation of highly differentiated, terminal cells. Moreover, a protein kinase with similar characteristics to mitogen activated protein kinases has recently been characterized in bull spermatozoa (Berruti 1994). We therefore investigated the presence of ERK2 in human sperm by using an experimental approach involving immunowestern blot analysis and immunohistochemical detection of the protein as well as immunokinase assay using both a monoclonal and a polyclonal antibody against ERK2. Immunostaining of adult human testis demonstrated a selective, intensive staining of ERK2 in the germ cells, while Sertoli and Leydig cells were negative. No staining was observed in the interstitial compartment. In the epididymis intense staining was present in the acrosome of maturing sperm and in tubular epithelial cells. In ejaculated spermatozoa, a selective localization of the protein to the acrosomal cap region and the miedpiece of the spermatozoa was demonstrated by immunostaining, while no staining was detected in the tail region. Interestingly, in acrosome reacted spermatozoa the protein was selectively localized to the equatorial segment, thus suggesting a redistribution of the protein during acrosomal exocytosis. Such finding prompted us to study the effect of a physiological stimulus of acrosome reaction, progesterone, on ERK2 activity by evaluating phosphorylation of a specific substrate with an immunokinase method. We found that progesterone stimulated a time-dependent 2.5-3 fold increase of ERK2 activity with a maximum between 5 and 10 minutes, suggesting a possible involvement of the kinase in the biological action of progesterone in human sperm. The occurrence of ERK2 in human sperm was also confirmed by Western blot analysis which revealed the presence of a major band with apparent molecular weight of 42 kDa. Such protein appears to be tyrosine phosphorylated during capacitation, as indicated by western analysis using a monoclonal antiphosphotyrosine antibody. In conclusion, our study suggests the presence of a functional ERK2 in the acrosomal region of ejaculated and maturing human spermatozoa; further studies will be necessary to establish a possible role of this enzyme in sperm maturation and in acrosome reaction

018

A BILATERAL EFFECT OF LEFT VARICOCELE (LV) ON EPIDIDYMAL SPERM ACROSIN PROFILES

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We investigated the effect of LV on bilateral caudal epididymal sperm physiology and function in the guinea pig.

The left testicular vein (LTV) of the guinea pigs used drained into the renal vein medial to the entrance of the adrenal vein. Ten animals were sham-operated (group A). LV was induced in 25 animals (group B) by partially ligating the renal vein medial to the entrance of the LTV. Eight weeks later, the induction of LV in group B was confirmed by laparatomy. At that time, animals in group A underwent a second sham-operation.

Fifteen weeks after the initial surgical procedure testicular vs intraabdominal temperature difference (ΔT;°C) was assessed bilaterally. Spermatozoa were collected from the left and right epididymal cauda and washed in Thyrode medium. Sperm motility was assessed. Sperm total acrosin activity (mIU/10^7 spermatozoa; STAA) was evaluated as previously described by Goodpasture and co-workers (Biol Reprod 1981, 25:44).

Group	Left side			Right side		
	ΔT	% motile sperm	STAA	ΔT	% motile sperm	STAA
A	4.1 [a]	88 [a]	113 [a]	4.0 [a]	84 [a]	118 [a]
B	2.3 [b]	36 [b]	58 [b]	2.4 [b]	53 [b]	83 [b]

Values are expressed as means. Within each column a vs b: $P<0.05$. Wilcoxon's test was applied for statistical analysis.

Our findings suggest that the induction of LV in the guinea pig results in an increase in testicular temperature bilaterally. As well, LV has a detrimental effect on bilateral caudal epididymal sperm motility and function.

020

PURIFICATION OF A HUMAN SPERM MATURATION-RELATED GLYCOPROTEIN, GP-83

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In epididymis, mammalian sperm interact with epididyma secretions, and acquire forward motility and ability to recogni and penetrate zona pellucida of eggs. Epididymal secretions related to sperm maturation are identified in rat, hamster, mouse, monkey and human. In human epididymis, our previous studies identified two sperm maturation-related glycoproteins, GP-83 and GP-39. In this study, GP-83 was purified from human seminal fluid by DEAE-ion exchange, gel filtration chromatography and gel elution. GP-83 was a glycoprotein which exhibited strong affinity to Con-A, PNA an WGA. The pI of GP-83 was 6.57. Monospecific antiserum to G 83 was induced in male New Zealand rabbits. On immunoblot antiserum was specific to GP-83. GP-83 was found in fluid an tissue of corpus and cauda epididymis, but not in the caput. Immunohistochemical localization revealed positive response principal cells and luminal contents of corpus and cauda epididymis. In the principal cells, GP-83 was found in supranuclear region and on cell membrane. In cultured principal cells of human corpus epididymis, GP-83 was found in Golgi apparatus. In conclusion, GP-83, a human sperm maturation-related glycoprotein, is secreted by principal cells and conjugated to sperm in corpus and cauda epididymis. (This study was supported in part by a grant from the Nation Science Council of the ROC, NSC 84-2331-B-016-091).

021 SPERM MEDIATED EFFECT ON MOUSE EMBRYO DEVELOPMENT.

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Exogenously added reactive oxygen species (ROS) have been shown to be detrimental or beneficial to mammalian spermatozoa depending on the type and level of ROS. Hydrogen peroxide (H_2O_2) is the primary ROS responsible for toxic effects on sperm motility. Since spermatozoa, even under physiological conditions, produce ROS, we investigated whether the low levels of H_2O_2 generated by spermatozoa could be detrimental to sperm motility, fertilizing ability and potential to support embryo development. The first study examined the effects of three concentrations of catalase (CAT) in the incubation medium for mouse spermatozoa, prior to in vitro fertilization (IVF). Mouse (CD-1) spermatozoa released from the cauda epididymis were treated with CAT (8, 14 and 32 µg/ml) at 37°C in MEM medium for one hour, washed on a mini-Percoll gradient and immediately used for insemination (with oocytes from 7-8 week old CD-1 mice) at a final concentration of 10^4 cells/ml. Motility parameters and fertilization rate were not significantly affected by CAT treatment. However, The rate of blastocyst and hatching blastocyst formation when CAT (16 µg/ml) treated spermatozoa were used, were significantly higher than those observed with non-treated spermatozoa (44% vs. 24%, 31% vs. 2%, $p<0.05$, respectively). Because sulhydryl groups on proteins are primary targets for H_2O_2, we have investigated whether, thioredoxine (TRX), a powerful sulhydryl reducing protein, could protect spermatozoa from the H_2O_2 they produce. When added to the medium for the 1h incubation prior to IVF, thioredoxin did not affect the percentage of motile cells and the fertilization rate, but caused an increase in hyperactivation from 16±3% to 32±4%. It also increased the rate of blastocyst formation from 6% to 16% to an extent similar to that triggered by CAT. These results suggest 1) that H_2O_2 produced by spermatozoa under normal physiological conditions can be detrimental to their potential to promote embryo development, 2) that these toxic effects of H_2O_2 are latent, appearing mainly 3-5 days after IVF, and 3) that CAT and TRX are efficient agents to protect the sperm potential to support embryo development.

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022 MOLECULAR CLONING OF THE GENE ENCODING GP-83, A HUMAN SPERM MATURATION-RELATED GLYCOPROTEIN

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Sperm maturation in epididymis is essential for sperm to develop fertility. Several genes of sperm maturation-related proteins, such as PH-30, EAP-1, HE 2 and 3 have been isolated and sequenced. DNA sequence of these molecules reveal proteinase- and disintegrin-encoding domains which are the same as those encoded in the genes of a variety of snake venom. Our previous studies identified a sperm maturation-related glycoproteins, GP-83, in human epididymis. In order to analyze DNA sequence and predict the biological significance, GP-83 encoding cDNA was isolated from cDNA library of human epididymis by immunoscreening using GP-83 specific antiserum. Three positive clones containing inserts of 1.5 Kb, 1.8 Kb and 4.0 Kb were isolated. Partial sequences of these inserts, up to 300-400 bp from 3' end, did not reveal any significant homology to known genes in the GenBank. In cDNA library of snake venomous gland, however, an ancrod-like sequence was identified. In conclusion, GP-83 may be a human epididymis-specific protein containing disintegrin-like domain. (This study wa supported in part by a grant from the National Science Council of the ROC, NSC 85-2331-B-016-097).

023 COCULTURE OF HUMAN SPERM WITH OVIDUCT CELLS

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Human, macaque and bovine oviduct epithelial cells (OEC) were recovered from females in the follicular phase. OEC explants were cultured on plastic, passaged and subsequently frozen. For use in this study, the thawed OEC from each species were grown to confluent monolayers on Matrigel in 2 cm^2 wells. Semen from four fertile men (i.e. normal semen analysis and children <2 yrs of age) was analyzed for CASA motility, viability (V) and hypo-osmotic swell (HOS). Sperm from each donor were then either used immediately (fresh) or routinely frozen for later use. Fresh or frozen sperm [12 million/500 ul Human Tubal Fluid (HTF)] were then placed across 5 treatment groups: bovine, macaque or human OEC; Matrigel (MG) alone; and HTF alone. All treatments were incubated at 37°C in 5% CO_2 and air. After one hour the sperm which had failed to attach to the different OEC or MG were removed by rinsing. Fresh HTF was placed over the the remaining sperm which were attached to OEC or MG in each group. The unattached sperm were counted, and analyzed for V, HOS and CASA. An aliquot of HTF control sperm was also analyzed. The attached sperm which remained in the coculture wells were qualitatively evaluated for flagellar motion. Sperm which detached from the OEC or MG during the initial 4 hours of culture were removed and evaluated as above. The motility of both attached and detached sperm in each treatment was then evaluated at 24 hour intervals until less than 5% of the sperm were motile. Sperm attached to all types of OEC, and the edges of the MG wells showed vigourous flagellar motion. Numbers of sperm attaching did not differ for any OEC, and tended to be the same for MG ($p=0.15$). However, fewer frozen sperm attached ($p=0.02$) than fresh sperm in all groups. Both fresh and frozen sperm survived longer ($p=0.05$) in coculture with bovine OEC than in HTF alone. The other treatments tended to support longer survival ($p \leq .13$) than the control. Frozen sperm survival was 48 hours less in coculture than that seen with fresh sperm for all groups. Sperm in coculture with OEC showed more ($p<0.05$) hyperactivation than did sperm with MG or control HTF. This tended to be higher in the macaque and human OEC groups ($p=.09$). The path velocity of fresh and frozen sperm which had detached from the OEC was faster ($p<0.5$) at 4 hours then that seen in the control group. These data demonstrate that sperm attachment to OEC in vitro exerts a beneficial effect on sperm survival, motility and capacitation. This effect was seen for both fresh and frozen sperm; however, frozen sperm did not attach as well to OEC or MG, or survive as long in coculture as fresh sperm. Funded by NIH FIRST to JEE.

024 PROTEIN KINASE A (PKA) MAY BE INVOLVED IN THE PLATELET ACTIVATING FACTOR (PAF) INDUCED HUMAN SPERM ACROSOME REACTION (AR).

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This study examined the role of PKA in the PAF-induced AR. The PKA inhibitor N-[2-(methylamino)ethyl]-5-isoquinolinesulfonamide dihydrochloride (H-8), and the PKA inducers forskolin and dibutyryl cyclic AMP (db_cAMP) have been used in preliminary studies to demonstrate PKA involement in the AR. In these two experiments, Percoll separated sperm were capacitated using 0.6% HSA in BWW and the effect of various inducers and inhibitors of the AR were examined using Pissum sativum lectin. In **Experiment I**, sperm were pre-incubated with H-8 (10µM) followed by a challenge with PAF (1pM), forskolin (10µM), or db_cAMP (10µM). PAF challenge of sperm not preincubated with H-8 (positive control) resulted in an AR of 18%; after H-8 exposure the AR was <8% in all groups following challenge. In **Experiment II**, sperm were pre-incubated with the PAF receptor antagonist, CV3988 (0.1µM) then challenged with PAF, forskolin, or db_cAMP. PAF challenge of sperm not pre-treated with CV3988 was used as a positive control. Preincubation of sperm with CV3988 reduced the AR (21%) in the antagonists-untreated group to less the 5% after antagonist exposure in all groups. These results implicate PKA in the PAF-induced AR.

025 COMPARATIVE STUDY OF SCREENING TEST FOR ANTISPERM ANTIBODY.

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Immunobead testing, the current standard for antisperm antibody detection, is time consuming and somewhat subjective. To overcome these limitations and maintain accuracy, two current available assay kits were studied.

The mixed antiglobulin reaction test(MAR) and the SpermCheck assay kit which are commercially available were performed. They were screened for antisperm antibody, and the procedures and diagnostic accuracy were then compared. A total of 61 samples ; 25 sperms and seminal plasmas from 23 infertile men and 2 prostatitis patients and seminal plasmas from 11 vasectomized cases were evaluated by both methods.

The results showed that 3 out of 25 sperm samples(12%) and 5 out of 36 seminal plasmas (13.9%) were positive in both method. On comparison of the procedures between these 2 assays, SpermCheck Assay took 20 minutes to detect, MAR took 3 minutes.

In this series, the rate of detection of sperm antibody was exactly same by both methods.

Above all, it is suggested that MAR is highly sensitive and a simple screening assay for detecting sperm antibody.

Therefore, MAR is the recommended test for the office setting.

027 A 18 kDA NUCLEASE IS ASSOCIATED WITH RAT SPERMATOCYTE APOPTOSIS INDUCED BY 2-METHOXYETHANOL (ME)

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Recent investigations in our laboratory revealed the appearance of a divalent metal cation-dependent endonuclease in testes from 24-day-old rats containing apoptotic spermatocytes in response to a single oral dose of ME. This nuclease was able to cleave a substrate DNA (in the form of chicken red blood cell nuclei) into a pattern of DNA fragmentation on agarose gels that consisted of ordered multiples of ~200 base pairs. The appearance of the DNA "ladders" was coincident with the appearance of morphologic spermatocyte degeneration and positive staining using an *in situ* 3' end-labeling procedure. Nuclear extract from control animals failed to degrade DNA. Further characterization of the nuclear extract from treated animals, using a radioactive gel nuclease assay, localizes the nuclease activity to an 18 kDA protein. This activity is dependent on calcium and is inhibited by both zinc and aurintricarboxylic acid (ATC). Interestingly, this nuclease activity co-localizes with both micrococcyl nuclease and cyclophilin A. Cyclophilin A recently has been demonstrated to be a calcium dependent nuclease, also inhibited by zinc and ATC. Cyclophilins are found in organisms as diverse as yeast and mammals, and in virtually every mammalian tissue examined, and are believed to play roles in protein folding and DNA degradation. Immunohistochemistry using antibodies to cyclophilin A demonstrates specific staining in pachytene spermatocytes, elongating spermatids, and in interstitial cells, but not in Sertoli cells. Our current hypothesis is that the nuclease activity we are observing is attributed to cyclophilin A. Protein sequence data are pending.

026 BINDING OF VASOACTIVE INTESTINAL PEPTIDE TO HUMAN SPERM SURFACE

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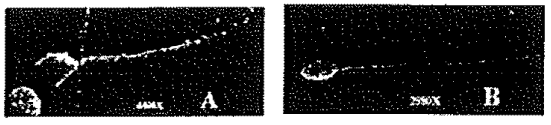
Vasoactive intestinal peptide (VIP), a 28-amino acid peptide, is present in many tissues. In target cells, VIP binds to specific surface receptor(s) and stimulates adenyl cyclase activity, leading to elevated intracellular levels of cyclic 3',5'-adenosine monophosphate (cAMP). Since the initiation and maintenance of sperm motility has long been known to be dependent on the effects of cAMP, we speculate that human sperm may be a target of VIP activity. In this study, using immunohistological techniques, we investigated if VIP binding sites are present on human sperm from 4 subfertile men and 4 normal men. Washed human sperm were incubated with and without 10 µg/ml VIP, and VIP binding to sperm surface or "receptors" identified using specific polyclonal rabbit anti-rat VIP antibodies. Antibodies were omitted in the controls. Presence of sperm surface VIP-antibody complexes was visualized by 1) goat anti-rabbit horseradish peroxidase conjugate binding with dimethyl benzidine (DAB) as substrate and 2) scanning electron microscopy (SEM) used in conjunction with anti-rabbit antibodies tagged with 20 nm gold particles (shown below). DAB staining was present on the surface of sperm incubated with VIP. In the controls, the sperm were devoid of staining.



A: Human sperm showing gold labelling of VIP bound to receptors (VIP present in media)
B: Absence of gold labelling (same sperm specimen incubated without VIP in media).

This is the first study to show the presence of VIP binding to the surface of human sperm. While the role(s) of VIP in sperm function is unknown, its reported stimulatory effects on adenyl cyclase activity suggests that VIP may participate in the initiation and attainment of maximal sperm motility important to successful penetration and fertilization of the oocyte.

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028 KINETICS OF MOUSE SPERM CELL LYSIS IN HYPOTONIC MEDIUM: IMPLICATIONS FOR DETERMINATION OF THE WATER PERMEABILITY (L_p) OF THE MURINE SPERM PLASMA MEMBRANE.

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Calculation of L_p values for mammalian sperm plasma membranes is usually done by the cell lysis method: cells are suspended in hypotonic media and the critical osmolality, Osm_{crit}, at which 50% of the cells lyse and the time, t_{crit}, to 50% lysis in a medium of $Osm << Osm_{crit}$ are measured. We have determined the kinetic form of the cell lysis reaction for mouse sperm suspended in medium of 3 mOsm, > 8-fold lower than Osm_{crit}, at 37°, 22°, 12°, 4°, 0°, and -3°C. The cells were pretreated with 5 µM cytochalasin D to disrupt the strong interaction between cytoskeleton and plasma membrane in mouse sperm. At the four higher temperatures, the kinetics were monophasic first order: values in sec^{-1} for the rate constant k_1 were: 0.048, 37°; 0.0092, 22°; 0.0112, 12°; 0.0045, 4°. Corresponding values in $\mu m \cdot atm^{-1} \cdot min^{-1}$ for L_p, calculated from the halftimes corresponding to k_1 and previously determined Osm_{crit} values, were: 0.111, 0.024, 0.019, and 0.0093, respectively. These values are calculated based on the low amplitude swelling model, shown previously to be the more valid one for mouse sperm. The Arrhenius plot for k_1 gave an activation energy, E_A, of 11.2 kcal/mol; that for L_p gave E_A of 12.3 kcal/mol. At 0° and -3°, the kinetics were biphasic first order, with k_1 in sec^{-1} for the rapid phase of 0.32 and 0.42, and for the slower phase of 0.0072 and 0.015, respectively. The fast phase accounted for 64% of the cells, the slow phase for 36%. This result supports our previous suggestion that a flexible-to-brittle membrane phase transition occurs between 4° and 0°. Cell lysis requires two sequential reactions: water influx into the cell and membrane rupture. L_p was calculated assuming that water influx is the rate-determining step, but the cell lysis method cannot distinguish between the two. We propose that the E_A values are consistent with a contribution from the membrane rupture reaction and that L_p values for mammalian sperm gotten by the lysis method be viewed with caution.

Supported by NIH grant HD-31757

029 **ISOLATED DEFECT OF ACROSOMAL FUNCTION IN MEN WITH OTHERWISE NORMAL SPERM MATURITY AND FERTILIZING POTENTIAL.** G. Huszar and L.Vigue*. The Sperm Phys. Lab., Dept. OB/GYN, Yale Univ. Sch. of Med., New Haven CT, USA

Aim: We have shown in blinded studies that the >10% creatine kinase(CK)-M isoform ratio, a marker of sperm cellular maturity, predicts the occurrence of pregnancies with a rate of >30% per IVF cycle. Sperm with low CK-M ratios were diminished in oocyte binding. In the present work we examined whether the ability of sperm to undergo capacitation and acrosome reaction (AR), in response to controlled exposure to calcium and progesterone, were related to sperm maturity and the CK-M ratios.

Methods:The washed semen samples of 46 husbands of infertile couples (conc: 67.8±6.5 x 10^6 sperm/ml, all data mean±SEM), were incubated at 37°C for 3 hours with 5mM $CaCl_2$ and subsequently 3μM progesterone was added. The rates of capacitation and AR were followed with chlortetracycline and FITC-PSA fluorescent patterns (200 live sperm at each time point) at 0 time and at 3 hours (prior to addition of progesterone), and after two hour and overnight incubations in the presence of progesterone.

Results: The incidence of acrosome intact sperm at the four time points were: 94±1%, 86±2%, 74±3% and 49.7±4% (N=44). In order to gain further insight, we have analyzed the overnight AR rates in relation to factors that are associated with diminished fertility. There were no differences in **oligospermic vs. normospermic** (34±13 vs. 56±7%), in **asthenospermic vs. normal motility** (53±10.9% vs. 51.8± 7.8%) samples or in men with **<10% vs. >10% CK-M ratio** (64.9±6.4% and 47.4±8%). Most revealing were the group of 17 **normospermic men with normal CK-M ratios:** 7 samples showed high rates and 10 samples low rates of AR after overnight incubation (80.3±9% vs. 21.4±2%, p=0.001). The chlortetracycline patterns indicated that capacitation occurred at a similar rate in both groups. The difference was due to the final step: to the loss of the acrosomal cap.

Conclusions: 1) In a defined environment there was a sample-to-sample variation in AR response following exposure to calcium/progesterone. 2) There was no relationship between AR response and semen parameters that are associated with diminished fertility. 3) As found previously with the hamster SPA and ARIC studies, there is a subpopulation of men who may have mature sperm and CK-M ratios, but show a diminished rate of AR activation in physiological conditions (Supported by HD-32902).

030 SINGLE INTRAMUSCULAR INJECTION OF 1000 MG TESTOSTERONE UNDECANOATE TO HYPOGONADAL MEN MAINTAINS PHYSIOLOGICAL SERUM TESTOSTERONE LEVELS FOR 7 WEEKS

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In hypogonadal men testosterone (T) substitution therapy is mandatory to induce and maintain physiological androgen actions. T enanthate (TE) (or T cypionate) is predominantly used but has to be injected every 2 to 3 weeks because of its relatively short elimination half-life of 4.5 days. Moreover, this ester results in supraphysiological T levels shortly after injection. While T buciclate (TB), with a half-life of 29.5 days, would be ideal for substitution (Behre & Nieschlag, J Clin Endocrinol Metab 1992;75:1204-10), its clinical development appears rather slow. In search of alternatives we recently demonstrated a prolonged duration of action of intramuscularly injected T undecanoate (TU) in cynomolgus monkeys (Partsch et al., Eur J Endocrinol 1995;132:514-9) and subsequently performed a clinical phase I-study on the single dose pharmacokinetics of TU. Hypogonadal patients were given intramuscular injections of 250 mg TU (group I; n=7) or 1000 mg TU (group II; n=7). Follow-up examinations were performed 1, 2, 3, 5 and 7 days after injection and then weekly up to study week 8. Whereas no prolonged increase of T was observed in group I, in group II serum levels of T increased from 4.8 ± 0.9 nmol/l (mean ± SEM) to maximum levels of 30.5 ± 4.3 nmol/l at day 7. T levels remained within the normal range (10 - 35 nmol/l) up to week 7 (13.5 ± 1.2 nmol/l). Serum estradiol showed a dose and time-dependent increase with maximum levels of 116.4 ± 13.9 pmol/l in group II 14 days after injection. Non-linear least squares regression analysis revealed a terminal elimination half-life for intramuscular TU of 20.9 ± 6.0 days. In conclusion, TU has an intermediate half-life between TE and TB and its favourable pharmacokinetics make it well suited for androgen substitution therapy and male contraception.

031

Pharmacokinetics and efficacy of transbuccal testosterone in the treatment of hypogonadal men. Adrian Dobs MD, MHS, Kathy Lesh RN, BS, EdM, MS, Donald Hoover PhD, Richard Allen PhD. From the Department of Medicine and Epidemiology. Baltimore, MD

Transbuccal administration of drugs provide a easy route of administration. To test the safety and efficacy of a novel testosterone (T) product, we performed a randomized, double-blind, placebo-controlled study at Johns Hopkins Hospital. Men with serum T levels <250 ng/dl were and administered either an active buccal tablet containing 10 mg of testosterone (n=7) or a placebo buccal tab (n=6) containing 3 mg of pseudoephedrine HCL for taste matching. Men were studied while on a standard T enanthate dose, after 6 weeks of a wash-out period, and after 8 wk of therapy. Pharmacokinetics data will be presented here. The treated and control groups were matched for age (41±16 vs.47 ±16 yrs.,mean ±SD) and type of hypogonadism (3 primary testicular failures in each group, with the remainder having a central etiology). Acute pharmacokinetic testing showed peak serum hormone levels 30 min after administering with serum T concentrations of 2688±147 ng/dl (range 1820 to 3770 ng/dl). Levels returned to baseline in four to six hours. Similar pharmacokinetics were observed for the hormone metabolites, bioavailable T, free T and estradiol. After 12 weeks of treatment, men on active drug had nocturnal penile tumescence studies comparable to that performed while on T enanthate therapy. In conclusion, transbuccal T therapy may provide adequate sexual function even though its pharmacokinetics profiles reveal rapid absorption. The benefits of short term exposure to circulating T will be discussed.

032 **DECREASED BONE MINERAL DENSITY AT THE HIP OF ADULT HYPOGONADAL MEN AS COMPARED WITH NORMAL PUBERTAL BOYS**

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Data comparing the bone mineral density (BMD) of the adult hypogonadal men and the normal pubertal boys are scarce.

The aim of this study was to compare the BMD and the influence of the total fat and lean body masses on the BMD, assessed by dual-energy X-ray absorptiometry (DXA), in adult hypogonadal men and in normal boys groups.

Material and methods: The BMD (g/cm^2) at the lumbar spine (L_2-L_4), at the femoral neck, at the trochanter and at the Ward's triangle and the body composition (Kg) measurements were performed by DXA, using a XR 26 Mark II-Norland system, in hypogonadal men (*n=13*, mean age = 42.5 years, range: 22-57 years), on androgen therapy just in adult age) and in normal pubertal boys (*n=13*, mean age = 16.3 years, range: 14.1-17.8 years) groups. Body mass index (BMI = Kg/m^2) was calculated. Data were evaluated by the One-Way Anova and the Spearman's correlation coefficient tests.

Results: The mean BMD at the trochanter and at the Ward's triangle were decreased in the adult hypogonadal men ($p<.03$). Diminished fat ($p<.03$) and lean ($p<.02$) masses and BMI ($p<.01$) were detected in the pubertal boys group.

Conclusions: These data suggest that the hypogonadal men, on androgen treatment during adult age, have an increased risk for osteoporosis; possibly, the decreased BMD is related with an inadequate bone mass peak. However, further studies should be performed in order to clarify the reduced BMD etiology in hypogonadal men on androgen therapy.

033 THE ROLE OF CADMIUM IN THE ETIOLOGY OF VARICOCELE ASSOCIATED INFERTILITY. S. Benoff[1], I.R. Hurley[1], M. Barcia[1,*], F.S. Mandel[2,*], D.Kostouros[1,*], G.W. Cooper[1,*] and A Hershlag[1,*], Depts of [1]Obstetrics & Gynecology and [2]Research North Shore University Hospital, Manhasset, NY 11030.

Our objective is to define mechanisms underlying the negative interaction between cigarette smoking and varicocele on sperm fertilizing potential. Smoking increases the level of cadmium ions (Cd^{2+}) in seminal plasma (from 0.44-0.55 ug/L to 0.89-1.44 ug/L) and also exacerbates the acrosome reaction insufficiency and abnormal sperm morphology associated with the presence of a varicocele. In somatic cells, the cytoskeleton regulates both cell shape and docking/fusion of secretory granules with the plasma membrane ("exocytosis") and Cd^{2+} disrupts the cytoskeleton by calcium substitution. It is thus inferred that cigarette smoke Cd^{2+} decreases normal sperm morphology and function. We have reported that [1] the sperm cytoskeleton is composed of polymerized actin (F-actin) which interacts with a superfamily of plasma membrane proteins containing myosin motors, and that [2] in motile sperm from infertile men with varicocele, cytoskeletal and membrane associated proteins decrease as the percentage of sperm with elongated or tapering head forms increases. The latter was observed even in non-smokers with varicocele. Therefore, we hypothesized that alterations in the normal levels of trace metals in the genital tract of infertile men with varicocele contributes to their decreased semen quality. Levels of Cd^{2+} and zinc (Zn^{2+}) in seminal plasma in 3 groups of men were quantitated by graphite furnace atomic absorption spectrometry. Although the average level of Zn^{2+} in seminal plasma from fertile donors (n = 9) was significantly greater than that observed in infertile men without varicocele requiring ICSI to achieve fertilization in IVF (n = 6) (respectively, 1.3 mM vs <0.5 mM, $P<0.0014$), both groups exhibited equivalent levels of seminal plasma Cd^{2+} which did not exceed 0.6ug/L ($P=0.4$,NS). In contrast, in a population of subfertile men presenting for fertility evaluation (n = 19), the majority of whom had a varicocele, both average Zn^{2+} and maximal Cd^{2+} levels in seminal plasma differed significantly from that of fertile donors (respectively, <1 mM, $P<0.0014$ and >1.0 ug/L, $P<0.0048$). Since Zn^{2+} is a stronger competitive cation than Cd^{2+}, these data indicate that sperm from infertile men with varicocele would be more susceptible to the effects of elevated Cd^{2+} because of their decreased Zn^{2+} levels. To test whether increased seminal plasma Cd^{2+} could contribute to the progressive depletion of F-actin associated with increased abnormal sperm morphology in infertile men with varicocele, aliquots of fertile donor sperm (n = 4) were incubated overnight in the absence or presence of 0.3 uM, 1.3 uM or 6.5 uM Cd^{2+}. A dose-response inhibition in the formation of F-actin in sperm was observed ($P<0.0001$). Varicocele is also associated with increased scrotal temperatures. The effects of Cd^{2+} (1.3 uM, 18 hrs) and heat (39°C, 30 min) on dissolution of a fully formed sperm cytoskeleton of F-actin and membrane associated elements in fertile donor sperm are independent and additive (n = 3; $P<0.0001$). As only about 1/6 of all men with a varicocele are infertile, these data strongly suggest that the infertility associated with a varicocele is not solely due to elevated temperatures but also to an existing defect in metals regulation or environmental exposures amplified by the varicocele. (Supported by NIH Grant No. ES 06180 to S.B.)

034 A CHALLENGE TO THE CONCEPT THAT THE USE OF CALCIUM CHANNEL BLOCKERS CAUSE REVERSIBLE MALE INFERTILITY

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There has been a case described where failed fertilization following in vitro fertilization (IVF) was attributed to the use of calcium channel blockers. Motile sperm were tested for their ability to bind fluorescein isothiamate-labeled, mannosylated bovine serum albumin as an index of the surface expression of mannose-ligand receptors associated with fertility potential and the sperm was found to not express head-directed mannose-ligand receptors at high frequency nor did they exhibit spontaneous acrosome loss. The failure to fertilize was hypothesized to the male's use of calcium channel blockers for hypertension since switching him to an alternative anti-hypertension medication improved the expression of mannose-ligand receptors. Further support for this hypothesis was the demonstration of similar surface sperm membrane characteristics in 9 other males taking calcium channel blockers. Similar normalization of mannose-ligand receptors were found in 3 of the males who were switched to another anti-hypertensive regimen. The authors (Benoff et al., Fertil Steril, 1994) concluded that therapeutic administration of calcium antagonists for hypertension control cause reversible male infertility associated with failure to fertilize oocytes following IVF. However, these conclusions were reached without the repetition of the IVF procedure in case 1 to prove that fertilization can now occur once off calcium channel blockers and no data from any IVF cycles were provided for the other 9 men. The study presented herein was a retrospective analysis of the fertilization outcome following IVF of couples whose male partners were taking calcium channel blockers for hypertension. There were 6 couples evaluated who had initiated 10 IVF cycles. Failed fertilization did not occur in any of the 10 cycles. There were 4 patients who only had 1 IVF cycle and the fertilization rates (FRs) of all oocytes retrieved were as follows: couple 1 - 8/8 (100%) oocytes fertilized; couple 2 - 2/3 (67%); couple 3 - 3/5 (60%); couple 4 - 22/34 (65%). The other 2 couples each had 3 cycles of IVF and the FRs were as follows: couple 1 - 3/6 (50%), 7/12 (58%), and 4/6 (67%); couple 2 - 13/16 (81%), 9/18 (50%), and 15/20 (75%). Thus, the retrospective analysis presented herein questions the conclusions reached about CA^{++} channel blockers causing reversible failed fertilization, since the previous study by Benoff et al., failed to prove Koch's postulates by repetition of IVF cycles.

035

PERCUTANEOUS TESTICULAR BIOPSY IN MEN WITH AZOOSPERMIA OR SEVERE OLIGOSPERMIA: RESULTS AND INTRAOPERATIVE FINDINGS

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Percutaneous testicular biopsy and touch imprint have been shown to correlate with open testicular biopsy and touch imprint. 13 men with either azoospermia or severe oligospermia (< 5 million/ml.) underwent percutaneous biopsy and touch imprint. The procedure is performed in the office with local anesthesia. The patient is prepped and draped in a sterile manner. Spermatic cord block is performed with 1% lidocaine with a 1.5 inch, 25 gauge needle. The skin is anesthetized and a small incision is made with the scalpel. An 18 gauge automatic biopsy gun is used. A touch imprint is made on a glass slide and placed in 95% alcohol and parenchyma placed in Bouin's solution. No patient developed scrotal hematoma, bleeding or infectious complications. Adequate tissue was obtained in all patients to permit a histologic and cytologic diagnosis. Histologic diagnoses included: maturation arrest (3); germ cell aplasia with focal spermatogenesis (3); germ cell aplasia (2); hypospermatogenesis (2); and normal (3). Cytologic evaluation revealed 8 with mature sperm and 5 with no mature sperm. 3 men underwent scrotal exploration and attempted ductal reconstruction. In these 3, no adhesions or ductal injuries were identified with small well-healed punctures in the tunica albuginea of the testes. 1 was reconstructed but the other 2 had no sperm in the epididymides. Percutaneous testicular biopsy is a safe, office-based procedure that provides adequate tissue for diagnoses. At subsequent scrotal exploration minimal or no fibrotic reaction is seen. With the advent of intracytoplasmic sperm injection (ICSI), men previously thought to have irreversible testicular failure are candidates for percutaneous testicular biopsy to determine the presence of mature sperm for use with ICSI.

036

ETHNIC DIFFERENCES IN SPERMATOGENIC POTENTIAL IN HUMANS. L. Johnson, L. Rodriguez, R.S. Swerdloff, X.H. Wang, and C. Wang. Dept. of Vet. Anatomy & Public Hlth., Texas A&M University, College Station, TX; Harbor-UCLA Medical Center, CA; Jiangou Family Planning Research Institute, Nanjing, China.

Asians appear to be more susceptible to androgen or androgen-progestagen supression of spermatogenesis than other ethnic groups. Our objective was to determine if ethnic differences exist in spermatogenic potential and daily sperm production. Testes from 12 Chinese men were compared to those from 8 Hispanic men and 13 Caucasian men of ages 29 ± 3, 30 ± 2, and 29 ± 3 yrs, respectively. Testes were fixed by vascular perfusion with glutaraldehyde, further fixed in osmium, embedded in Epon, and evaluated by stereology using 0.5 μm sections stained with toluidine blue. Homogenates of fixed testes were evaluated for the number of Sertoli cells ($x10^6$) and daily sperm production ($x10^6$) based on pachytene primary spermatocytes (PDSP) or spermatids with spherical nuclei (DSP). Paired parenchymal weight was less ($p<0.05$) in Chinese men than Hispanic or Caucasian men (23 ± 2, 45 ± 3, and 38 ± 2 g, respectively). PDSP/g parenchyma (6.5 ± 0.6, 7.4 ± 0.6, and 9.0 ± 0.9) and DSP/g (5.7 ± 0.8, 6.7 ± 0.7, and 7.4 ± 0.7) tended to be lower in Chinese men. The PDSP/man (155 ± 25, 334 ± 37, and 294 ± 30) and DSP/man (136 ± 24, 309 ± 43, and 312 ± 34) was lower ($p<0.05$) in Chinese than Hispanic or Caucasian men. The number of Sertoli cells/g was higher ($p<0.05$) in Chinese or Caucasian men (31 ± 2, 21 ± 2, and 28 ± 2), but number/man tended to be lower in Chinese men (733 ± 102, 916 ± 65, and 971 ± 52). The volume of seminiferous tubules was less ($p<0.05$) in Chinese than Hispanic or Caucasian men (13 ± 1, 24 ± 2, and 23 ± 2 ml). These normal Chinese men had smaller testes, reduced Sertoli cell number, reduced PDSP/man and DSP/man, and reduced volume of seminiferous tubules than other ethnic groups. Hence, the potential for ethnic differences in their response to contraceptive drugs may be related to a lower spermatogenic potential of Asians. NIH AG00465-04

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REPRODUCTIVE ASSESSMENT OF MILITARY PERSONNEL ASSOCIATED WITH MILITARY DUTY ASSIGNMENTS

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As a follow-up to the pilot study of semen quality of soldiers with various military assignments (Weyandt et al, 1992, JOA 13:29P) a larger, more complete study was conducted. Soldiers were recruited at Fort Hood, Texas. Thirty-three men were exposed to radar as part of their duty assignment in the signal corps, 57 men were involved with firing the 155 howitzer (potential lead exposure), and 103 soldiers had neither lead nor radar exposure and served as the comparison control group. Both serum and urinary FSH and LH and serum, salivary, and urine testosterone levels were analyzed in all men. A complete semen analysis was conducted on each soldier. For statistical analysis, the primary study variables were: sperm concentration, sperm/ejaculate, semen volume, percent normal morphology, percent motile, percent viable (both vital stain and hypoosmotic swelling), curvilinear velocity, straight-line velocity, linearity, sperm head length, width, area, and perimeter. Variables were adjusted for significant confounders (e.g. abstinence, sample age, race). No statistical differences ($p<0.05$) were observed in any measurement. While these results are in agreement with 2 previous studies assessing soldiers firing the 155 howitzer (Weyandt et al, 1992, JOA 13:29P; Weyandt et al, 1994, JOA 14:P54), they contradict the previous report indicating that radar exposure caused a significant decrease in sperm numbers. A possible explanation is that the radar exposure in this study was that used in signal corps operations while the men in the previous study were using radar in military intelligence. The data presented here on men firing the 155 howitzer combined with the previous studies confirms that there are no deficits in semen quality in these men. The contradiction between the radar exposure indicates that more data are needed on different types of military radar and male reproduction.

039

ALTERATIONS OF THE PARAMETERS OF SEMEN ON PATIENTS WITH VARICOCELE PRE AND POST VARICOCELECTOMY

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OBJECTIVE: To characterize the alterations of semen on patients suffering varicocele and changes observed after surgery at: 3, 6, 9 and 12 months.

MATERIAL AND METHODS: We evaluated 209 samples of semen of 53 patients with varicocele. All patients were subjected to internal spermatic vein ligation.
The samples of semen were analyzed under OMS criteria of 1992. Morphology was evaluated under Strict Kruger criteria.
Semen analyses were made every 3 months after surgery.

RESULTS: Basal evaluation showed: 11 azoospermic patients, 9 with sever oligozoospermia, 11 with moderate oligozoospermia and 22 with sperm concentration equal or more than 20 millions/ml; 25 patients presented sperm motility less than 50% of type A and type B degrees, or less than 25% of type A degree.
Three months after surgery semen analyses showed no favorable changes in 95% of patients. After 6 and 9 months 80% of patients increased sperm concentration.
Sperm motility improve in all patients after 12 months.

CONCLUSIONS: The major percentage of recovery in sperm concentration was achieved at 9 months after surgery, sperm motility improve at 12 months. For this reason we considered theses patients potentially fertile.
Although severe alterations as to concentration were not modified in 20% of the patients, maybe because the varicocele was not the principal conditioner for infertility.

038

POLYMERASE CHAIN REACTION (PCR)-BASED QUANTITATION OF SEXUALLY TRANSMITTED PATHOGENS IN SEMEN OF INFERTILITY PATIENTS WITH LEUKOCYTOSPERMIA

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The purpose of this study was to reinvestigate the possible association between leukocytospermia (>10^6PMN/ml semen) and genital tract infection, using a highly sensitive new molecular technique (PCR-ELISA). Sexually transmitted viruses (CMV, EBV, HBV, HHV6, HSV) and chlamydia, STD pathogens that can "silently" infect the male genital tract, were investigated.
Materials and Methods: Semen samples from 97 leukocytospermic patients attending the BWH infertility clinic were tested. Seven negative control semen samples from fertile seronegative (HIV, CMV, chlamydia) donors were obtained from the New England Cryo Bank. Semen DNA and DNA standards for the respective pathogens (4 to 40000 copies) were amplified by PCR in the presence of digoxigenin-labeled nucleotides, and internal controls (mimics) for each pathogen. PCR products (pathogen and internal control) were quantitated by ELISA, which provided a dynamic range of 4-40,000 DNA copies per PCR sample.
Results: Out of 97 semen samples from leukocytospermic patients, 2 were positive for chlamydia DNA (copy numbers/ejaculate 10,000 and 460,000), 14 for CMV DNA (range 110-580,000 copies), 1 for EBV (210 copies), 4 for HHV6 (250-3,500,000 copies) and 4 for HSV (510-15,000 copies). HBV was not detected. Only two patients had more than one detectable pathogens (2 each). Semen from one out of seven seronegative controls contained CMV DNA.
Conclusions: The prevalence of STD pathogens in semen of leukocytospermic infertility patients is considerable (24%). This prevalence may be even higher, as our panel did not include HPV, genital mycoplasma, N. gonorrheae or T. pallidum. CMV was the most frequently detected pathogen. As three of the pathogens responsible for 80% of detected infections in our study are potentially treatable (chlamydia, CMV and HSV), further studies should be performed on the pathophysiology, diagnosis and treatment of these genital tract infections in infertility patients.

040 **TEST KIT TO DETERMINE SPERM COUNT IN SEMEN.**

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Microscopic determination of sperm concentration in semen is commonly used for the diagnosis of male infertility. A concentration below 20 million sperm/mL is diagnostic of oligospermia (WHO criteria) when confirmed by analysis of at least two samples on two separate days. In this study, a test kit to determine sperm count in semen is described and compared to microscopic analysis. Thirty five samples were obtained from males consulting a gynecologist for infertility. Sample preparation: a fraction of the semen sample was washed twice in Earle's medium and resuspended in the same medium to obtain the original sperm concentration in semen. The seminal plasma was centrifuged at 4,000g for 10 minutes and transferred to a test tube. Aliquots of 8μL of the liquefied semen were used to obtain sperm concentration by microscopic analysis. Test kit analysis: a 50μL aliquot of the liquefied semen, washed sperm or sperm-free seminal plasma were added separately to test tubes containing a 0.5mL solution of Rhodamine-123 in distilled water (0.5mg/mL) and incubated at room temperature for 1 minute. A 50μL aliquot was then added to a Whatman microfibre glass filter (3 mm in diameter and 2.7 μm pore size) mounted on a liquid reservoir, allowed to drain, and the presence of color on the filter compared to a color chart. Absence of color on the filter was considered a negative test (less than 20 million/mL); faint color borderline (20-30 million/mL); and intense color positive (above 30 million/mL). Results: Sperm concentrations in semen as determined by microscopic analysis ranged between 1 and 180 million/mL. We divided these samples into 3 groups: Group I true negative samples (less than 20 million/mL) (n = 12); Group II true borderlines (20-30 million/mL) (n = 10) and Group III true positives (above 30 million/mL) (n = 13). Eleven of the twelve samples from Group I were correctly identified by the test kit as negative and 1 incorrectly identified as borderline. Nine of the 10 samples in Group II were correctly identified as borderline and 1 as positive. Twelve of the 13 samples in Group III were correctly identified as positive and 1 as borderline. Total test kit time was 3 minutes. These results confirm the sperm test kit to be convenient and accurate for the determination of sperm concentration in semen, potentially for use in both physician office or at home.

041 EVALUATION OF SUBNORMAL SEMEN PARAMETERS AND SUBSEQUENT ZONA PELLUCIDA (ZP) THICKENING FOLLOWING IN VITRO FERTILIZATION (IVF)

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There are data demonstrating that there may still be a male factor present despite the appearance of normal fertilization resulting in poor pregnancy rates (PRs) and the transfer of normal looking embryos. This discrepancy has been found in males with hypo-osmotic swelling (HOS) test scores below 50% and in males who have <80% recovery of initial sperm motility after subjecting the sperm to heat and shaking "stress". The mechanism responsible for lower PRs is not known. One cause of failure to achieve pregnancy despite transfer of embryos is zona thickening which inhibits the embryo from hatching despite initial cell cleavage. The phenomenon has been associated with the age of the female partner. The study presented herein was designed to evaluate whether there is any association with zona thickness and some subnormal semen parameter. We considered that some factor may be released from abnormal sperm which prevents thinning of the ZP. The embryos were photographed on the day of embryo transfer with a camera connected with the Omnex Imaging System software package. The computer would then measure 4 points on the ZP which were averaged and the mean thickness/embryo was analyzed. 1479 embryos from 429 IVF cycles were analyzed; cycles using donor sperm or ICSI were excluded. Since the distribution of the average zona thickness was normal with a mean of 17.3um and a standard deviation (SD) of 3.1, the embryos were divided into 3 categories: thin (> 1 SD below mean); average (within 1 SD of mean) or thick (>1 SD above mean, i.e., >20.4um). There were no differences in the median levels of HOS scores or strict morphology (SM) in the 3 thickness groups. No differences were found either according to median sperm volume or percent motility. However, significant differences ($p<.05$, Kruscal Wallis) were noted according to median concentration of sperm, total motile sperm and motile density (MD) in that these values were the highest in the group of embryos with the thinnest ZPs and lowest in the group with the thickest ZPs. Since we have previously demonstrated no benefit of assisted embryo hatching of embryos from couples where the HOS was low and have not found much association with low SM and poor PRs, it is not surprising that there was no association with these parameters and zona thickness. Future studies are needed to determine if this impairment in zona thinning may be associated with decreased PRs.

042 USE OF SEMEN AS BIOPSY MATERIAL FOR ASSESSMENT OF HEALTH STATUS OF THE MALE REPRODUCTIVE TRACT.

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Routine light microscopic evaluation of semen for motility and morphological features of sperm does not fully utilize the diagnostic and prognostic potential of seminal samples. We examined seminal samples from eight stallions with fertility problems, using a procedure that facilitated cytopathologic evaluation by light and electron microscopy. Other parameters evaluated included seminal volume, concentration and motility of sperm. Gel-free semen was fixed in 4% buffered glutaraldehyde. Aliquants of fixed semen were centrifuged for 5 min at 100 xg; resulting cell pellets were post-fixed in 1% osmium tetroxide, embedded in Poly/Bed, sectioned and stained for evaluation by light and transmission electron microscopy. Additional samples of fixed semen were stained with 0.5% toluidine blue in 1% sodium borate and used to evaluate morphological features of sperm by light microscopy.

Counts (per ejaculate; $x10^9$) of 1) total sperm, 2) morphologically normal sperm, and 3) progressively motile sperm varied widely among stallions and ranged from 1.04 to 10.93, 0.14 to 2.34, and 0.34 to 1.93, respectively. In several instances, we found that not all morphologically normal sperm were motile or that not all motile sperm were morphologically normal. Examination of sections of pelleted cells revealed, in addition to abnormal sperm, presence of prematurely shed germ cells (6 stallions), a variety exfoliated somatic cells originating from testes (6) efferent ducts (1), epididymides (1), vesicular glands and/or prostate (2), and bacteria and associated inflammatory cells (3); in several cases, cellular debris from more than one source was observed. Presence of these cell types in ejaculated semen indicates several disease conditions such as degeneration and inflammation.

Although immature germ cells were identified in seminal smears stained with toluidine blue, identification of other exfoliated cells was difficult. In contrast, light and transmission electron microscopy of pelleted cell preparations allowed identification of several types of exfoliated somatic cells and facilitated further characterization of subtle lesions in sperm. Thus, this method provided useful diagnostic and prognostic information in assessment of potential fertility. Compared to other common biopsy procedures, use of semen as biopsy material is noninvasive, more representative, and less expensive.

043 THE USE OF IODIXANOL AS A DENSITY GRADIENT MATERIAL FOR THE ISOLATION OF MOTILE, MORPHOLOGICALLY NORMAL HUMAN SPERM FROM SEMEN.

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Although highly desirable, the efficient isolation of motile, morphologically normal spermatozoa from semen has been limited due to the unavailability of an iso-osmotic gradient material with a density higher than human sperm. A new gradient material, Iodixanol (60% w/v, OptiPrep™, Nycomed), with its relatively high osmolality (260 mOsm) and density (1.32 g/ml), may be suitable for this purpose. Iodixanol solutions, 1.154 g/ml and 1.054 g/ml (pH 7.3), were prepared using modified Human Tubal Fluid (m-HTF) medium. Osmolarity was adjusted to 300 mOsmol with NaCl. Semen specimens (n=15) were obtained from 10 normozoospermic donors. Semen volume, sperm concentration (C), motility (%M), normal forms (%N; Tygerberg strict criteria), total motile (TM), and total normal (TN) sperm were determined. Semen was diluted 4:6.5 with OptiPrep and layered under the 1.154 and 1.054 g/ml solutions (3 ml each) in 4 tubes. The discontinuous gradient was centrifuged for 40 min at 1,500 x g during which time sperm rose to the 1.154/1.054 g/ml interface. The sperm were collected from the interface and diluted 1:5 in m-HTF, centrifuged for 15 min at 500 x g and then resuspended in 1.0 ml of m-HTF. Final volume, C, %M, forward progressive velocity (V_f), TM, %N and TN were determined. To identify any possible cytotoxic effect of Iodixanol, an aliquot of prepared sperm suspension was diluted to 10 x 10^6 /ml in 1 ml of m-HTF supplemented with 0.4% human serum albumin and incubated under mineral oil at 37°C in air for 24 h after which %M and V_f were again determined. Values are expressed ± SE. Following centrifugation, a mean of 32.0 ± 3.8 x10^6 motile sperm were recovered from the 1.154/1.054 g/ml interface, representing 21 ± 1.8% of the motile and 19.5 ± 2.5% of the morphologically normal sperm. Following 24 h of incubation at 37°C, %M and V_f had decreased 18.1 ± 2.4% and 14.3 ± 1.7 µ/s respectively. The results of this preliminary study show that Iodixanol may be used to obtain motile, morphologically normal sperm for IUI, IVF or ICSI. The relatively small decline in %M and V_f at 24 h after recovery indicates Iodixanol was not toxic to the sperm. Since the use of Iodixanol as a gradient material appears promising, future studies will involve fine tuning the gradient densities to enhance the yield of motile, morphologically normal sperm.

044

QUALITY CONTROL OF THE MEASUREMENT OF ALFA-GLUCOSIDASE ACTIVITY IN SEMINAL PLASMA.

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Alfa-glucosidase is considered to be an important marker of epididymal secretion. Several methods for the measurement of alfa-glucosidase activity are available. The aim of this study is to investigate the reliability of three of these methods.

Methods and materials : Three different methods were compared : Episcreen (FertiPro), the WHO method (NAG) and the method described by Chapdelaine et al (TAG). Episcreen and TAG measure total alfa-glucosidase activity, whereas NAG only measures neutral alfa-glucosidase activity.

Samples were collected from 20 patients consulting our unit for male infertility (including 2 vasectomized males). Each sample was analysed three times. In between the different analyses the samples were frozen at -20 °C. The limits for normality are >= 20 mU/ejac for TAG and NAG, and > 20 mU/mL for Episcreen.

Results : The mean coefficients of variation (CV) of the different methods are : 3.15 % for Episcreen, 17.61 % for NAG and 7.41 % for TAG.

A lower number of samples were considered normal according to Episcreen (7/20) compared to TAG (13/17) and NAG (13/17).

The samples of the vasectomized males were considered abnormal by all three methods.

Discussion : The Episcreen and TAG methods have an acceptable mean CV of respectively 3.15 % and 7.41 %. A lower number of samples were considered normal according to Episcreen compared to TAG. This is due to the limit for normality proposed by Episcreen which does not consider the volume of the ejaculate. The WHO method for neutral alfa-glucosidase activity shows a high mean CV (17.61 %).

Alfa-glucosidase activity can be analysed reliably using Episcreen and the method described by Chapdelaine et al, although the criteria for normality need to be revised, especially in the case of Episcreen.

Chapdelaine P., Tremblay R. R., Dubé J.Y. Clin Chem 1978; 24:208-211.

045 Development And Validation Of A Procedure To Determine The Concentration Of Testosterone Dihydrotestosterone and Estradiol In The Human Ejaculate.

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Objectives: To develop and validate a procedure to determine the concentrations of Testosterone (T), Dihydrotestosterone (DHT) and Estradiol (E_2) in seminal plasma(SP) of the normal human ejaculate.
Design: T, DHT and E_2 were extracted from the SP of 19 men with normal sperm parameters and then measured by radioimmunoassay (RIA).
Materials and Methods: Briefly, ejaculates were collected, allowed to liquify, and semen parameters determined. The ejaculates were centrifuged at 1000 x g for 10 minutes to separate the SP from the spermatozoa. The SP was divided into 1 mL aliquots and frozen in sealed vials for later analysis. The specimens were extracted in ethyl ether, then purified on a silica gel column to separate T from DHT. The fractions were then assayed for T, DHT and E_2 RIA.
Results: The concentrations ± SEM of T (0.46±0.13 ng/mL) and DHT (1.00±0/39 ng/mL in the SP were much lower than corresponding levels in blood plasma, but the ratio of DHT:T was higher. SP E_2 (33.6±5.3 pg/mL) was to similar blood plasma concentrations, making the E_2:T ratio much higher than that in blood plasma.
Conclusion: The SP concentrations of T and DHT are lower than that found in blood plasma but, the DHT:T was greater in SP than in blood plasma. E_2 was similar in both compartments. These findings may aid to directly relate the effects of testicular steroids on spermatogenesis and sperm quality.

046 NITRIC OXIDE SYNTHASE (NOS) ACTIVITY IN HUMAN SEMINAL PLASMA

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Recent studies have demonstrated the presence of NOS in the epithelium of the male reproductive tract and have shown that nitric oxide (NO) can modulate human sperm function *in vitro*. The objectives of the present study were to determine whether human seminal plasma possesses NOS activity, and whether if present, this activity correlates with parameters of a standard semen analysis.

NOS activity was measured in human seminal plasma by the conversion of L-arginine to L-citrulline as described by Bredt and Snyder (*Proc Natl Acad Sci USA* 87: 682-685). Semen samples obtained by masturbation, were collected from 51 unselected men presenting for infertility evaluation at our institution. After a standard semen analysis was performed, semen samples were centrifuged at 10,000 x g and the seminal plasma was collected, and stored at -20°C for later evaluation of calcium-dependent and calcium-independent NOS activity. Controls in which the incubation was arrested immediately and those in which the NOS inhibitor L-NAME was added to the medium, were evaluated in parallel with each sample.

NOS activity was detected in 49 of 51 samples tested. The mean calcium-dependent NOS activity was 6.3 fmol/min/ml and the mean calcium-independent NOS activity was 3.2 fmol/min/ml of seminal plasma. No significant correlation was found between NOS activity in seminal plasma and either sperm concentration, sperm motility or leukocyte concentration.

To the best of our knowledge, this study is the first to demonstrate the presence of NOS activity in human semen and validate the possible role of NO on sperm function by documenting the presence and putative source of NOS in semen. The finding that NOS activity was detected in the semen samples of men after vasectomy but not in the 2 men with congenital bilateral absence of the vas deferens suggests that the seminal vesicle is a probable source of this activity and that the prostate is unlikely to be a significant source of NOS activity.

047 THE BIOLOGICAL BASIS OF THE RESAZURIN REDUCTION TEST

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The reduction of the blue stain Resazurin to the pink stain Resorufin is the chemical reaction used in the first kit for home semen analysis (FertilityScore, Androscore Corp, USA). When monitored by spectrophotometry, this reaction is correlated with the concentration of Percoll-gradient selected vital spermatozoa ($r=0.99$) and the proportion of vital spermatozoa in different mixtures of vital and dead spermatozoa ($r=0.94$). In whole ejaculates the reduction is correlated with the concentrations of spermatozoa ($r=0.65$)and of ATP ($r=0.65$), and it is inversely correlated with the generation of reactive oxygen species by spermatozoa stimulated by phorbol ester ($r=-0.51$). The reducing capacity of seminal plasma is correlated with prostate function as assessed by the activity of gamma glutamyl transferase ($r=0.43$). The reduction reaction can be blocked by addition of dicoumarol, a specific inhibitor of the diaphorase enzyme, and is stimulated in a dose dependent manner until saturation by the addition of $NADH_2$ to spermatozoa or seminal plasma. It is concluded that the FertilityScore kit-result depends on the concentration of metabolically active spermatozoa which generate $NADH_2$, that reacts with resazurin as shown:

$$\text{Resazurin} = NADH_2 \rightarrow \text{Resorufin} + NADH + H_2O.$$

These results corroborate the validity of the Resazurin reduction test for the assessment of sperm quality.

048

PRESENCE OF ISOFORMS OF LH AND FSH IN SERUM OF MAN WITH TESTICULAR DAMAGE

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OBJECTIVE: Characterize the chromatographic profiles of FSH and LH in the serum of males with testicular damage and the influence of gonadal steroids on these profiles.

MATERIAL AND METHODS: 17 patients aged 20 to 50 years: 8 with testicular damage, high concentrations of FSH and LH and low T; 9 patients with normal testicular function and normal hormon levels, both groups without hepatic desorders or thyroid and/or metabolism of lipids. Each serum was analised by filtration in gel using Sephadex G -100. Seventy fractions were collected to determinate on each one of the concentration of LH, FSH and sialic acid.
The chromatographic profile was obtained graphing the volume of each fraction versus the concentration of LH or FSH in mIU/ml, characterizing the isoforms for their partition constant (Kav), molecular weight and elution of volume.

RESULTS: The men with testicular damage showed chromatographics profiles of LH with a predominance of isoforms of 60 KDa, 45 KDa and free subunit (21-15 KDa) and absence of isoforms of major molecular weight (100 - 66 KDa).
The FSH isoforms predominated were of 100-66 KDa type followed by 45 KDa and free fragments (< 15KDa). The isoforms of 60 KDa were not present in the patients with testicular damage, with exception of one patient with testicular cancer.

CONCLUSIONS: The gonadal steroids influence on the degree of glicosilation of the isoforms; this could affect the presence, absence and/or proportion of the isoforms found.

049

Decreased Spermatogenesis as the Result of an Induced Autoimmune Reaction Directed Against the Gonadotropin Receptors in Male Rats

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Given the significant role played by the gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in testicular development, it is possible that an autoimmune reaction directed against their receptors (FSHR or LHR) would lead to clinically significant infertility. Therefore, development of an experimental autoimmune reaction resulting in male infertility could not only provide a model for the study and diagnosis of idiopathic infertility but could also lead to a nonsurgical alternative for long term contraception.

The first study consisted of 5 treatment groups with 4 males per group. They were actively immunized with FSHR or LHR in various forms. These forms included: 1) FSHR extracellular domain linked to Glutathione S-Transferase as a fusion protein (FSHR-GST); 2) N-terminal FSHR (10 amino acids) peptides linked to a lysine core to produce a "multi-antigenic protein" (MAP); 3) N-terminal FSHR linked to Keyhole Limpet Hemocyanin (FSHR-KLH); 4) N-terminal LHR (14 amino acids) linked to KLH. An initial injection of 400 µg of antigen + Freund's complete adjuvant was given subcutaneously to 18 day old rats. Three boosters were given 2 weeks apart beginning 2 weeks after the initial injection using 200 µg antigen + Freund's incomplete adjuvant. Control animals received only adjuvant. Animals were sacrificed at 70 days of age.

The second study consisted of 4 treatment groups with 5 males per group. Again, there was a control group and the FSHR-GST was used. Two new treatments were added: 1) FSHR peptide linked to a promiscuous T cell antigen from tetanus toxoid (FSHR-TT); 2) LHR peptide linked to the piece of tetanus toxoid (LHR-TT). The injection schedule was the same except 1 mg of antigen was used for each injection and animals were sacrificed at 120 days of age.

Testes were fixed and processed for light microscopy. Up to 37% of tubule cross-sections were aspermatogenic in males treated with FSHR-GST while males in the LHR-KLH treatment group had up to 16% aspermatogenic tubules. All 5 males treated with LHR-TT showed aspermatogenic tubules. Testosterone levels were elevated and sperm counts were reduced up to 40% in all immunized animals.

The induced autoimmune reaction against the FSHR or LHR had a significant effect on normal spermatogenesis. This phenomenon indicates the important role that FSH and LH play in reproductive development and provides a means for the study of idiopathic infertility. (supported by NIH Grant HD 10808 to MDG)

050 **CYCLOPHOSPHAMIDE (CPA) INDUCES APOPTOSIS IN THE GERM CELLS OF RAT TESTES IN A CELL AND STAGE SPECIFIC MANNER.** L. Cai*, B. F. Hales*, and B. Robaire, Dept. of Pharmacol. & Therap., McGill University, Montréal, Québec, Canada

The administration of CPA to male rats results in adverse effects on progeny outcome. These effects are dependent on the length of treatment and thus on the specific phases of germ cell development that are exposed. However, the extent and nature of germ cell death induced by this drug have not been determined. Apoptosis is a process by which cells commit suicide. The objective of this study was to determine whether CPA can induce apoptosis in rat germ cells, and, if so, to identify which germ cells and what stages of spermatogenesis are most vulnerable. To determine the dose-response relationship, adult Sprague-Dawley rats were treated with saline or CPA (2, 7, 20 and 70 mg/kg) and the testes were perfused-fixed 12 hrs after treatment. To study the timing of the response, male rats were treated with a single dose (70 mg/kg) of CPA; the testes were perfused-fixed at 0, 4, 8, 12, 18, 24 and 48 hrs after treatment. Apoptosis was assessed by TUNEL detection *in situ* using an Apotag-peroxidase kit and by determining DNA fragmentation with agarose gel electrophoresis. A low rate of germ cell apoptosis was observed in untreated rats (46.0 ± 2.4 apoptotic cells/300 tubules); the apoptotic cells were predominantly pre-meiotic germ cells of stages I-IV and XI-XIV of the seminiferous tubules. Thus, apoptosis occurs spontaneously in pre-meiotic germ cells in a stage-specific pattern. DNA fragmentation was detected in the form of a DNA ladder. Exposure to CPA doses greater than 20 mg/kg significantly induced apoptosis. After CPA treatment, the incidence of apoptosis increased progressively at 4h and 8h, reached a peak at 12 hrs (3.5-4 fold of control) and then decreased rapidly to reach control levels by 48 hrs. CPA-induced apoptosis was significantly elevated in spermatocytes at stages I-IV and XI-XIV. In CPA-treated rats (70 mg/kg), twice as much DNA was fragmented in the form of a DNA ladder as in controls. Thus, CPA induces apoptosis in male germ cells in a cell and stage specific manner. Apoptosis may be important in preventing the transmission of damage caused by CPA in pre-meiotic germ cells to subsequent generations. Supported by MRC of Canada.

051 ANDROGEN-INDUCED GROWTH FACTOR (AIGF/FGF-8) IS EXPRESSED IN CANINE PROSTATE AND TESTIS: CLONING OF cDNA ENCODING CANINE AIGF/FGF-8. H. Canatan[1,3*], S.K. Kulp[1*], W.Y. Chang[1*], F. Shidaifat[1*], Y.Sugimoto[1*], R.W. Brueggemeier[2,4*], and Y.C. Lin[1,3,4]. [1]Colleges Vet. Med. and [2]Pharm., [3]Program in Molec., Cell. and Devel Biol., and [4]The Ohio State Univ. Comprehensive Cancer Ctr. The Ohio State Univ., Columbus. OH, 43210-1092 USA.

AIGF/FGF-8 was originally isolated from the conditioned medium of the androgen-dependent mouse SC-3 cell line. AIGF/FGF-8 expression is known to be induced by hormones and to have oncogenic capacity. The testis plays a pivotal role in the etiology of human and canine benign prostatic hyperplasia (BPH). Both the presence of testes and aging are required for development of spontaneous BPH in humans and dogs. Although testicular androgens and estrogens affect prostatic cell growth and development, evidence suggests that the testis produces nonsteroidal factor(s) that also influence prostatic cell growth. Therefore, we investigated the expression of AIGF/FGF-8 mRNA in canine prostate and testis. Total cellular RNAs were isolated from mature and immature canine prostate tissues, cultured canine prostatic stromal cells (CPSCs) and canine prostatic epithelial cells (CPECs), mature canine testicular tissue, and Leydig cell-enriched canine testicular cells. The cDNAs were generated from total RNA by reverse transcription (RT) reaction. The AIGF/FGF-8 primers used for PCR were derived from published human and mouse cDNA sequences and were identical to each other. The canine AIGF/FGF-8 transcript was detected by RT-PCR in both young and mature canine prostate tissues, CPSCs and CPECs, mature canine testicular tissue, and Percoll-enriched canine Leydig cells. The authenticity of the AIGF/FGF-8 amplicon was confirmed by Southern blot analysis using human AIGF/FGF-8 cDNA as probe. The canine AIGF/FGF-8 cDNA (248 bp) was cloned into pCR 3 vector. The nucleotide sequence of the canine AIGF/FGF-8 cDNA was determined and, excluding the primer sequences, was found to be 90.7% and 89.7% identical to its human and mouse counterparts, respectively. The deduced amino acid sequence of canine AIGF/FGF-8 is 100% identical to corresponding human and mouse amino acid sequences. Studies to obtain full length canine AIGF/FGF-8 cDNA(s), as well as continuing efforts to determine the cellular origin of the AIGF/FGF-8 transcript within the testis, are underway utilizing the canine-specific AIGF/FGF-8 cDNA. The expression of AIGF/FGF-8 in prostate as well as in testis suggests that therapeutic castration for BPH will eliminate only the testis-derived AIGF/FGF-8, not local production of AIGF/FGF-8. Supported by NIH grants DK45916 and CA16058.

052 **ALTERATIONS IN CATALASE AND GLUTATHIONE PEROXIDASE mRNA EXPRESSION IN THE RAT TESTIS AFTER SURGICAL CRYPTORCHIDISM AND EFFERENT DUCT LIGATION.** A. Zini, M. Goldstein and P. N. Schlegel. James Buchanan Brady Foundation, Department of Urology, The New York Hospital-Cornell Medical Center and The Population Council, Center for Biomedical Research, New York, NY.

The testis is known to be highly sensitive to a number of physical stresses. We suspect that as in other organ systems under pathologic conditions, oxidative stress may be an important mediator of testicular injury. To address this, we have evaluated the effects of experimental cryptorchidism and efferent duct ligation on the mRNA expression of antioxidant enzymes.

Prepubertal rats (20 day-old, n=8) were rendered unilaterally cryptorchid and 40 days after the procedure, cryptorchid and contralateral testes, and testes from sham operated rats (n=4) were harvested for RNA extraction. Adult rats (n=10) were subjected to unilateral efferent duct ligation and the obstructed testes were harvested at 1, 4, 8, 16 and 28 days after the procedure. Glutathione peroxidase and catalase mRNA expression was assessed by northern blot analysis using ^{32}P-labeled DNA probes derived from known cDNA sequences for classical cellular glutathione peroxidase (GSHPx), phospholipid hydroperoxide glutathione peroxidase (PHGPX) and catalase. Relative mRNA levels were expressed as a function of total RNA, not per testis, and were calibrated using a glyceraldehyde 3-phosphate dehydrogenase probe as control.

Cryptorchidism resulted in a 72% reduction in testis weight compared to control testes ($p<.05$). GSHPx mRNA levels were reduced by 39% in cryptorchid testes compared to control testes ($p<.05$). Likewise, and more significantly, PHGPX mRNA levels were also reduced (by 98%, $p<.05$) in cryptorchid compared to control testes. On the other hand, catalase mRNA levels increased (30%) in cryptorchid testes compared to control testes, although this was not statistically significant.

At 1, 4 and 8 days after efferent duct ligation, testis weight was significantly increased (29%, 52% and 38% respectively, $p<.05$), but by 16 days after obstruction, testis weight had declined significantly (by 38%, $p<.05$), relative to controls. GSHPx mRNA levels increased 1 day post-ligation, but subsequently decreased relative to control testes. Likewise, PHGPX mRNA levels increased 1 day post-ligation, but subsequently decreased to undetectable levels by 28 days post-obstruction. On the other hand, the relative levels of catalase mRNA initially decreased in obstructed testes, but by 16 days post-obstruction, the levels had increased compared to control testes.

This study demonstrates that the mRNA expression of antioxidant enzymes in the testis is differentially altered in response to physical stresses. This suggests that oxidative stress may be implicated in abnormal spermatogenesis and tumorogenesis.

053 THE PROLIFERATIVE CAPACITY OF RAT LEYDIG CELLS DECLINES DURING PUBERTAL DEVELOPMENT

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Postnatal differentiation of Leydig cells can be conceptually divided into three distinct stages of differentiation: first they exist as mesenchymal-like Leydig cell progenitors (PLC) by day 21; secondly, as immature Leydig cells (ILC) by day 35, they acquire steroidogenic organelle and enzyme activities but metabolize most of the testosterone they produce; thirdly, as adult Leydig cells (ALC) by day 90 they actively produce testosterone. The aim of the present study was to determine whether changes in proliferative capacity are associated with progressive differentiation of the Leydig cell. PLC, ILC and ALC were isolated after collagenase dispersion by Percoll gradient centrifugation and elutriation. The cells were cultured in DMEM/F12 for 24 h followed by an additional 24 h in the presence of hormonal factors that have been previously shown to promote Leydig cell differentiation: LH (1 ng/ml), insulin-like growth factor-I (IGF-I, 70 ng/ml) or 7α-methyl-19-nortestosterone (MENT, 50 nM), a synthetic androgen that is not metabolized by 5α-reductase. Proliferative capacity was measured by assay of the uptake of [^{3}H] thymidine during the last 2 h of culture, and scored as cpm/10^4 cells. The results showed that [H] thymidine incorporation by ILC and ALC was 18% and 3% of PLC, respectively, indicating that Leydig cells progressively lose their proliferative capacity during pubertal differentiation. LH and IGF-I increased proliferation in PLC by 1.4- and 2-fold, respectively ($P < 0.05$), while MENT was without effect compared to untreated controls. In contrast, MENT increased [^{3}H] thymidine incorporation by ILC 1.8-fold, as did LH and IGF-I (2.1- and 1.9-fold respectively, $P < 0.05$). No effects of hormonal treatment were observed in ALC. We conclude that the proliferative capacity of Leydig cells is highest during the progenitor cell stage, and that leydig cells become less sensitive to hormonal stimulators of cell division as they differentiate.

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054 PHYSIOLOGICAL CONCENTRATIONS OF GLUCOCORTICOID REGULATE SERUM TESTOSTERONE AND 11β-HYDROXYSTEROID DEHYDROGENASE IN PURIFIED LEYDIG CELLS.

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It has been shown that rat Leydig cells contain 11β-hydroxysteroid dehydrogenase (11β-HSD), an enzyme that oxidatively inactivates glucocorticoids. The function of 11β-HSD in Leydig cells is to protect against inhibitory effects of high, stress levels of glucocorticoids on testosterone production. However, the role of lower, physiological concentrations of glucocorticoid in regulating Leydig cells has not been established. Glucocorticoids are known to control 11β-HSD activity in some (e.g., brain and liver), but not all tissues (e.g., kidney). The aim of the present study was to investigate whether physiological levels of glucocorticoid modulate testosterone production and Leydig cell 11β-HSD. Male rats (200-250 grams b.w.) were bilaterally adrenalectomized (ADX), and controls were subjected to sham-surgery (SHAM). ADX rats received either corticosterone (B, 1 mg/100 gm) or vehicle (coin oil) by intraperitoneal injection. Serum testosterone was measured by radioimmunoassay in trunk blood on day 7 after surgery, 3 h after injection of B or vehicle and 1 h after injection of LHRH (50 ng/100 gm) to synchronize LH. In another set of experiments, 11β-HSD enzyme activity and steady-state mRNA levels were measured in purified Leydig cells on day 7, after receiving B or vehicle on the preceding 3 days. Serum testosterone was unaffected by B in SHAM rats, elevated after ADX (1.5-fold, $P < 0.05$), and remained at control levels in ADX animals that received B. 11β-HSD activity declined (to 31% of control, $P < 0.05$) after ADX and was maintained at control levels after treatment with B. Steady-state 11β-HSD mRNA followed a similar pattern. We conclude that physiological levels of B exert a tonic, negative control on serum testosterone levels, and are responsible for maintaining normal activity of 11β-HSD activity in Leydig cells. The results support the hypothesis that 11β-HSD is thus a significant determinant of Leydig cell steroidogenic capacity.

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055 DEVELOPMENTAL EXPRESSION AND DIFFERENTIAL REGULATION OF CU/ZN SUPEROXIDE DISMUTASE, Mn SUPEROXIDE DISMUTASE, GLUTATHIONE PEROXIDASE AND CATALASE mRNAs DURING SPERMATOGENESIS IN THE MOUSE. W. Gu and N.B. Hecht. Department of Biology, Tufts University, Medford, MA 02155.

The coordinate expression of four antioxidant enzymes [Cu/Zn superoxide dismutase (SOD-1), Mn superoxide dismutase (SOD-2), catalase (CAT) and glutathione peroxidase (GSHPx)] plays a significant role in protecting testicular cells against reactive oxygen species. To start to understand the mechanisms regulating their expression in the testis, we have examined the steady-state levels of mRNAs encoding these four enzymes in spermatogenic cells. Three different sizes of SOD-1 mRNAs of about 0.73, 0.80 and 0.93 kB are detected. The 0.73 kB SOD-1 mRNA is present in somatic cells and in early stages of male germ cells, whereas the 0.80 and 0.93 kB SOD-1 mRNAs are exclusively detected in post-meiotic germ cells. Similarly, we find in the testis three SOD-2 mRNAs of about 2.2, 1.2 and 1.0 kB that are developmentally regulated, with the maximal level of expression in early post-meiotic germ cells. In contrast, one major GSHPx mRNA of about 0.8 kB and one CAT mRNA of about 2.4 kB are found in testes. The levels of GSHPx and CAT mRNAs are relatively constant in prepuberal and adult mouse testes and lower in pre-meiotic, meiotic, and post-meiotic germ cells. Analysis of the translational status of SOD-1, SOD-2, GSHPx and CAT mRNAs reveals that the GSHPx and CAT mRNAs are primarily polysome associated implying efficient translation whereas the 0.93 kB SOD-1 mRNA and the 1.2 kB SOD-2 mRNA are primarily present in the non-polysomal fraction indicating translational regulation. We have identified a RNA-binding protein that binds to the 5′ untranslated region of the translationally regulated 0.93 kB SOD-1 mRNA and represses its translation "in vitro". We propose that the translational regulation of SOD-1 and SOD-2 mRNAs provides a means to fine tune the levels of these critical enzymes in differentiating male germ cells.

056 *IN SITU* EVALUATION OF GAMMA-GLUTAMYL TRANSPEPTIDASE mRNA EXPRESSION IN THE RAT EPIDIDYMIS FOLLOWING LOSS OF TESTICULAR FACTORS. Daniel B. Rudolph, Cynthia M. Barber[1], Mark H. Stoler[1] & Barry T. Hinton. Departments of Cell Biology and Pathology[1], University of Virginia Health Sciences Center, Charlottesville, Va 22908.

Glutathione (GSH, γ-glu-cys-gly) and gamma-glutamyl transpeptidase (GGT) are involved in the protection of sperm in the epididymis from the deleterious effects of compounds such as reactive oxygen species. GSH serves as an oxygen free radical scavenger in the epididymal lumen and GGT, an integral membrane protein, is the only known enzyme capable of hydrolyzing the γ-glutamyl bond of GSH. Four GGT mRNAs are transcribed from four different promoter sites on the single copy rat GGT gene, differing only in their distal 5′ untranslated regions (UTRs). The expression of these mRNAs varies throughout the different regions of the epididymis and is regulated in some cases by circulating androgens and/or other testicular factors. The goal of this study was to use *in situ* hybridization analysis to examine the expression of the four GGT mRNAs in the rat epididymis and to examine any changes in response to the removal of testicular factors by 12 hr, 24 hr or 3 day efferent duct ligation (EDL). Male Sprague-Dawley rats were subjected to unilateral 12 hr, 24 hr or 3 day EDL. Animals were then perfused with 10% formalin and the initial segment, caput, corpus and cauda epididymides were paraffin imbedded and sectioned. Each tissue was probed with [^{3}H]cytosine and [^{3}H]uracil labeled antisense RNA probes to the four different GGT mRNA 5′ UTRs. Results showed that all four GGT mRNAs were detected throughout the epididymis. Regional variations were detected in some of the mRNAs that were examined, particularly $mRNAs_{IV}$ which exhibited less signal in distal epididymal regions than in proximal regions. Localization of certain mRNAs to apical or basal cytoplasmic aspects was also detected. GGT $mRNAs_{I,II,III}$ in the cauda exhibited higher apical expression while $mRNA_{III}$ showed signs of higher basal expression in the initial segment. EDL induced the reduction of the signal from GGT $mRNA_3$ in the caput after 24 hr. Our working hypothesis is that the multiple promoters of the single copy rat GGT gene allow for differential regulation of GGT expression in the various regions of the epididymis.

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057 **ORCHIDECTOMY INDUCES A WAVE OF APOPTOTIC CELL DEATH IN THE EPIDIDYMIS.** X.P. Fan* and B. Robaire, Dept. of Pharmacol. & Therap., McGill University, Montréal, Québec, Canada

The epididymis is the site where spermatozoa are matured and stored. After orchidectomy this tissue loses up to 80% of its weight. Approximately half of this decrease is caused by the loss of fluid and spermatozoa from the lumen of the duct, while the rest of the weight loss is caused by androgen withdrawal and is associated with cellular atrophy. In the prostate, androgen withdrawal is associated not only with cell atrophy but also with apoptotic cell death. The objective of this study is to investigate whether apoptotic cell death is involved in the androgen dependant weight loss found in the orchidectomized rat epididymis. Adult male Sprague-Dawley rats were orchidectomized and the epididymides were perfused-fixed after 12h, 18h, and 1 2, 3, 4, 5, 6, and 7 days (n=4/time point). Apoptotic cells were identified by in situ labelling of the 3'OH groups in endonuclease cleaved DNA (TUNEL staining). No indication of apoptosis was seen 12 hrs after orchidectomy. Apoptosis first appeared in the epithelium of the **initial segment** of the epididymis 16 hrs after orchidectomy, reached a maximum (56% of the tubules had apoptotic cells) on day 2 and disappeared by day 5 post-orchidectomy. In the **caput** epididymidis, apoptosis was first found after 24 hrs, reached a maximum by day 3 (49% positive tubules) and was detectable until day 5. In the **corpus** epididymidis, apoptosis was first seen on day 4, peaked on day 5 (29% positive tubules) and was undetectable by day 6 post-orchidectomy. In the **cauda** epididymidis, apoptosis was first seen on day 5, peaked on day 6 (25% positive tubules) and was occasionally detected on day 7. This time course is coincident to the movement of the luminal contents through the epididymal duct. Throughout the rat epididymis, apoptotic cell death was only seen in the epithelium, and localized specifically to principal cells. The number of tubules with cells staining positively for apoptosis was higher in the initial segment and caput epididymidis than in the corpus or cauda epididymides. Thus, a wave of apoptosis occurs along the rat epididymis after orchidectomy. We speculate that this wave of apoptosis is induced by withdrawal of specific component(s) from the luminal compartment. Supported by MRC of Canada.

059 EVIDENCE FOR TOXICANT-INDUCEDACCELERATION IN E PIDIDYMAL TRANSIT TIME. J.D. Suarez* and G.R. Klinefelter. U.S Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, MD # 72, , Research Triangle Park, NC. 27711

Previously we established that a four-day exposure to chloroethylmethanesulphonate (CEMS) resulted in a significant decrease in cauda epididymal sperm reserves in adult male rats without a concommitant decrease in the number of homogenization-resistant spermatids within the testis. This epididymis-specific alteration occured whether or not circulating and caput/corpus testosterone (T) levels were maintained using T-filled Silastic implants. An initial study was designed to determine whether this epididymis-specific decrease in sperm number was the result of decreased epididymal transit time. The vas deferens was ligated at its midpoint just prior to the first of four daily ip injections of either vehicle (30 % DMSO in water) or 12.5 mg/kg CEMS. In addition, CEMS-treated animals received a 2.5 cm T-filled Silastic capsule to control for the CEMS-induced decline in serum T. On day 5, three tissues: testis, caput/corpus epididymidis, and cauda epididymidis/proximal vas deferens were removed and frozen for subsequent enumeration of sperm. In theory, if epididymal sperm were accelerated due to treatment, there would be fewer sperm in the caput/corpus and more sperm in the cauda/vas of the treated animals compared to control. The number of sperm (x 10^6) in the caput/corpus decreased significantly ($p < 0.05$) from 127 in vehicle animals to 82 in CEMS + T animals (N=8), while the number of sperm in the cauda/vas increased significantly from 445 to 510. Daily sperm production (DSP; x 10^6/gr parenchyma) was not affected by treatment; 17.5 and 18.8 for vehicle and CEMS + T treated animals. By contrast, caput/corpus transit time (x 10^6/DSP) was decreased significantly from 7.7 to 4.4 days in vehicle and CEMS + T animals, respectively. Next, to determine whether testicular fluid played a role in the epididymis-specific decline in sperm numbers, the study was repeated but the efferent ducts were ligated at the same time the vas deferens was ligated. The number of sperm in the caput/corpus decreased significantly from 27 in vehicle animals to 14 in CEMS + T animals (N= 12); and there was a reciprocal increase in the number of cauda/vas sperm in the CEMS + T group relative to controls. Finally, while the epididymis-specific decrease in sperm seemed unrelated to T status, perturbation of an androgen-mediated process might account for the alteration. To address this, animals were castrated and implanted with 2.5 cm T capsules, a treatment previously shown to maintain a sufficient level of T in the epididymis to support sperm maturation (i.e. fertilizing ability). The vas deferens was ligated and animals received either vehicle or 25 mg/kg of the antiandrogen hydroxyflutamide (HF) for four days. Once again, the number of sperm in the caput/corpus decreased significantly from 25 in vehicle animals to 12 in HF + T animals (N=8); and there was a reciprocal increase in cauda/vas sperm. Taken together, these data suggest that both CEMS and HF produce an epididymis-specific decrease in transit time. This is not influenced by T status, testicular fluid, or the testis; but may involve a lesion in androgen-dependent epididymal function.

058 **LOCALIZATION OF TESTINS IN SERTOLI CELLS AND GERM CELLS IN VITRO** K.E. Muffly and D.F. Cameron. Department of Anatomy, University of South Florida College of Medicine, Tampa, FL

Testins are Sertoli cell secretory proteins which, in vivo, are associated with Sertoli-Sertoli junctional complexes and with elongating spermatids, possibly Sertoli-spermatid junctional complexes. We determined the expression and localization of testins in Sertoli and germ cells in vitro by immunocytochemistry using an antibody provided by Drs. Bardin and Cheng. Mixed populations of isolated germ cells were incubated for 24h in blank medium, Sertoli pre-condition medium (SPM) and SPM in which Sertoli cells had been incubated with FSH and testosterone. Sertoli cells alone were incubated for 24h with or without FSH, testosterone or FSH+testosterone. Results showed that spermatogonia and spermatids (round and elongating) expressed testins, apparently unaffected by hormones or media soluble Sertoli cell products. Results also showed that testins expression in Sertoli cells was influenced by FSH, but not testosterone. Testins immunostaining in untreated or testosterone (T) treated Sertoli monocultures resulted in a diffuse pattern of testins localization in the cell. FSH or FSH+T treatment resulted in the peripheral distribution of testins in the Sertoli cell in a fashion similar to the FSH-stimulated peripheral distribution of junction related actin and vinculin.

Results suggest that FSH, but not testosterone, predisposes the Sertoli cell to testins-related junctional interaction with some germ cells while testins expression in germ cells is hormone independent.

060 **EXPRESSION AND FUNCTIONS OF CYCLIN A AND CYCLIN A-DEPENDENT KINASES IN THE MOUSE TESTIS** [1]*Stuart E. Ravnik and* [2]*Debra J. Wolgemuth.* [1]Dept. of Cell Biol. & Biochem., Texas Tech University, Lubbock, TX 79430 and [2]Dept. Genet. & Devel., Columbia Univ. Coll. of Phys. & Surg., New York, NY 10032.

Our previous work on the mRNA expression of the A-type cyclins and their possible cyclin dependent kinase partners revealed striking specificity of expression in different germ cells in the testis. Cyclin A1 (CycA1) mRNA in present at highest levels in diplotene spermatocytes in prophase I just prior to the first meiotic division. Cyclin A2 (CycA2) mRNA is present at highest levels in pre-leptotene spermatocytes, prior to the entry into the meiotic prophase. While both Cdc2 and Cdk2 are thought to be the predominant cyclin dependent kinase partners for CycA1 and CycA2, the highest levels of Cdc2 and Cdk2 mRNA were observed in cells in pachytene of prophase I. Here, we report the distribution of CycA1 and CycA2 and Cdc2 and Cdk2 proteins in the mouse testis. Cyclin A1 protein was found in late pachytene and diplotene cells and remarkably, only in cells undergoing the first, but not the second meiotic division. In contrast, CycA2 protein was detected in some spermatogonia in interphase, but was most abundant in all pre-leptotene cells in stage VII tubules; these cells are making the commitment to enter the meiotic pathway. While both Cdc2 and Cdk2 were detected in some spermatogonia, their highest levels of expression were later in the meiotic prophase. Cdc2 was present in the nucleus of early pachytene cells, but Cdc2 levels declined as cells entered the diplotene phase and was barely detectable in cells carrying out the meiotic division. In contrast, Cdk2 was highly expressed throughout the pachytene and diplotene phases. Notably, in cells undergoing the reduction divisions, Cdk2 appeared to be localized specifically to the chromatin. Spermatogonial cells undergoing mitotic division did not express Cdk2. Immunoprecipitation analysis revealed that CycA1 could bind both Cdc2 and Cdk2 in the testis, but CycA2 appeared to complex only with Cdk2. These results suggest that the expression of the A-type cyclins and their cdk partners is specifically regulated in different stages of germ cell differentiation and that these cyclin/cdk complexes have different functions in the initiation of and passage through meiosis.

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HUMAN TESTIS AND RAT TESTIS ARE EQUALLY SENSITIVE TO 2-METHOXYETHANOL: A DIRECT *IN VITRO* COMPARISON

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2-Methoxyethanol (2-ME) is one of the glycol ethers which are widely used as organic solvents. It has been shown that 2-ME produces reproductive effects in humans at exposure levels that are ≈ 60-fold lower (2.6 mg/m^3) than those that are toxic to rats (167 mg/m^3), suggesting that humans are much more sensitive to the testicular toxicity of 2-ME than rats. To test whether human testis *per se* is more sensitive to 2-ME than rat testis, we directly compared the responses of cultured human seminiferous tubules (hSTs) and rat (rSTs). This is the only *in vitro* model in which the 2-ME lesion can be faithfully reproduced. *In vitro* studies with glycol ethers require the use of methoxyacetic acid (MAA), the active metabolite of 2-ME *in vivo*. In rSTs, exposure to ≥1mM MAA for 24 hr induced spermatocyte cell death. The dying cells showed necrotic-like morphology, as seen *in vivo*. The same dose-response pattern was found in hSTs; for both species, doses lower than 1 mM were without visible effect. Interestingly, the dying human germ cells appeared apoptotic. Agarose gel electrophoresis on DNA samples from both species showed DNA fragmentation after MAA treatment, indicating that MAA induced apoptosis in both human and rat germ cells, though the dying cells showed different morphology. Furthermore, MAA-induced germ cell apoptosis in both species could be significantly attenuated by plasma membrane-active calcium channel blockers such as nifedipine or verapamil. In summary, these data show that (1) human testis and rat testis respond similarly to MAA, and at the same concentrations; (2) MAA induces germ cell apoptosis both in human and rat, probably through similar, calcium-dependent mechanism(s). The precise steps in this germ cell apoptosis are under investigation.

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CHARACTERIZATION OF THE PROTEIN OSTEOPONTIN IN HUMAN MALE REPRODUCTIVE TISSUES AND CELLS.

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Osteopontin (OPN) is a highly negatively charged, glycosylated phosphoprotein that has been identified in many human epithelial tissues, and has been found in secreted form in milk, gallbladder, and kidney tubular fluid. OPN has been characterized also in bull semen and rat epididymal fluid. The function of OPN is not clearly understood in non-mineralized tissues. It is of interest in the male reproductive tract because it contains a cell adhesion sequence and has a high binding capacity for calcium. Here we report the initial characterization of OPN in human male reproductive tissues. Using monoclonal antibody MPIIIB$_{10}$ directed against purified rat bone OPN, we identified OPN by indirect immunofluorescence in the human epididymis. OPN was localized primarily to the epididymal epithelium, most strongly in the distal caput and corpus regions as compared to either the proximal caput or distal cauda regions. Some staining of connective tissue elements was also observed. Western blot analysis of kidney and testis tissue homogenates, and fluid samples taken from the distal caput, corpus, and proximal cauda regions of the epididymis detected a single, diffuse band migrating at Mr 26-30 kDa. Very faint bands at 26-30 kDa and 44 kDa were observed in muscle tissue homogenate. Examination of washed, human ejaculated sperm by indirect immunofluorescence indicated faint staining of the posterior acrosomal region of the sperm head extending, in some sperm, to the midpiece. These results are the first to be reported on the presence of OPN in the human epididymis and on human ejaculated sperm. This work was supported by funds from the Minnesota Medical Foundation and by USPHS grant HD-11962 to D.W.H.

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ADULT ESTROGEN RECEPTOR KNOCK-OUT MICE HAVE ABNORMAL SEMINIFEROUS TUBULES, RETE TESTES AND EFFERENT DUCTULES

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The function of estrogen in the normal adult male reproductive system is unknown. However, it is known that exposure to estrogenic compounds, such as DES, during the perinatal period induces abnormal development and function of the male reproductive system. To investigate the f nctional role of estrogen in the male tract, we used the transgenic estrogen receptor knock-out mouse (ERKO), which was recently reported to cause infertility in both males and females (Lubahn et al., 1993; Proc Natl Acad Sci, 90:11162). This study examined controls (+/+), heterozygotes (+/-) and ERKO (-/-) mice for testis weight, daily sperm production, cauda sperm concentration, % abnormal cauda sperm, and morphology of the testis, efferent ductules and epididymis. Mean testis weight was found to be greater in the ERKO mice, which also had the greatest variability compared to +/+ and +/-. Mean daily sperm production per testis for +/+, +/- and ERKO was 5.57x10^6, 6.62x10^6 and 2.85x10^6, respectively. ERKO had the greatest number of abnormal sperm in the caudal fluid. Spermatogenesis appeared normal in many seminiferous tubules of the ERKO mice, but the tubules were swollen with e larged lumens and germ cells often appeared to have been sloughed. Spermatogenesis was normal in +/+ and +/- mice. ERKO rete testis appeared swollen and abnormally extended into the testis. The efferent ductules were also swollen in the ERKO but other regions of the tract did not exhibit an enlarged lumen. The efferent ductal epithelium showed the greatest difference compared to all other control (+/+) ductal epithelia. The epithelium in efferent ductules of ERKO was decreased in height, with decreases in apical vesicles, endocytotic apparatus and number of dense lysosomal bodies in nonciliated cells. Ciliated cells were small and contained fewer cilia than in +/+ or +/- mice. However, the +/- mice exhibited an intermediate effect on the efferent ductal epithelium, the nonciliated cells having fewer numbers of vesicles and a slightly smaller height than +/+. ERKO efferent ductules appeared similar to blind-ending tubules that receive no luminal fluids, and have small nonciliated epithelial cells with fewer lysosomes and endocytotic vesicles (Gulttoff et al., 1992). ERKO efferent ductules also had thickened basement membranes and an increase in the number of smooth muscle layers, compared to +/+. The +/- mice showed moderate effects on the connective tissue. Epididymal regions showed minor effects in ERKO, such as more narrow clear cells with fewer vacuoles. These data indicate that estrogen is responsible for proper cellular function in the efferent ductules. Disruption of these functions could affect fluid resorption and lead to swollen seminiferous tubules and a decrease in normal sperm production.

064

ULTRASTRUCTURE OF A SERTOLI LEYDIG CELL TUMOR OF THE OVARY

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Sertoli-Leydig cell tumors of the ovary arise from abnormal differentiation of the sex cords and stroma toward Sertoli and Leydig cells, instead of granulosa and theca cells. These tumors are rare, comprising less than 0.2% of ovarian neoplasms. At the gross level, they tend to be nodular and solid. The histopathologic features range from well differentiated tubules, Sertoli and Leydig cells to an undifferentiated sarcomatoid pattern.

We report a case which showed a mixed pattern on light microscopy. Tubular structures could be identified, but they were mostly solid and closely packed. Only Sertoli cells were noted within the tubules. There were scattered Leydig cells in the intertubular tissue.

Transmission electron microscopy confirmed the presence of tubular structures containing only Sertoli cells, showing characteristic deep indentation of the nuclei and prominent nucleoli. Within the cytoplasm, there were attenuated profiles of smooth endoplasmic reticulum, elongated mitochondria, lipid vacuoles and droplets and some intermediate filaments. Charcot-Bottcher crystalloids were not observed. In the extra tubular tissue, there were varying number of Leydig cells with well developed smooth endoplasmic reticulum, mitochondria with tubular cristae and small myelinoid figures, but no Reinke crystals.

065 EFFECT OF INCREASED SCROTAL TEMPERATURE ON SPERM PRODUCTION IN NORMAL MEN. C. Wang, V. McDonald*, A. Leung*, L. Superlano*, L. Hull*, R.S. Swerdloff, Division of Endocrinology, Department of Medicine, Harbor-UCLA Medical Center, Torrance, CA 90509.

Increased testicular temperature has been known to result in suppression of spermatogenesis. A recent study showed that marked suppression of spermatogenesis to azoospermia occurred in all 14 men after they wore polyester scrotal supporters for a period of 4 to 5 months (Shafter, 1992). This suggested that a simple, physical method of male contraception may be developed. We studied the effect of increased scrotal temperature in 21 normal, healthy men. Subjects in group 1 (n=7) wore athletic supporters lined by a single layer of polyester; in group 2 (n=7), the athletic supporters were lined by a layer of polyester and a second layer of aluminum impregnated polyester; and in group 3 (n=7) the athletic supporters were lined by 2 layers of polyester. Scrotal and rectal temperatures were determined by a thermistor attached to a digital thermometer. The subjects had 4 semen analyses performed during the pre-treatment phase (6 weeks) before the application of the polyester supporters. They were then studied at monthly intervals for 6 to 12 months before entry into a recovery phase. In all groups, the scrotal temperature increased by about 1°C (and the rectal-scrotal temperature decreased about 1°C)in all three groups of subjects after wearing polyester lined athletic supporters (Table).

Scrotal Temp.°C	Pre-Treatment	Treatment Months 3	6	9	12	Recovery
Gp 1	34.2 ± .2	34.9 ± .2	35.3 ± .3	35.2 ± .2	35.1 ± .2	33.9 ± .3
Gp 2	34.3 ± .2	34.8 ± .2	34.9 ± .3	35.0 ± .2	35.6 ± .2	34.7 ± .5
Gp 3	34.3 ± .4	34.8 ± .2	35.3 ± .2	-	-	-

Mean sperm concentration, sperm motility and percent spermatozoa with normal spermatozoa showed decreasing trends with treatment. These decreases were small, not consistent in all subjects, and did not reach statistical significance during most of the treatment period. We conclude that the small increase in scrotal temperature induced by polyester lined athletic supporters was insufficient to cause significant suppression of spermatogenesis. (Supported by the CONRAD Program CSA-93-122 and NIH MO1 RR00425)

066 CRYPTORCHIDISM AND MALE INTERSEX - CLINICAL ANALYSIS AND HISTOLOGICAL AND ENDOCRINOLOGICAL INVESTIGATIONS-

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As previously reported in 1989(J.Urol.,141:1166), cryptorchidism is suggested to have some primary testicular dysgenesis. The relation between cryptorchidism and male intersex was investigated histologically by counting the number of spermatogonia(S/T) of the testis, and endocrinologically by using the human chorionic gonadotropin (hCG) test.

The subjects are 16 cases of bilateral cryptorchidism and 14 of male intersex, 3-8 years old. In this study, 30 testicular biopsies were carried out at the time of operation, and hCG tests were performed on each case.

For male intersex without cryptorchidism, normal testes were found in 3 out of 10(30.0%), and with cryptorchidism in 2 out of 12(16.7%). These rates were lower than that of cryptorchid cases without any complications. The hCG test of 16 cases of prepubertal bilateral cryptorchidism showed that 9 of these cases(56.3%) were recognized as having a less than normal reaction. Comparing cryptorchidism and male intersex, the response pattern of hCG was very much correlated to S/T and in order following : high hCG-reactive cryptorchidism > male intersex without cryptorchidism > low hCG-reactive cryptorchidism > male intersex with cryptorchidism.

These results suggest that cryptorchidism has some primary testicular dysgenesis and is related to the phenomenon of feminization, male intersex.

067 ADRENOMEDULLIN INDUCES PENILE ERECTION IN THE CAT. Hunter C. Champion*, José A. Santiago*, Run Wang, David H. Coy*, William A. Murphy*, Philip J. Kadowitz*, Wayne J.G. Hellstrom. Tulane Medical School, New Orleans, LA 70112

Adrenomedullin, a novel hypotensive peptide that was first isolated in human pheochromocytoma cells, has been shown to cause vasodilation by increasing intracellular levels of cAMP in smooth muscle cells. The peptide has been found in human plasma and may act as a circulating hormone. This study investigates the ability of intracavernosal injections of adrenomedullin to induce penile erection in the cat.

A 30-gauge needle was placed into the right corpus for administration of the peptide into the penis. A 25-gauge needle was placed midway into the left corpus to measure intracavernous pressure. The response was characterized by changes in intracavernosal pressure, duration of the maximum pressure, total duration of the drug effect, change in penile length, and alterations to the systemic arterial blood pressure. The reference drug combination (1.65 mg papaverine, 25 μg phentolamine and .5 μg PGE_1) was injected intracavernosally after each experiment for control.

Intracavernosal injection of adrenomedullin caused penile erection in a dose-dependent manner in cats. The maximum effect on intracavernous pressure (74.0 ± 10.0 mmHg from baseline 11.8 ± 7.3 mmHg) was about 74% of that obtained by the standard drug combination. The maximum increase in penile length (23.3 ± 1.2 mm from baseline 16.3 ± 0.8 mm) was comparable to that caused by the standard drug combination. The duration of maximum pressure (4.7 ± 1.3 mins) and total duration of the drug effect (15.6 ± 4.1 mins) was more abbreviated than that caused by the standard reference combination (30.0 ± 8.0 and 43.0 ± 12.0 mins., respectively, $p<0.01$). Intracavernous injections did not significantly decrease systemic blood pressure whereas the reference drug combination decreased systemic blood pressure by 45.3 ± 9.5 mmHg ($p<0.01$).

The present study supports the potential clinical investigation of adrenomedullin to induce penile erection via intracavernous injection.

068 CESSATION OF SMOKING PRODUCES RAPID IMPROVEMENT IN ERECTILE FUNCTION.

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There is a well known association between cigarette smoking and cardiovascular disease. Tobacco abuse is felt to be as common as diabetes or hypertension in causing erectile dysfunction, and has been shown to be an independent risk factor. Besides long-term vascular blockage attributed to cigarette smoking, acute intrapenile abnormalities are felt to occur such as arterial spasm or venous leakage. Sleep lab monitoring has shown that not only are nocturnal erections abnormal in smokers but that penile rigidity was inversely correlated with the number of cigarettes smoked per day. We studied ten male smokers, ages 32-62 yrs, who smoked one pack or more per day. Nocturnal penile activity was monitored using the RigiScan portable home monitor. Two nights were monitored, the first while smoking and the second not smoking for only 24 hours. Multiple parameters monitored showed statistically significant improvement in nocturnal tumescence and rigidity. Four men were monitored while using a nicotine patch off cigarettes with continued or enhanced improvement suggesting that nicotine is not the ingredient responsible for erectile dysfunction.

PARAMETER	ON CIGS	OFF CIGS	p
	BASE LEAD		
RAU	29.3+/-9.4	51.5+/-11.2	0.000078
TAU	25.8+/-10.0	45.8+/-10.7	0.0012
	TIP LEAD		
RAU	15.8+/-2.2	41.2+/-9.8	0.024
TAU	12.1+/-2.2	33.0+/-6.6	0.007

RAU=rigidity activity units
TAU=tumescence activity units

069 COMPARISON OF TRANSURETHRAL AND INTRA-CAVERNOSAL ADMINISTRATION OF PGE_1 TO INDUCE ERECTIONS IN CATS. Run Wang, Hunter C. Champion*, Nikolaos Sofikitis*, Wayne J.G. Hellstrom New Orleans, LA, Paul Doherty*, Menlo Park, CA.

Intracavernosal PGE_1 is used for the pharmacologic treatment of impotence. The medicated urethral system for erection (MUSE) delivers vasoactive agents transurethrally. This study compares PGE_1 administered by these two methods in cats.

PGE_1 was administered by either a 30-gauge needle intracavernosally or by a 20-gauge Jelko™ transurethrally. The response was characterized by changes in intracavernosal pressure, duration of maximum pressures, total duration of drug effect, changes in penile length, and alterations in systemic arterial blood pressure. The reference standard drug combination (1.65 mg papaverine, 25 μg phentolamine and 5 μg PGE_1) was given intracavernosally for control comparison.

PGE_1 caused penile erection in a dose-dependent manner by both methods. The maximum effects on cavernosal pressure (86.4 ± 16.0 mmHg from the baseline of 9.2 ± 1.6 mmHg) and penile length (23.8 ± 1.6 mm from the baseline of 16.4 ± 1.1mm) obtained by 0.5 mg PGE_1 transurethrally were comparable to those caused by 30 μg PGE_1 intracavernosally and by the control. The duration of the maximum pressure (23.2 ± 5.0 min) and the total duration of the drug effect (38.7 ± 7.0 min) transurethrally were shorter than the control (30.0 ± 8.0 min and 43.0 ± 12.0 min respectively, $p<0.05$). The dose of PGE_1 needed to cause maximum response transurethrally was approximately 17 times higher than intracavernosally. PGE_1 decreased systemic blood pressure by 9.2 ± 2.0 mmHg while the control decreased it by 62.0 ± 17.0 mmHg ($P<0.01$).

Transurethral delivery of PGE_1 can induce comparable penile erections to those caused by intracavernosal injections of PGE_1 or by intracavernosal injection of the standard combination

Abstract withdrawn.

071 LONG-TERM PASSIVE SMOKING IN THE RAT REDUCES PENILE NITRIC OXIDE SYNTHASE WITHOUT IMPAIRING THE ERECTILE RESPONSE TO ELECTRICAL STIMULATION OF THE CAVERNOSAL NERVE Y. Xie*, H. Garban*, Ch. Ng*, J. Rajfer and N.F. Gonzalez-Cadavid. UCLA School of Medicine, Department of Surgery, Division of Urology, Harbor-UCLA Medical Center, Torrance, CA

Smoking is a risk factor for erectile dysfunction. Penile erection is triggered by nitric oxide (NO), which is synthesized in the penis by the neuronal nitric oxide synthase (nNOS). To determine whether long-term smoking impairs erectile function and whether this is caused by a decrease in penile NOS, adult (5 month-old) and old (20 month-old) Fischer 344 rats were exposed or not (n=12) to daily passive smoking for 60 days. Three days later, half of the animals were submitted to electrical field stimulation (EFS) of the cavernosal nerve and the maximum intracavernosal pressure (MIP) and mean arterial pressure (MAP) were determined and expressed as mm Hg+/-S.E.. NOS activity in the penile cytosol was determined by the arginine/citrulline assay, and nNOS content was estimated by western blot. As compared to controls, the smoking rats had a higher MAP in both the adult (120 vs 90) and old (175 vs 140) rats, but surprisingly the MIP increased from 77 to 111 (adult rats) and from 58 to 83 (old rats). Smoking reduced penile NOS activity by 73% (adult rats), and 62% (old rats). The 160 kD nNOS band was also reduced by 43% and 50%, respectively. Neither exposure to nicotine (1mM) nor to cotinine (0.5 mM) for 5 days inhibited nitrite release by cavernosa slices in vitro, or by rat penile smooth muscle cells (RPSMC) up to 10 passages. These results indicate that in the rat: a) chronic smoking induces hypertension and augments the erectile response to EFS; b) smoking reduces penile NOS; c) nicotine does not seem to be responsible for the penile NOS impairment.

072 CLINICAL AND LABORATORY TREATMENT OF RETROGRADE EJACULATION

R. Donald Eward, MD

The patient with retrograde ejaculation presents a clinical problem in the collection of viable sperm for use in intrauterine insemination in the spouse. The timing of ovulation also adds to the complexity of the clinical management of the male. Three patients diagnosed with retrograde ejaculation were evaluated and treated with sodium bicarbonate tablets. Concurrently the spouses were treated with ovulation induction and inseminated with Percoll washed sperm when appropriate. Four term pregnancies have been achieved with the methods employed. Discussion of the clinical and laboratory treatment along with the pre and post wash semen results will be presented.

073 DECLINE IN SEMEN QUALITY: GLOBAL OR SELECTIVE?

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Recent reports have appeared suggesting that human semen quality has steadily declined over the past 50 years. Environmental toxins that possess estrogenic properties and chemicals such as DDT have been held responsible. We examined our data on 510 normal men that we had collected over the past 20 years to address this issue. The mean age was 29 years, body weight 171 lbs., and a median of 6 semen samples (range 4-30) were collected per man. The men were employed in non-manufacturing jobs or attended college. No intervening illnesses were observed during the semen collection period and the men were controls for other studies. Semen analyses were performed using WHO standards, but no results that showed "low" counts were excluded from analysis. We established statistical analytic methods for sperm concentration using log transformed data (Berman et al., J. Andrology, in press). The geometric mean for our male volunteers ranged between 49.20 (C.I. 42-57) and 68.17 (C.I. 56-83) million/ml for 195 men in 1973-7 and 51.96 (C.I. 43-62) to 76.17 (C.I. 52-112) million/ml in studies between 1989-93 involving 70 subjects. No decrease in sperm concentration with time was noted between 1977-1989 in the remaining men. Semen volume also did not decrease with time. We conclude that no decline in semen quality could be detected in the data from the male volunteers residing in our greater Seattle metropolitan area. Based on other reports that semen deterioration occurs, then it would follow that it is geographically selective. If this is true, the public health implications are obvious.

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074 SUBMICROSCOPIC DELETIONS OF Y CHROMOSOME OUTSIDE THE DAZ REGION IN A SUBSET OF INFERTILE MEN IMPLICATE ADDITIONAL Y-SPECIFIC GENES IN THE PATHOGENESIS OF MALE INFERTILITY. H. Najmabadi, V. Huang, M. Subarao, P. Yen, D. Bhasin, S. Naseeruddin, W. Taylor, D.M. DeKretser, K. Loveland, H.W.G. Baker, R.I. McLachlan, S. Bhasin. Department of Medicine, Charles R. Drew University of Medicine and Science, Los Angeles, CA 90059

Genes on the long arm of Y chromosome (Yq), deletion interval 6, have been postulated to play a critical role in human spermatogenesis. Cytogenetically detectable deletions of this region are associated with azoospermia, but are uncommon. Recent reports suggest that submicroscopic deletions of Yq interval 6, that are not detected by karyotyping, may be more common in infertile men. The objective of this study was to determine by using a sequence-tagged site (STS)-mapping method what proportion of men with idiopathic azoospermia or severe oligozoospermia carry microdeletions in Yq interval 6. Another aim was to assess if Yq deletions in infertile men include the DAZ (*d*eleted in *a*zoospermia) gene, a Y-specific gene that was proposed as a candidate for azoospermia by the Page laboratory. Genomic DNA was extracted from peripheral leukocytes of 60 infertile men with idiopathic azoospermia or severe oligozoospermia. Controls included 16 normal fertile men, 7 females, and 15 patients with the X-linked disorder ichthyosis. Polymerase chain reaction (PCR) analysis of 26 Y-specific STSs showed no deletions in any of the normal men or patients with ichthyosis. Female DNA failed to amplify any of the STSs. Of the 60 infertile men typed, 11 (18%) failed to amplify one or more STSs. The deletions were predominately clustered in two regions of Yq interval 6: a distal region extending from sY149 to sY158 that overlaps with the deletion region from which DAZ gene was isolated by the Page laboratory (DAZ region); and a proximal region extending from sY131 to sY139 that does not overlap with the DAZ region. Four of these 11 patients had proximal Yq microdeletions that did not overlap with the DAZ region. Presence of DAZ sequences in 3 of these 4 infertile men with proximal Yq deletions was confirmed by PCR, using DAZ-specific primers. Conclusions: These data confirm the high prevalence (18%) of Yq microdeletions in infertile men with azoospermia or severe oligozoospermia. Presence of deletions in some of the infertile men in the proximal region of Yq interval 6 that does not include the DAZ gene, suggests that additional Y-specific genes, other than the DAZ, are associated with a subset of male infertility.

075 CONTRACEPTIVE VACCINE DEVELOPMENT: THE B-CELL EPITOPE OF A NOVEL TESTIS ANTIGEN

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Our strategy to develop a contraceptive vaccine involves using molecular cloning technology to identify antigenic determinants (epitopes) recognized by anti-sperm antibodies. These epitopes may then be synthesized chemically for use as a vaccine. To identify candidate antigens, a human testis cDNA expression library was screened with serum containing antisperm antibodies obtained from an infertile woman (serum 629; Clarke, G.N. *et al.*, 1988, *Fertil. and Steril.* 49, 1018-1025). This serum completely inhibited *in vitro* fertilization of human oocytes and identified a cDNA clone, designated C2. Clone C2 recognized a 2.1 kb mRNA specific to testis, and not in somatic tissues. This clone did not show significant homology to any DNA or protein sequences in *Genebank*. An 872 bp EcoRI fragment of clone C2 was subcloned into the pGEX 4T-1 vector and expressed. The purified glutathione-S-transferase fusion protein was recognized by the original 629 serum on a Western blot. To map the epitopes of C2 fusion protein, unidirectional nested deletion constructs were generated to produce truncations from the 3'-end of the cDNA. Fusion proteins were produced and purified from each deletion construct and analyzed by Western blot with 629 serum. A single 29 amino acid region containing an 18 amino acid B-cell epitope was identified near the carboxy-terminus of the protein. Sera from 2 other infertile patients recognized this determinant. This peptide has been synthesized and will be tested for immunogenicity and contraceptive efficacy in the non- human primate.

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076 SPERM CHROMATIN STRUCTURE ASSAY (SCSA) PREDICTS HUMAN FERTILITY POTENTIAL. [1]D. Evenson, [2]M. Zinaman*, 3E. Clegg and [1]L. Jost*, [1]Olson Biochemistry Laboratories, South Dakota State University, Brookings, SD, [2]Department OB/GYN, Loyola University Medical Center, Maywood, IL and [3]US EPA, Washington, DC.

Multiple semen samples were obtained from 200 couples participating in a very comprehensive male-factor fertility study at the Georgetown Medical Center, Washington, D.C. (M. Zinaman, PI). 450 semen samples were measured by the SCSA which defines abnormal chromatin structure as an increased susceptibility to DNA denaturation in situ and is quantitated by the expression alpha t [α_t = red/(red + green) fluorescence]. SCSA variables used were Meanα_t, SDα_t and Cells Outside the Main Population (COMPα_t). In addition, percent of cells with high green fluorescence (lack of chromatin condensation) were determined. Using SCSA data on semen samples from couples successfully pregnant by 3 months as the "fertile standards", the mean SCSA variables and percent high green fluorescence values were all significantly higher (lower quality, $p<0.001$) from those requiring up to 9 more months to achieve pregnancy, which in turn were significantly different from those that were not pregnant after 12 months. The SCSA is a rapid, sensitive, precise and statistically robust method for evaluating human males for potential fertility.

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077 **EFFECTS OF A NONSCROTAL TESTOSTERONE TRANSDERMAL SYSTEM ON SEXUAL FUNCTION OVER 6-12 MONTHS OF TREATMENT IN HYPOGONADAL MALES.** S Arver*,[1] AS Dobs,[2] AW Meikle*,[3] S Sanders*,[4] NA Mazer*,[4,5] [1]Karolinska Inst. Stockholm; [2]Johns Hopkins U., Baltimore MD; Dept [3]Medicine and Pathology, and [4]Pharmaceutics. U. Utah, Salt Lake City, UT; [5]TheraTech Inc, Salt Lake City, UT.

Introduction and Objectives: We report the results of two open-label multicenter studies that investigated the effects on sexual function of treatment with a nonscrotal testosterone transdermal (TTD) system (Androderm®).

Methods: Previously treated (Study 1) and untreated (Study 2) hypogonadal males used TTD systems daily for 6-12 months. Effects on sexual function were measured objectively by RigiScan® monitoring and subjectively by patient-completed Watts and Davidson Questionnaires. Evaluations during TTD systems treatment were compared with those obtained during prior intramuscular testosterone (IM) treatment and androgen-free periods (Study 1) or a prior treatment-free period (Study 2).

Results: 29 previously treated males and 12 previously untreated virilized males completed the studies. Mean serum testosterone levels measured monthly during treatment were within normal range in 93% of TTD-treated patients in Study 1, and 100% in Study 2. Overall measures of sexual function in Study 1 showed significant ($p \leq 0.003$) improvement during treatment with TTD systems compared with the treatment-free period, and did not differ from those seen during the IM treatment period. In Study 2, sexual function was maintained, with improvement in Watts score ($p < 0.05$).

Study	Period	RigiScan® Base/Tip†	Watts‡	Davidson‡
1	Hg Baseline	6.5(0-30)/ 4.5(0-23)	52.5(1.6)	2.3(0.6)
	TTD Systems	12.7(1-35)/11.3(1-37)	60.4(1.5)	7.8(1.1)
2	Pt Baseline	13.7(3.8-25.9)/ 8.9(0.9-20.0)	54.0(1.5)	5.8(2.5)
	TTD Systems	13.1(7.1-22.3)/ 8.7(4.3-15.6)	60.0(2.5)	7.0(2.3)

†Erectile Index (range) = (total events/h) x (mean event duration) x (mean rigidity); ‡Questionnaire, mean score (SEM); Hg=hypogonadal; P=pretreatment

Conclusions: TTD systems normalized testosterone levels and improved sexual function in both previously treated and previously untreated males who used the systems over intervals of 6 to 12 months.

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NO DECLINE IN DAILY SPERM PRODUCTION IN A GROUP OF NORTH AMERICAN MEN OVER A SEVEN-YEAR PERIOD. L. Johnson, R. Levine, J.J. Barnard, and W.B. Neaves. Dept. of Vet. Anatomy & Public Hlth., Texas A&M University, College Station, TX; National Institute of Child Health and Human Development, Bethesda, MD; Depts. of Pathology and Cell Biol./Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX.

Studies indicating diminished spermatogenic potential in humans have been based on declining sperm count in human ejaculates. The large variation in ejaculated sperm numbers make it difficult to estimate spermatogenic potential in men. Hence, a more direct, non-ejaculate method of determination of spermatogenic potential is needed. Daily sperm production (DSP) estimates spermatogenic potential by counting cells in the process of spermatogenesis. The objective was to determine if DSP/g parenchyma and DSP/man decline between years 1979 and 1986 in testes obtained at autopsy in Dallas, Texas. Testes were perfused with glutaraldehyde, further fixed with osmium, embedded in Epon, and evaluated by stereology for volume density of seminiferous tubules. DSP was estimated from number of spermatids in homogenates of fixed testicular tissue. For years '79, '80, '82, '83, '84, and '86, fairly large numbers of men (54, 39, 14, 58, 18, and 9, respectively) were used, and these had similar mean ages (28 ± 1, 28 ± 1, 29 ± 1, 30 ± 1, 32 ± 1, and 31 ± 3 yr). Paired testicular parenchymal weight (29 ± 1, 31 ± 1, 41 ± 2, 38 ± 1, 40 ± 3, and 32 ± 4 g), percentage of seminiferous tubules (70 ± 4, 74 ± 1, 59 ± 2, 64 ± 2, 61 ± 3, and 60 ± 5), DSP/g (6.0 ± 0.5, 5.7 ± 0.6, 5.4 ± 0.6, 4.0 ± 0.2, 4.4 ± 0.3, and 5.8 ± 0.9 x 10^6), and DSP/man (181 ± 18, 177 ± 19, 224 ± 29, 159 ± 11, 186 ± 20, and 191 ± 45 x 10^6) indicated no pattern of diminished spermatogenic potential from 1979 to 1986. Based on direct measures of sperm production potential in these North American men, there appears to have been no decline in spermatogenic potential during this 7-year period. **NIH K04 AG00464-04**

079 **MEDIA AND DILUTION PROCEDURES FOR PROCESSING HUMAN, RABBIT AND BULL SPERM FOR COMPUTER-ASSISTED SPERM ANALYSIS.** R.H. Foote and P.B. Farrell*, Dept. of Animal Science, Cornell University, Ithaca, NY 14853.

Proper handling of semen prior to computer-assisted sperm analysis (CASA) is critical, if the analysis is to be representative of the fresh sample. The effects of diluting medium or dilution and holding time before CASA on multiple sperm characteristics were studied. Four replicates of semen from each of eight human donors were diluted with PBS-glucose plus BSA (PBSGB), and compared with TALP and with high potassium TALP to a concentration of approximately 25 x 10^6 sperm/ml. Diluted human semen was held for 0, 1 and 2 hours in capped tubes on a warming plate at 37°C before CASA. There was little difference between the three diluters in all 12 variables measured, with a decline of 3 to 6% in the proportion of motile sperm during 2 hours ($P<0.05$). Donors were the largest source of differences ($P<0.05$). Rabbit sperm (5 bucks x 4 ejaculates per buck) were processed similar to the human sperm. There was a major effect of media. The average percentages of motile sperm over 2 hours in TALP, K-TALP and PBSGB were 76, 42 and 29, respectively ($P<0.05$), with a decline of only 3% in TALP during 2 hours. Hyperactivity and other characteristics were affected by treatment. Donors were a large source of variation. Bull semen (10 bulls x 2 ejaculates per bull) either was not diluted or diluted with TALP 1:1 or 1:3 and held for 0, 1 and 2 hours at 37°C and then diluted to 25 x 10^6 sperm/ml with TALP. There was little change in most sperm characteristics during the 2 hours, although many were statistically significant. The percentage of motile sperm in the undiluted semen declined from 87 to 82% during this time. The best procedures tested maintained sperm from all species with little change for at least 1 hour. Modified TALP was a suitable diluting medium for sperm from all three species, and a simple PBS-glucose-BSA medium can be used for human sperm.

080 **HUMAN SPERM MOTILITY AND OTHER CASA VARIABLES MEASURED DIRECTLY ON SPECIMENS TREATED WITH HOECHST 33342 DYE.** R.H. Foote[1], P.B. Farrell[1]* and M.J. Zinaman[2]. Dept. of Animal Science[1], Cornell University, Ithaca, NY 14853 and Dept. of Ob/Gyn[2], Loyola University Medical Center, Maywood, IL 60152.

The accurate measurement of the proportion of motile sperm, as well as the determination of total sperm concentration, using computer-assisted sperm analysis, requires that static particulate matter and nonmotile sperm be clearly distinguishable. A Hamilton Thorne IVOS unit equipped with optics for fluorescence, was used to distinguish sperm stained with DNA-specific Hoechst 33342 dye from non-nuclear particles. The standard phase contrast option was used to evaluate sperm with no dye. Fresh semen and semen diluted with buffered egg yolk before and after freezing were examined. Hoechst stain (0, 5, 10 and 20 μg/ml) was included in the phosphate-buffered saline with glucose and BSA used to dilute the semen to 25 x 10^6 sperm/ml for CASA analysis. The effect of dye on semen from nine donors was examined after 0, 0.5, 1 and 2 hours of holding time in this medium. There was no significant effect of 5 μg/ml of Hoechst dye for 0.5 hours and there was little effect over the 2 hours of exposure on any variables measured. The percentage of motile sperm exposed to 5 μg/ml of dye was 34 at both 0 and 0.5 hours and with 10 μg/ml of Hoechst dye after 0.5 h it was 29 ($P<0.05$). However, 10 μg/ml of dye stained sperm sufficiently to evaluate the samples immediately and there was no effect on sperm motility (37%) at 0 hours. With egg yolk added for storing or freezing sperm a dye concentration of 40 μg/ml was required for proper staining and this did not affect motility of sperm. Thus, sperm concentration and the proportion of motile sperm can be determined accurately with Hoechst dye in fresh specimens and those processed with egg yolk for storing or freezing semen.

081

ENERGY AND SPERM MOTILITY IN SEMINAL HYPER VISCOSITY. G.R.Mendeluk, M.J. Munuce*, C. Carizza, M. Sardi* and C. Bregni*. Medicina Reproductiva, Rosario y Departamentos de Bioquimica Clinica y Tecnologia Farmacéutica, Facultad de Farmacia y Bioquimica, Universidad de Buenos Aires.

Seminal adenosine triphosphate (ATP) content (bioluminescence), objective spermatic motility (Hamilton Thorne Research) and the rapid progressive spermatozoa (grade "a") recovery after swim-up were studied in hyperviscous samples.

The ATP concentration per living spermatozoa in normal samples was significantly lower than in asthenospermic samples ($\bar{X} \pm 2SEM$ pmol per million living spermatozoa, 317.2 ± 101.3 (n:7) and 486.3 ± 77.1 (n:38) respectively, $p<0.05$). On subdividing the latter group according to consistency, the mean sperm ATP concentration was significantly higher in the hyperviscous samples than in the semeo samples with normal consistency (normal: 449.4 ± 65.1 (n:29) and high: 605.1 ± 242.8 (n:9); $p<0.05$). Average path velocity (VAP), straight line velocity (VSL), curvilinear velocity (VCL), amplitude of lateral head displacement(ALH) and linearity (LIN) were significantly higher in normal samples than in athenozoospermic samples (35.2 ± 3.1 um/sec, 30.5 ± 2.9 um/sec, 46.6 ± 3.1um/sec, 6.3 ± 0.4 um, 61.2 ± 3.6% (n:13) and 23.3 ± 1.8, 18.8 ± 1.7, 35.9 ± 2.6, 4.1 ± 0.7, 52.4 ± 3.6 (n:36); $p<0.05$). Classifing the astheozoospermic group according to consistency ALH was significantly lower in hyperviscous semens (normal: 4.6 ± 0.7 um (n:20) and high: 3.5 ± 1.2 um (n:16); $p<0.05$).The rapid progressive spermatozoa recovery after swim-up was significantly higher in the hyperviscous group (normal: 71.0 ± 38.0% (n:14) and high: 181.3 ± 108.9% (n:6); $p<0.02$).

We conclude that spermatozoa from hyperviscous ejaculates may adapt themselves efficiently to the surrounding medium, diminishing the employment of cinetic energy when the medium is adverse and being able to utilize it under improving conditions.

082 EFFECT OF LIPOPOLYSACHARRIDE (LPS) AND INTERFERON-γ (IFN-γ) ON SPERM MOTION IN VITRO. Run Wang, Lance S. Estrada*, Hunter C. Champion*, Mahadevan Rajasekaran, Suresh C. Sikka, Wayne J.G. Hellstrom. Tulane Medical School, New Orleans, LA 70112

It is well recognized that cytokines such as interferon are elevated in the semen of men with genitourinary inflammation. Whether this increase in cytokines is the major mechanism by which some men with genitourinary inflammation suffer from infertility is under current investigation. The present study was designed to determine the in-vitro effect of lipopolysacharride (LPS) and interferon-γ (IFN-γ) on sperm motility, viability, and motion parameters.

Washed sperm form normal volunteers (n=7) were incubated in the presence/absence of LPS (0.1 μg/ml) plus IFN-γ (0.1 μg/ml). The sperm motility, viability, and video sequences were recorded at different time intervals 0, 30, 60, and 180 minutes. The sperm motion parameters were analyzed using computer assisted semen analysis.

Our results demonstrated a time-dependent negative correlation of LPS plus IFN-γ on sperm motility, viability, straightline velocity (VSL), and lateral-head displacement (ALH). The maximum decrease in sperm motility (47.1 ± 4.8), viability (45.5 ± 5.01), and ALH (4.4 ± 0.2) was observed at 120 minutes and was significant compared to the control samples (60.2 ± 4.8, 62.4 ± 2.9, and 5.3 ± 0.4, respectively, $p<0.05$). The maximum detrimental effect on sperm VSL (57.6 ± 3.7) when compared with control (69.7 ± 4.7) ($p<0.05$) was observed at 60 minutes.

This study suggests that the detrimental effects of LPS plus IFN-γ on sperm motility, viability, and motion parameters will influence the fertilizing capacity of human sperm. These mechanisms are likely operational in cases of unexplained infertility in men with genitourinary inflammation.

083 OPTIMIZING MOTILITY FOLLOWING CRYOPRESERVATION OF EPIDIDYMAL AND TESTICULAR SPERM. A.H. Amin*, B.T. Storey, L. Blasco*, J.L. Murmar# and S. Heyner. Department of Obstetrics and Gynecology, University of Pennsylvania, Philadelphia PA 19104. # Department of Urology, Cooper Hospital, Camden NJ 08103.

There is an increasing need to cryopreserve both epididymal and testicular sperm for ART techniques in the management of male infertility. We describe the results of comparing two different media to cryopreserve these sperm obtained by biopsy. One is the standard TEST-buffer + egg yolk (TBY) medium and the other is HTF medium containing 5mg/ml BSA and 6% glycerol (HTFBG). Ten epididymal aspirates obtained via micro-surgical epididymal aspiration, and three testicular samples obtained by testicular biopsy were cryopreserved using the two media above. The concentration of the epididymal sperm was 7.5 ± 4.5 x 10^6 and the motility was 47% ± 15 after swim-up. Testicular sperm concentration was 6.5 ± 26 x 10^6 and motility was 27% ± 9. Following cryopreservation, we assessed recovery of motility of the epididymal and testicular spermatozoa, at 10, 20, 40, 60, and 120 min., respectively. In the TBY method, the initial recovery of epididymal sperm motility was rapid; the % motility then decreased with time up to 2 hr post thaw. (At 10 min, 30 ± 11; 20 min, 27 ± 10; 40 min, 25 ± 9; 60 min, 22 ± 8; and at 120 min, 20 ± 7). In the HTFBG method, initial recovery of motility of the epididymal spermatozoa was slow, followed by gradual improvement of motility with time. (At 10 min, 18 ± 7; 20 min, 26 ± 8; 40 min, 29 ± 9; 60 min, 30 ± 10; and after 120 min, 26 ± 9). With regard to testicul r spermato oa there was no significant difference in the pattern of the recovery of motility between TBY and HTFBG except by 2 hr post-thaw. (At 10 min, 11 ± 4 vs 7 ± 3; 20 min, 10 ± 3 vs 8 ± 1; 40 min, 8 ± 2 vs 10 ± 4; 60 min. 7 ± 3 vs 10 ± 3 (n.s.); and after 120 min, 3 ± 1 vs 9 ± 6 ($p < 0.001$)). We conclude that HTFBG is a superior medium for cryoprotection, and permits recovery of viable, motile epididymal and testicular sperm. Supported by NIH grant 25867 and a grant from the Egyptian Government to AHA

084 MORPHOLOGICAL AND MORPHOMETRIC COMPARISONS OF RABBIT SPERMATOZOA EXPOSED TO LEAD

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Morphology and morphometric measurements were performed on stained spermatozoa from 35 Dutch Belted rabbits. Baseline ejaculates were collected weekly for five weeks prior to 15 weeks of subcutaneous injections of lead acetate to maintain blood lead levels of 0, 50, 70, 90, and 110 ug/dl. Five weeks before the end of the study, ejaculates were again collected and compared to the baseline data. Morphometric measurements were made with the Image Processor (Image Technology Corporation, Deer Park, NY) and morphology was evaluated by a trained technician. Rabbit semen appears to have few abnormally shaped sperm heads, thus a comparison of the two measurements should show a relationship. A random intercepts linear model was fit to these data and statistically significant linear trends were discovered for all morphology and morphometry measurements (p-values range from 0.0015 to 0.0001). The most prominent trends in morphology is a decrease in percentage normal, a decrease in percent of cells with normal acrosomes, and an increase in the proportion of small cells. In morphometry, the average length, area, and perimeter of sperm cell heads show a large decline. Predicted values estimated from the random intercepts model are presented in the following table:

	MORPHOLOGY			MORPHOMETRY		
Blood Lead Level	Percent Normal Sperm	Percent Normal Acrosome	Percent Small Sperm	Average Head Length	Average Head Area	Average Head Perimeter
0 μg/dl	85.2	42.1	4.0	8.36	29.1	22.1
50 μg/dl	66.2	33.4	10.5	8.15	28.2	21.6
100 μg/dl	47.3	24.8	17.0	7.94	27.2	21.1

Lead exposure has a consistent effect on morphology and morphometry variables; an increase in lead exposure is associated with small sperm cells and defective/missing acrosome caps.

085

DOES ELEVATED BLOOD LEAD INCREASE SPERM VELOCITY?

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As part of a large lead project to validate the rabbit model, sperm motion analysis was conducted on 700 ejaculates. Longitudinal study design involved 5 weeks of baseline followed by 15 weeks of lead dosing with the last 10 weeks at plateau target values. Dutch Belted rabbits (n=35) were randomized into five dose groups (n=7/group): 0.0 μg/dl, 50 μg/dl, 70 μg/dl, 90 μg/dl, and 110 μg/dl. Motion analysis was conducted on the first ejaculate. The ejaculates were maintained at 37°C for no more than 10 minutes until dilution in Ham's F-10 tissue culture medium, then examined by microscopy and video taped for computerized motion anelysis. Analysis was performed using Celltrack VP110 by Motion Analysis. The parameters evaluated included curvilinear velocity, straight line velocity, and percent motility. We found that there was a statistically significant decrease (p=0.0001) in the number of motile cells in the lead treated groups. We also noted as did Osorio et al. (1992) and Wildt et al. (1983) an increase in sperm velocity in the lead treated groups. The increase in curvilinear velocity was statistically significant with a p-value of 0.0001. There was a slight temporal increase in the average curvilinear velocity in the controls, but this temporal trend was not statistically significant with a p-value of 0.315. Each of the lead treatments showed an increase even after adjustment for the temporal trends in the controls. The increase in straight line velocity was also statistically significant (p=0.016). There was a slight temporal increase in average straight line velocity in the controls which was statistically significant (p=0.042) and differences among pre-exposure means at each target level, but this effect was not statistically significant (p=0.090). The increase in both curvilinear and straight line velocities was unexpected. Possible explanations for the increase include:

1. Decreased sperm head size, could have improved hydrodynamics.
2. Change in proportion of motile cells such that lead treatment shifts slow cells to immotile cells
3. Change in frequency distribution of cell velocity types.

086

PRO-INFLAMMATORY CHEMOKINE (IL-8) MEDIATED EFFECTS ON HUMAN SPERM MOTILITY, VIABILITY, AND INTEGRITY

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Inflanunation of the male genitourinary (GU) tract is a potential cause of infertility. Our recent studies have demonstrated that GU inflammation elaborates significantly high levels of pro-inflammatory chemokines specifically IL-8 and GRO-α in human seminal plasma. Three-fold higher IL-8 levels in seminal plasma were observed in infertile men with leukocytospermia compared to normal controls. However, it is not known whether these pro-inflammatory cytokines have any direct detrimental effects on human sperm. The present study was designed to evaluate the in-vitro effect of IL-8 on human sperm motion parameters, hypoosmotic swelling test (HOST) and viability. Washed sperm from normal volunteers (n=9) were incubated with human recombinant IL-8 (0, 100, 300 ng/ml). The motility, viability, and motion parameters were recorded at 0, 30, 60, 120, and 180 minutes. The sperm motion parameters were analyzed using computer assisted semen analysis (CASA). Our results showed a time and dose dependent effect of IL-8 on sperm motility and viability. A maximum 18% decrease in sperm motility and a 25% decrease in straight line velocity was observed ($P<0.05$) at 120 minutes with 300 ng/ml dose compared to control. Viability and HOST evaluations did not show any detrimental effects on sperm membrane under present conditions. Thus, this pro-inflammatory chemokine does not have a significant detrimental effect on sperm integrity and function under these conditions. Decreased sperm motility may be caused by the effects of high IL-8 in vitro on sperm flagellar mechanisms. These data imply that additional intermediary mechanisms are involved in the decreased sperm function observed during clinical GU inflammation.

087

SPERM PARAMETERS IN MEN WITH VARICOCELES (Vx) PRIOR TO AND AFTER SURGERY (Sx) COMPARED TO MEN WITHOUT VARICOCELES.

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There are numerous conflicting studies on the effects of Vx on sperm parameters and function, and the efficacy of surgical repair. The purpose of this study was to compare sperm parameters in men with Vx before and after Vx ligation. Men were examined manually using valsalva maneuver or doppler ultrasound. Semen specimens were examined at 0.5h after ejaculation by computer-aided analysis (CASA; HTM-2030) using standard set-up parameters. At least 3 analyses were done per patient per group (before and after surgery). The mean follow-up time after Sx was 6.5 months. Sperm count (SC) and motility (MOT), hypoosmotic swelling (HOS) test and morphology by Pap stain were examined in the following: Group 1 - Vx, no Sx (n=8); Group 2 - Vx, Sx (n=13); no Vx (n=4); known fertile donors, no Vx (n=20). The mean (SD) for each measured parameter was as follows:

	SC(mill/ml)	MOT (%)	% Normal	% HOS
Grp 1(no Sx)	42.1 (26.1)	22.1(12)	46.1(12.9)	53.3(16.7)
Grp 2 (Pre)	48.0 (40.7)	22.4(6.2)	48.1(20.5)	69.8(12.8)
(Post)	46.6 (45.4)	26.8(10.1)	53.3(17.3)	60.9(24.9)
No Vx	28.7 (21.1)	32.8(17.4)	55.9(10.4)	59.4(19.0)
Donors	168.5 (67.9)	66.7(8.7)	78.3(7.3)	80.3(4.9)

There were no differences in the SC, MOT, HOS and % Normal between the patient groups. There was no difference in the SC prior to and after Sx. However, four patients showed a mean of 42.5% (34.1) increase in SC, 8 showed a mean 35% (11.7) increase in MOT and 5 showed a mean 29.4% (17.7) increase in % normal after Sx. In conclusion, although the numbers are small, this study did not show a significant and consistent improvement in sperm parameters following Vx surgery by 6.5 months in the entire group, but did show a trend towards improvement in SC, MOT and normal forms in some patients examined. Repair of Vx may be useful in a subset of infertile men who do not respond favorably to hormone therapy or intrauterine insemination.

088

FLOW CYTOMETRIC TECHNIQUES TO IDENTIFY AND ENUMERATE VIABLE SPERM CELLS.

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Sperm cells from fresh and/or cryopresered bull, boar, ram, mouse, human, and turkey semen were analyzed and enumerated with the Bryte HS flow cytometer (Bio-Rad). Semen samples, particularly those diluted in milk- or egg-based cryopreservation media, may contain large numbers of particles which cannot be distinguished from sperm cells by light scatter measurements alone. Therefore, a labeling system capable of distinguishing sperm cells from numerous extraneous particles is needed. In addition, one also needs a labeling system that can dietinguish "live" and "dead" sperm cells if one intends to assess seminal quality. Two fluorescent labeling systems were evaluated, namely, combinations of SYBR-14 or Hoechst 33342 with propidium iodide (PI). SYBR-14 (*Fertil*Light, Molecular Probes, Eugene, OR) binds to nucleic acid, selectively accumulates in *intact* cells, is excited by blue (~490nm) light and produces green (~530nm) fluoreseence. Hoechst 33342 stoichiometrically binds to DNA in both intact and damaged cells, is excited by UV light (~350nm) and fluoresces blue (~460nm). PI also binds to nucleic acids but it selectively labels cells with *damaged* membranes. PI can be used with either SYBR-14 or Hoechst 33342 since it can be excited by UV or by blue light. PI labeled damaged cells produce red (~620nm) fluorescence so that SYBR-14 (green) or Hoechst 33342 (blue) labeled intact cells can be readily distinguished. The Bryte HS flow cytometer has an arc lamp as its light source and one can alter the excitation and emission wavelengths simply by exchanging optical filter blocks. It also uses a calibrated micro-syringe to inject the sample so that *absolute* cell concentrations can be determined in addition to the percentages of live and dead cells. Staining and/or analysis conditions, *e.g.*, dye concentration, incubation time, sample flow rate, *etc.*, were evaluated. Semen samples were rapidly measured flow cytometrically, *i.e.*, 30-60 samples/hr, and the resultant data files were then analyzed by *SuperCyt Analyst* (Sierra Cytometry). The results showed that although both labeling systems could identify and enumerate intact sperm cells, the Hoechst 33342-PI combination had the advantage of being less affected by particulate materials in egg- or milk-based semen extender media.

089 **CHANGES IN SPERM MOTION PARAMETERS FOLLOWING PERCOLL SPERM WASH FOR MOTILE SPERM CELL SEPARATION.**

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Computer automated semen analysis (CASA) appears to be the most objective way to investigate changes in sperm parameters. This study was designed to compare pre- and post- percoll treated sperm motion parameters. A paired-sample comparison was used to analyze parameters before and after motile cell separation in three groups: fresh semen (group 1); chymotrypsin-galactose treated semen (for men with known sperm antibodies; group 2) and cryopreserved semen (group 3). One hundred thirty-six fresh semen specimens, twenty chymotrypsin galactose treated specimens and thirty-two cryopreserved specimens were analyzed. Each semen specimen was layered onto a 1cc 80% Percoll gradient and washed with modified Human Tubal Fluid (HTF) medium supplemented with Human Albumin 5 mg/mL. Pre- and post- wash sperm concentration and motion parameters were determined using a Motion Analysis system with CellTrak/S software/ The pre vs. post data was analyzed by paired t-test. A one-way analysis of variance was used to analyze differences between the three groups. Sperm motility increased in 79% of fresh specimens processed; 70% of chymotrypsin-galactose treated specimens; and 53% of cryopreserved specimens. Straight line velocity (VSL) increased significantly in the fresh and cryopreserved specimens. Curvilinear velocity (VCL) increased in all three groups, however, statistical significance could only be demonstrated in group 1 and group 3. Linearity (LIN) and amplitude of lateral head displacement (ALH) decreased significantly in all 3 groups post wash. Velocity of the average path (VAP) increased significantly in the fresh semen (group 1). Analysis of variance between the groups yielded the following results. Group 3 (cryopreserved) was found to have a signicantly lower % motility, VCL, and ALH following sperm washing. VSL, VCL, and VAP were significantly higher before sperm washing in the chymotrypsin treated group (2). VAP was signicantly different between all 3 groups with fresh (1) > chymotrypsin treated (2) > cryopreserved (3). Further analysis of post wash specimens may in the future lead to a better understanding of the changes in motion parameters that are necessary for capacitation and fertilization. A follow up study investigating motion characteristics exhibited by sperm that lead to pregnancy is necessary. In addition, further investigation into the mechanism of action of chymotrypsin galactose for treatment of sperm antibodies also needs investigation.

090 EFFECT OF PRE-FREEZE PERCOLL WASH AND ARTIFICIAL MOTILITY STIMULATION ON CRYOPRESERVED HUMAN SPERMATOZOA. R. K. Sharma, S. Kohn*, A. J. Thomas Jr. and A. Agarwal, Andrology Research & Clinical Laboratories, Department of Urology, Cleveland Clinic Foundation, Cleveland, OH 44195.

Sperm preparation methods and artificial stimulants improve sperm motility and other motion parameters in fresh ejaculate. Cryopreservation results in poor sperm motility, which correlates with poor fertilization rates. Sperm motility must be maintained for a minimum period for intrauterine insemination and other assisted reproductive procedures. The present study compares the data for improvement in sperm motion parameters after sperm preparation alone and with artificial stimulation. Semen samples from normal healthy volunteers were divided in two groups. Group I samples were frozen without further treatment. Samples in group II was processed by density-gradient separation. The freezing medium was TEST-yolk-buffer and immersed in liquid nitrogen (-196°C) before use. Sperm motility and sperm kinematics (curvilinear velocity [VCL], straight line velocity [VSL], average path velocity [VAP], amplitude of lateral head displacement [ALH], and linearity [LIN]) were analyzed on a motion analyzer. Sperm viability was evaluated by eosin-nigrosin staining. Membrane integrity was determined with the hypoosmotic swelling test. After thawing, samples from both groups were incubated in modified human tubal fluid (5% human serum albumin) at 37°C for a period of 24 h. Aliquots from each group were removed at 1 h (short period), 6 h, and 24 h after incubation (extended period). All parameters were analyzed at these time intervals and compared with 0 h (base line). Pentoxifylline at a concentration of 2.5 mM and 5 mM and 2-deoxyadenosine at a concentration of 2.5 mM dissolved in modified human tubal fluid (5% human serum albumin) was added to the sperm suspension. Semen analysis was done before the addition of the stimulants and after 60 minutes of incubation. Results were compared between specimen without stimulants versus stimulants as well as between the different concentrations of pentoxifylline and 2-deoxyadenosine. For short periods (less then 1h) of incubation, both untreated and Percoll-wash results were comparable. Motility loss for longer periods is reduced in Percoll wash samples. Pentoxifylline increases only motility, whereas 2-deoxyadenosine improves motility and all other motion parameters except linearity. Motility increases significantly differ for both stimulants at both incubation times (0 and 60 minutes), compared to no stimulant at 0 minute's incubation. Compared with the untreated samples, Percoll-wash samples had no advantage in regard to artificial stimulation. Motility loss is less over an extended time in Percoll-treated cryopreserved spermatozoa. Motility is also improved by incubation with pentoxifylline and 2-deoxyadenosine and can be maintained for 60 minutes. This stimulation of motility coupled with a reduced loss over time may be useful in improving fertilization outcome.

091 Effect of pentoxifylline on the intrinsic force of human sperm

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Objective: It is still controversial whether in vitro exposure of sperm to pentoxifylline increases sperm motility and vigor. Sperm vigor is the product of velocity by beat frequency of the tail, as assessed by CASA method. Laser optical tweezers have been used to evaluate sperm force. Therefore, the aim of this study was to determine whether the exposure of human sperm to pentoxifylline has any effect on sperm intrinsic forces.
Design: Prospective study
Setting: Laser Biology Institute at Beckman Laser Institute, Irvine, Ca
Subjects: Twelve healthy subjects: 10 with normal semen parameters and 2 with asthenospermia.
Intervention: Each semen specimen was washed, resuspended in human tubal fluid (HTF) and divided in two aliquot. One was incubated with pentoxifylline for 30 min. (final concentration=3.6mM) and the second, without pentoxifylline, served as control.
After 30 min. the pentoxifylline treated aliquot was divided in two halves: one portion was washed to remove the pentoxifylline and the other was left in prolonged coincubation.
Main Outcome Measure: Sperm intrinsic force in milliwatts (mW), by means of a non invasive infrared laser optical trap created by a continuous wave 1064 nm Nd:YAG laser beam directed in an inverted microscope.
Results: Exposure to pentoxifylline consistently increased sperm relative escape force from a laser optical trap. The increase ranged from 33% to up 154% over baseline fotce and compared to controls. The average absolute increase in sperm force rose from 37 mW to 79mW. Specimens with sperm having an initial low relative escape force, gained the highest relative increase. The effect of pentoxifylline on sperm force was already apparent after 5 minutes, reached a peak at 30 minutes and persisted for up to three hours either in sperm left in coincubation or washed off of the pentoxifylline.
Conclusion: Pentoxifylline significantly increases sperm intrinsic relative force in normozoospermic and asthenospermic samples. This experiment confirms that optical tweezers can provide an accurate determination of sperm force in in vitro conditions. Clinical data have now to establish if an increase in sperm force is an important parameter for sperm fertilizing capacity.

092 SPERM QUALITY IN THE VARIOUS STAGES AND HISTOLOGIC TYPES OF TESTICULAR CANCER. M. Tolentino Jr., I. Ayzman*, A. J. Thomas Jr., and A. Agarwal, Andrology Research & Clinical Laboratories, Department of Urology, Cleveland Clinic Foundation, Cleveland, OH 44195.

Many patients with testicular cancer have low sperm density. In addition, cryopreservation techniques invariably lead to diminution of sperm quality. Thus, cryopreserved sperm from these patients produces lower pregnancy rates than sperm from fertile men. It is still unclear, however, whether sperm from these patients are inherently defective or if they lose their motility after thawing. This study was conducted to demonstrate whether differences in testis tumor histology following cryopreservation are related to the decrease in sperm quality and whether disease stage has any bearing on this effect. All testis cancer patients referred for semen cryobanking were retrospectively grouped according to histology and stage of disease. Records from 34 newly diagnosed patients were reviewed. Seventeen percent of patients presented at stage I, 29% stage II and 21% stage III. Semen analyses were done by using a computer-assisted semen analyzer before and after cryopreservation. The nitrogen-vapor technique using Test-yolk buffer with glycerol as a cryoprotectant was used for freezing. Various sperm motion characteristics (motility, curvilinear velocity [VCL], linearity, amplitude of lateral head displacement [ALH], and motility index [MI] together with the post-thaw motile sperm count (MSC) were evaluated. Mean age, semen volume and both pre-freeze and post-thaw semen characteristics were not statistically different between patients with stages I to III cancer. Pre-freeze semen quality tended to worsen as the disease stage progressed, especially with respect to MSC, VCL, and motility. Following thawing, the same trend was seen with MSC, motility and MI. Stage III patients also had the worst pre-freeze ALH and MI, and post-thaw VCL and ALH. With respect to tumor histology, improved semen quality was seen among patients with pure seminoma (28%) > pure embryonal (22%) > mixed germ cell tumors (50%). However, 71.4% of patients with mixed tumors presented with stage III disease, whereas all patients with seminoma presented with stage I disease. The disease stage at presentation may affect semen quality to a greater extent than tumor histology. With the availability of new micromanipulation techniques in assisted reproduction, improvements and continued research in cryopreservation of sperm from testis cancer patients is needed.

TIME COURSE OF IN VITRO SPERM ACROSOME REACTION.

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The acrosome reaction (AR) is an exocytotic process essential for sperm penetration of the zona pellucida and binding to the oocyte (DeJonge CJ. Repr.Med.Rev. 3:159-178,1994). Evaluation of in vitro AR can suggest fertility potential. The Acrobeads Test (Fertility Technologies, Natick,MA) uses paramagnetic beads coated with MH61, a monoclonal antibody which binds to acrosome reacted sperm. The purpose of this study was to determine AR as a function of time after removal of sperm from the seminal fluid. Specimens were acquired from known fertile donors (n=7) and IVF patients (n=6) on the day of IVF, and processed at 30 min after ejaculation. The semen was processed by percoll gradient centrifugation. An aliquot was then divided into two portions: one was tested using the Acrobeads test and one was held at room temp for 24h prior to running the test. The sperm were diluted to approx. 4.0 mill/ml in BWW with 3.5% HSA and then serially diluted into each of 4 wells of a microtiter plate. MH61 beads were added and the plate placed in a humidified incubator at 37°C, 5% CO_2 and assessed at 6 and 24h. Five fields/well were examined. If all beads were bound and agglutinated with sperm, the field was considered positive; if three or more fields were positive, the well was positive for AR sperm. A score of 1 was given for each positive well, for a total score of 4. If one or more beads were unbound to sperm, the field was negative. Initially, no specimens were positive at 6h, therefore all further analyses were done at 24h after incubation with beads. The mean motility was 56.4% at 24h, 42.2% at 48h. At 24h post removal from the semen, donor and IVF patient sperm were positive for the test, with a mean score of 3.1. The mean fertilization rate for the IVF patients was 64.4% (range 33-90). When sperm were held for 24h prior to the test (48h after removal from semen), there was little or no bead binding, and hence a negative test (mean score of .8). These data suggest that completion of the acrosome reaction occurs by 24-48 hours after removal of the sperm from the seminal fluid.

CAPACITATION OF CLOUDED LEOPARD SPERM: II. EFFECT OF CALCIUM AND CYCLIC AMP ON ACROSOME REACTION AND ZONA PENETRATION.

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The clouded leopard (*Neofelis nebulosa*), an endangered Asian felid, produces a high proportion (>80%) of morphologically abnormal sperm, low rates of acrosome reaction (<35%) and low penetration of the zona pellucida (ZP; <40%) and perivitelline space (PVS; <3%). In this study, we analyzed the effects of both dibutyryl cAMP (dbcAMP) and extracellular calcium (Ca^{2+})on clouded leopard sperm function by evaluating: 1) sperm motility; 2) Ca^{2+} ionophore-induced acrosome reaction; and 3) penetration of salt-stored, domestic cat oocytes. Electroejaculates from 4 males were assessed for sperm motility, diluted in Ham's F10 (+ 5% clouded leopard serum), washed and resuspended in Ham's F10 containing either 100 µM dbcAMP and 0.3 mM Ca^{2+} (LowCa) or 100 µM dbcAMP and 1.8 mM Ca^{2+} (HiCa). At 0, 1, 2 and 4 h, sperm aliquots were exposed to 4 µM Ca^{2+} ionophore for 30 min (38°C; 5% CO_2). Sperm motility was assessed immediately before and after incubation. Acrosomal status was determined with FITC-conjugated peanut lectin (PNA, 100 µg/ml) and scored as: 1) acrosome-intact (AI); 2) malformed acrosome-intact (MAI); 3) partially ecrosome-reacted (PAR); or 4) acrosome-reacted (AR). Following co-incubation (38°C, 5%CO_2) with 2 x 10^6 motile cells/ml for 6 h, oocytes (n= 140) were assessed for % ZP (sperm in inner ZP) and % PVS penetration and the average number of ZP or PVS sperm. Overall, sperm capacitation and AR were enhanced (p<0.01) following incubation in HiCa. At 1, 2 and 4 h, AI sperm were lower (p<0.01) in HiCa (range, 19-37%) than LowCa (range, 47-49%), and MAI sperm also were decreased (p<0.01) in HiCa (range, 14-19%) compared to LowCa (range, 24-27%). In contrast, AR sperm in HiCa were greater (p<0.01) at 1h (40%), 2 h (51%) and 4h (63%) than LowCa (22%, 24% and 27%, respectively). No differences (p>0.05) were observed in PAR sperm between treatments. Sperm motility patterns over time indicated that differences in % AR were not confounded by cell death. Sperm-oocyte interaction was enhanced (p<0.01) following incubation in HiCa. Although % ZP penetration (HiCa, 67.0%; LowCa, 51.2%) was similar (p>0.05), % PVS penetration was greater (p<0.01) in HiCa (43.5 %) compared to LowCa (10.4%). The number of ZP and PVS sperm was 4 to 13-fold higher in HiCa (ZP, 14.2; PVS, 3.1) than LowCa (ZP, 3.3; PVS, 0.2). These results demonstrate, forthe first time, that dbcAMP and increased extracellular Ca^{2+} promotes clouded leopard sperm capacitation, acrosome reaction and oocyte penetration. (NIH grant HD 23853; Smithsonian Institution Scholarly Studies Program)

CAPACITATION OF CLOUDED LEOPARD SPERM: I. EFFECT OF PROTEIN ON ACROSOME REACTION AND ZONA PENETRATION.

095 J.A. Long†, B.S. Pukazhenthi†, D.E. Wildt*‡, S. Murray*‡, S. Barrett*§ and J.G. Howard†. †National Zoological Park and ‡Conservation and Research Center, Smithsonian Institution, Washington, DC 20008; §Nashville Zoo, Nashville, TN 37080.

Clouded leopard (*Neofelis nebulosa*) ejaculates are teratospermic (< 20% normal sperm/ejaculate) and contain high numbers of sperm with malformed acrosomes (> 35%/ejaculate). We examined the effect of 2 protein sources on clouded leopard sperm capacitation by evaluating: 1) the Ca^{++} ionophore-induced acrosome reaction; and 2) zone pellucida (ZP) penetration of salt-storad, domestic cat oocytes. Electroejaculates from 6 males were split into 2 aliquots, diluted 1:1 with Ham's F10 containing: 1) 5% clouded leopard serum (CLS); or 2) 20 mg/ml human serum albumin (HSA). Following centrifugation (300 x *g*; 10 min), pellets were resuspended in 100 µl of the same medium and maintained at 25°C for 6 h. At 0, 3 and 6 h, sperm aliquots were exposed to: 1) 0.1% DMSO (control); or 2) 4 µM Ca^{++} ionophore (CI) for 30 min (38°C; 5% CO_2). Sperm motility was assessed immediately before and after incubation with DMSO or CI. Acrosomal status was determined with FITC-conjugated peanut lectin (100 µg/ml) and scored as: 1) acrosome-intact (AI); 2) malformed, acroaome-intact (MAI); 3) partially acrosome-reacted (PAR); or 4) acrosome-reacted (AR). Following co-incubation (38°C; 5% CO_2) with 2x10^6 motile sperm/ml for 6 h, oocytes (HSA, n=91; CLS, n=89) were assessed for % ZP penetration (sperm within inner ZP). At 0 h, no differences (p>0.05) were detected between control end CI-treated sperm for either protein. At 3 h, HSA had no influence (p>0.05) on AR sperm (control, 10.5 ± 2.3%; CI, 14.7 ± 3.0%); however, in CLS, a higher (p<0.05) proportion of AR sperm were observed in CI-treated samples (33.5 ± 5.8%) than controls (13.9 ± 6.6%). At 6 h, although no differences (p>0.05) in AR sperm were observed between control (16.5 ± 14.7%) and CI-treated (20.2 ± 4.9%) HSA samples, PAR and AR sperm were greater (p<0.05) in CI-treated (14.3 ± 3.7%, 34.9 ± 7.3%, respectively) than controls (6.8 ± 1.3%, 12.6 ± 3.1%, respectively) in CLS samples. Similar (p>0.05) percentages of MAI sperm were observed for both HSA end CLS, regardless of time point (range, 36.8 ± 7.5% - 51.6 ± 11.3%). Sperm motility patterns over time indicated that differences in % AR were not confounded by cell death. Clouded leopard sperm penetrated domestic cat ZP in the presence of both proteins; however, % inner ZP penetration was higher (p<0.05) in CLS (37.8 ± 11.5%) than HSA (10.4 ± 5.6%). Results indicate that: 1) CLS promotes a higher level of sperm capacitation than HSA; and 2) clouded leopard sperm capacitation occurs within 3 h after exposure to homologous serum. (NIH grant 23853; Smithsonian Institution Scholarly Studies Program)

FERTILIZING POTENTIAL OF FROZEN/THAWED EPIDIDYMAL SPERMATOZOA FROM FRESH CADAVERS.

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We have recently shown that spermatozoa retrieved from some cadavers have fertilizing potential and that retrieval within three hours after death yields sperm of optimal quality (Fertil Steril 1995, S178). However, conceptions in these situations depend upon the use of assisted reproductive techniques utilizing cryopreserved spermatozoa. In the present study we investigated the fertilizing capacity of frozen/thawed caudal epididymal spermatozoa recovered from fresh cadavers.

Sperm samples were obtained from five cadavers two hours after death and washed with BWW medium. Washed sperm samples were then mixed (1:1) with a zwitterion-citrate-egg yolk extender containing TES and TRIS, sodium citrate (12.5%), fresh egg yolk (20%), and glycerol (14%). Sample-extender mixtures were refrigerated at 2 to 4 °C for 60 minutes to allow a slow cooling (0.5 °C/min). Samples were then allowed to freeze slowly in liquid nitrogen and finally taken to -196 °C. Six months later the samples were slowly thawed, spermatozoa were resuspended in BWW medium supplemented with human serum albumin (3%), and processed for zona free hamster oocyte sperm penetration assay (SPA).

SPA outcome for the individual cadaveric sperm samples was 13%, 19%, 33%, 67%, and 78%. When fresh or frozen/thawed spermatozoa from fertile donors were processed for SPA in our facilities, mean penetration rates of 83% and 61% were achieved, respectively.

Our results suggest that frozen/thawed spermatozoa obtained postmortem may have fertilizing capacity.

097 PROFILE OF DONOR REJECTION IN A SPERM BANK PROGRAM. I. Ayzman*, S. Kachoria*, C. Curtis*, R.K. Sharma, A.J. Thomas Jr. and A. Agarwal. Andrology Research & Clinical Laboratories, Department of Urology, Cleveland Clinic Foundation, Cleveland, OH 44195.

Using frozen donor semen for artificial insemination is an established practice throughout the world. Donor recruitment for a sperm bank program is difficult and slow because of high dropout rates and a high rejection rate of men who fail to meet the guidelines established by American Society for Reproductive Medicine and American Association of Tissue Banks for selection of donors for sperm bank programs. This study determined the profile of successful donors in our sperm bank and documented the reasons for rejection. A total of 199 men were screened from 1986 to 1994; 173 (87%) men were rejected as they failed to meet the minimum guidelines or dropped out. The 26 accepted donors (group I) and 17 rejected men (group II, selected on the basis of two consecutive semen analyses) were included. Sperm quality variables (volume, motility, velocity, linearity, motile sperm count (MSC), amplitude of lateral head displacement and abstinence) demographic data (age, race, religion, marital status, education, occupation, smoking, alcohol, caffeine, and drug use) were compared between the groups. Accepted donors had significantly better semen quality compared to rejected donors: motility ($P<0.01$); velocity ($P<0.001$); MSC ($P<0.0001$); linearity ($P<0.001$); and ALH ($P<0.001$). Ejaculate volume and length of abstinence did not differ between the groups. Analysis of demographic information revealed significant differences in marital status: more rejected donors were single (n=15) than accepted donors (n=15) ($P<0.045$). Group I consumed more caffeine ($P<0.001$) and the difference in educational level (college or more) was near-significant ($P<0.055$) with Group I being higher. No significant differences were found in regard to other demographic data. Our results indicate that poor semen quality is one of the most important causes of rejection of men in a donor sperm bank program. The percentage decline in semen quality after cryopreservation between accepted and rejected men was not significant.

098 A STUDY OF FAILED FERTILIZATION - RELATIVE FREQUENCY OF MALE VS FEMALE FACTORS AND THERAPEUTIC OPTIONS IN NEXT CYCLES
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When IVF results in failed fertilization, the possibility exists that there was a problem with either sperm, oocyte, or both. Any problem with gametes could be transient or more persistent. For the next cycle the couple could choose donor oocytes, donor sperm, change in sperm preparation (use of chymotrypsin-galactose or pentoxifylline) or oocyte insemination technique (e.g., cumulus removal, addition of 20% follicular fluid, increased sperm concentration, or the use of ICSI). The study presented herein retrospectively evaluated all IVF cycles with failed fertilization from 10/30/91 to 6/11/94 (before we were performing ICSI). The objective was to determine, based on presumptive diagnosis of combined female (poor oocyte production or quality) and male factor (parameters below WHO standards) - (gr 1); or female factor only (gr 2); or male factor only (gr 3); or no obvious male or female factor, what was the relative frequency of these problems. Also, based on the diagnosis which option would they choose for the next cycle - donor oocytes, donor sperm, or change in oocyte/sperm insemination technique. 86 couples had failed fertilization and 37 (43%) attempted another cycle within the study period. The percent of patients with fertilization in the next cycle was 71% for 7 couples in gr 1, 77% for 13 couples in gr 2; 80% for 10 couples in gr 3, and 57% for those with unexplained failure. For groups 1 and 3 (male factor groups) 7 patients used donor sperm with fertilization in 86% versus 70% in 10 patients changing sperm/oocyte insemination. Respective pregnancy rates (PRs) were 66% for donor sperm vs 0% for change in technique. Donor oocyte were chosen by only 3/20 (15%) with suspected oocyte factor and all 3 had fertilization but none conceived versus 2/11 (18%) who tried their own gametes the second time. Some of the patients with fertilization chose to freeze the embryos because of insufficient number or poor endometrial development. No one in group 4 chose donor oocytes and 4 (57%) had fertilization but no pregnancies achieved. Thus, though change in technique can lead to improved fertilization in couples with suspected oocyte factor, or unexplained failure, the subsequent PRs are poor. How well ICSI can improve this group remains to be determined. For suspected oocyte factor it seems reasonable to try once again with modification of technique. About 50% of failed fertilization appears to be, at least in part, male factor related.

099 VIABLE PREGNANCIES WITH INTRACYTOPLASMIC SPERM INJECTION FOR POOR HYPO-OSMOTIC SCORES
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Previous data has shown that it is extremely unusual for a couple to achieve a pregnancy following intercourse or even with timed intrauterine insemination when the hypo-osmotic swelling (HOS) test is <50%. When evaluating male partner's sperm two independent studies, one using a matched control protocol and the second using a shared oocyte program, found normal fertilization rates but poor pregnancy rates (PRs) with subnormal HOS scores. In contrast there were excellent PRs in the controls whose HOS scores were ≥50%. Recently we have seen improved HOS scores following treatment of sperm with chymotrypsin/galactose, and subsequently higher PRs in couples with men with poor HOS scores. This had led to the hypothesis that sperm may secrete some protein-like factor that not only impairs the functional integrity of the sperm membrane, but perhaps in some way damages the oocyte membrane leading to normal cleavage but poor implantation rates. If this hypothesis is correct, intracytoplasmic sperm injection (ICSI) should result in excellent PRs, since there is only exposure to 1 sperm instead of the 10,000 to 25,000 with conventional in vitro fertilization (IVF). Four couples with HOS scores <50% had ICSI performed. The HOS scores were 35, 41, 43 and 48%. The sperm was processed on a 3 layer discontinuous Percoll gradient. One ul of sperm suspension at 1-2 million/mL was added to 1 ul of 10% PVP under oil. A motile, normal appearing sperm was selected for ICSI. Selection of the sperm according to HOS was random since the membranes can only be seen to swell when in the HOS solutions, which, of course, were not used. Thus, we could not identify a sperm for ICSI that had normal HOS. Once selected the sperm was injected into the oocyte. The fertilization rate with ICSI of sperm from males having low HOS scores were similar to the fertilization rate of those with normal HOS scores. Viable pregnancies were established. The couple with the 48% had 2 cycles with one viable pregnancy. The couple with the lowest HOS score (35%) also conceived a viable pregnancy. Thus, there was a PR of 40% per transfer following ICSI. Though the series is small, since the previous studies found no viable pregnancies with HOS <50%, these data support the hypothesis of a possible sperm toxic factor causing a discrepancy between fertilization and PRs. ICSI may be an alternative if treating sperm with low HOS scores if chymotrypsin/galactose fails to improve the HOS percentage.

100 ANTIOXIDANTS IMPROVE TURKEY SPERM VIABILITY, MEMBRANE INTEGRITY AND MOTILITY DURING LIQUID STORAGE A.M. Donoghue*. Germplasm and Gamete Physiology Laboratory, ARS, USDA, Beltsville, MD 20705

Aerobic conditions are required to maintain the viability of turkey sperm in vitro. In mammalian sperm, excess oxygen during storage results in lipid peroxidation causing membrane damage and reduced motility and fertility. In this experiment the effect of adding antioxidants (AO) to turkey sperm during liquid storage was studied. Semen was collected and pooled from 10 toms on 12 occasions and AO were tested six times at six concentrations. Semen was diluted into Beltsville Poultry Semen Extender and supplemented with either: no AO (Control); Tocopherol (VITE, 1-320 ug/ml); Butylated Hydroxytoluene (BHT, .02-1.25 mM); Tempo (TE, 0.39-.312 uM); or vitamin C (VITC, 1-400 ug/ml) and stored at 5°C for 48 hr. Semen was evaluated at 0, 24 or 48 hr for sperm viability, membrane integrity and motility. Flow cytometric analysis was done using the live/dead stain combination (SYBR-14/propidium iodide) for sperm viability and membrane integrity was assessed using a hypoosmotic stress test. After 24 hr storage, viability was higher for several concentrations of VITE and TE ($P<.05$) compared to controls. After 48 hr storage, viability, membrane integrity and motility were lower in the controls ($P<.05$) compared to the 24 hr time point, however, these parameters were maintained for VITE, BHT and TE at all levels tested. For example, Means ± SEM for Control, 10 ug/ml VITE, .16 mM BHT and .04 uM TE at 48 hr were 54.7±14.9, 84.3±3.7, 82.0±4.1 and 78.6±1.4% for viability; 19.4±8.8, 38.4±3.3, 44.6±3.0 and 38.7±1.6% for membrane integrity and 19.6±8.0, 73.5±4.1, 74.8±2.1 and 68.8±.7% for motility, respectively. VITC treatments were similar to controls. Addition of antioxidants VITE, BHT and TE to turkey semen improves sperm survival, membrane integrity and maintains motility after 48 hr storage and appears to reduce the damaging effects of lipid peroxidation during in vitro storage.

101 **INTRACYTOPLASMIC SPERM INJECTION (ICSI) IS TREATMENT OF CHOICE FOR SUBNORMAL TOTAL SPERM ACROSIN ACTIVITY**

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Total acrosin activity cannot be predicted from standard semen analysis and subnormal acrosin activity is a fair predictor of poor fertilization in vitro using zona-intact insemination.

The purpose of the present study was to determine whether subnormal acrosin activity predicts fertilization success after ICSI. ICSI was performed in cases of previous poor results in andrology profiles. Total acrosin activity (AcroScreen™, Fertility Technologies Inc., Natick MA) was measured in the same ejaculates used for ICSI (n=12). Mean (± SD) fertilization rates in normal acrosin (acrosin activity index ≥ 5) and subnormal acrosin (acrosin activity index < 5) were 75 ± 23% and 63 ± 25% respectively. There was no difference (P=0.88) in fertilization rates between normal and subnormal acrosin groups.

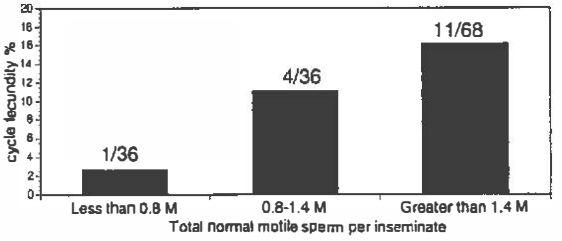
In conclusion, subnormal acrosin is a guide to therapy. It is suggested that subnormal acrosin activity is an indication for recommending ICSI rather than zona-intact insemination.

102 **THE USE OF THE HEMIZONA ASSAY (HZA) AS AN ADVANCED DIAGNOSTIC TOOL IN ASSISTED REPRODUCTION.** M.C. Mahony, S. Oehninger. The Jones Institute for Reproductive Medicine, Dept Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, VA 23507

Management of male infertility currently relies on a thorough history and physical examination with repeated semen analyses. Andrological evaluation should be a sequential, multi-step diagnosis and include sperm-zona pellucida binding tests (particularly the HZA) which have a high predictive value for IVF. The objectives of the studies were to: (A) examine practical aspects of the HZA to standardize methodology and maximize sensitivity, i.e., determine the impact of oocyte type (immature, non-inseminated (P1) vs. mature, inseminated for IVF but unfertilized (NF) oocytes), and semen storage (fresh (Fr), frozen with cryoprotectant-thawed (Fz) or refrigerated in test yolk buffer for 18 hrs (Rf)) on HZA results (HZI: # sperm bound for patient/control x 100) (B) assess the capacity of the HZA to predict IVF outcome for severe infertile men; and (C) investigate the impact of the HZA on patient management for IVF and oocyte micromanipulation. Results: (A) P1 oocytes had a higher binding capacity than NF oocytes (27 ± 2 vs. 17 + 2, $p < 0.002$); fertile donors had similar HZI when used Fr vs Fz; infertile men had similar binding capacity when used Fr vs. Rf. (B) HZI was the highest predictor of semen parameters for successful IVF (rank order correlation, $p<0.001$). As a predictor of successful IVF (>65%), a HZI cut-off value >35 had a sensitivity of 100%, and a positive and negative predictive values of 79% and 100%, respectively. (C) for patients with prior IVF failure subjected to PZD and/or SUZI, fertilization was better ($p<0.06$) in patients with deficient HZI than in patients with superior HZI. There was no correlation between HZI and ICSI outcome. Conclusions: The HZA can be performed in a standardized fashion; as such the HZA is highly predictive of sperm fertilizing potential and aids in patient selection for oocyte micromanipulation.

103 THE PREDICTIVE VALUE OF MOTILE SPERM WITH NORMAL MORPHOLOGY IN DONOR INSEMINATION. CL Gnatuk, RS Legro*, J Moessner*, J Miller* and WC Dodson*. Hershey Medical School, Department of Obstetrics and Gynecology, Pennsylvania State University, Hershey, PA 17033

Various post thaw parameters have been studied to attempt to predict in vivo fertilizing capability of frozen donor sperm. In this study we examined whether or not there is predictive value for cycle fecundity rates in the percentage of motile sperm with normal morphology in inseminates. Inseminates were prepared by washing the thawed donor semen with Ham's F10 with bicarbonate and a protein source, centrifugation, and resuspension of the pellet in the same medium. An aliquot of sperm prepared for insemination was examined for motility and count by the WHO criteria and for normal morphology based on strict Kruger criteria. Morphology was examined by two authors independently. Interobserver coefficient of variation was 11%. The average of the two observations was used to define % normal forms. Data from 142 IUI cycles were collected. Sixteen pregnancies resulted. There were no tertile differences in cycle fecundity rates according to motility, % normal forms, or total number of motile sperm in the inseminate. However, when we examined total motile normal (= number motile x % normal forms), we found a significant trend by chi square analysis for improved success in the best tertile of motile normal sperm (chi square = 3.86, $p<.05$). The odds ratio (OR) of pregnancy in this upper group was 6.53 compared to the lowest group and in the middle group 4.83 compared to the lowest group. These data suggest that the total number of motile sperm with normal morphology in the post thaw, prepared inseminate has better predictive value than motility, % normal morphology or total number of motile sperm alone to assess the expected cycle fecundity for cryopreserved donor sperm.



104 IMPACT OF HUMAN SPERM MORPHOLOGIC ABNORMALITIES ON INTRAUTERINE INSEMINATION (IUI) PREGNANCY RATES D.S. Karabinus and T.J.Gelety*. University of Arizona Health Sciences Center, Dept. of OB/GYN, Tucson, AZ 85724

Intrauterine insemination can be used to circumvent the adverse effects on fertility associated with low quality semen. However, seminal characteristics indicative of IUI success are poorly defined. For human IVF, sperm morphology, as judged by strict criteria, has been shown as an indicator of fertilization rate. Recent evidence suggests that levels of morphologically normal sperm, as judged by strict criteria, do not affect IUI outcome. This retrospective study was conducted to determine whether evaluation of specific human sperm morphology traits can be used as an indicator of IUI success. Semen quality and sperm morphology data were evaluated for 380 IUI cycles from 150 patients. Sperm concentration, motility and kinematics in raw and washed semen were analyzed by computer assisted semen analysis. Sperm morphology was evaluated at 1000 X under oil from stained smears prepared from raw semen; concomitant with evaluation using strict criteria evaluation, specific morphology characteristics were recorded. Data were analyzed by least-squares methods. Pregnancy rates did not differ when strict criteria results were divided among eight strata in five percentage point increments, and pregnancy rates were compared. The six most prevalent abnormal sperm morphologies were then identified and their incidence was compared for pregnant and non-pregnant cycles. Incidence of normal sperm, pyriform heads, tapered heads, elongated heads, asymmetrical heads, large oval heads, and amorphous heads did not differ between non-pregnant and pregnant cycles, respectively (19.8±0.1% vs 20.0±0.4%, 10.5±0.4% vs 9.7±1.3%, 7.3±0.4% vs 7.8±1.1%, 7.3±0.4% vs 7.8±1.2%, 8.3±0.4% vs 9.7±1.2%, 30.6±0.9% vs 29.0±0.4%, and 6.0±0.3% vs 6.2±9.9%). No differences existed among pregnancy rates when each abnormal morphology was stratified by incidence of occurrence. Results provide further evidence that sperm morphology in the raw semen has little impact on IUI pregnancy rates.

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ICSI WITH PERCUTANEOUS ARTIFICIAL SPERMATOCELE SPERM ASPIRATION(ASSA-ICSI) IN FAILURE CASES OF MESA-ICSI.

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Objectives : In case of surgically uncorrectable obstructive azoospermia or congenital absence of vas doference (CAVD), conventional IVF or IUI was done utilizing spermatozoa aspirated from artificial spermatocele which had been implanted in the previous operation. However, the results of sperm parameter aspirated from artificial spermatocele were so poor and pregnancy rate was so low, also. Thus, this technique has now been abandoned. The purpose of this stude was to see the efficiency of ICSI with sperm aspirated percutaneously from artificial spermatocele.

Design : Retrospective analysis of ICSI with spermatozoa aspirated from artificial spermatocele from May 1994 through April 1995.

Materials and Methods : At first, MESA with IVF had performed with surgically uncorrectable obstructive azoospermia 5 men and CAVD 4 men. At that time artificial spermatocele(polytetrafluroethylene) was attached to the epididymis. When pregnancy had failed in the first IVF using MESA-ICSI, ICSI was performed in 14 cycles using sperm percutaneously aspirated from artificial spermatocele at outpatient department(OPD).

Results : The results are summarized in the table below. The time of sperm aspiration was between 2 and 40 months after implantation of artificial spermatocele.

Conclusion : The quality of sperm aspirated from artificial spermatoclele is so poor. However, fertilization and pregnancy was successful with ICSI, and sperm was easily achieved on OPD base percutaneously. It is concluded that ICSI with sperm aspirntion form artificial spermatocele is good treatment tool after failure of MESA-ICSI in surgically uncorrectable obstructive azoospermia or CAVD.

No. of Patients	No. of Cycles	No. of Oocytes	No. of Fertilization (%)	No. of Clinical Pregnancy (%)
9	14	109	74 (67.9)	6 (42.9)

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MODIFIED GUANIDINIUM THIOCYANATE METHOD IS IDEAL FOR HUMAN SPERM DNA PREPARATION TO BE USED FOR EVALUATION OF THE X AND Y COMPOSITION OF THE SPERM POPULATION.

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DNA analysis has been indicated to be the most reliable confirmatory test in assessing the success of the procedures that entich semen for either X or Y sperm. Since DNA in mammalian spermatozoa, including human, is highly condensed, isolating sperm DNA can be a formidable task. Procedures that produce high quality DNA from other eukaryotic cells fail to yield quality sperm DNA. In this study we modified the guanidinium thiocyanate method (Nucleic Acids Res 1988, 16:1208) to optimize human sperm DNA preparation to be used for the evaluation of the composition of the sperm population. The commonly used eukaryotic DNA isolation procedures involve homogenization, proteinase-K digestion, phenol-chloroform extraction, ethanol treatment and centrifugation. The modified guanidinium thiocyanate procedure (MGTP) involved washing the sperm (0.3 M NaCl, 2 mM EDTA), lysis (6 M guanidinium thiocyanate, 25 mM sodium citrate, 0.5% Sarkosyl, 0.25 mg/ml proteinase-K and 2-mercaptoethanol) at 50° C, precipitation of DNA in the lysis cocktail by addition of 0.8 volumes of isopropanol, immediate removal and wash of DNA in distilled water and dissolving in TE buffer. DNA extracted by MGTP method and two conventional methods were evaluated by measuring O.D, mini gel analysis, restriction enzyme digestion and Southern blot analysis. MGTP produced degradation free DNA, while DNA prepared by other methods exhibited considerable degradation as revealed by mini gel analysis. Although the protein contamination in MGTP produced DNA was slightly higher compared to that of other procedures (O.D 1.6 vs > 1.7) it did not affect the restriction endonuclease digestion. *TaqI* (frequently used for simultaneous generation of X and Y specific DNA fragments), *EcorI* and *HindIII* indiscriminately digested DNA prepared by all three procedures (mini gel evaluation). *TaqI* / pDP34 (DXYS1) and *HindIII* / Inhibin-*a* restriction enzyme-probe combinations revealed 14.6 / 11.8, and 17.0 / 3.2 kb DNA fragments of satisfactory quality, respectively. Quantitation of X and Y by DNA probe analysis requires intact high molecular weight DNA. Since MGTP is a simple one step procedure which produces degradation free DNA, it could be the procedure of choice for human sperm DNA preparation to be used for this specific purpose.

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IN VITRO LABORATORY TREATMENTS HAVE IMPACT ON HYPOOSMOTIC SWELLING PATTERN WHEN APPLIED TO HUMAN SPERM

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Hypoosmotic swelling (HOS) test originally designed for evaluating the functional integrity of sperm membrane (Reprod Fert 1984, 70:219)[1] is now considered as an important sperm function test. Human sperm undergo laboratory treatment(s) such as freeze/thaw, wash, centrifugation, capacitation, hyperactivation, incubation, temperature shock etc before they are used for *in vivo* or *in vitro* insemination. In this study we have assessed the impact of some of these laboratory treatments on the sperm tail swelling pattern (swelling types a-g)[1] which is monitored in HOS test. Aliquots of 23 semen samples with no apparent male factor underwent the above mentioned laboratory treatments before they were evaluated by HOS test. The untreated fresh semen aliquots served as control. The test procedure involved incubation of sperm sample at 37° C in hypoosmotic solution (150 mosmol fructose and 150 mosmol sodium citrate) at 1:9 dilution for 1 hr and then types of tail change (a-g swellings)[1] were assessed under the microscope. In some aliquots observation continued for an hour at 5 minute intervals from the start of incubation. Fresh semen exhibited a, b, c, d, e, f and g types of swelling at a rate of 32±20, 18±10, 11±10, 1±3, 1±1, 4±5 and 32±13 %, respectively. A time course study indicates that the hypoosmotic swelling initiates with the type b swelling in control as well as treatment conditions (only type b swelling at 5 min incubation). The relative abundance of d and e types of swelling were not influenced by any treatment. A significant shift between a and g types occured due to Percoll wash, freeze/thaw and temperature shock. The pattern of tail changes between 24 hr old semen sperm and washed sperm were remarkably different. Swelling subtyping (both origin and developmental changes) seem more effective in differentiating semen samples than by total swelling counts. Intermixing between a and f swelling types is the important source of false HOS signals. Since the consistency of the HOS test like other sperm function tests is still in a state of flux, our these observations can be of useful in proper application of HOS test in obtaining reproducible result.

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ONE OF THE FRACTIONS OF PERCOLL ELIMINATED SPERM CAN BE ADDED TO PERCOLL RECOVERED SPERM TO INCREASE SPERM YIELD IN PERCOLL WASH PROCEDURE.

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Assisted conception requires the preparation of a sperm population enriched with morphologically normal, motile sperm free of seminal plasma. Sperm preparation by Percoll is one of the most widely used techniques, however, it has the disadvantage of producing a low final yield of motile sperm. In this study, we have compared the quality of different fractions of Percoll eliminated sperm with that of Percoll recovered sperm to determine if all the benefits of Percoll wash were being derived. Semen specimens were fractionated by discontinous Percoll column. This resulted in four sperm fractions: sperm retained in the semen (SRS), sperm clumped at the interface of 40 and 80% Percoll (SCI), sperm scattered in the column (SSC) and Percoll recovered sperm (PRS) that formed the pellet. The sperm fractions were evaluated for sperm count, percent motility, grade of motility, viability and vitality. Eighty two percent of the sperm were lost during Percoll wash; 37% of those did not even enter the column. The Percoll sperm recovery was thus 18%. If SSC and PRS fractions are combined, the total sperm recovery in Percoll wash becomes 52%. The SRS exhibited the lowest motility, viability and vitality (40±15, 68±16 and 64±11 %, respectively), which were significantly different ($p < 0.05$) from that of SCI, SSC and PRS. The motility, viability and vitality differences among SSC and PRS did not reach statistically significant levels. The satisfactory motility quality (grades a and b) was noticed in SSC (72%) when compared to PRS (83%). The pattern of changes in motility and viability in SSC and PRS over 120 hr were identical. Morphological evaluation did not reveal any difference between the lower part of SSC and PRS fraction. These data suggest that in the Percoll wash procedure, the SSC fraction, particularly the lower part which maintains the physical contact with the Percoll recovered sperm can be pooled together to increase the final sperm yield without compromising the quality of the sperm to be used for insemination.

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ACQUISITION OF TESTICULAR SPERM FROM MEN WITH NON-OBSTRUCTIVE AZOOSPERMIA. J.L. Marmar[1], M. Gibbs[1], Susan Heyner[2]. Division of Urology, Robt. Wood Medical School at Camden, Camden, NJ[1] and Department of OB-GYN, Univ. of Pennsylvania School of Medicine, Philadelphia, PA[2]

Testicular sperm from some men with non-obstructive azoospermia have used successfully for intracytoplasmic sperm injection (ICSI). However, sperm extraction from these men is always a challenge because there are limited number of sperm in the seminiferous tubules and it is always uncertain whether sufficient sperm can be extracted for ICSI. Therefore, improved methods of testicular aspiration, tissue preparation and sperm acquisition seem desirable for these cases. In this report, we will describe an effective protocol that has been developed at our institution for the acquisition of testicular sperm from men with non-obstructive azoospermia. The study group consisted of 10 men who's biopsies revealed hypospermatogenesis (5 cases) and Sertoli Cell Only (5 cases). At least 5% of the tubules demonstrated some sperm in each case. Approximately 50-100 mgs of testicular tissue were aspirated using an 18 gauge 1 1/4" angiocath and a 20 cc syringe in a pistol grip. The tissue was microdissacted into small pieces and equal quantities were placed in two vials containing 0.5 ml, 6mM pentoxifylline. One vial was incubated at room temperature for one hour, whereas the other was incubated overnight for 24 hours. Following incubation, the visible tissue was compressed and removed with a wooden applicator stick. The specimen was centrifuged at 300Xg for 20 minutes and the pellet was resuspended in 0.1 ml solution. A droplet was examined with a slide and cover slip. In each case, sperm were present with 10-15% vibratory motion and 2-10% forward progression. In the one hour preparation, there were 0-3 sperm per high power field, but in the 24 hour preparation there were 4-8 sperm per high power field. In four cases, this protocol was utilized to extract sperm for ICSI (2 hypospermatogenisis, 2 Sertoli Cell Only). In three of these four cases, there was fertilization leading to one pregnancy. In conclusion, testicular sperm with motility may be extracted from aspirated testicular tissue of men with hypospermatogenesis and Sertoli Cell Only following incubation in 6mM pentoxifylline. Increased numbers of sperm saem available after 24 hours of incubation. These preparations may be used successfully for ICSI.

110 DOUBLE FLUORESCENT STAIN FOR ACCURATE ASSESSMENT OF HUMAN-HAMSTER ICSI OUTCOME. R. Dolgina, P Studney*, L Ross, GS Prins, C Niederberger. Department of Urology, University of Illinois School of Medicine, Chicago, IL 60614.

Recently, a variation in the Sperm Penetration Assay (SPA) was introduced for predicting intracytoplasmic sperm injection (ICSI) outcomes during in vitro fertilization (IVF) in subfertile males (Gvakharia, Lipshultz, Lamb, ASRM, 1995). This procedure involves microinjection of human sperm into zona-intact hamster oocytes and positive outcome is determined by assessing sperm head decondensation rate in a minimum of 10 oocytes. However, ICSI itself can be detrimental to egg viability in 20-30% of injected oocytes resulting in a negative outcome independent of sperm function. Thus it becomes critical to score decondensation outcomes in only viable eggs following the microinjection procedure. To aid in this assessment, we herein describe a double fluorescent staining procedure for simultaneous analysis of oocyte viability and sperm head decondansation. Oocytes were collected from superovulated hamsters and the cumulus mass was removed with hyaluronidase. Sperm from fertile donors and subfertile patients were pretreated in 15mM TRIS and used for single-cell ICSI into zona-intact eggs using standard procedure. Oocytes were next cultured in 50 µl BWW with 0.3% BSA under oil in a 5% CO_2 incubator at 37C. After 3 hr, 1 µl of stock fluorescein diacetate (FDA; 5 x 10-4 M) and 1 µl stock bisbenzimide fluorochrome (Hoechst 33258; 1 mg/ml) solutions were added to the initial microdrops and oocytes were incubated for 5 minutes at 37C. Oocytes were washed with fresh BWW and gently pressed on a microscopic slide. Stained eggs were examined by ultraviolet epi-fluorescence illumination (excitation 365 nm). Oocytes which survived the ICSI procedure stained bright green (FDA) while dead eggs did not fluoresce. Sperm heads stained blue (Hoechst) and decondensation was readily apparent by size and density analysis of sperm chromatin. Final decondensation rates were calculated by a ratio of decondensed heads/viable eggs. Results with 629 ICSI oocytes show a 72.5 ± 3.6% decondensation rate when using viability staining versus 53.6 ± 5.1% without evaluation of viability (P=0.012). We conclude that this dual stain approach results in a more accurate assessment of human-hamster ICSI outcome. This animal model is very useful for technician training and quality control for human ICSI-IVF procedures and also has application for chromosome karyotyping of individual sperm.

111 MANNOSE BINDING ASSAY IN DUTCH BELTED RABBITS

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Alpha-D-mannose has been shown to be the predominant sugar residue playing a key role in the binding of human sperm to a human egg. A recent report by Benoff et al (ASRM 1995 Abstract 1) indicated several metal ions including lead could alter mannose binding when exposed to human sperm in vitro. This could affect fertilization. An animal model to verify these effects in vivo and the ultimate effects on fertilization is needed. Our lab has extensive experience studying effects of lead on Dutch Belted rabbit sperm and explored mannose binding on the rabbit sperm. The Mannose Binding Assay was conducted with kits (Fertility Technologies, Inc., Natick, MA) that measure the ability of spsrm to bind to glycoproteins similar to alpha-D-mannose glycoproteins found on the zona pellucida of the human oocyte. Human sperm were analyzed according to the kit instructions. Rabbit sperm were analyzed according to the kit instructions for humen sperm except for the capacitation method. Two different methods were used for rabbit sperm capacitation. Sperm were collected from the rabbit using an artificial vagina. In the first capacitation method, the sperm were allow to capacitate overnight. The second capacitation method utilized short term high salt treatment (hyper osmotic treatment - Brackett and Oliphant, 1975, BOR 12:260). The alpha-D-mannose binding was clearly apparent on the human sperm but binding could not be visualized on rabbit capacitated with the overnight method or hyper osmotic treatment. Thesa data suggest that mannose binding observed in human sperm may not be universal in all mammals. Further work is needed to identify the sugar residue involved in rabbit sperm and egg interaction.

112 DISCONTINUOUS PERCOLL OPTIMIZES SPERM POPULATIONS USED FOR ASSISTED REPRODUCTIVE TECHNOLOGIES (ART)

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Percoll gradients (P) are frequently used as an alternative to conventional swim-up (SU) methods for separating motile spermatozoa from seminal plasma. This study was performed to determine if the population of sperm selected using P is superior to that recovered with SU for use in any ART. **Design:** This study was divided into 2 experiments. Data regarding semen samples from all patients undergoing evaluation for IVF, intrauterine insemination and unexplained infertility were pooled and included in *Experiment I* (N=124). In this experiment, sperm motility and morphology (Strict criteria) were analyzed pre- and post-P only. The % of motile sperm recovered was based on the initial TMS count *Experiment II* included semen analyses from patients undergoing IVF only (N=69). Parameters of sperm recovered using either P or SU were compared in this experiment. Specifically, % recovery, motility, fertilization and # of cleaving embryos 72 hr post-fertilization were analyzed and retrospectively compared between the P and SU groups. Oocytes were inseminated no later than 6 hr post-retrieval for both groups. **Methods:** All semen samples, including frozen specimens, were evaluated and those with $\leq 10^6$ total motile sperm (TMS)/ejaculate were excluded. Motile sperm were recovered using the SU procedure, following a 1 hr incubation at 37°C of a washed sperm pellet overlaid with HTF + 7.5% protein. Motile sperm were also separated using P by layering liquefied semen over a discontinuous (47%/90%) gradient and centrifuging for 15 min. at 350 x g at room temperature. **Results:** *Experiment I*: Motility (56.9 pre vs 84.0% post, p<.0001), and morphology (14.6% pre vs 26.9% post, p<.001) increased after P. Motile sperm recovery was 18.1 ± 1.8%. *Experiment II*: % recovery of motile sperm with SU was the same as with P (18.0 ± .02%). There were also no differences in the # of oocytes collected or in the # of sperm used for insemination between the P (n=47) and SU (n=22) groups. The % of oocytes fertilized at 18 hr post-insemination (74 vs 54%, p<.04) and the # of cleaving embryos (9.2 ± .8 vs 6.1 ± 1.0, p<.02) were higher for the P group (vs SU). No differences in embryo grade or in the mean number of blastomeres were observed between groups. **Conclusion:** Use of percoll gradients results in a very motile and more morphologically normal population of sperm. The data presented in this study also suggest that fertilization of oocytes by sperm derived from percoll separation is superior to that of sperm from swim-up. Percoll could, therefore, be considered the preferred sperm-separation technique for ART.

113 MICROSURGICAL EPIDIDYMAL SPERM ASPIRATION AND IVF OUTCOME. [1] SM Tarchala, [1]RG Rawlins, [1]SR Mack, [1] E Radwanska, [1] Z Binor, [1]M Wood Molo, [1] BA Soltes, [2]L Levine, [2]E Lenting. [1] Dept of Ob/Gyn, [2] Dept. of Urology, Rush Medical Center, Chicago, IL.

Objective : Microsurgical epididymal sperm aspiration (MESA) has been used in combination with in vitro fertilization (IVF) in the treatment of male factor infertility due to azoospermia. In comparison to ejaculated sperm, sperm retrieved from the epididymis generally shows decreased sperm number, decreased sperm motility and decreased forward progression. In addition, sperm retrieved through microsurgical aspiration are often contaminated with red blood cells and cellular debris. In this study, we describe the results of patients undergoing MESA in combination with IVF.
Design : A retrospective analysis of patients undergoing MESA plus IVF.
Materials and Methods : The patient population consisted of 6 couples undergoing 8 cycles of MESA in conjunction with IVF. The population was divided into three groups depending on the diagnosis of the male factor infertility. Group I (n=3) was diagnosed with neurogenic anejaculation ; Group II (n=3) was diagnosed with congenital absence of the vas deferens; Group III (n=2) was diagnosed with ejaculatory duct obstruction . Sperm from the epididymis was recovered from the proximal caput epididymis. The recovered sperm were processed over a Mini-Percoll gradient . The eggs were inseminated with 200,000 - 1 x 10^6 sperm under oil.
Results : No significant differences were observed between the groups when comparing sperm counts and forward progression in both the initial and postwash samples. However, the neurogenic anejaculatory group (Group I) was significantly different from the group exhibiting congential absence of the vas deferens (Group II) in overall motility (X +/- S.D.; 83.3 +/- 5.5 vs. 21.7 +/- 10.4 respectively, p< 0.05). The overall motility in all other groups was not statistically different. In Group I, 3/3 cycles resulted in a positive pregnancy; in Group II, 0/3 cycles resulted in pregnancy; and in Group III, 1/2 cycles resulted in pregnancy . The overall pregnancy rate per cycle was 50% in patients undergoing MESA in conjunction with IVF. Also, it was observed that sperm processed over the Mini-Percoll gradient resulted in a postwash sample that was free of cellular debris and red blood cells.
Conclusion : The reults of this small series show that MESA in conjunction with IVF, is a successful procedure for acheiving pregnancy, particularly in cases of neurogenic anejaculation and ejaculatory obstruction. In addition, the method of sperm processing used is beneficial in removing cellular debris and red blood cells from the initial specimen.

114 FERTILIZATION AFTER INTRACYTOPLASMIC INJECTION OF ROUND SPERMATID FROM CRYPTORCHID MOUSE
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Cryptorchidism is one of the representative disorders for azoospermia. Recently, human pregnancy was reported following intracytoplasmic injection of round spermatids in semen from men with unexplained azoospermia (Tesarik et al., N Engl J Med 333: 529, 1995). We investigated fertilization after intracytoplasmic injection of round spermatids from cryptorchid mice. Mature male mice (B6D2F1, 8-12 weeks old) were made artificially cryptorchid. Before surgery and at 3, 7, 10 and 14 days after surgery, spermatid nuclei were injected into oocytes (B6D2F1, 8-12 weeks old) after electro-stimulation (Kimura and Yanagimachi, Development 121: 2397, 1995). The oocytes injected spermatids which were collected from testes before surgery each had two large pronuclei and one second polar body. Approximately 50 percent of them developed into blastocysts in vitro. Rates of oocyte activation and development into blastocysts were the same when spermatids from cryptorchid testes were used for injection. Normal offspring was born when 2-cell embryo was trnsferred to foster female. This study suggests that intracytoplasmic injection of round spermatid is an effective treatment for azoospermic patients with cryptorchidism.

115 PREGNANCIES IN BLACK-FOOTED FERRETS AND SIBERIAN POLECATS AFTER LAPAROSCOPIC ARTIFICIAL INSEMINATION WITH FRESH AND FROZEN-THAWED SEMEN. [1]J.G. Howard, [2]D.R. Kwiatkowski*, [2]E.S. Williams*, [3]R.W. Atherton*, [3]R.M. Kilchin*, [2]E.T. Thome*, [1]M. Bush and [1]D.E. Wildt*. [1]National Zoological Park and Conservation and Research Center, Smithsonian Institution, Washington, DC 20008; [2]Wyoming Game and Fish Department and [3]University of Wyoming, Laramie, WY 82071.

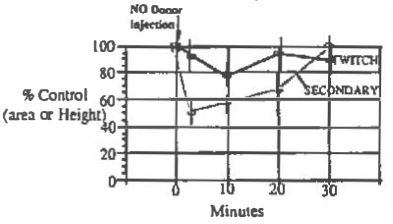
The black-footed ferret (*Mustela nigripes*) is an endangered species, once considered extinct until 18 ferrets were rediscovered in Wyoming in 1981. Species recovery is focused on natural breeding, but assisted reproduction would be useful for incompatible individuals, distribution of gametes and storage of valuable germ plasm. Objectives here were to: 1) determine an optimum semen cryotechnique in the closely related Siberian polecat (*Mustela eversmanni*) prior to application to the black-footed ferret; 2) compare the two cryomethods resulting in maximum domestic ferret sperm cryoprotection (PDV diluent/pellet freezing/37°C thaw versus TEST diluent/straw freezing/60°C thaw); and 3) assess the efficacy of laparoscopic intrauterine artificial insemination (AI) in Siberian polecats and black-footed ferrets using fresh or cryopreserved semen. Electroejaculates from 8 Siberian polecats and 9 black-footed ferrets were assessed for % sperm motility, forward progression (scale, 0-5; 5=best), % normal sperm (NS), % intact acrosome (IA) and sperm motility index [SMI=(% sperm motility)+(forward progression X 20) divided by 2]. Seminal aliquots were diluted in cryodiluent, cooled for 30 min at 5°C and pelleted on dry ice or frozen in 0.25 ml straws (20°C/min to -100°C). After thawing, SMI and IA were assessed. Parous females (7 Siberian polecats; 8 black-footed ferrets) with maximum vulvar swelling were given 90 i.u. human chorionic gonadotropin and laparoscopically inseminated *in utero* with fresh or frozen-thawed sperm. Mean pre-freeze SMI and IA were similar between Siberian polecats (72.5, 96.8%) and black-footed ferrets (63.6, 82.1%); however, % NS were lower (P<0.5) in ferrets (50.1%) than polecats (74.5%). Post-thaw sperm viability was influenced by cryomethod. In Siberian polecats, sperm survival and acrosome protection were greater (P<0.01) in PDV diluent/ pellet/37°C thaw (SMI, 67.5; IA, 61.3%) than TEST diluent/straw/60°C thaw (SMI, 31.6; 8.6%). Using the PDV/pellet method, SMI and IA in black-footed ferret sperm were 40.6 and 34.9%, respectivaly. Six of the 7 (85.7%) Siberian polecats inseminated with fresh (n=1) or PDV/pellet frozen (n=5) semen became pregnant (mean litter size, 5.3). Four of the 6 (66.6%) black-footed ferrets inseminated with fresh (n=3) or PDV/pellet frozen (n=1) semen became pregnant (mean litter size, 2.3). The results demonstrate the: 1) biological competence of fresh or frozen-thawed ferret sperm; and 2) potential for germ plasm cryopreservation and AI in black-footed ferrets. (Funded by a grant from the U.S. Fish & Wildlife Service)

116 NITRIC OXIDE DEPRESSES VAS DEFERENS CONTRACTILITY IN THE RAT IN VIVO P.J. Turek and K. Aslam*. Department of Urology, University of California, San Francisco, San Francisco, CA 94143

Nitric oxide (NO), a non-adrenergic, non-cholinergic neurotransmitter, may be involved in autonomic nervous system control of the male reproductive tract. Evidence from in vitro studies indicates that NO has an excitatory effect on sympathetic nerve-induced contractile response of the rat vas deferens. We addressed the in vivo rat vas deferens response to several NO donors in a newly developed microsurgical model.

After pentobarbital anesthesia, the vas deferens of 13 Wistar rats was isolated and cannulated microsurgically with PE-50 tubing. Intravasal pressures were transduced and recorded. Carotid arterial blood pressure was also monitored. After hypogastric nerve stimulation, a biphasic vasal pressure response was measured as twitch (height, mm) and secondary (area under curve) components. Intravenous saline (n=5 rats), or either of two NO donors (sodium nitroprusside 2.5mg/kg or L-arginine 2.3mg/kg, n=4 rats each) was given and vasal responses were measured after repeated, timed stimulations.

In saline controls, multiple stimulations of the vas did not decrease either phase of the vas response (SD 5%). Sodium nitroprusside decreased both twitch and secondary responses of the vas (see figure); the effect was more profound on the secondary response. This effect was independent of alterations in systemic blood pressure. Recovery of contractility was observed with time after NO donor injection. Similar findings were observed with L-arginine.



This is the first in vivo demonstration of the effect of NO on vas deferens activity. Contrary to in vitro observations but in agreement with NO effects in other tissues, it appears that NO depresses vas deferens contractility in vivo. This suggests a role for the NO pathway in the male reproductive tract.

117 IGFBP-5 EXPRESSION IS ASSOCIATED WITH INVOLUTION OF THE VENTRAL PROSTATE IN CASTRATED AND FINASTERIDE-TREATED RATS. R.S. Rittmaster, L. Thomas* and P. Cohen*. Dalhousie University, Halifax and U. of Pennsylvania.

Insulin-like growth factor binding protein (IGFBP)-5 has been proposed as a signal for apoptosis, possibly by competing with the IGF receptor for the potent mitogen IGF-I. To investigate the association of IGFBP-5 with apoptosis during regression of the androgen-deprived prostate, rats were left intact, castrated or treated with the 5α-reductase inhibitor finasteride for 4, 9, 14 and 21 days. The ventral prostate tissue was immunostained for IGFBP-5. Apoptotic cells were identified by end-labeling broken DNA with dATP using the TUNEL technique. In intact rats, 0.7 ± 0.3% of epithelial cells stained positive for DNA breaks and 7.3 ± 5.4% of epithelial cells demonstrated staining for IGFBP-5.

Percent of prostate epithelial cells staining for DNA breaks and IGFBP-5

	Day 4	Day 9	Day 14	Day 21
TUNEL, castration	5.6 ± 0.7	3.9 ± 0.09	2.6 ± 0.6	0.7 ± 0.04
TUNEL, finasteride	0.7 ± 0.3	0.2 ± 0.02	0.2 ± 0.08	0.2 ± 0.09
IGFBP-5, castration	39 ± 12	96 ± 2.1	3.0 ± 2.5	---
IGFBP-5, finasteride	22 ± 14	64 ± 13	0.3 ± 0.3	4.0 ± 2

TUNEL staining peaked at Day 4 with both castration and finasteride treatment, while IGFBP-5 staining peaked at Day 9. The delayed expresssion of IGFBP-5 compared to TUNEL staining suggests that IGFBP-5 is not a signal for apoptosis. To examine the relationship between IGFBP-5 expression and apoptosis, mirror image serial sections of the prostate were examined. Comparison of TUNEL and IFGBP-5 stained cells was accomplished by photographing identical fields with the two staining procedures and superimposing the photographs using Adobe Photoshop. TUNEL and IGFBP-5 staining usually were not expressed in the same cells and often not in the same areas. We observed several atrophied prostatic ducts in which 90 - 100% of cells were positive for IGFBP-5 staining, but no cells showed evidence of apoptosis. Atrophied ducts (those demonstrating flattening of epithelial cells) were always heavily stained for IGFBP-5. Conversely, we observed many healthy looking ducts with one or more apoptotic cells, but no IGFBP-5 staining. Prostatic involution after androgen deptivation involves both programmed cell death and inhibition of cell growth. Because of the delayed expression of IGFBP-5, we postulate that this binding protein functions as an inhibitor of cell proliferation, rather than a signal for cell death.

118 NEONATAL ESTROGEN EXPOSURE LEADS TO PROSTATE LOBE-SPECIFIC DYSPLASIA AND ADENOMAS IN THE AGING RAT. GS Prins, L Birch*, SH Ye*, V Ray*, Dept of Urology, University of Illinois School of Medicine, Chicago, IL 60612

Brief exposure of male rats to estrogens during the neonatal critical period is known to permanently imprint growth and function of the adult prostate gland. Our previous work has shown that the effects of neonatal estrogenization are prostate lobe-specific. While all lobes are hypoplastic at day 90, only the ventral and dorsal lobes exhibit epithelial hyperplasia, differentiation blockade and reduced androgen receptor (AR) expression. The lateral lobe appears histologically normal and expresses normal levels of AR and secretory genes. The objective of the present study was to examine prostatic growth abnormalities upon aging in these estrogenized animals. Male Sprague-Dawley rat pups were given 25 μg estradiol benzoate (EB) or oil on days 1, 3 and 5 of life and were housed until 22 months of age. Upon sacrifice, the prostate lobes were dissected and paraffin-embedded. Sections were either stained with H&E for histologic analysis by a pathologist or were stained for AR by immunohistochemistry using microwave antibody retrieval and PG-21 antibody against rat AR. Seven rats (4 controls and 3 neoEB) survived to 22 months of age. Oil-treated control prostates appeared histologically normal with epithelial AR staining in each lobe. In the ventral prostate, neonatal estrogen treatment was associated with prostatic adenoma in 2/3 rats and extensive epithelial hyperplasia and dysplasia in the third rat. Dorsal lobes exhibited extensive hyperplasia and dysplasia in 2/3 estrogenized rats. All dysplastic foci were AR negative in the ventral and dorsal lobes. The lateral lobes of all estrogenized rats appeared histologically normal and epithelial cells were strongly AR positive. These data indicate that the lobe-specific imprints in differentiation and AR expression due to early exposure to estrogens directly correlate with abnormal prostatic growth upon aging. These findings support earlier claims that neonatal estrogen exposure may lead to prostatic dysplasia and adenomas in the aging animal. (NIDDK40890)

119 QUANTITATION OF 5α-REDUCTASE TYPE 1 AND 2 mRNA IN NONHUMAN PRIMATE MALE REPRODUCTIVE TISSUES. M.C. Mahony, K. Gordon*. The Jones Institute for Reproductive Medicine, Department of Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, VA, 23507.

Maturation of spermatozoa within the epididymis is under androgen control, via the 5α-reduced metabolite of testosterone, DHT. Two genes encode two isozymes of the 5α-reductase enzyme. The objective of this study was to investigate the presence and abundance of reductase types 1 and 2 mRNAs in nonhuman primate male reproductive tissues using quantitative reverse transcriptase-polymerase chain reaction with cyclophilin mRNA as an internal standard. mRNA was extracted from testis, prostate, kidney, and the proximal caput (PCp), caput (Cp), midcorpus (Co), and distal cauda (Cd) regions of the epididymis (Ep) from three breeder male cynomolgus monkeys (*Macaca fascicularis*). Reductase type 1 and 2 mRNAs were reverse transcribed and then each co-amplified with cyclophilin primers in a PCR reaction spiked with ^{33}P-dCTP. Reductase type 1 and 2 and cyclophilin abundance was measured by scintillation spectroscopy of excised bands. Data are expressed as the cpm-ratios of reductase 1 and 2/ cyclophilin mRNA and were analyzed by ANOVA. Reductase type 1 mRNA was most abundant in the testis (5.65±1.6 cpm ratio) and significantly decreased distally along the Ep (PCp=2.3±0.4; Cp=2.3±0.4; Co=1.7±0.4; Cd=0.6±0.09) ($p<0.001$). No expression was observed in monkey prostate or kidney. Reductase type 2 mRNA was undetectable in testis and kidney but was present in monkey prostate and throughout the Ep. No difference in reductase 2 mRNA abundance was detected along the Ep (PCp=10.7± 1.9; Cp=8.8±1.0; Co=9.2±1.5; Cd=7.5± 2.4). These data demonstrate that 5α-reductase type 1 mRNA is differentially expressed along the nonhuman primate epididymis while 5α-reductase type 2 gene expression is constant. (Supported by a Jeffress Foundation Research Grant)

120 SEMINAL FLUID FINDINGS IN MEN WITH NONBACTERIAL PROSTATITIS OR PROSTATODYNIA Charles H. Muller, Richard E. Berger, Susan O. Ross*, Ivan Rothman* and John N. Krieger*. University of Washington School of Medicine Department of Urology, Seattle, WA 98195.

There is considerable confusion about the effects of prostatitis syndromes on male reproductive physiology. Therefore, we studied seminal fluid and expressed prostatic secretions (EPS) of 100 men attending a prostatitis clinic. These men had symptoms of prostatitis but no evidence of urethritis, bacterial prostatitis, or significant urological abnormalities. All evaluations included lower urinary tract localization studies, EPS and semen analyses (SA) with Bryan-Leishman staining. Results were compared in men with inflammation ($\geq 10^6$ leukocytes/mL) in EPS, i.e., nonbacterial prostatitis (n=21), and men without inflammation in EPS, i.e., prostatodynia (n=79); and in men with or without leukocytospermia (LCS, $\geq 10^6$ leukocytes/mL). Of the 21 men with nonbacterial prostatitis, only 6 (29%) had LCS and 15 (71%) did not have LCS. Of the 79 men with prostatodynia, 17 (22%) had LCS and 62 (78%) did not have LCS (NS, not significant). Of 23 men with LCS, only 6 (26%) had nonbacterial prostatitis (mean leukocyte concentration 8.6 ± 9.4 x 10^6/mL of semen) and 17 (74%) had prostatodynia (mean leukocyte concentration 6.2 ± 7.0 x 10^6/mL, NS). Of 77 men who did not have LCS, 15 (19%) had nonbacterial prostatitis (mean semen leukocyte concentration 0.1 ± 0.2 x 10^6/mL) and 62 (81%) had prostatodynia (0.1 ± 0.2 x 10^6 leukocytes/mL, NS). These findings did not change substantially when we used other definitions for inflammation. Men with nonbacterial prostatitis had lower percent motile sperm (45% vs 60% for men with prostatodynia, $p = 0.08$) and sperm velocity (median 3 vs 4 for men with prostatodynia, $p = 0.03$). Men with LCS had higher sperm concentrations (median 122 M/mL vs 55 M/mL for men without LCS, $p < 0.0001$), and a higher proportion of normal morphology (62%, vs 37% for men without LCS, $p < 0.05$).

In summary, a minority of men had LCS, even among men with nonbacterial prostatitis. There was poor correlation between inflammation in the prostatic secretions and in the semen. Non-bacterial prostatitis, but not LCS, was associated with reduced sperm motility. Thus, prostatitic inflammation may reduce sperm function independent of leukocyte counts in the semen. Seminal leukocytes may remove abnormal sperm. Our findings highlight technical issues and the importance of investigating different sites and samples, including the urethra, EPS, and seminal fluid.

Abstract withdrawn.

122 CHARACTERIZATION OF TWO SERINE PROTEASE INHIBITORS IN THE SECRETIONS OF THE RAT VENTRAL PROSTATE

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The prostate gland secretes a number of proteases into the semen. Since endogenous inhibitors are important in regulating protease activities and function, our objective was to examine protease inhibitor activities in secretions of the ventral, lateral, and dorsal lobes of the rat prostate using reverse zymography. Proteins from these secretions were separated by electrophoresis in 12.5% polyacrylamide gels made with and without gelatin copolymerized with the acrylamide. The gelatin gel was incubated in trypsin (3 micrograms/ml in 10 mM Tris-HCl buffer, pH 7.5) for 75 minutes and then stained with coomassie blue. Gelatin was digested except where inhibitors were located. Two inhibitors of typsin were found in the secretions of the ventral prostate, but none in secretions of the lateral or dorsal lobes. These inhibitors were approximately 34 and 63 kDa in size with isoelectric points of 5.8 and 6.8, respectively, as determined by preparative isoelectric focusing. The protease specificity of these separated inhibitors was examined by determining their effect on the activities of different proteases assayed with specific chromogenic peptide substrates. The 34 kDa/pI 5.8 inhibitor strongly inhibited trypsin (84%) and was less active towards chymotrypsin (44 % inhibition) and plasmin (19 % inhibition). In contrast, the 63 kDa/pI 6.8 inhibitor had a stronger preference for chymotrypsin (35% inhibition) than trypsin (24% inhibition) or plasmin (4% inhibition). The ventral lobe of the rat prostate, in difference to the lateral and dorsal lobes, specificly secretes a number of proteases in addition to these serine protease inhibitors. These inhibitors may function to control activities of secretory serine protease in the ventral lobe glands as well as in the semen (Supported in part by research funds of the Dept. Veterans Affairs).

123 SEMINAL PLASMA PEPTIDASE ACTIVITIES AS INDICATORS OF PROSTATE FUNCTION IN MEN

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The prostate gland secretes a number of peptidases into the seminal plasma (SP). Although their function(s) remain unknown, 2 peptidases are elevated in benign prostate hyperplastic (BPH) tissue: leucine aminopeptidase (LAP) and angiotensin converting enzyme (ACE). We undertook this study to determine whether LAP and ACE activities measured in seminal plasma could be useful indicators of normal prostate function in vasectomized and nonvasectomized men, ages 25-50 years, without evidence of prostate disease. ACE and LAP activities were measured in the same seminal plasmas using hippuryl-L-histidyl-L-leucine and leucine-p-nitroanalide as substrates, respectively. ACE and LAP activities were slightly reduced, 34 and 17% respectively, in vasectomized individuals (N=58 for LAP; N=27 for ACE) compared with those with normal sperm counts (N=16). This suggests the prostate is a major source of these peptidases in SP. We found only a 13 and 15% variation in LAP and ACE activities in repeat SP samples from the same vasectomized men (N=7 men), indicating that a peptidase measurement in a single SP sample can be representative for the individual. Also, in vasectomized men there was a strong correlation of ACE activities with those of LAP and there was no change in either peptidase activity in men from ages 30 to 50 years. This indicates there is an intimate concordance of secretion of these activities by the prostate and that the prostatic secretion of ACE and LAP does not change in the years before the onset of clinical symptoms of BPH or cancer. These data suggest that LAP and ACE activities may be useful indicators of prostate function, but different normal ranges may need to be developed for men who are vasectomized. They also establish the foundation for future studies of peptidase markers as signals for the onset of the age-related prostate diseases BPH and cancer. (supported in part by Dept. Veterans Affairs and Merck, Inc.)

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CENTRAL HYPOGONADISM: DIFFERENTIATING IDIOPATHIC HYPOGONADISM (IH) FROM PITUITARY TUMORS (PT). **M Wiederkehr, G Wand MD, AS Dobs MD, from the Department of Medicine, The Johns Hopkins University, Baltimore, MD**

The evaluation of men with low testosterone (T) levels can be time-consuming and expensive, often requiring an MRI to yield a definitive diagnosis. Men presenting with central hypogonadism have borderline-low serum T levels, inappropriately normal FSH/LH levels, and normal prolactin levels. These men may have benign IH or compression from a PT. We compared 27 cases of IH to 37 controls of PT (34 PT, 1 germinoma, 1 meningioma, 1 glioma) in a case-control study to determine if there were criteria that would help differentiate these two populations. We reviewed the charts of all patients seen by the endocrine faculty within the past 15 yrs, who had CAT or MRI documentation and available lab values. Although men with PT had similar mean serum T levels (235.2ng/dl ± 30.4, vs. 223.5ng/dl ± 20.2), compared to IH, they were more likely to have levels <100 ng/dl (39% vs. 15%, p<0.05). In addition, PT had significantly higher serum levels of LH (6.4 IU/L ± 0 vs. 3.5 IU/L ± 0.4; p = 0.007), FSH (8.2 IU/L ± 1.1 vs. 5.0IU/L ± 0.4; p = 0.01), and prolactin (20.3ng/ml ± 23 vs. 7.1ng/ml ± 0.8; p = 0.0001) compared to men with IH. Significantly, more men with PT presented with visual field deficits (67.6% vs. 3.7%; p = 0.0001), headaches (32.4% vs. 7.4%; p = 0.04) and hypothyroid symptoms (56.8% vs. 25.0%; p = 0.03). In contrast, the men with IH complained significantly more of erectile dysfunction (78.3% vs. 39.1%; p = 0.017), and depression (66.7% vs. 6.7%; p = 0.002) than men with PT tumors. IH men shared comparable rates of fertility (75.0% vs. 77.4%) and ages of presentation (52.6 vs. 56.3 yrs) with men with PT. In conclusion, men with PT, as compared to those with IH, were more likely to have lower serum T, higher serum FSH/LH and prolactin levels, and visual field abnormalities, and less likely to have sexual dysfunction.

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ARTIFICIAL STIMULATION OF CRYOPRESERVED HUMAN SPERMATOZOA IN CANCER PATIENTS.

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Dramatic improvements in patient survival with almost total reversal in testicular cancer and Hodgkin's disease have led them to resume their pre-illness lifestyles. However, the very treatment that cures them can also render them sterile. Cryopreservation of semen offers a chance of fertility in these patients. Though, poor semen quality after cryopreservation is a major impediment to future fertility. We studied how cryopreserved semen samples from such patients prior to cancer treatment respond to artificial motility stimulants and whether this response was related to patient age, type of disease, or extent of disease. Semen samples from 17 cancer patients were stimulated with pentoxifylline (2.5 mM and 5 mM), and 2-deoxyadenosine (2.5 mM). Sperm viability and semen motion characteristics (curvilinear velocity, straight line velocity, linearity, average path velocity, and amplitude of lateral head displacement) were assessed before adding the stimulant, and after 60 minutes. Compared with baseline values, sperm motion was significantly greater both immediately after adding either stimulant ($P<0.017$) and at 60 minutes ($P<0.05$). There was no significant correlation between sperm motion characteristics before or after stimulation or to patient age or type of cancer. Extent of the disease (stage III and IV) negatively correlated with the percentage of stimulation. Therefore, it is important that cryopreservation in these cancer patients be encouraged. These patients can benefit from artificial stimulation in conjunction with assisted reproductive techniques and have the option of initiating a successful pregnancy.

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ANTIOXIDANTS IMPROVE THE QUALITY AND SURVIVAL OF CRYOPRESERVED BOVINE SPERM.

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There is a 50% decrease in the fertility of bovine sperm following commercial cryopreservation techniques. To address the hypothesis that the addition of antioxidants to the semen would improve the survival of the sperm under physiological conditions, this study investigated the effects of various antioxidants added to the semen prior to freezing. Antioxidants were added directly to the egg yolk extender (6% glycerol) to which the semen was added directly. Treated semen was then processed by normal industry standards. The dose responses of 5 different antioxidants were tested: fatty acid-free BSA (0, 1, 10, 100 mg/ml), α-tocopheryl + ascorbic acid combined (0+0, 25+50, 25+500 μM, 2.5+5.0 mM respectively), superoxide dismutase (SOD; 0, 10, 100, 1000 U/ml), catalase (0, 100, 1000, 10000 U/ml); SOD + catalase combined (0+0, 20+80, 200+800, 2000+8000 U/ml respectively). Sperm function over time was evaluated by motility using phase contrast microscopy and as detected by a Hamilton-Thorne IVOS Motility Analyzer, % viability as assessed by SYBR-14 and propidium iodide (Mol Probes Inc) and intracellular ATP concentration. BSA improved sperm survival somewhat as cells treated with 10 mg/ml were slightly more motile than controls at time 0 (39 vs 30%) and 5 h (32 vs 27%) post-thaw. Controls contained more ATP than any of the BSA-treated sperm at 0 h, but after 4 h, internal [ATP] remained slightly higher in sperm treated with 10 mg/ml BSA (.15 vs .20 μg/7 million sperm). All doses of tocopheryl + ascorbic acid improved sperm motility vs controls at 0 and 5 h post-thaw, although viability was not improved to such a degree. ATP levels were best maintained after 4 h in sperm treated with 25+500 μM, 2.5+5.0 mM tocopheryl + ascorbic acid. SOD alone had negative effects on sperm function. All catalase levels improved motility and viability at 0 and 5 h relative to controls and reduced motility and ATP loss over time. The superior antioxidant treatment, SOD + catalase combined, dramatically improved sperm motility, velocity, viability and ATP level at all times, as well as reducing the loss of function over time.

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RESPONSE OF FROZEN-THAWED BOVINE SPERMATOZOA TO THE HYPOOSMOTIC SWELLING TEST AT VARIOUS TEMPERATURES.

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The objective of this study was to assess the sperm membrane functional status and permeability during the HOS test incubation of frozen-thawed bovine spermatozoa processed at various temperatures. Processing of post-thawed spermatozoa at temperatures below 37°C seems to reduce the detrimental effects associated with dilution and reduction in osmotic pressure of the cryopreservation medium. The hypoosmotic swelling (HOS) test can be used to measure changes in sperm membrane functional status and permeability. Frozen-thawed specimens from 5 bull (ABS, Inc., Madison, WI, USA) were used in this study. The specimens (0.5-mL) were thawed at 37°C for 10 sec and transferred to a water bath at 37°C (Aliquot 1), 21°C (Aliquot 2) or 5°C (Aliquot 3) to completely thaw (1-2 min). The specimens were maintained and processed at these temperatures for additional 5-10 min. Specimens were slowly diluted 1:1 (v/v) and washed with Ham's F-10 media containing 3% BSA (SpermPrep™ media; ZBL, Inc., Lexington, KY, USA). The percentage of spermatozoa with coiled tails was assessed before the performance of the HOS test. The HOS test was performed by adding 0.1-mL of the sperm sample to 1.0-mL of a 100 mOsm/L HOS diluent. The following treatments were performed: 1) Aliquot 1 (control), specimens were incubated in HOS solutions at 37°C for 5 min; 2) Aliquot 2, specimens were incubated in HOS solutions at 21 and 37°C for 5 min, and 3) Aliquot 3, specimens were incubated in HOS solutions at 5 and 37°C for 5 min. Samples were obtained from the sperm-HOS diluent mixtures at 1 min intervals (during the 5 min incubation period), fixed and assessed for sperm swelling patterns. The sperm response to the HOS test for specimens

Processing temperature (°C)	HOS temperature (°C)	% sperm swelling Incubation Time (min)					
		0	1	2	3	4	5
37	37	47±3.3	59±1.5	60±2.2	60±2.1	61±2.0	61±2.0
21	37	60±2.3	65±1.5	65±1.2	65±1.2	67±2.6	67±5.3
	21	50±3.8	55±1.0	58±2.3	58±1.5	58±2.0	60±3.8
5	37	50±2.1	54±.6	54±.6	54±1.0	54±1.0	55±.6
	5	46±3.8	50±1.0	51±1.2	51±.6	51±1.5	52±1.0

processed at temperatures below 37°C was higher when samples were added and incubated with HOS diluents at 37°C. This finding indicates that the potential for sperm swelling (measurement of sperm membrane functional status) can be maintained when spermatozoa are processed at temperatures below 37°C. The highest response to the HOS test was observed in spermatozoa processed at 21°C and incubated in an HOS solution at 37°C. This response was superior to the one observed in specimens maintained and processed at 37°C throughout. Thawing of spermatozoa at 37°C, followed by processing at 21°C seems to reduce the negative effects associated with osmotic shock and results in the preservation of the sperm membrane functional status during the in vitro handling of post-thawed bovine spermatozoa.

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PREPARATION OF FROZEN-THAWED BOVINE SPERMATOZOA VIA VARIOUS SPERM SELECTION TECHNIQUES EMPLOYED IN ASSISTED REPRODUCTIVE TECHNOLOGIES.

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A number of manipulative techniques for fresh semen are currently available to remove the undesirable spermatozoa, debris, and other factors and to increase sperm quality. These various techniques include Swim-up, Ficoll centrifugation, Percoll density gradient centrifugation, glass-wool fiber and Sephadex filtration. Many of these manipulative techniques often increase sperm quality at the expense of numbers of recovered spermatozoa. Also, none of these techniques has been employed successfully with frozen-thawed spermatozoa because of the low quality and quantity of spermatozoa recovered, from a sample that is already compromised in quality and life expectancy. The use of motility stimulants, such as caffeine, could optimize the recovery of high quality frozen-thawed spermatozoa processed by a variety of sperm selection techniques. Frozen-thawed specimens from 5 bulls (ABS, Inc., Madison, WI, USA) were slowly diluted and washed with Ham's F-10 medium (ZBL, Inc., Lexington, KY, USA) containing 0 mM or 2 mM caffeine. Aliquots containing 100×10^6 spermatozoa were used for conventional sperm wash, Swim-up, Percoll density gradient centrifugation (80,70,55 and 40% Percoll gradients) and SpermPrep™ filtration. The total processing time for the selection techniques was 60 min (Swim-up), 20 min (Percoll) and 5 min (SpermPrep™). Spermatozoa were washed, resuspended and assessed after processing. Parameters evaluated included: count ($x10^6$), percentage of motility, grade (0 to 4), percentage of spermatozoa with coiled tails and response to the hypoosmotic swelling test (HOS; percentage of swollen spermatozoa). The HOS test was performed by adding 0.1-mL of the sperm sample to 1.0-mL of a 100 mOsm/L HOS diluent. Spermatozoa recovered via Swim-up or Percoll centrifugation showed a slight improvement in qualitative characteristics when compared to control and washed specimens ($P>0.05$). Also, the numbers of harvested spermatozoa were low (13 to 20% Swim-up; 17 to 19% Percoll). These treatments seemed to be traumatic for the harvested spermatozoa (head-to-head agglutination) due to possible dilution (Swim-up) and/or centrifugation effects (Percoll). Spermatozoa selected via the SpermPrep™ filtration method (45 to 55% sperm recovery) were qualitatively superior to spermatozoa obtained by other methods ($P<0.05$). Treatment of spermatozoa with 2 mM caffeine followed by SpermPrep™ filtration resulted in the recovery of higher numbers of motile spermatozoa with biochemically active membranes ($P<0.05$). This effect was not seen for spermatozoa processed via the other techniques. Considering that the viability of frozen-thawed spermatozoa is already limited, SpermPrep™ filtration is the method of choice over other techniques evaluated in this study because it provides significantly greater numbers of high quality sperm in a much shorter time under less stressful conditions for the frozen-thawed sperm.

129 DILUTION ASSOCIATED CHANGES IN OSMOTIC PRESSURE OF THE CRYOPRESERVATION MEDIUM DURING WASHING OF FROZEN-THAWED BOVINE SPERMATOZOA. J.R. Correa and P.M. Zavos. Department of Animal Sciences, University of Kentucky and Andrology Institute of Lexington, Lexington, KY 40546.

Frozen spermatozoa are usually washed after thawing to remove the cryopreservation medium that contains the cryoprotective agent and replace it with fresh culture medium. Glycerol is the most commonly used cryoprotective agent for bovine sperm freezing. The cryopreservation medium usually contains 7% (v/v) glycerol, which increases the osmotic pressure of the medium to approximately 1500 mOsm/L. The osmotic pressure of most commercially available culture media is in the range of 280 to 320 mOsm/L. Dramatic changes in osmotic pressure of the cryopreservation medium could occur depending on the rate of dilution. The objective of this study was to assess the changes in osmotic pressure of the cryopreservation medium that occur during sperm wash procedures and subsequent effects on sperm viability. Frozen specimens (0.5-mL) from 5 bulls (ABS, Inc., Madison, WI) were thawed and diluted 1:1 v/v with Ham's F-10 medium (ZBL, Inc., Lexington, KY) in one step or by a drop-wise method (0.1-mL/min). The osmotic pressure of the cryopreservation medium diluted in one step was reduced abruptly from 1500 to 890 mOsm/L. The osmotic pressure of the medium diluted slowly was also reduced to 890 mOsm/L, but the decrease in osmotic pressure was gradual (approximately 123.7 mOsm/L per min). Changes in osmotic pressure of the cryopreservation medium were measured after addition of 0.5-mL Ham's F-10 in a single step and at each 0.1-ml addition during dropwise dilution (5 min). The spermatozoa were centrifuged and the cryopreservation medium was replaced with fresh Ham's F-10. Spermatozoa were incubated (2 h at 37°C) and assessed for % motility, grade (0 to 4), occurrence of osmotic shock (% spermatozoa with coiled tails) and response to the hypoosmotic swelling test (HOS; % swollen spermatozoa). The HOS test was performed by adding 0.1-mL of the sperm sample to 1.0-mL of a 100 mOsm/L HOS diluent. Spermatozoa diluted via the fast dilution method were less viable (reduced motility) and a high incidence on the occurrence of osmotic shock was evident during incubation. Also, these spermatozoa were less reactive to the HOS test. Differences between the 2 populations of diluted spermatozoa were due to abrupt changes in osmotic pressure of the cryopreservation medium during the sperm wash procedure. It seems that spermatozoa diluted slowly are able to gradually adjust their internal (intracellular) osmotic pressure with that of the surrounding (intercellular space) medium, and to prevent the cellular damage associated with osmotic shock. The results obtained in this study indicate that frozen-thawed spermatozoa should be diluted at a rate that enables the removal of the cryopreservation medium without an abrupt reduction in osmotic pressure which could bring about reduction in sperm viability and possibly subsequent reduction in their fertilizing ability.

130 A SIMPLIFIED APPROACH TO THE SWIM-UP METHOD: USE OF A NEW ONE STEP SWIM-UP/SWIM-DOWN STANDARDIZED TECHNIQUE. P.M. Zavos and P.N. Zarmakoupis-Zavos. Andrology Institute of Lexington, Lexington, KY 40546.

Treatment of infertility recently has greatly increased the number of options available for infertile couples and the infertility specialist. For IVF and other forms of assisted reproduction, the natural process has been largely bypassed since gametes are obtained and manipulated outside of the normal means for conception. For the male, improvements in sperm quality have involved the use of the Swim-up technique which involves the swimming abilities of a small percentage of spermatozoa within a sperm population. The current study examined the enhancement of the routine semen parameters of samples obtained either via the traditional Swim-up (SU) or the Zavos Swim-up Column (ZSC™) as compared to the unprocessed sample. The ZSC sample was obtained via the use of a standardized self-contained system (ZSC™; ZBL, Inc., Lexington, KY), which was designed to harvest almost all overlayered media at the end of the procedure and to maximize the sperm harvest. The ZSC™ device consists of a column with a conical cavity located on the bottom where the semen is placed, and from where the sperm rise into the overlayered medium then swim down into the peripherally situated medium and are subsequently recovered at the end of the procedure. The ejaculates (N=10) were assessed and prepared (0.5 mL each) for either the Swim-up or ZSC™ method. The fluid (non-pelletted) samples were overlayered with 1.0 mL modified Ham's F-10 (SpermPrep™ media; ZBL, Inc., Lexington, KY) and processed for 1.0 h. The mean semen parameters assessed prior to and after being processed via the SU and ZSC methods are shown below:

Treatments	Count	Motility	Grade	Morphol.	HOS
Control	195.4	52.3	3.1	54.4	63.4
SU	9.6	86.7	3.6	81.3	89.0
ZSC™	14.2*	85.8	3.6	82.7	87.8

The results point out that the ZSC™ harvested specimens were similar to those of the Swim-up. However, the ZSC™ recovered specimens yielded greater numbers of spermatozoa (36.3%; P<0.05)*. The superiority of the ZSC™ technique over the Swim-up was due to the design of the ZSC™ which enables the harvesting of up to 100% of the overlayered medium compared to only 80% for the Swim-up, without disturbing the seminal plasma-Ham's F-10 interface. The ZSC™ technique enables the harvesting of the medium closest to the underlayered semen maximizing the number of sperm recovered which can be further used in all ART procedures. This technique could be beneficial for harvesting sperm from patients with spermatogenic deficiencies and could also be time saving in processing normospermic specimens. Furthermore, the ZSC™ provides a simple, fast, reproducible and error free one-step procedure for the infertility specialist.

131 EFFECT OF SPERM CAPACITATION BEFORE FREEZING AND ITS INFLUENCE ON THE EXTENT OF SPONTANEOUS ACROSOME REACTION. S.C. Esteves*, R.K. Sharma, A.J. Thomas Jr. and A. Agarwal, Andrology Research & Clinical Laboratories, Department of Urology, Cleveland Clinic Foundation, Cleveland, OH 44195.

Capacitation and acrosome reaction are a pre-requisite for fertilization. This process is synchronized *in vivo*, however, it may occur prematurely in patients with idiopathic infertility. The aim of this study was to examine how capacitation prior to cryopreservation influenced the acrosomal status of the spermatozoa. A highly motile sperm population was prepared from 15 normal donors by the swim-up technique. Each specimen was divided into two aliquots. The first portion was capacitated by incubating the sample in a HEPES modified BWW medium with 5 % human serum albumin at 37°C under 5% CO_2 in air for 3 hours. The second aliquot received no subsequent treatment. TEST-yolk buffer was used as a cryoprotectant. The samples were stored in liquid nitrogen. Spontaneous acrosome reaction in non-capacitated and capacitated samples was assessed using fluorescent-peanut lectin labeling. Viability was assessed by Hoechst-33258 dye. Before freezing, spontaneous acrosome reaction was significantly higher in capacitated sperm preparations ($P<0.02$). However, the percentage of viable cells showing acrosome reaction significantly increased after cryopreservation ($P<0.0025$). The amount of increase in acrosome reaction was similar in both capacitated and non-capacitated samples ($P<0.05$). Incubation under capacitating conditions can optimize acrosome reaction; however, under *in vitro* conditions it may not be a prerequisite for normal human spermatozoa. Capacitation does not provide any added advantage in reducing spontaneous acrosome reaction after cryopreservation. Cryopreservation-induced spontaneous acrosome reaction may involve a complex mechanism rather than merely a physiological change.

132 THE RELATIONSHIP BETWEEN QUALITY OF CRYOPRESERVED DONOR SEMEN AND PREGNANCY IN ARTIFICIAL INSEMINATION. A. Agarwal, I. Ayzman*, C. Curtis*, S. Kachoria*, A.J. Thomas Jr. and R.K. Sharma, Andrology Research & Clinical Laboratories, Department of Urology, Cleveland Clinic Foundation, Cleveland, OH 44195.

Cryopreserved donor sperm is routinely used in patients with male or female factors of infertility. We studied the relationship between donor semen quality and pregnancy rate in women undergoing artificial insemination by the intrauterine insemination. Data were analyzed from 203 women who became pregnant after more than one cycle as a result of artificial insemination using cryopreserved donor sperm (n=54) from a total of 422 women who underwent artificial insemination at our center from 1987 to 1994. A total of 299 pregnancies occurred (some women became pregnant more than once; n=75) but only data from first pregnancies were analyzed. The results of semen analysis (prefreeze and post-thaw) of donor semen used for artificial insemination in the pregnant women were analyzed. The women were divided in four groups based on the number of insemination cycles: Group I, 1 to 3 cycles; Group II, 4 to 5 cycles; Group III, 6 to 10 cycles; and Group IV, more than 10 cycles. Semen samples resulting in pregnancy were compared to samples from the same donor that did not result in a pregnancy in the same woman during the previous insemination cycle. The two inseminations were done in a 3-month period. Semen characteristics (motility, velocity, linearity, amplitude of lateral head displacement) abstinence time, and semen volume were analyzed. Most variables did not significantly differ between samples that led to pregnancy and samples that did not. However, the post-thaw velocity and linearity in Group I patients were the only semen characteristics for which the pregnant group was significantly higher ($P=0.033$, $P=0.006$) than the nonpregnant group. Semen quality was similarly high between the four groups. The maximum number of pregnancies occurred in Group I (42%) patients as compared to Group II (26%), Group III (22%), and Group IV (9%). Because distinct differences were not present in semen quality in specimens leading to pregnancy versus those that did not, we postulate that pregnancy in a donor insemination program is influenced more by female factors of infertility than by semen characteristics. In summary, good quality cryopreserved donor semen is a necessary but not sufficient requirement for induction of pregnancy in a donor insemination program.

133 EFFECT OF CENTRIFUGATION SPEED OR INTERMEDIATE BUFFER ON IMPROVEMENT IN POST-WASH FROZEN SPERMATOZOA. S. Kohn*, S. Vemulapalli*, R.K. Sharma, O.P. Padron*, A.J. Thomas Jr. and A Agarwal, Andrology Research & Clinical Laboratories, Department of Urology, The Cleveland Clinic Foundation, Cleveland, OH 44195.

Pregnancy rate in assisted reproductive procedures is higher with freshly ejaculated semen samples. However, fear of AIDS and other infectious diseases has replaced the use of freshly ejaculated spermatozoa in most artificial inseminations and other assisted reproductive programs with frozen spermatozoa. Cryopreservation results in a significant decrease in post-thaw sperm motility as cryopreservation medium has to be removed before the insemination procedure. This results in a further reduction in sperm motility of the washed specimen due to osmotic shock and centrifugation. Semen samples from 16 healthy normal volunteers were analyzed for routine semen analysis by a computer assisted semen analyzer for motion characteristics. Samples were frozen using the liquid nitrogen vapor method and divided into 6 aliquots. We compared the effect of an intermediate buffer (TEST yolk-buffer without glycerol) and three centrifugatioin speeds (X 800, 1200 and 1600 rpm) with modified human tubal fluid with 5% human serum albumin (mHTF) on sperm motility, viability and other motion characteristics. Results were analyzed for improvement in motility, viability, and other motion characteristics. Compared with HTF alone, the addition of the TEST yolk-buffer medium did not result in improved sperm motion characteristics. However, HTF alone resulted in a significant improvement in curvilinear velocity and amplitude of lateral head displacement at 1200 rpm compared to other centrifugation speeds ($P<0.05$). No improvement was seen in other semen characteristics. Reduction in spenn motility and other motion characteristics is an intrinsic outcome of cryopreservation. This change cannot be reversed by speed of centrifugation alone or by using an intermediate buffer. Cryopreservation techniques that result in a higher recovery of motile spermatozoa are needed.

134 CLINICAL APPLICATION AND LIMITATION OF MONOCLONAL ACROBEAD TEST IN FRESH AND CRYOPRESERVED SEMEN SAMPLES. R.K. Sharma, O.F. Padron*, A.J. Thomas Jr. and A Agarwal, Andrology Research & Clinical Laboratories, Department of Urology, The Cleveland Clinic Foundation, Cleveland, OH 44195.

Evaluation of the acrosome reaction is important for male infertility diagnosis. Disturbance in the acrosome renction is suggested as one of the reasons for decreased fertility potential which can not be measured by a routine semen analysis. Human spermatozoa expressed CD46 molecule on its head after acrosome reaction. Acrobead assay is reported as a simple, reproducible, cost-effective test that does not involve animal handling, and is a good predictor of the acrosomal status of the sperm. The test can be performed in a clinical andrology laboratory in conjunction with other tests. We performed the Acrobead test to determine the optimum capacitation time in fresh and frozen specimens. Sperm motion analysis was done on fresh specimen. Swim-up method was used to process one aliquot and the second unprocessed aliquot was frozen by the liquid nitrogen vapor method using the TEST- yolk buffer as the freezing medium. Both fresh and frozen specimens were prepated by swim-up in mBWW medium containing 0.3% human serum albumin (HSA) and then resuspended in mBWW containing 3.5% HSA. One hundred µl of the semen samples (4 X 10^6/mL) was added in a serial dilution (1:1, 1:2, 1:4, and 1:8) to a 96 well tissue culture plate. Ten-µL of 1.5 X 10^6/mL immunobeads coated with anti-CD46 monoclonal antibodies (MH-61 beads) were added to each well. Acrosome reacted spermatozoa formed a sperm-bead complex (agglutination) that was observed under a phase-contrast inverted microscope. Positive sperm agglutination was identified by the absence of attachment to the MH61 beads. The test was considered negative by the presence of unattached beads. The wells were scored at 0, 1, 3, 6, and 24-h incubation period on a scale of 0 to 4. An acrobead score of ≥ 2 was considered normal. A higher acroscore reflects a high acrosome reaction. In the fresh specimens, 7% (1 h), 53% (3 h), 80% (6 h), and 100% (24 h) of the donors had an acroscore of ≥2. These results indicate that a capacitation period of 6 h is adequate for the spermatozoa to undergo acrosome reaction. However, significantly higher number of frozen specimens had an acroscore of ≥2 even after a capacitation period of 1 h (62%), and after 3 h (77%), and 6 h (92%) indicating that in a majority of these specimens acrosome reaction and membrane damage occured during the cryopreservation process. No correlation was seen with sperm count, motility, and acroscore in our study as semen specimens were obtained from normal healthy donors. An incubation period of 6 h can be used clinically to screen individuals who may present normal semen parameters but have acrosome insufficiency. Acrobead test is not reliable in evaluating the acrosomal status in frozen specimens due to increased spontaneous acrosome reaction and membrane induced damage.

135 COMPARISON OF POST-CRYOPRESERVATION SPERM QUALITY IN PATIENTS WITH TESTICULAR CANCER, HODGKIN'S DISEASE, AND LEUKEMIA. O.F. Padron*, R.K. Sharma, A.J. Thomas Jr. and A. Agarwal, Andrology Research & Clinical Laboratories, Department of Urology, Cleveland Clinic Foundation, Cleveland, OH 44195.

Cryobanking is an established strategy to preserve fertility in young patients before treatment of testicular cancer, Hodgkin's disease and leukemia. Whether cryobanking should be offered to Hodgkin's patients is controversial because of poor pre-freeze semen quality. It is generally accepted that the semen quality worsens with higher disease stage. This study retrospectively determined whether cancer stage, disease type, and patient response to treatment (if the patient survived following cancer therapy) correlated to pre-freeze and post-thaw semen quality. The clinical records, results of pre-freeze and post-thaw semen analysis of 106 cancer patients (testicular cancer n=46; Hodgkin's n=49; leukemia n=10) since 1983 were reviewed. Semen analysis was done using a computer-assisted semen analyzer. The nitrogen-vapor technique using TEST-yolk buffer with glycerol as a cryoprotectant was used for freezing. Sperm motion characteristics (motility, curvilinear velocity, straight-line velocity, average path velocity, linearity, and amplitude of lateral head displacement) were evaluated. The average total sperm count, pre- and post-thaw motility, and other motion characteristics did not correlate with the disease stage or treatment outcome. Pre-freeze and post-thaw semen quality did not differ between testicular cancer and Hodgkin's patients. Leukemia patients had higher total motile sperm count than other groups (pre freeze $P<0.01$; post-thaw $P<0.03$). Disease type, stage, and treatment outcome are not correlated to pre-freeze and post-thaw semen quality. Although, leukemia patients had advanced disease and worse prognosis, they still had a significantly higher total motile sperm count. Semen cryopreservation should not be denied to cancer patients because of type or extent of disease.

136 **EFFECT OF CRYOPRESERVATION ON ACTIVABLE PROACROSIN LEVELS IN BOVINE SPERM.** **D.L. Garner[1], C.A. Burner*[1], Doug Redelman*[2], D. Hudig*[1], A.S. Abuelyaman*[2], C.-M. Kam*[2], and J.C. Powers*[2]. [1]School of Veterinary Medicine, University of Nevada, Reno 89557; [2]Sierra Cytometry, Reno, NV 89509 and [3]School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA, 30332.**

Activable proacrosin levels were determined before and after cryopreservation of semen samples from six bulls. The biotinylated isocoumarin serine protease inhibitor, Bt-Aca-Aca-OMe-IC (BIC) was used to determine the proportion of sperm with activable proacrosin. The acrosin on sperm was localized by secondarily labeling the inhibitor bound to the acrosin with avidin florescein conjugate (Neutralite™) (FL) and counterstaining with propidium iodide. The green and red fluorescence of the labeled sperm were quantified simultaneously using flow cytometry. Among the bulls, significant differences ($P<0.01$) were found in the proportion of sperm with exposed proacrosin both before and following cryopreservation. The proacrosin within the acrosomes of intact sperm was exposed by treating with Triton X-100 to disrupt membranes. Differences between the amount of sperm labeled with FL before and after detergent treatment reflected the activable proacrosin levels in intact acrosomes. The mean proportion of the 24 hr stored semen samples with exposed acrosin before detergent treatment was 78.8 ± 2.8%, while the mean proportion of sperm after cryopreservation was only 55.8 ± 4.1% reflecting cryopreservation damage. The activable proacrosin levels, i.e., percentage difference before and after detergent treatment, between the 24 hr stored and cryopreserved samples were 33, 18, 44, 9, 10 and 24% for the six bulls. These results indicate that the irreversible isocoumarin serine protease inhibitor, BIC, can be used to determine the proportion of sperm having retained their activable proacrosin following cryopreservation.

137 MORPHOLOGIC VARIATION AND MEGACEPHALY IN PRE- AND POST-CRYOPRESERVED HUMAN SPERM

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Strict morphology of pre- and post-cryopreserved human sperm was recorded for multiple samples, collected over a 6-week time period, from a group of 20 donors. Cryopreservation involved post-liquefaction washing of samples and adding an egg yolk, glycerol-containing, buffer-based medium equal to half of the sample volume. After 30 min. of refrigeration, cryopreservation proceeded in liquid nitrogen vapor (4 cm. above the liquid) for 30 min. followed by plunging the sample into the liquid nitrogen and leaving for storage. An aliquot was thawed 24 hrs. later for morphology evaluation. Morphology slides were prepared by placing a 5µl droplet of whole semen (pre-) or semen with cryoprotectant (post-cryopreserved) at one end of a glass slide and drawing the droplet out with a second slide. All slides were Papanicolaou stained. Morphologic evaluation of cryopreserved sperm has been reported by a number of authors in the past with no significant effects from cryopreservation using WHO morphology criteria. Using strict morphologic criteria, with 1,500 x magnification, confirmed the past results on these samples. However, there was an overall trend for decreased morphologic values after cryopreservation, the significance of which will be proven over the next year's data collection. There was a clearly differentiated dual population of sperm in the cryopreserved-thawed samples. Normal-sized heads measured between 2-3 µm width (W) and 4-6 µm length (L) with well-differentiated staining characteristics. Megacephalic heads measured between 3-5 µm W and 6-8 µm L with a uniform dark pink stain. All donors did not have the observed megacephalic population of cells but in those donors where this population was observed the appearance was consistent through the collection period in all samples collected from that donor. We attribute this characteristic, which can be a functional marker, to some sperm being more susceptible to cryopreservation damage. Significance of these findings in relation to pre- and post-thaw semen parameters and pregnancy rates will be reported.

138 IMPROVED RESULTS OF THAWED SPERM CRYOPRESERVED WITH STAGE COOLING WITH A CELLEVATOR

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Successful pregnancies following intrauterine insemination (IUI) of cryopreserved sperm have been disappointingly low when using sperm frozen prior to testicular ablative procedures, e.g., chemotherapy, radiation therapy or surgery. Thus, there is a need to still develop better techniques of cryopreservation. The study presented herein compared semen parameters of count/mL and hypo-osmotic swelling (HOS) changes, percent progressive motility, and percent grade A sperm following freeze-thaw with the standard vapor freezing technique versus a new method using a slower stage cooling in a cellevator (a device originally used to freeze lymphocytes). The mean baseline sperm count was 74.3 with a range of 32.0 to 125.0. The mean HOS score was 75.2% and the range was 52.0 to 87.0%. The mean % progressive motility was 71.6 with a range from 44.4 to 90.0. The mean percent motility was significantly higher post-thaw in specimens frozen with the cellevator (49.3±14.3%) than with vapor freezing (33.4±12.9%, p<.01). The percentage of sperm with grade A quality was 17.8±13 vs 9.2±8.2% (p<.01). The mean HOS score following thawing of sperm cryopreserved with the cellevator was 57.5±9.7 vs 45.8±10.9 with vapor freezing (p<.01). The number of specimens at or above the critical level of 50% was 2 of 11 (18%) following vapor freezing vs 6 of 11 (55%) with freezing with cellevator, but there were inadequate numbers to show significance (Fisher's exact test, p>.05). The results using stage cooling with a cellevator demonstrate improved motility and HOS test scores compared to liquid nitrogen vapor freezing. These data support other studies using commercial semi-programmable freezers. However, the advantage of the cellevator is that it is much cheaper than commercial freezers and thus it would be available to more andrology centers. It will be interesting to determine if the improvement in post-thaw parameters, especially the improved HOS scores, might allow a higher pregnancy rate following IUI.

139 COMPARISON OF THAWING TEMPERATURE ON SEMEN PARAMETERS OF CRYOPRESERVED SPERM

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A study by Leffler et al., (Pacific Coast Fertility Society, 1995) demonstrated that comparing sperm motility post-thaw of seven cryopreserved specimens using a rapid liquid nitrogen vapor technique for freezing found a higher percentage of motility when the specimens were thawed at 37°C (34%) vs room temperature (22.4%) for the initial thaw. The study presented herein attempted to corroborate or refute these findings but in addition determine if the thawing temperature similarly affects the percentage of sperm demonstrating hypo-osmotic swelling (HOS) changes. Seven specimens from six donors were equally divided and frozen according to the same methodology. The means were compared using t-test for paired data. The medians were compared using Friedman's analysis of variance. The initial mean sperm count was $60.8\pm23.5\times10^6$/mL and dropped to 24.7±22.6 for room temperature thaw and 24.0±22.3 for 37°C thaw. The comparable median levels were 52, 16 and 14 (p.470, p.450). The initial mean percent motility was 70.4% and dropped to 41±12.9% at room temperature and 36.1±14.7 at 37°C (p=.065). The median percent motility initially was 74 dropping to 44 and 31, respectively (p=.059). The initial mean (±SD) and median HOS scores were 69.2±9.7 and 73, respectively vs 46.3±13.4 and 50 at room temperature and 46.0±12.7 and 47 at 37°C (p=.936 and .705, respectively). Thus, we are not able to corroborate the findings of Leffler et al., that thawing at warmer temperature improves semen parameters nor did it improve HOS scores. In fact, if there was even a trend toward improved percent motility, it was with thawing at room temperature and not 37°C.

140 INTRA ACROSOMAL EVENTS AND DESTRUCTION OF SINGLE STRANDED DNA SPERM DURING HUMAN EPIDIDYMAL SPERM MATURATION PROCESS.

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Epididymal sperm maturation is a multistep process. It is known that changes in the sperm cytoskeleton and cell surface occur during epididymal sperm passage (ESP) and these events may be associated with the development of sperm fertilizing ability. We have recently shown that a part of monkey sperm proacrosin (PRO) is activated and forms free acrosin (FA) during ESP (J Urol, 1993, 309A). In the present study we evaluated alterations in the human sperm acrosin system and ratio single/double stranded DNA sperm with epididymal sperm maturation.

Sperm samples were collected from the head (H-E), body (B-E), and tail (T-E) of the epididymis from 23 men with epididymal tail obstruction undergoing epididymal microsurgical procedures. The human sperm acrosin system was evaluated as previously described (J Androl 1:16). The % of single-stranded DNA spermatozoa (%SS-DNA-S) in H-E, B-E, and T-E samples was also evaluated as we previously described (Fertil Steril 1993, 59:690).

There was no significant difference in the total acrosin activity of spermatozoa (TAAS) among H-E, B-E, and T-E samples (P>0.05:analysis of variance). The mean value of the % of the TAAS in PRO form was significantly lower(P<0.05) in T-E samples (93%) than in H-E (100%) or B-E samples (98%). In contrast the mean value of the % of the TAAS in FA form was significantly higher (P< 0.05)in T-E samples (7%) than in H-E (0%) or B-E (2%) samples. After in vitro activation of PRO, acrosin inhibitor was found sufficient to inhibit 100%, 94%, and 91% of the TAAS in H-E, B-E, and T-E samples, respectively. The mean %SS-DNA-S in H-E, B-E, and T-E samples was significantly reduced in the order of 28%, 19%, and 9%, respectively.

We suggest that destruction of single-stranded DNA spermatozoa and alterations in all the components of the acrosin system (PRO, FA, and acrosin inhibitor) occur during the human ESP.

141 HYDROPEROXIDE REDUCING CAPACITY OF GLUTATHIONE PEROXIDASE/REDUCTASE SYSTEM OF HUMAN SPERM. K.A. Thompson*, J.G. Alvarez‡ and B.T. Storey. Department of Obstetrics & Gynecology, University of Pennsylvania, Philadelphia, PA 19104; ‡Department of Obstetrics & Gynecology, Harvard Medical School, Beth Israel Hospital, Boston, MA 02215.

We have shown previously that the coupled enzyme system: glutathione peroxidase(GPX)/glutathione reductase (GRD)/glutathione (GSH) acts as a major antioxidant defense in human sperm by reducing reactive hydroperoxides, formed by lipid peroxidation, to unreactive hydroxylipid. Maximal GPX and GRD activities are quite constant in samples from healthy donors and high relative to peroxidation rates, but the antioxidant capacity of the defense system depends on the rate at which GSH, the reductive substrate for GPX, can be regenerated by GRD-catalysed reduction of oxidized glutathione (GSSG). In this study, we utilized sperm samples obtained by a swim-out method that excludes all non-motile cells to strictly avoid leucocyte contamination; only samples with cells of normal morphology were deemed acceptable for rate determinations. We have shown that the reductive substrate for GRD is NADPH; NADH is not utilized. At 37°C, V_{max} for NADPH oxidation in the GRD-catalysed reduction of GSSG was 36±7 nmol/min-10^8 cells (n=12); K_m was 15±4 μM for NADPH (n=7) and 150±20 μM for GSSG (n=5). At 37°C, V_{max} for glucose-6-phosphate dehydrogenase (G6P-DH) activity, the source of NADPH, was fairly constant, 3.5±0.3 nmol/min-10^8cells (n=6); hexokinase (HK) activity was more variable, 14±4 nmol/min-10^8 cells (n=6); all assays were carried out in medium containing 1.5 mM glucose. No NAD/NADP transhydrogenase activity could be detected in these cells. We conclude that the capacity of the GRD/GPX/GSH system to reduce lipid hydroperoxides is set by the rate at which G6P-DH activity can supply NADPH reducing equivalents to GRD. The extent to which the rate of hydroperoxide formation exceeds V_{max} for G6P-DH would thus determine the rate of sperm lipid peroxidation and the time to complete loss of motility in the sperm sample.

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142 EFFECT OF INTERACTION WITH OVIDUCTAL EPITHELIUM ON INTRACELLULAR CALCIUM LEVELS OF HAMSTER SPERM. Susan S Suarez and Margaret C Lo*, Dept of Anatomy, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.

Prior to ovulation, sperm are trapped in a reservoir in the caudal isthmus where they bind to isthmic oviductal epithelium (IOE). Hyperactivation occurs while sperm are bound to IOE and involves an increase in the intracellular calcium concentrations (Ca_i) of sperm. We proposed that, at ovulation, IOE induces a rise in the Ca_i of attached sperm to hyperactivate them. Epithelium was obtained from female hamsters shortly after ovulation and used immediately. Using the fluorescent calcium indicator indo-1AM and the ESIMAGE imaging system, the Ca_i of sperm bound to IOE and of control sperm stuck to the coverslip were determined in the acrosomal (acr), post-acrosomal (pacr), proximal midpiece (pmid), and distal midpiece (dmid) regions of the sperm (Table 1). The Ca_i of sperm bound to ampullar oviductal epithelium (AOE) were determined as well (Table 2).

Table 1. Ca_i in control sperm and sperm attached to IOE [nM]

Sperm Region:	acr	pacr	pmid	dmid
control	131.5±14.5	120.0±10.4	94.2±9.2	77.9±6.3
bound to IOE	120.5±10.7	105.8±13.0	86.0±9.8	71.5±7.6

Table 2. Ca_i in control sperm and sperm attached to AOE [nM]

Sperm Region:	acr	pacr	pmid	dmid
control	124.3±7.3	130.8±9.4	87.4±4.3	95.5±5.7
bound to AOE	133.1±11.3	127.6±8.9	97.4±6.3	98.5±5.8

(n=4; 10 sperm/treatment/experiment)

For all four regions, the Ca_i of sperm bound to oviductal epithelium did not differ from the Ca_i of control sperm ($P>0.05$). Thus, direct interaction with the periovulatory oviductal epithelium does not raise Ca_i in attached sperm. Other factors, such as secretions of the epithelium, may hyperactivate sperm, thereby enabling them to fertilize. *Funded by NIH HD 19584.*

143 NEW CHEMICAL MARKERS OF SPERM FUNCTION

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In a set of semen samples we have confirmed the increased generation of reactive oxygen species (ROS) in cases with oligo (n=32, 70 fold higher than controls), or asthenozoospermia (n=22, 11 fold), with immunological infertility (n=10, 16 fold), or with inflammation (n=24, 17 fold), and we found a parallel increase of seminal plasma concentration of Interleukin-6 in the latter (r=0.71, n=104). Il-6 concentration was as powerful as ROS in discriminating between absence or presence of inflammation (area under the ROC curve: IL-6: 0.916, ROS: 0.903), and the former was also increased in subfertile patients with varicocele. The essential fatty acid composition of the sperm membrane of fertile and subfertile spermatozoa separated on a Percoll gradient presented significant differences, with higher concentration of saturated fatty acids (16:0 and 18:0) and lower polyunsaturated fatty acids (22:6w3) in the 90% layer. Changes in the fatty acid composition can be brought about by lipid peroxidation which can be monitored by the generation of malondealdehyde. The C-MET receptor for hepatocyte growth factor/scatter factor (HGF/SF) is expressed on the adluminal aspect of spermatogonia, on spermatocytes, spermatids and spermatozoa. On the latter the expression is inversely correlated (r=-0.80, n=23) with sperm concentration but the concentration of HGF/SF in semen is correlated with sperm concentration (r=0.33, n=23). Taken together, these data and their relation with the outcome of intra uterine insemination and IVF, suggest that complex interactions between cytokines, sperm maturation, ROS generation, fatty acid composition, and receptor expression of the sperm membrane may influence their fertilising potential. Our results should stimulate new approaches to male infertility treatment.

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THE IMMUNOHISTOCHEMIAL LOCALIZATION OF UROKINASE-TYPE PLASMINOGEN ACTIVATOR (uPA) AND UROKINASE-TYPE PLASMINOGEN ACTIVATOR RECEPTOR(uPAR) IN HUMAN SPERMATOZOA

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This paper focuses on the distribution of urokinase-type plasminogen activator (uPA) and urokinase-type plasminogen activator receptor(uPAR). uPA and uPAR were determined in the ejaculated spermatozoa of health man by immunohistochemistry and immunoelectron microscopy. Based on these findings it is suggested that uPA is distributed in the outer and inner acrosomal membrane and the plasma membrane of the head of the spermatozoa. The plasma membrane of spermatozoa tail contained uPA. The uPAR was found in the position where uPA was located in the membrane of the spermatozoa. It is suggested that sperm-bound uPA on the uPAR may play an important role in spermatozoa motility, semen liquefaction and fertilization. All of these factors are known to play an action role in human reproduction.

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$(CA^{2+})_i$ INCREASE AND ACROSOME REACTION IN RESPONSE TO PROGESTERONE PREDICT FERTILIZATION SUCCESS IN IN VITRO FERTILIZATION (IVF).

C. Krausz*, L. Bonaccorsi*, P. Maggio*, M. Luconi*, B. Fuzzi§*, S. Pellegrini§*, L. Criscuoli§*, G., G. Forti & E. Baldi*. Dipartimento di Fisiopatologia Clinica, Unita' di Andrologia and Istituto di Ginecologia ed Ostetricia§, Universita' di Firenze. Although IVF is an important therapeutic option for many infertile couples, the percentage of fertilization failure is rather high in cases where the male partner is subfertile. Therefore the introduction of simple tests able to identify specific sperm disfunctions and thus to predict IVF outcome, is of high interest both for diagnostic and therapeutic reasons. We have recently reported, in a small casistic, that sperm responsiveness to progesterone (P) is highly correlated with fertilization rate in subjects undergoing IVF cycles (Krausz et al, 1995). In the present study we extended the previous results to a larger casistic of 92 infertile couples undergoing IVF cycle. In a fraction of the same (post swim-up) capacitated sperm samples used for insemination, we evaluated P-induced $[Ca^{2+}]_i$ increase with two different concentrations of the steroid (0.1 and 1.0 ug/ml) and acrosome reaction (ARPC) (P=4 ug/ml), as well as acrosome reaction induced by the calcium ionophore A23187 (10 μM-ARIC). Sperm motility parameters were also evaluated using a computerized image analyzer (Hamilton -Thorn). In order to establish the clinical significance of these assays we calculated their sensitivity, specificity, positive and negative predictive values, by establisliung a cut off value for each parameter. The $[Ca^{2+}]_i$ increase in response to P, acrosome reaction in response to P and A23187 were statistically significantly correlated with the fertilization rate (FR). The most predictive assays appeared to be the increase of $[Ca2+)_i$ induced by 0.1 ug/ml of P and ARPC (respectively with sensitivity of 0.82 and 0.76, specificity of 0.80 and 0.78, positive predictive value of 0.95 and 0.92 and negative predictive value of 0.50 and 0.50). In cases where increase of Ca^{2+}, ARPC, and ARIC or 2 out of the 3 parameters gave positive results (over the cut off value) the predictive value for the fertilization success was 100%. In all cases of negative results (below the cut off value) for the three parameters, failed fertilization was always observed, indicating that the simultaneous measurement of these parameters increases prediction of IVF outcome. $[Ca^{2+}]_i$ increase in response to P did not show intraindividual variations with time. Indeed, a highly significant correlation was found between response in the IVF day and in a previous semen analysis ($r=0.81$, $P<0.001$, $n=17$). In conclusion, our data suggest that the combination of P responsiveness with the ARIC is a reliable tool in identifying samples which contain functionally normal and thus fertile spermatozoa.

We invite you to join our Society!

Background

Founded in 1975, the American Society of Andrology is composed of clinicians and basic research scientists involved with the physiology and pathology of male reproductive functions. The research interests of andrologists encompass several basic science and clinical disciplines including biochemistry, urology, immunology, psychology, genetics, pathology, pharmacology, internal medicine, and animal science. The Society fosters a multidisciplinary approach to the study of male reproduction. The goals of the Society are to promote scientific interchange and advance the understanding of andrology by sponsoring annual meetings, postgraduate courses, and the publication of the *Journal of Andrology.*

Journal of Andrology

The *Journal of Andrology* is a major source of scientific reports and state-of-the-art reviews covering all aspects of male reproduction. The *Journal of Andrology's* contents are indexed in Current Contents and Index Medicus.

Annual Meeting

The annual meeting of the American Society of Andrology is designed to serve the interests of its diverse membership. Scientific sessions stimulate the interchange of ideas. State-of-the-art lectures, clinical workshops, and basic science symposia provide updates in the basic and clinical sciences. The Society also hosts a postgraduate course held in conjunction with the annual meeting: continuing medical education credits are offered to qualified attendees of the annual meeting and postgraduate course. In addition to general social events, a special Mixer and Colloquium are scheduled for Students.

Awards

The American Society of Andrology has established two annual awards to recognize individuals who have made significant contributions to the progress of andrology: the **Distinguished Andrologist Award,** the highest honor presented by the Society, and the **Young Andrologist Award,** presented to an active member who is less than 40 years of age. In addition, the **Serono Award Lecture** is presented by an outstanding andrologist. Students with the best abstracts and presentations at the annual meeting will be awarded the **New Investigator Award** (First place) and **Student Merit Awards.**

Membership

Membership in the American Society of Andrology is open to individuals interested in the progress and development of andrology. All members receive a subscription to the *Journal of Andrology.*

There are three types of individual membership:

Active Member: any professionally qualified physician or scientist with evidence of scientific contributions in the field of andrology

Student Member: any student in a program leading to a degree, postdoctoral fellow, medical intern, or resident interested in the progress of andrology

Life Member: anyone eligible for active members status who has paid at one occasion the sum of 20 times the annual dues

Organizations or individuals supporting the Society at specific financial level are designated as either **Corporate** or **Sustaining Members.** Please contact the Society at the address provided in this brochure for more information about these special memberships.

AMERICAN SOCIETY OF ANDROLOGY

MEMBERSHIP APPLICATION

Please type or print

NAME: FIRST MIDDLE INITIAL LAST

DEGREE(S):*

*You will receive a certificate with Name and Degree

INSTITUTION MAILING ADDRESS:

TITLE: DEPARTMENT:

CITY, STATE, ZIP CODE:

S.S. #

TELEPHONE NUMBER (for Directory):

E/MAIL #:

FAX #:

EDUCATION (undergraduate, graduate, postdoctoral – in chronological order):

INSTITUTION	DEGREE	YEAR

MEMBERSHIP REQUESTED:

☐ Active ☐ Student ☐ Life

SPECIALTY OR AREA OF RESEARCH INTEREST:

SPECIALTY (indicate one):

- ☐ *BR* Basic Research
- ☐ *U* Urology
- ☐ *E* Endocrinology
- ☐ *OG* Obstetrics/Gynecology
- ☐ *IM* Internal Medicine
- ☐ *P* Pediatrics

CLINICAL PRACTICE (indicate one)

- ☐ *C* Clinical Practice
- ☐ *O* No Clinical Practice

DISCIPLINES (Choose up to 4)

- ☐ *a* – Advance Diagnostic Techniques
- ■ *b* – Biochemistry
- ☐ *c* – Immunology
- ☐ *d* – IVF/GIFT
- ☐ *e* – Impotence
- ☐ *f* – Electroejaculation
- ☐ *g* – Cellular Biology
- ☐ *h* – Sperm Banking
- ☐ *i* – Infectious Diseases
- ☐ *j* – Infertility
- ☐ *k* – Oncology
- ☐ *l* – Nutrition
- ☐ *m* – Sperm Anatomy/Biology
- ☐ *n* – Testis Anatomy/Biology
- ☐ *o* – Accessory Glands
- ☐ *p* – Toxicology
- ☐ *q* – Endocrinology

EACH APPLICATION MUST INCLUDE:

☐ Completed form

☐ Check, drawn on a US bank and payable in U.S. dollars only to: *American Society of Andrology* for the annual dues of:

	Active Member	Student Members	Life Member (Single payment)
Residents of North America:	$85/yr	$30/yr	$1,700
Residents outside of North America:	$95/yr	$40/yr	$1,900

☐ Surcharge to receive ***Journal of Andrology*** by *Air Mail:* (U.S. $)

Mexico or Canada	$12
South America, Europe or North Africa	$28
South Africa, Middle East, Australia, New Zealand or Asia	$35

☐ Letter from advisor for any student member, stating that he/she is a student seeking any degree or is a postdoctoral fellow, intern, or resident.

☐ Signatures of sponsor.

MAIL TO: **American Society of Andrology**
P.O. Box 15171
Lenexa, KS 66285-5171
1-913-541-9077
FAX: 913-541-0156

Checks must be in U.S. $ written on a U.S. bank or money order payable in U.S. dollars.

Total Amount Enclosed: U.S. $_____

All memberships include an annual subscription to the *Journal of Andrology*.

Signature of Sponsor

Signature of Applicant Date

Additional biographical data may be enclosed if you deem it necessary.

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