American Society of Andrology
Annual Meeting

American Society of Andrology
12th Annual Meeting

Program and Abstracts

March 6-9, 1987
Denver, Colorado
<table>
<thead>
<tr>
<th>Time</th>
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<tbody>
<tr>
<td>0800</td>
<td><strong>NEUROENDOCRINOLOGY</strong> Neuronal anatomy and Animal Studies; A. Plant</td>
<td>0800</td>
<td>Session A, Opening R. P. Amann W. D. Odell</td>
<td>0800</td>
<td>Session K, Clinical Andrology; Papers 62 to 69</td>
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<tr>
<td>0850</td>
<td>Pituitary Tumors; P. Snyder</td>
<td>0810</td>
<td>The Serano Lecture R. V. Short The Androgens</td>
<td>BREAK</td>
<td><strong>BREAK</strong></td>
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<td>1000</td>
<td>Regulation of Gonadotropin in Man: W. Bremner</td>
<td>1015</td>
<td>Session G, State of the Art Lecture R. P. Michael Neuroendocrine and Behavioral Aspects</td>
<td>1020</td>
<td>Awards Ceremony</td>
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<tr>
<td>1050</td>
<td>Therapy with GnRH and Analogs; W. Crowley</td>
<td>1145</td>
<td>Session L, The Serano Lecture, R. V. Short, The Size of the Testes</td>
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<tr>
<td>1200</td>
<td>LUNCH</td>
<td>1200</td>
<td>LUNCH ON YOUR OWN</td>
<td>1245</td>
<td>Adjourn</td>
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<tr>
<td>1315</td>
<td>Overview of AID; R. L. Urry</td>
<td>1330</td>
<td>Session C, Sperm Evaluation; Papers 8 to 14</td>
<td>1245</td>
<td>Adjourn</td>
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<td>1330</td>
<td>Donor Selection; N. Alexander</td>
<td>1330</td>
<td>Session H, State of the Art Lecture E. O. Price Evolutionary Aspects of Male Behavior</td>
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<td>1400</td>
<td>Sexually Transmitted Disease Screening; M. E. Guinan</td>
<td>1430</td>
<td>Session I, Posters coffee and soda; Papers 77 to 116</td>
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<td>1505</td>
<td>Legal and Liability Aspects of AID; D. Young</td>
<td>1530</td>
<td>Session D, Infertility; Papers 15 to 21</td>
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<td>1550</td>
<td>Screening Couples; K. Jones</td>
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<td>Break</td>
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<td>1620</td>
<td>Sperm Freezing; R. L. Urry</td>
<td>1620</td>
<td>Break</td>
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<td>1650</td>
<td>Insemination and Results; K. Jones</td>
<td>1730</td>
<td>Student Colloquium, G/H Colorado Ballroom; Speaker: Stephen W. Byers</td>
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<td>1745</td>
<td>Exhibitor Tutorials, Pennrose &amp; Pomeroy Rooms (on L3)</td>
<td>1730</td>
<td>Exhibitor Tutorials, I &amp; J Colorado Ballroom</td>
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<tr>
<td>1800</td>
<td>Student Mixer, Matchless Room (on LL1)</td>
<td>1900</td>
<td>Break</td>
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<tr>
<td>1900</td>
<td>Opening Reception, Denver Ballroom</td>
<td>1900</td>
<td>Cocktails in Denver Ballroom</td>
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<td>1930</td>
<td>Banquet In Denver Ballroom</td>
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PRESIDENTIAL MESSAGE

On behalf of the American Society of Andrology, welcome to our Twelfth Annual Meeting. This year's meeting, held in beautiful Denver, Colorado, commences with a Postgraduate course addressing two important topics: one physiologic, the neuroendocrinology of male reproduction; the other, socio-legal, the collection and use of reproductive samples. The following three days comprise presentation of 124 scientific papers, four lectures by three distinguished andrologists, and a workshop on animal welfare and animal use in biologic research. This year's Serono lecturer is Roger Short, Professor of Physiology at Monash University, and the State of the Art lecturers are Richard Michael, Professor of Psychiatry at Emory University and Edward Price, Professor of Animal Science at the University of California at Davis. We hope all will enjoy, learn from, and profit by this broad program. I encourage all attendees to actively participate in the exchange of data and ideas.

William D. Odell
President, American Society of Andrology

AMERICAN SOCIETY OF ANDROLOGY
OFFICERS

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Vice President
Larry L. Ewing
Secretary
Joel L. Marmar
Treasurer
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COMMITTEE CHAIRPERSONS

Awards .......................................Terry T. Turner
Constitution and Bylaws ....................Anthony V. Boccabella
Finance .....................................Arnold M. Belker
Future Meetings ............................Luis J. Rodriguez-Rigau
International Liaison ......................Philip Troen
Liaison ......................................Balwant Ahluwalia
Local Arrangements ........................Rupert P. Amann
Membership ..................................Larry Johnson
Nominating ...................................Claude J. Migeon
Postgraduate ................................Richard Horton
Program .....................................Rupert P. Amann
Publication ..................................David W. Hamilton
Student Affairs .............................Barry T. Hinton
Editor, Journal of Andrology .................Marie-Claire Orgebin-Crist
Welcome to the 1987 Annual Meeting of the American Society of Andrology. It is a pleasure to have you join us in Colorado. I hope you will take this opportunity to visit with clinicians and scientists located along the Front Range. Your Local Committee has worked diligently to insure that our meeting is unique and rewarding. The Program Committee has strived to develop an outstanding program and assemble the submitted abstracts in logical groups. Hopefully, they will elicit discussion and improve our understanding of both basic and clinical aspects of andrology of man and animals of economic importance, as well as the principles of reproductive biology established using appropriate animal models. The strength of the American Society of Andrology is in bringing together clinicians and scientists with diverse backgrounds so that each may learn from others. I challenge you to approach our Twelfth Annual Meeting with this inquisitive and comparative approach. Only in this way will andrology best serve society. I am thankful to the members of both the Program and Local Committees, the many individuals who helped make the meeting a success, our corporate sponsors and exhibitors, and for the support of the College of Veterinary Medicine and Biomedical Sciences of Colorado State University.

Rupert P. Amann, Ph.D.
Local and Program Chairman
Animal Reproduction Laboratory
Colorado State University
Fort Collins, Colorado

Local Committee
Robert D. Atherton
Bruce D. Schanbacher
Francis R. Tekpetey
Rupert P. Amann

Program Committee
William Bremner
William D. Odell
Bruce D. Schanbacher
Ronald L. Urry
Rupert P. Amann

AMERICAN SOCIETY OF ANDROLOGY
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
GENERAL INFORMATION

Headquarters
Marriott City Center
18th St. and California St.
Denver, CO
Tel. (303) 297-1300

On-site Registration
Conference Center (Lobby, Lower Level Two)
Thursday, March 5
4:00 to 7:00 P.M.
Friday, March 6
7:15 A.M. to 5:00 P.M.
Saturday, March 7
7:15 A.M. to 5:00 P.M.
Sunday, March 8
7:30 A.M. to 5:00 P.M.
Monday, March 9
7:30 to 10:30 A.M.

Registration Fees
Postgraduate Course
With CME credit $125.00
Without CME credit $125.00
Students (w/o lunch) $ 30.00

Annual Meeting
Non-member $95.00*
Regular member $80.00*
Student member $45.00*

*Includes the $15.00 late registration fee for individuals who did not preregister by mail prior to February 14, 1987.

Transportation
Taxi (about $8) and Limousine Service (about $5) from Denver's Stapleton Airport to the hotel are available outside the baggage area. The Denver Marriott DOES NOT provide pick-up service.

Annual Business Meeting
Monday, March 9, 10:50 A.M. Any motions should be sent to the society Secretary prior to the meeting.

The Press Room is located in the Silverton Room (lower level 1)
The Slide Preview Room is the Homestake Room (lower level 1)

Sponsors and Supporters of the 1987 Annual Meeting
Animal Reproduction Laboratory, College of Veterinary Medicine & Biomedical Sciences, Colorado State University
Serono Laboratories, Inc.

Beverages during the morning and afternoon breaks are provided through the courtesy of the commercial exhibitors. Please express your thanks to them.

Sustaining Members of the Society
The following organizations are sustaining members of the American Society of Andrology. The Society is very grateful for their support.

Buckeye Urological Associates
Knoll Pharmaceutical Company
National Medical Enterprises, Inc.
Ortho Pharmaceutical Corporation
Serono Laboratories, Inc.

Syntex Company
Syva Company
TAP Pharmaceuticals
The Upjohn Company
West Michigan Reproductive Institute
DISTINGUISHED ANDROLOGIST AWARD

The American Society of Andrology presents its 1987 Distinguished Andrologist Award to Emil Steinberger, M.D., in recognition of his vision and deep commitment to the development of andrology. He was a founder of the American Society of Andrology and served as its first president. His contributions in basic and clinical research have left an indelible mark on the discipline and have given direction to the work of others. Dr. Steinberger, President of the Texas Institute for Reproductive Medicine and Endocrinology in Houston, and Clinical Professor of Endocrinology, Department of Medicine, at the University of Texas Medical School at Houston, was born in Germany and received his early education in Europe. He emigrated to the United States in 1948 and obtained an M.Sc. in Anatomy and Endocrinology and an M.D. from the State University of Iowa in 1955. Dr. Steinberger’s research has encompassed all cellular components of the seminiferous tubule, the kinetics of spermatogenesis, the function and interaction of the Leydig and Sertoli cell, genetic, hormonal and metabolic factors influencing testicular development and function, as well as the endocrinology of the testis and the development of male contraceptives. Additionally, he is a dedicated teacher and superb clinician. He has championed two important causes: that treatment of infertility must involve understanding the fertility potential of the couple, and the integration of various disciplines into the area of reproductive medicine.

1987 ... Emil Steinberger, M.D.

YOUNG ANDROLOGISTS

1982 ... L.J.D. Zaneveld, D.V.M., Ph.D.
1983 ... William B. Neaves, Ph.D.
1984 ... Lonnie D. Russell, Ph.D.
1985 ... Bruce D. Schanbacher, Ph.D.
1986 ... Stephen J. Winters, M.D.
1987 ... Ilpo T. Huhtaniemi, M.D., Ph.D.

SERONO AWARD LECTURESHIP

Dr. Roger V. Short, Professor of Reproductive Biology at Monash University, Clayton, Australia, has a distinguished career in reproductive biology. He attended the University of Bristol School of Veterinary Medicine and received his B.V.Sc. and his M.R.C.V.S. in 1954, his Ph.D. degree from the University of Cambridge in 1958, and his Sc.D. in 1969. He was an honorary member of the Agricultural Research Council Unit of Reproductive Physiology and Biochemistry at Cambridge until 1972, when he was appointed Director of the Medical Research Council Unit of Reproductive Biology in Edinburgh, a position he held until leaving for Australia in 1982. Dr. Short has published approximately 235 manuscripts in the area of reproductive biology and is widely respected as a teacher. Together with Professor C.R. Austin, he edited a series of texts entitled “Reproduction in Mammals,” which have been reprinted and translated into several languages. As Serono Lecturer, Dr. Short will present two lectures on comparative andrology.

YOUNG ANDROLOGIST AWARD

Dr. Ilpo T. Huhtaniemi, M.D., Ph.D., was educated in Finland and currently is Professor and Chairman of the Department of Physiology at the University of Turku. His research has centered on the endocrine regulation of development and function of the fetal, neonatal and adult testis. This research has resulted in more than 125 papers in refereed journals. He also is an outstanding teacher. In recognition of his elucidation of testicular endocrinology, the American Society of Andrology is pleased to present Ilpo T. Huhtaniemi its 1987 Young Andrologist Award.
NEW INVESTIGATOR AWARD
(Prior to 1986 called the Student Presentation Award)
Donated by the West Michigan Reproductive Institute

This winner of this award will be announced at the Awards Ceremony, Monday, March 9, 10:20 a.m.

Previous Recipients
1983 ... Thomas T. Tarter
1984 ... Peter S. Albertson
                  Randall S. Zane
1985 ... none
1986 ... Mark A. Hadley

STUDENT INFORMATION

Student Mixer ................. Friday, March 6, 6:00 p.m. to 7:00 p.m. in the Matchless Room, lower level 1.

Student Colloquium .......... Saturday, March 7, 5:30 p.m. to 6:45 p.m. in G/H Colorado Ballroom

Speaker ...................... Stephen W. Byers
Topic ......................... In Vitro Studies of the Male Reproductive System (Organized by the Student Affairs Committee)

Student Travel Awards ......... Five awards of $100 each, authorized for payment from the general fund by the Executive Council, will be presented to five students at the Awards ceremony. Winners were selected by the Awards Committee.

Placement Service ............. Available throughout the meeting at a board near the registration desk. Joanne Killinger will answer questions.

SPECIAL EVENTS

Opening Reception
Friday, March 6, 7:00 p.m.
Denver Ballroom on the conference level (LL2)
This event is free to all registrants at the Annual Meeting and their guests. Hot and cold treats will be provided and extra beverages (after the first hour) will be available for purchase.

Banquet
Sunday, March 8, 7:30 p.m.
The banquet will be preceded by cocktails, beginning at 7:00 p.m., in the Denver Ballroom (left end). Entertainment will be provided. Special banquet tickets are required for both registrants and their guests.

Awards Ceremony
Monday, March 9, 10:20 a.m.
A brief introduction will be given by President William Odell.
Five student travel awards will be made and awards will be presented to the New Investigator, the Young Andrologist and the Distinguished Andrologist.
SPECIAL EVENTS

SATURDAY
8:10 A.M.  THE SERONO AWARD LECTURE I.
Roger V. Short
Department of Reproductive Biology
Monash University, Clayton, Australia

9:00 A.M.  STATE OF THE ART LECTURE
Neuroendocrine and Behavioral Aspects of Male Reproduction
Richard P. Michael
Departments of Psychiatry and Anatomy
Emory University

SUNDAY
1:30 P.M.  STATE OF THE ART LECTURE
Evolutionary Aspects of Male Sexual Behavior in Domestic Ungulates, Laboratory Rodents and Primates
Edward O. Price
Department of Animal Science
University of California at Davis

4:30 P.M.  WORKSHOP ON ANIMAL RIGHTS
Speaker and Moderator:
Bernard E. Rollin
Departments of Philosophy and Physiology
Director, Bioethical Planning
Colorado State University

MONDAY
11:45 A.M.  THE SERONO AWARD LECTURE II.
The Size of the Testes
Roger V. Short
Department of Reproductive Biology
Monash University, Clayton, Australia

COMMERCIAL EXHIBITORS
As this program goes to press, a complete list of commercial exhibitors is not available. However, an Exhibitors' Program and Buyers Guide will be included in the material distributed to all registrants at the Postgraduate Course or the Annual Meeting. The commercial exhibits are in I/II Denver Ballroom, and will be open Friday through Sunday.
TWELFTH ANNUAL MEETING
POSTGRADUATE COURSE IN ANDROLOGY
Friday, March 6, 1987
Conference (lower) Level, Marriott City Center, Denver, CO
Richard Horton, M.D., Course Director

NEUROENDOCRINOLOGY OF MALE REPRODUCTION—Richard Horton, Chairperson,
LAC/USC Medical Center, Los Angeles, CA
  8:00  Neuroanatomy and Animal Studies—Anthony Plant, University of Pittsburgh Medical
         School, Pittsburgh, PA
  8:50  Pituitary Tumors—Peter Snyder, University of Pennsylvania School of Medicine,
         Philadelphia, PA
  9:40  Break
10:00  Regulation of Gonadotropin in Man—William Bremner, VA Medical Center,
         Seattle, WA
10:50  Therapy with GnRH and Analog—William Crowley, Jr., Massachusetts General
         Hospital, Boston, MA
11:40  General Discussion
12:00  Lunch

CURRENT ISSUES WITH DONOR INSEMINATION—Ronald L. Urry, Chairperson,
University of Utah School of Medicine, Salt Lake City, UT
  1:15  Overview of AID—Ronald L. Urry, University of Utah School of Medicine,
         Salt Lake City, UT
  1:30  Donor Selection—History, Physical and Genetic Screening, and Population Pools—
         Nancy Alexander, Eastern Virginia Medical School, Norfolk, VA
  2:00  Sexually Transmitted Disease Screening for AID—Mary E. Guinan,
         Center for Disease Control, Atlanta, GA
  2:45  Break
  3:05  Legal and Liability Aspects of AID—David Young, Attorney-at-Law,
         Salt Lake City, UT
  3:50  Screening Couples and Psychological Aspects—Kirtly Jones, University of Utah
         School of Medicine, Salt Lake City, UT
  4:20  Sperm Freezing, Thawing and Preparation for Insemination—Ronald L. Urry,
         University of Utah School of Medicine, Salt Lake City, UT
  4:50  Insemination Techniques, Results of AID, and Other Facets to Consider—Kirtly Jones,
         University of Utah School of Medicine, Salt Lake City, UT
  5:20  General Discussion

The School of Continuing Medical Education, University of Utah School of Medicine, has
designated this Postgraduate course for 8 credit hours in Category 1 of the Physicians Recogni-
tion Award of the American Medical Association.
# TWELFTH ANNUAL MEETING

**FRIDAY evening, March 6**

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<tr>
<td>6:00</td>
<td>Student Mixer in the Matchless Room on lower level (LL1).</td>
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<tr>
<td>7:00-9:00</td>
<td>Opening Reception in the Denver Ballroom.</td>
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**SATURDAY, March 7**

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<tr>
<th>Time</th>
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<tr>
<td>8:00</td>
<td><strong>Scientific Session A, E/F Colorado Ballroom</strong></td>
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<td>8:10</td>
<td>The Serono Lecture I. Roger V. Short, Monash University, Clayton, Australia—THE ANDROGENS:</td>
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<td>NATURE, RED IN TOOTH AND CLAW</td>
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<td>9:00</td>
<td>State of the Art Lecture Richard P. Michael, Emory University, Atlanta, GA</td>
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<td>9:50</td>
<td><strong>NEUROENDOCRINE AND BEHAVIORAL ASPECTS OF MALE REPRODUCTION</strong></td>
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<td>10:15</td>
<td>**Estradiol (E2), but not testosterone (T), allows for the development of LHRH self-priming in</td>
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<td>the adult male rat. P.M. Grosser,* Z. Xue* and B. Robaire.</td>
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<td>10:30</td>
<td>**Role of testosterone metabolites in the maintenance of the self-priming effect of LHRH in</td>
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<td>pubertal male rats. S.J. Nazian.</td>
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<td>10:45</td>
<td>**Photoperiodic maintenance of the ram testis during exposure to contrasting stimulatory and</td>
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<td>inhibitory daylengths. Bruce Schanbacher.</td>
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<td>11:00</td>
<td>**Purification and comparative structural analysis of a FSH and testosterone responsive</td>
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<td>glycoprotein from rat and mouse primary Sertoli cell culture media. C. Yen Cheng, Josephine</td>
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<td>Grima, Kenneth S.K. Tung and C. Wayne Bardin.</td>
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<td>11:15</td>
<td>**Germ cells bind testicular 57Fe-transferrin following the transferrin mediated transport of</td>
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<td>57Fe across Sertoli cells. Daniel Djakiew and Martin Dym.</td>
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<td>11:30</td>
<td>**Germ cells influence the polarized secretion of androgen binding protein (ABP), but not</td>
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<td>transferrin (TRF) by Sertoli cells in vitro. Andrzej Janecki,* Andrzej Jakubowiak* and Anna</td>
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<td>Steinberger.</td>
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<td>11:45</td>
<td>**Effect of age on composition of human seminiferous tubule boundary tissue and on the volume of</td>
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<td>each component. J.G. Abdo,* L. Johnson and W.B. Neaves.</td>
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<td>12:00-1:30</td>
<td><strong>Lunch—on your own</strong></td>
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**Scientific Presentation Session C, SPERM EVALUATION**

*Chairpersons: A.M. Belker and R.H. Hammerstedt*

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<td>1:30</td>
<td><strong>Relationship of human sperm DNA stability and fertility potential.</strong> J.E. Sokoloski, M.M.</td>
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<td>Quigley* and A.J. Thomas</td>
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<td>1:45</td>
<td>**Studies on the function and regulation of sulphydrys in caput epididymal sperm induced to</td>
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<td>2:00</td>
<td><strong>Automated semen analysis: reproducibility studies.</strong> Kenneth A. Ginsburg* and Ernest L.</td>
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<td>Abel.*</td>
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<td>2:15</td>
<td><strong>Comparison of fully automated and traditional methods of semen analysis.</strong> D. Vantman, M.</td>
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<td>Zinaman and R. Sherins.</td>
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<td>2:30</td>
<td><strong>Analysis of prevasectomy ejaculates by the hypoosmotic swelling (HOS) test.</strong> L.J.D. Zaneveld,</td>
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*Not member of the American Society of Andrology.
2:45 13 Relationship between the hypoosmotic swelling (HOS) test and the sperm penetration assay (SPA). C. Bastias,* T. Thompson,* A. Buck,* M. Hinson* and B.J. Rogers.


3:15 Break—coffee and soda in the central lobby and exhibition area

Scientific Presentation Session D, INFERTILITY
Chairpersons: N.J. Alexander and K.D. Smith

3:30 15 Lymphopenia in male partners of infertile couples. Allan R. Glass.


4:00 17 Evidence for antibody binding specificity to donor sperm. Stephen E. Howe and Dona M. Lynch.

4:15 18 Inability of sperm washing to remove antisperm antibodies. Gilbert G. Haas, Jr., Osmond D'Cruz* and Fayez Nahhas.*

4:30 19 Clomiphene and tamoxifen in oligospermic infertility. W.S. Maxson, B.J. Rogers, C.M. Herbert, C. Markinson and F.K. Kirchner, Jr.


5:30 Student Colloquium—G/H Colorado Ballroom
Speaker—Stephen W. Byers, IN VITRO STUDIES OF THE MALE REPRODUCTIVE SYSTEM

Scientific Presentation Session E, Denver Ballroom

7:00-9:30 Papers 22 to 61 and wine and cheese.
Presenters of posters should ensure that their poster is in place by 1:30 p.m. to allow casual viewing during the afternoon and should be at their poster during the evening session.
Posters must be removed by 10:00 p.m.

22 Effects of prenatal irradiation on testicular LH receptors in adult Syrian hamsters. A.G. Amador, A. Bartke, V. Chandrashekar* and H.G. Klemcke.*

23 Changes in serum testosterone and luteinizing hormone levels in male rats with adjuvant-induced arthritis. Jeffrey W. Clemens* and Brent C. Bruot.*


25 Effect of seasonal changes in Leydig cell (LC) number on the volume of LC smooth endoplasmic reticulum (SER) and intratesticular testosterone content (IT). Larry Johnson and D.L. Thompson, Jr.

26 Influence of 35°C and melatonin (MEL) on testosterone (T), testis and accessory sex organs (ASO) after HCG in juvenile deer mice. Pat Fail.

27 Computerized analysis of sperm motion: effect of stage temperature and storage at room temperature and 4°C. A.B. Stockwell,* P.J. Burns and D. Douglas-Hamilton.*


29 Correlations between fertility and computer-determined swimming parameters of bull sperm. P.R. Budworth* and R.P. Amann.

*Not member of the American Society of Andrology.
30 Automated semen analysis in large epidemiologic studies. Frank DeStefano,* Joseph L. Annest,* Marcie-j o Kresnow,* Melinda L. Flock and Steven M. Schrader.


34 Employment of the hypoosmotic swelling (HOS) test to assess the functional integrity of equine sperm membrane. Panayiotis M. Zavos and Gary W. Gregory.*

35 The P and G pattern in patients with a normal sperm morphology < 14%. T.F. Kruger,* K.F. Simmons* and A.A. Acosta.


38 Usefulness of scanning and transmission electron microscopy in clinical andrology. Paul W. Musselman, Anthony J. Thomas, Jr.," James T. McMahon and Howard S. Levin.*

39 Paper withdrawn

40 The role of sperm morphology evaluated by strict criteria in the hamster ova/human sperm penetration assay (SPA). R. James Swanson, Thinus F. Kruger,* Mary Hamilton,* Kathy F. Simmons* and Arabal A. Acosta.


45 Use of Penetrak to study sperm penetration patterns. Richard M. Harrison and Ronald W. Lewis.


47 Predictive value of quantitative and qualitative bacteriologic studies in zona-free hamster egg penetration test failure. Laszlo Sogor, Hunter Hammill,* Doina Glavan* and Arlette Coulter.*


49 New observations on the morphological features of the guinea pig sperm acrosome reaction. S.P. Flaherty,* V.P. Winfrey* and G.E. Olson.

50 Analysis of sperm fertilizing ability in the stallion. E. Bustos-Obregon, Hector Rodriguez* and Sylvia Leiva.*


52 Regional differences in androgen binding by ram epididymal tissue. F.R. Tekpetey and R.P. Amann.

53 The effect of varying FSH pulses on transferrin secretion in superfused Sertoli cell cultures. Andrzej Jakubowia k,* Andrzej Janecki* and Anna Steinberger.

*Not member of the American Society of Andrology.
SATURDAY, March 7

54 Neutral amino acid absorption by rat epididymis. B.T. Hinton and H. Hernandez.
55 Direct and indirect immunologic response following acute testicular torsion. Johnny B. Roy, James Mays,* Gilbert G. Haas, Sharon Einfeldt,* Brian Lansford* and Fayez Nahhas.*
56 Incorporation of (14C)gossypol and formation of (14C)gossypol-conjugates in mouse transformed Sertoli, TM4, cells. Nongnuj Tanphaichitr* and Lisa Fitzgerald.*
58 Doppler evaluation of varicocele. C.B. Dhabuwala and Anil Kumar.*
60 Effects of cyclosporine on testicular structure and function. L. Seethalakshmi, D.A. Diamond and M. Menon.

SUNDAY, March 8

Scientific Presentation Session F, CLINICAL ANDROLOGY
Chairpersons: A.R. Glass and R.Z. Sokol

8:00 62 Physiological attributes of episodic follicle stimulating hormone secretion in normal men. J.D. Veldhuis, J.C. King,* R.J. Urban,* A.D. Rogol and M.L. Johnson.*
8:15 63 Effect of altering sex steroid levels with human chorionic gonadotropin or testolactone on sex hormone binding globulin level. Richard V. Clark, James F. Dunn* and Richard J. Sherins.
8:30 64 Subnormal prolactin (PRL) response to thyrotropin-releasing-hormone (TRH) does not necessarily support the diagnosis of hypogonadotropic hypogonadism. Duarte Pignatelli, Davide Carvalho* and Manuel Hargreaves.*
8:45 65 Effects of chronic endurance exercise training on male reproductive hormonal profiles. A.C. Hackney* and W.E. Sinning.*
9:00 66 The role of sexual stimulation and ejaculate characteristics during the clinical use of a seminal collection device (SCD). Panayiotis M. Zavos.
9:45 68 Induction of acrosome reactions by the human zona pellucida. N.L. Cross,* P. Morales,* J.W. Overstreet and F.W. Hanson.*
9:45 69 Factors affecting microinjection of mammalian spermatozoa into hamster eggs. R.N. Clarke and L.A. Johnson.*
10:00 Break—coffee and soda in the central lobby and exhibition area

Scientific Presentation Session G, TOXICOLOGY AND AGEING
Chairpersons: B. Robaire and J.L. Fourcroy

10:30 71 Increased degeneration of germ cells late in meiosis and no loss during spermiogenesis in aged men. Ali Bagheri* and Larry Johnson.

*Not member of the American Society of Andrology.


Lunch—on your own

Scientific Session H, E/F Colorado Ballroom

State of the Art Lecture

Edward O. Price, University of California, Davis, CA

EVOLUTIONARY ASPECTS OF MALE SEXUAL BEHAVIOR IN DOMESTIC Ungulates, Laboratory Rodents, AND PRIMATES

Scientific Poster Presentation Session I, Denver Ballroom

Papers 77 to 116 and coffee and soda

Presenters of posters should ensure that their poster is in place by 8:00 a.m. to allow casual viewing during the morning and mid-day and should be at their poster during the afternoon session. Posters must be removed promptly at 4:30 p.m.


78 Is nocturnal penile tumescence (NPT) a test of pituitary-testicular function? F.T. Murray, M. Sciadini* and H. Wyss.*

79 Hydroflex penile prosthesis: early experience and recommendations. H. David Mitcheson.


81 Testicular biopsy in the evaluation of male infertility. Miguel Diaz, Fernando Rivas, Lidia Garcia, Horacio Rivera and Jose Maria Cantu.


83 Influence of macroagglutinating (MA), microagglutinating (MI) and immobilizing (I) anti-sperm antibodies on the hamster ova/human sperm penetration assay (SPA). Mary Hamilton,* Anne Bogerart,* Mahmood Morshedi,* Steven Ackerman and R. James Swanson.


85 Association of sperm agglutination and immobilization tests with immunobead (IMB) assay. M. Morshedi,* S. Ackerman, A. Bogerart,* A. Acosta and R.J. Swanson.

86 Comparison of the sperm penetration assay (SPA) with objective motility parameters. L.R. Kossoy,* T. Thompson,* A. Buck,* M. Hinson,* B. Brodie,* W. Vaughn,* A.C. Wentz* and B.J. Rogers.

87 Intracornual semen deposition at different depths and subsequent fertilization and embryonic development in superovulated cows. John Fernandez-Vancleve,* Panayiotis M. Zavos and Roger W. Hemken.*

88 Experience with intrauterine insemination in infertile couples. Christine L. Cook,* Leigh T. Price* and Arnold M. Belker.

89 Effect of neuraminidase on sperm separation for sex selection. Hugh C. Hensleigh* and Denise Truzinski.*

90 Increased rate of human sperm fertilization ability by Percoll-gradient centrifugation. N. Tanphaichitr,* A. Agulnick, D. Daz, J. Hill, L. Fitzgerald* and D. Anderson.

91 Ability of glass wool filtration to isolate fertile sperm fractions. W.J. Holmgren, R.S. Jeyendran, M.R. Neff, M. Perez-Pelaez.

92 Sperm preparation for artificial insemination using rayon filters. Hugh C. Hensleigh* and Denise Truzinski.*

*Not member of the American Society of Andrology.

12-P
93 ATP decline of infertile patient semen after swim-up separation method (SSM). M. Morshed,* R. Acosta,* P. Pleban,* A.A. Acosta, J. Yuan* and R.J. Swanson.

94 Polylactosamine on the mouse sperm surface during maturation and capacitation. Charles H. Muller.


96 Interactions between selected fatty acids and the activation of palmitic acid in spermatozoa. Robert E. Jones and Stephen R. Plymate.

97 Changes in cytosolic protein and phospholipase activity during in vitro capacitation of sperm from fertile and subfertile bulls. Margaret Henault, Gary Killian and David Chapman.*

98 Glycospidase activities in bovine sperm during in vitro capacitation and the acrosome reaction. Tamara McNutt,* Gary Killian and David Chapman.*


100 Immunological comparisons between the boar and human proacrosin-acrosin proteinase systems. Mark S. Siegel,* Dana S. Bechtold,* Janet L. Willand* and Kenneth L. Polakoski.*

101 Inhibitory effect of zinc on protein phosphorylation in the sperm head membranes in spisula solidissima. Balwant Ahluwalia and George Nolan.*

102 Oxygen consumption rate varies with the concentration of bull spermatozoa. Carole Wegner,* Gary Killian and David Chapman.*

103 Movement characteristics of cynomolgus monkey sperm recovered from the cervix and uterus. E. Behboodi,* D. Katz, J. Overstreet and A. Hendrickx.*

104 Pulsatile LHRH-treatment in male patients with severe oligospermia and selectively elevated FSH. W. Aulitzky,* J. Frick,* F. Hadziselimovic.


107 Leydig cell function in the cryptorchid rat. Tom O. Abney.


110 Microsurgical accessory gland ablation: The effects on fertility potential in the rat. William Blank, Eric Yousha* and Marc Goldstein.

111 Protein contributions of the accessory organs to the composition of human seminal plasma as determined by high resolution two-dimensional electrophoresis. Edward E. Gaunt, Anibal A. Acosta, Steven B. Ackerman, Patricia A. Pleban,* John F. Stecker and James H. Yuan.*

112 Characteristics of the accessory glands of the male reproductive tract of some native California rodents. B. Peitz and S. Membreno.*


114 Cloning and sequence analysis of human testis-specific lactate dehydrogenase C. Erwin Goldberg, Catherine E. Driscoll* and Jose L. Millan.*

115 Evidence for polymorphic sperm antigen expression in inbred mice. Chong Xu* and Deborah J. Anderson.


*Not member of the American Society of Andrology.
SUNDAY, March 8

Scientific Session J, E/F Colorado Ballroom
4:30-6:15 WORKSHOP ON ANIMAL RIGHTS
Bernard E. Rollin, Colorado State University, Ft. Collins, CO

COCKTAILS AND BANQUET, Denver Ballroom
7:00-7:30 Cocktails (cash bar)
7:30-9:30 Banquet

MONDAY, March 9

Scientific Presentation Session K, EPIDIDYMIS
Chairpersons: J.L. Marmar and M.-C. Orgebin-Crist.

8:00 117 Androgen movement into the rat seminiferous and epididymal tubules perfused in vivo. T.T. Turner.
8:15 118 Protein secretion by rat epididymal epithelial cells growing in bicameral culture chambers. S.W. Byers.
8:30 119 Maturation-related glycoproteins in the mouse epididymis. Hwan-Wun Liu* and Charles H. Muller.
8:45 120 Correlations between changes in rat sperm membrane lipid composition and the temperature-dependence of the membrane physical state during epididymal maturation. Joseph C. Hall* and Laura M. Edgerly.*
9:00 121 The role of superoxide dismutase in preventing lipid peroxidation and loss of motility in human ejaculated spermatozoa. Juan G. Alvarez,* J.C. Touchstone,* Luis Blasco and Bayard T. Storey.
9:30 123 Results of specific tubule microsurgical vasoepididymostomy. Sherman J. Silber.
10:00 Break

Special Session, E/F Colorado Ballroom
Awards Ceremony—William D. Odell, presiding
The New Investigator Award
Student Travel Awards
The Young Andrologist Award
Ilpo T. Huhtaniemi, M.D., Ph.D., Helsinki, Finland
The Distinguished Andrologist Award
Emil Steinberger, M.D., Houston, TX
10:50 Annual Business Meeting of the American Society of Andrology
William D. Odell, presiding

Scientific Session L, E/F Colorado Ballroom
11:45 The Serono Lecture II.
Roger V. Short, Monash University, Clayton, Australia—THE SIZE OF THE TESTES
12:45 Adjourn Meeting

*Not member of the American Society of Andrology.
ABSTRACTS

1. EXTRADUOIN (E), BUT NOT TESTOSTERONE (T), ALLOWS FOR THE DEVELOPMENT OF LH-RH SELF-PRIMING IN THE ADULT MALE RAT. P.M. Grosser1, L. Zuel1 and B. Rohaie1. Centre for the Study of Reproduction and Depts. of Pharm/Therap and Ob/Gyn, McGill University, Montreal, Canada; and Dept. of Urology, University of Heiing, Beijing, Peoples Republic of China.

T and E2, administered simultaneously to adult male rats, synergize to suppress spermatogenesis. Although exploited for the development of a male contraceptive, the mechanism responsible for this steroid interaction at the hypothalamic-pituitary complex is not known. The present studies examined the effects of T (2.5 cm) and E2-filled (0.1 cm) subdermal Silastic implants, alone or in combination, on pituitary LH responsiveness to LH-RH and LH-RH self-priming. Male SD rats (300-325 g) were equipped with jugular catheters. Blood samples for RIA of plasma LH were obtained every 10 min for 7 hrs, 1 day before and 1.3, 7 and 14 days after giving T, E2 or T+E2 implants. LH-RH (600 ng/kg) was given at 90 min (pituitary LH responsiveness) and at 240 and 270 min (LH-RH self-priming) after initiation of sampling. Within 14 days, T treatment reduced LH response to LH-RH by more than 70%; LH-RH self-priming (ratio of 3rd to 2nd LH peak) was reduced from 133% in controls to 108%; E2 alone reduced pituitary responsiveness by less than 20%. However, LH-RH self-priming was increased markedly from 120% in controls to 201% by day 14. The combination of T and E2 or E2 alone were additive with respect to pituitary responsiveness. In contrast, the self-priming response was greater than with E2 alone (338% by day 14). Thus T and E2 act differently on the hypothalamic-pituitary complex. T suppresses pituitary responsiveness while E2 primarily sensitizes the pituitary to LH-RH self-priming. These complementary actions may account, in part, for the synergistic action of T and E2. Supported by NCI and FCAC.

2. ROLE OF TESTOSTERONE METABOLITES IN THE MAINTENANCE OF THE SELF-PRIMING EFFECT OF LH-RH IN PUBERTAL MALE RATS. S.J. Hanly, Department of Physiology & Biophysics, College of Medicine, University of South Florida, Tampa, FL 33612.

Pubertal male rats release more LH in response to LH-RH if primed with small doses of LH-RH than if pretreated with saline. Castration eliminates this self-priming effect. Treatment of castrated rats with Silastic capsules containing testosterone (T), dihydrotestosterone (DHT) or estradiol (E2) will maintain the effect. It has been suggested that the E receptor plays a role in this process because the DHT metabolite 5α androstan-3β-17β-diol (B-DIOl) is known to bind to it. To examine this, pubertal male rats were castrated and implanted with one of two sizes of capsule filled with B-DIOl or its 3α isomer. 4 days later these animals were anesthetized and primed with 10 μg LH-RH/100g BW or saline iv at 1/2 hr intervals. 30 min after the 3rd injection a blood sample was collected by heart puncture and all animals received a 50 ng LH-RH/100g BW challenge injection. A final blood sample was collected 10 min later. Neither isomer was capable of maintaining a self-priming effect. B-DIOl seemed to reverse the effect; saline primed rats released more LH in response to LH-RH challenge than did LH-RH pretreated animals. Additional studies indicated that neither isomer had any effect on pituitary sensitivity to a single LH-RH injection. When administered to intact rats for 4 days neither a 5α reductase inhibitor (V-MA) nor an aromatase inhibitor (AIN) was capable of blocking the self-priming effect. When given to castrated rats bearing T capsules capable of maintaining the effect, AIN did not block it. These data suggest that both aromatization and 5α reduction of T will maintain the self-priming effect and that in the absence of one, the other is sufficient. Supported by NIH15313.


The Suffolk ram is characterized by a cyclic reproductive response to changes in day length. Seasonal responses of the ovine testsis can be experimentally driven by alternating exposure to contrasting short (8L:16D) and long (16L:8D) days (D'Occhio et al., Biol. Reprod. 30:1039-1054, 1984). Twelve nature Suffolk rams were assigned to two groups and exposed for a period of one year to a 56-d (hexaneutral) photoperiod cycle of either 8L:16D·40:16D·8L or 8L:16D·40:16D·8L. All rams entered the experimental late in the breeding season (Nov) when mean testis diameters (6.4 cm) and mean serum testosterone levels (2.6 ng/ml) were decreasing. Testis diameters and serum testosterone for gps I and II were similar and paralleled each other over the course of the study with minimum average values (5.8 cm and 1.0 ng/ml) recorded during the first two cycles (Dec-March). Thereafter, both testis diameters and serum testosterone increased abruptly and were maintained near 7.5 cm and between 5 and 10 ng/ml over the next four cycles. Twenty-four hour secretory patterns of melatonin in these rams accurately reflected day length exposure with summary, we have (1) identified a 5α-reductase inhibitor; four-fold higher than those during the day. In view of the evidence that testicular regression requires weeks of exposure to inhibitory long days, these findings suggest that 56-d cyclic stimulation of the pineal-pituitary-gonadal continuum is effective at preventing normal photo-induced testicular regression in rams. Preliminary evidence also indicates that these rams produce good semen and display sexual aggressiveness suitable for year-round mating.

4. PURIFICATION AND COMPARATIVE STRUCTURAL ANALYSIS OF A FSH- AND TESTOSTERONE-RESPONSIVE GLYCOPROTEIN FROM RAT AND MOUSE PRIMARY SERTOLI CELL CULTURE MEDIA. C. Yan Cheng, Josephine Grima, Kenneth S. Tung*, and C. Wayne Bardin. The Population Council, 1230 York Avenue, New York, NY 10021, and Department of Pathology, School of Medicine, University of New Mexico, Albuquerque, NM 87131.

During a search for hormonally responsive proteins from primary Sertoli cell-enriched cultures established from 20-day-old rats we noted a FSH- and testosterone-responsive protein designated CMB-21 (Cheng et al. Endocrinology 118:80-98, 1986). CMB-21 has been purified to apparent homogeneity using sequential anion-exchange, chromatofocusing, gel permeation, and hydrophobic HPLC from batches of 2 litres of Sertoli cell culture medium. Also a monospecific antiserum has been raised against CMB-21. CMB-21 is a heterogeneous glycoprotein of Mr ~25,000 consisting of two subunits with identical electrophoretic mobility of Mr ~5,000. It is reactive to wheat germ agglutinin but not to concanavalin A on lectin-blot. Using Sertoli cell cultures established from prepubertal mice at 20 days of age, it was noted that it consisted of a CMB-21 immunoreactive macromolecule demonstrated by immunoblot. We have therefore partially purified this immunoreactive CMB-21 protein from the mouse Sertoli cell cultures by HPLC and shown that the mouse immunoreactive CMB-21 is a glycoprotein of Mr 84,000 consisting of two subunits of Mr 42,000. Peptide maps were generated for these two proteins and visualized by either immun- or lectin-staining. These analyses indicate that even though these proteins have some distinctive features, they have similar peptide maps and share common immunodeterminants. In summary, we have (1) identified a FSH- and testosterone-responsive protein in rat primary Sertoli cell cultures and (2) this protein shares epitopes with a similar macromolecule in the mouse primary Sertoli cell cultures.
5 GERM CELLS BIND TESTICULAR 59Fe-TRANSFERRIN FOLLOWING THE TRANSFERRIN MEDIATED TRANSPORT OF 59Fe ACROSS SERTOLI CELLS. Daniel Dijkstra & Martin Osm. Dept. of Anat. & Cell Biol., Georgetown Univ., Washington D.C. Using confluent epithelial sheets of Sertoli cells (3.5x10^6 cells/chamber) grown in bicameral culture chambers (0.38 cm^2) we have shown the transferrin (TF) mediated transport of 59Fe across Sertoli cells (I). We now report the effect of coculturing germ cells on Sertoli cells during this process. Two populations of germ cells (primary spermatocytes, round spermatids) were isolated from adult rats by unit gravity sedimentation and cocultured (5x10^5 germ cells/chamber) with epithelial sheets of Sertoli cells. When 10^7 pho of human 59Fe-TF were placed in media bathing the basal aspect of Sertoli cells alone for 6 hours, 3.7 pm of testicular 59Fe-TF was immunoprecipitated from apical media. However, coculture of spermatocytes or spermatids on Sertoli cells reduced the percentage reduction in immunoprecipitable 59Fe-TF in apical media in the presence of germ cells, 2.4x10^7 spermatozoids or 1.2x10^7 spermatocytes will bind all the 59Fe-TF secreted apically by Sertoli cells. Since in vivo there are a number of unoccupied TF receptors which may act as a sink for the passage of Fe across Sertoli cells, a concentration gradient from blood (high concentration of Fe) to germ cells (lower concentration of Fe) will bind all the Fe-TF secreted apically by Sertoli cells. Since spermatocytes and spermatids contain 3.1x10^7 and 10.7x10^7 TF receptors, respectively, on their cell surface, the reduced immunoprecipitable 59Fe-TF in apical media of cocultures indicates that some of the 59Fe-TF secreted apically by Sertoli cells is subsequently bound to germ cells. From the percentage reduction in immunoprecipitable 59Fe-TF in the presence of germ cells, 2.4x10^7 spermatids or 1.2x10^7 spermatocytes will bind all the 59Fe-TF secreted apically by Sertoli cells. Hence, less than one germ cell per Sertoli cell will bind all the 59Fe-TF secreted apically by Sertoli cells. In vivo there are a number of unoccupied TF receptors which may act as a sink for the passage of Fe across Sertoli cells, a concentration gradient from blood (high concentration of Fe) to germ cells (lower concentration of Fe) during the transferrin mediated transcellular transport of Fe across Sertoli cells. (I) Dijkstra et al, (1986). J. Androl. 7:355-366.

6 GERM CELLS INFLUENCE THE POLARIZED SECRETION OF ANDROGEN BINDING PROTEIN (ABP) BUT NOT TRANSFERRIN (TRF) BY SERTOLI CELLS IN VITRO. Andrzej Janecki*, Andrzej Jakubowicz, and Anna Steinberger. Department of OB/GYN and Reproductive Sciences, University of Texas Medical School at Houston, 6431 Fannin, Houston, TX 77030

The polarity of ABP secretion in the rat testes changes with age, possibly due to varying germ cell populations. In the present study, we investigated the influence of germ cells (GC) on the polarized secretion of ABP and TRF by rat Sertoli cells (Sc) maintained in two-compartment culture chambers (Janecki and Steinberger, J. Androl. 7, 1986). Sc from 14-day-old rats secreted ABP bi-directionally, the outer:inner compartment ratio (OC/IC) being highest (+2.0) at Days 3-4 of culture when the monolayers became essentially free of contaminating GC. This ratio was not influenced by FSH (200 mg/ml). Addition of GC (> 85 k platey spermatocytes) reduced the ratio to 0.7, but only in the presence of FSH. GC-culture with GC did not influence the TRF OC/IC ratio. In cultures of Sc from 32-day-old rats, intentionally containing many original GC, the ABP OC/IC ratio was ~ 0.6. However, if these cultures were treated with hypotonic buffer, to remove the residual contaminating GC, the ABP OC/IC ratio changed to ~ 1.3, independent of FSH. The hypotonic treatment did not influence the TRF OC/IC ratio (~ 0.7). These data suggest that GC may influence the polarity of some Sc secretions in vitro, the effect being FSH-dependent in Sc obtained from 14-day-old rats but not from 32-day-old animals.

7 EFFECT OF AGE ON COMPOSITION OF HUMAN SEMINIFEROUS TUBULE BOUNDARY TISSUE AND ON VOLUME OF EACH COMPONENT. J. G. Ando*, L. Johnson, and W.B. Leaves. University of Texas Health Science Center at Dallas, Dallas, TX 75235.

Tubular boundary tissue thickens with age. In a small group of men, the percentage of myoid cells in tubular boundary tissue was not influenced by age, and the composition of extracellular components remains unknown. The objective was to characterize the composition of boundary tissue in 16 younger (20 to 29y) and 18 older (51 to 84y) adult men. Testes were perfused with glutaraldehyde, placed in osmium, and embedded in Epon. Volume of boundary tissue was calculated from the percentage boundary tissue determined by point counting 0.5um sections and the paired parenchymal volume. Percentage of myoid cells, collagen, microfibrils, or other extracellular component in boundary tissue was determined from electron micrographs taken randomly on predetermined sites of EM grids. Volume of each component was calculated from the volume of boundary tissue/cell and the percentage of boundary tissue occupied by each component. There was no difference (P>0.05) between testes from younger and older men in the percentage of boundary tissue occupied by myoid cells (35.2±1.5 vs 31.8±1.8%), collagen (27.0±2.0 vs 26.2±1.5%), microfibrils (22.1±1.3 vs 24.1±1.5%), and other (14.8±1.8 and 15.0±1.9%). The volume of boundary tissue/cell (3.3±0.2 vs 3.3±0.3um^2), myoid cell volume/cell (1.2±0.1 vs 1.1±0.1um^3), collagen volume/cell (0.8±0.08 vs 0.97±0.14um^3), microfibrils/cell (0.74±0.06 vs 0.80±0.10um^3), and volume of other (0.6±0.06 vs 0.5±0.06um^3) were not different (P>0.05) between age groups. This study confirms that age-related thickening of boundary tissue is not due to the deposition of collagen or other boundary tissue components. Supported by NIH grant AG02260.

8 RELATIONSHIP OF HUMAN SPERM DNA STABILITY AND FERTIL­ITY POTENTIAL. Sokoloski*J.R., Qiugley* H.-M. and Thomas, A.J. Dept. of Gynecology and Urology, Cleveland Clinic Foundation, Cleveland, OH 44106.

Male fertility potential cannot be predicted by microscopic semen analysis alone. To augment the conventional analysis various in vitro tests have been developed to better delineate the possible male factors involved in an infertile union. One of these tests takes advantage of the fluorescent chromatic properties of Accidine Orange (AO). AO, when bound to native DNA, fluoresces green and when bound to denatured DNA glows red. This report profiles a population of fertile men and infertile patients whose sperm has been subjected to AO analysis. 72 patients were evaluated in this preliminary study. The AO score (a green) displayed a bimodal histogram with an arithmetic range of 2 to 100% (x=89.2±17.1) and was taken as the hypothetical "normal" range. One of these tests takes advantage of the fluorescent chromatic properties of Acridine Orange (AO). AO, when bound to native DNA, fluoresces green and when bound to denatured DNA glows red. This report profiles a population of fertile men and infertile patients whose sperm has been subjected to AO analysis. 72 patients were evaluated in this preliminary study. The AO score (a green) displayed a bimodal histogram with an arithmetic range of 2 to 100% (x=89.2±17.1) and was taken as the hypothetical "normal" range. Ten of the 72 samples fell into the "abnormal" range. There was no significant difference between the two groups in terms of total sperm count, ejaculate and % motility. The sperm of the 10 abnormal pts and 23 of the normal AO group was subjected to the Hamster Egg Assay with no significant differences in the % eggs penetrated (37.4±33.1 vs 33.1±7.2). Seventeen pts were in the IVF-ER program. 6 had abnormal AO and fertilized only 13% (4/30) of their wives eggs while the normal AO partners fertilized 47% (39/83) (p<0.05). These data suggest that AO analysis may provide a new, unimodal range of 54.9-100% was generated and a new, unimodal range of 54.9-100% was generated. The spermatozoa from each sample were evaluated using a bimodal histogram with an arithmetic range of 2 to 100% (x=89.2±17.1) and was taken as the hypothetical "normal" range. Ten of the 72 samples fell into the "abnormal" range. There was no significant difference between the two groups in terms of total sperm count, ejaculate and % motility. The sperm of the 10 abnormal pts and 23 of the normal AO group was subjected to the Hamster Egg Assay with no significant differences in the % eggs penetrated (37.4±33.1 vs 33.1±7.2). Seventeen pts were in the IVF-ER program. 6 had abnormal AO and fertilized only 13% (4/30) of their wives eggs while the normal AO partners fertilized 47% (39/83) (p<0.05). These data suggest that AO analysis may provide a new, unimodal range of 54.9-100% was generated and a new, unimodal range of 54.9-100% was generated.

10-P
9 STUDIES ON THE FUNCTION AND REGULATION OF SULFHYDRYLS IN CAPUT EPIDIDYMAL SPERM INDUCED TO ACQUIRE PROGRESSIVE MOTILITY IN VITRO. G. Cornwall, S. Tilloian* and T.S.K. Chang, Dept. of Urology, The Johns Hopkins Hospitals, Baltimore, Md.

To study the function and regulation of sulfhydryls (SH) in epididymal sperm maturation, immature caput epididymal sperm were induced to acquire progressive motility by incubation in induction medium (IM) containing 15% seminal plasma and 50 mM theophylline. However, 95% of these sperm exhibited a flagellar angularity characterized by a 90-180° bend at the neck or midpiece. Addition to IM of diamide, a sulfhydryl oxidant which forms disulfide (S-S) bonds, completely prevented the flagellar bending in mature-induced caput sperm. The peak effect of diamide however was transient; 180 min. after addition of diamide 85% of the sperm again exhibited flagellar angularity. Subsequent studies suggested that sperm glutathione reductase activity may be involved in the recurrence of flagellar bending by increasing the levels of SH in diamide-treated sperm. In these studies addition of nitrofurantoin (NF), a GR inhibitor, reduced caput sperm SH activity by 83% and prevented sperm flagellar bending. Flow cytometric studies using a SH fluorescent stain were used to monitor sperm SH levels in IM containing diamide and NF. These studies revealed that: 1) motile-induced sperm with angular flagella contained high levels of SH; 2) these SH levels were significantly reduced by diamide; 3) sperm SH levels in diamide-treated sperm increased over time paralleling the increase in flagellar angulation; and 4) the presence of NF prevented increases in sperm SH as well as flagellar bending. Taken together these studies suggest that the interaction between intracellular sperm sulf hydryls and concentration, motility, linearity, velocity, head displacement (Al.H), concentrations of 

Conclusions: 1) Sperm distribution on microscope slides may be a significant source of variation for linearity and BCF-reaction but not for concentration, motility, velocity and Al.H. This variation may need to be taken into consideration in developing a routine profile of the sperm population from a single sperm analysis. 2) A sampling method with predetermined areas done in triplicate, calculating mean±SE, gives the least within-slide variation. 3) To change in results occur after analysis of 200 cells. 4) The CASA gives highly reproducible results with an overall mean±SE of 1.3% for all parameters reasured, and should thus be suitable for detecting small changes in sperm motile characteristics in both a research and clinical setting.

10 AUTOMATED SEMEN ANALYSIS: REPRODUCIBILITY STUDIES. Kenneth A. Ching and Ernest L. Abel*, Wayne State University C.S. Kott Center, Detroit, Ml, 48201.

Computer-assisted videomicrographic systems permit analysis of sperm movement with unprecedented speed and accuracy. We investigated the variability and reproducibility of such systems, with emphasis on computer analysis of sperm smears in seminal plasma using the CellSoft Automated Seven Analyzer (CAS), CytoResources, HF under a Metler chamber. 10 specimens with sperm concentrations of 31 to 121,000 were studied in 3 different protocols, and concentration, motility, linearity, velocity, head displacement (Al.H) and beat frequency (BF) were determined. 50,000 sperm were evaluated per slide in each of 3 slides per slide. Correlation coefficient for sperm counts was determined by regression to examine differences between individual and pooled analyses with 5 repetitions per method. In Method B, multiple fields were examined and data pooled, and in Method C, 4 predefined fields were examined and average results determined. 2) Effects of number of cells analyzed on the stability of sperm motility and density. When sperm were analyzed in triplicate as described in Method B, sperm SMV, but not Al.H, BCF or head displacement, was significantly affected. To test the impact of cell density on sperm motility and velocity, 90-180° arc of the flagellum were induced to acquire progressive motility by incubation in induction medium (IM) containing 15% seminal plasma and 50 mM theophylline. However, 95% of these sperm exhibited a flagellar angularity characterized by a 90-180° bend at the neck or midpiece. Addition to IM of diamide, a sulfhydryl oxidant which forms disulfide (S-S) bonds, completely prevented the flagellar bending in mature-induced caput sperm. The peak effect of diamide however was transient; 180 min. after addition of diamide 85% of the sperm again exhibited flagellar angularity. Subsequent studies suggested that sperm glutathione reductase activity may be involved in the recurrence of flagellar bending by increasing the levels of SH in diamide-treated sperm. In these studies addition of nitrofurantoin (NF), a GR inhibitor, reduced caput sperm SH activity by 83% and prevented sperm flagellar bending. Flow cytometric studies using a SH fluorescent stain were used to monitor sperm SH levels in IM containing diamide and NF. These studies revealed that: 1) motile-induced sperm with angular flagella contained high levels of SH; 2) these SH levels were significantly reduced by diamide; 3) sperm SH levels in diamide-treated sperm increased over time paralleling the increase in flagellar angulation; and 4) the presence of NF prevented increases in sperm SH as well as flagellar bending. Taken together these studies suggest that the interaction between intracellular sperm sulf hydryls and concentration, motility, linearity, velocity, head displacement (Al.H), concentrations of 

Conclusions: 1) Sperm distribution on microscope slides may be a significant source of variation for linearity and BCF-reaction but not for concentration, motility, velocity and Al.H. This variation may need to be taken into consideration in developing a routine profile of the sperm population from a single sperm analysis. 2) A sampling method with predetermined areas done in triplicate, calculating mean±SE, gives the least within-slide variation. 3) To change in results occur after analysis of 200 cells. 4) The CASA gives highly reproducible results with an overall mean±SE of 1.3% for all parameters reasured, and should thus be suitable for detecting small changes in sperm motile characteristics in both a research and clinical setting.


Recent technology for fully automated computer-assisted analysis of sperm count and motility characteristics (CellSoft) potentially offers an objective method for semen analysis. We tested the accuracy of this new method by comparing estimates of sperm count and motility in seminal plasma obtained by two methods: Cell Soft and traditional semen analysis using hemocytometer, for cell density and subjective assessment for motility and sperm velocity. Sperm from 63 sperm positive and 7 azospermic men were provided after 3-4 h's abstinence (40±10 cells/field). CellSoft precision (coeff. of var.,mean±SE) for count and motility are shown using U, p<0.05 EXPRESSION Concentration of 8 motility cells/field 0 1-10 11-20 >20 Hemocytometer 0 9.5±1.3 9.4±1.3 9.7±1.3 9.7±0.9 CellSoft 1.3±0.1 12.8±1.0 21.9±1.3 90±1.0 9.0±0.3 p<0.05 p<0.05 NS NS NS

Correlation coefficient for concentration: r=0.87, l<0.01 and velocity: r=-0.56, l<0.01. We concluded that CellSoft provides acceptable accuracy and precision when analyzing more than 10 cells/field (15 million/ml).

However, sperm velocity may require more detailed analysis.

12 ANALYSIS OF PREVASECTOMY EJACULATES BY THE HYPOOSMOTIC SWELLING (HOS) TEST. L.J.D. Kanevel, R.S. Jeyendran, H.P.P. de Castro and P.J.M. Silveira*, Dept. of Obst/Men, Rush University, Chicago, IL, USA and Propter Institute, Sao Paulo, Brazil.

The hypoosmotic swelling (HOS) test assesses the biochemical integrity of the sperm membranes by determining the influx of water under hypoosmotic conditions, resulting in the expansion of the sperm. The HOS test was shown to be more reliable in predicting the outcome of human in vitro fertilization (IVF) than the more standard semen analyses. To assess the validity of these values in vivo, ejaculates from 1,680 men who had fathered children at one or more times in their life and requested a vasectomy, were analyzed by the HOS test. 96% of these men gave a positive HOS test (360 up to 2,000 sperm), 25% gave a negative test (less than 50 sperm per 200 sperm) and 10% fell into the 'grey' area. By comparison, 13% of the ejaculates had less than 50% motile sperm and 68% possessed 60% or more motile sperm. Assuming that the large majority of these men were still fertile at the time of evaluation, the results suggest that the HOS test correlates well with the fertility status of the male and should be a worthwhile addition to the semen analysis.

Supported by NIH HD 19555 and support from Rush-Presbyterian-St Luke's Medical Center.
13 RELATIONSHIP BETWEEN THE HYPOOSMOTIC SHELLING (HOS) TEST AND THE SPERM PENETRATION ASSAY (SPA). C. Bastias,* T. Thompson, A. Buck, H. Hinson, and B.J. Rogers, Dept. OB/GYN, C-FARR, Vanderbilt University School of Medicine, Nashville, Tn. 37232.

The (HOS) test has been proposed as a useful assay in the diagnosis of the infertile male. A good correlation between the HOS and SPA in fertile and normal semen samples was initially presented but subsequently no significant correlation was demonstrated with fertile and infertile patients. To validate the potential usefulness of the HOS test clinically, we have evaluated 68 patients using both the HOS and SPA as well as traditional semen parameters. The methodology for the HOS test was as originally described by Jeyendran et al. The SPA was performed by the original procedure utilizing an 18 hour preincubation period. Values of >60% for the HOS and >10% for the SPA were considered positive for HOS and 0% for SPA were considered negative. The gray area (50 - 59%) for HOS and (1 - 9) for SPA were excluded. The sensitivity of the HOS test was 74% and the specificity was 85% for predicting the SPA results. The false positive rate in the HOS test was 22% and the false negative rate was 18%. The HOS had an 80% success for correct prediction of SPA results. Correlation coefficients were: HOS vs SPA - 0.44 (P<0.02); vs motility - 0.50 (P<0.01); vs count - 0.41 (P<0.05). Due to the high degree of success of the HOS test in predicting the SPA results and the ease of performance of the HOS test we believe that it is a useful adjunct to evaluation of standard semen parameters as part of a comprehensive male fertility assessment.

14 HUMAN SPERM ACROSIN AS A FERTILITY MARKER. H.H. Vander Ven, W.P. Kennedy, J.H. Kaminiski, R.S. Jeyendran and L.J.D. Zaneveld, Dept. OB/Gyn, Univ. of Bonn, FRG and Dept. OB/Gyn, Rush University, Chicago, Il. The acrosin activity of 36 ejaculates obtained from men in an IVF program was compared with the fertilizing capacity of spermatozoa in order to ascertain whether acrosin could be used as a fertility marker. Total acrosin was determined using a recently developed simple assay system (Kennedy et al, American Fertility Society Abstracts, 87, 1985). Briefly, spermatozoa from each ejaculate were washed free of seminal plasma by mild centrifugation over ficoll. The washed spermatozoa were then incubated with the substrate, N-benzoyl-dl-arginine p-nitroanilide (BAPNA; 1mg/ml) and 0.1% triton X-100 detergent (which causes dispersal of the sperm acrosomal membranes and releases acrosin) at pH 8.0. After 3 hrs of incubation, benzamidin, a potent acrosin inhibitor was added to quench the reaction and the absorbance of the supernatant was read at 410 nm after mild centrifugation. This simple assay was reported to be linear in response to sperm numbers in the range of 2-10 million and also compared well with the standard acrosin assay. A significant difference in the total acrosin activity was noted between the 26 fertile (mean ± S.E. 0.34 ± 0.03 mU) and 10 infertile (0.19 ± 0.03 mU) ejaculates. Moreover, the acrosin levels observed in the individual ejaculates significantly correlated with their fertilizing capacity (r=0.475; p<0.01). The data demonstrate the clinical applicability of the human acrosin assay for determining the fertilizing capacity of spermatozoa. Supported in part by NIH HD 19555.

15 LYMPHOPENIA IN MALE PARTNERS OF INFERTILE COUPLES. Allan R. Glass, Walter Reed Hospital, Washington, DC 20070.

The etiology of oligospermia in most infertile men is unknown. To explore whether the process that disrupts spermatogenesis in infertile men might also affect another rapidly proliferating cell population, namely blood cells, we conducted a retrospective study of blood counts in 72 unselected male partners of infertile couples attending our clinic and in 119 healthy controls undergoing routine periodic health examinations. There was no difference between infertility clinic patients and controls in mean levels of hemoglobin, hematocrit, or red cell volume, or in percentage of subjects with abnormal values for these parameters.

Platelet counts were also similar in both groups. However, the mean total lymphocyte count was lower in the infertility clinic group (5818±1728/mcL) vs 6397±174/mcL; p<.05). The infertility clinic group also had lower total lymphocyte count than controls (1697±69 vs 2060±80 per mcL; p<.001) but did not have lower neutrophil counts (3331±144 per mcL; control=3678±139). In the male partners of infertile couples, there was no correlation between sperm density and either lymphocyte count or total leukocyte count and no difference in mean values of these parameters between those with sperm density above or below 20 million per cc. Preliminary results from an ongoing prospective study of infertile, oligospermic men (currently n=6) and fertile controls (n=4) show no difference between the groups in counts of total T lymphocytes, helper lymphocytes (T4), or suppressor lymphocytes (T8). We conclude that the lymphopenia seen in infertile male partners have significant lymphopenia without other changes in blood count, and this lymphopenia is not clearly related to reduction in sperm density. Whether lymphopenia in this population reflects an underlying immune defect which predisposes to infertility remains to be determined.


The male and female reproductive tracts are immunologically dynamic tissues containing immune response cells (lymphocytes and macrophages) that elaborate soluble factors upon activation (lymphokines and monokines, respectively). The majority of these soluble immune factors provide intercellular signals that potentiate and regulate the immune response; in addition many lymphokines and monokines have been shown to be cytotoxic to nonimmune cells that are targets of immune attack. Experiments were conducted to evaluate the effects of supernatants from activated leukocyte cultures, and purified lymphokine and monokine preparations on sperm motion. An automated semen analyzer was used to obtain accurate measurements of several parameters of sperm motion. Supernatants from activated peripheral blood leukocyte cultures significantly decreased sperm motility. In studies of effects of individual lymphokines and monokines, significant antinmotility effects were observed when spermatozoa were incubated with the lymphokine gamma-interferon, and the monokine tumor necrosis factor. Subnormal fertility may result from defective sperm function caused by lymphokines and monokines elaborated by activated lymphocytes and macrophages residing in the reproductive tracts of infertile men and women.
Evidence for Antibody Binding Specificity to Donor Sperm.

Stephen E. Howe and Donna M. Lynch, Department of Pathology, Rose Medical Center, Denver, Colorado 80202.

Differences in antisperm antibody binding have been demonstrated between husband sperm and donor sperm in some infertile women. To investigate the contribution of antisperm antibody binding specificity may play in noncorrelations between a traditional indirect functional assay and an indirect ELISA method, sera from infertile patients with positive immobilization assays were investigated. The immobilization assay was chosen for comparison since it is believed to be more specific than agglutination assays in the detection of antisperm antibody. Sera from 15 infertile patients with positive immobilization assays (SIV > 2.0) were evaluated as follows: (1) ELISA assays with target sperm from 3 individual sperm donors, (2) SIF and ELISA assays performed in parallel using the same sperm donor specimens, and (3) ELISA assays using pooled sperm from four different donors. When the 15 sera were tested using the SIT against 5 individual random sperm donors, the correlation was improved to 80% (36/45). These data were comparable to our findings in a larger group of 292 infertile patients. In this group, 64 positive immobilization assays were found and when compared an ELISA using pooled target sperm, 80X (52/64) correlated positively. These data suggest that there are significant differences in antibody binding between individual sperm samples, probably due to antibody specificity, which may effect correlations between sperm antibody tests when different sperm donors are used. These differences may be minimized by utilizing appropriately selected sperm pools with significant antigen excess.

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21 CANETE | INTRA-FALLOPIAN TUBE TRANSFER (G.I.F.T.): INFLUENCE OF THE MALE FACTOR ON THE SUCCESS RATE.
Luis J. Rodriguez-Riga,1 George M. Grunert,2 Reu M. Woodard2, Eberhard C. Lotze, Joseph R. Feate, William Gibbons,3 Keith D. Smith,3 and Emil Steinberger.1
1Texas Institute for Reproductive Medicine and Endocrinology, 2Woman’s Hospital, 3Baylor College of Medicine, Houston, TX.

In vitro fertilization (I.V.F.) and G.I.F.T. have been proposed as therapeutic approaches in infertile couples where a significant male factor is present. To date, no data have been published relating the success rate of G.I.F.T. to the severity of the male factor. In this report we analyzed the results of our first 100 G.I.F.T. cases. The I.V.F. Center, Clay Center, NE 68933.

22 EFFECTS OF PREGNATAL IRRADIATION ON TESTICULAR LH RECEPTORS IN ADULT SYRIAN HAMSTERS. A.G. Amador, A. Bartke, V. Chandrashekar4 and H.G. Klencker4. Dept. Physiology, The S.U. School of Medicine, Carbondale, IL 62901-4531;

23 CHANGES IN SERUM TESTOSTERONE AND LUTEINIZING HORMONE LEVELS IN MALE RATS WITH ADJUVANT-INDUCED ARTHRITIS. Jeffrey W. Clemens* and Brent C. Bruet*. Department of Biological Sciences, Kent State University, Kent, OH 44242

It has been suggested that androgens may protect males from autoimmune diseases such as rheumatoid arthritis. This study was designed to examine the effects of adjuvant-induced arthritis (AA) on serum testosterone (T) and luteinizing hormone (LH) levels. Male Lewis rats were made arthritic by injection of 1 mg Mycobacterium butyricum in Freund’s adjuvant into the right hind footpad. Groups of arthritic and non-injected control rats (n=10/group) were sacrificed on days 18, 21, 24 and 27 post-injection. Body and thymus weights, left and right hind paw volume, and serum T and LH were determined. Adjuvant injection resulted in significant L/LH concentrations in those irradiated for 4 min than in those from any other group. Furthermore, LH receptor (LH-R) content was significantly higher in those irradiated for 3 or 4 min. However, LH-R content was significantly higher in those irradiated for 4 min. Significant correlations were observed between pregnancy and sperm count (S.C., million/ml), total sperm count (T.S.C., million/ ejaculate), % motility, motile sperm count (M.S.C., million/ml) and total motile sperm count (T.M.S.C., million/ ejaculate). Significant relationships were observed between pregnancy and S.C., T.S.C., M.S.C., and T.M.S.C. The best correlations were noted with M.S.C. and T.M.S.C. Pregnancy rates over 30% occurred in groups with M.S.C. over 40 million/ml or T.M.S.C. over 100 million/ ejaculate. With M.S.C. below 10 and T.M.S.C. below 25 the pregnancy rates were 12.5% and 9.5%, respectively. No correlation was found between % motility and pregnancy rate. In a subpopulation of 24 patients results of sperm penetration assay (S.P.A.) using zona-free hamster eggs were available. No correlation between S.P.A. and pregnancy rate could be demonstrated.

24 THE EFFECTS OF CASTRATION AND TESTOSTERONE ON PLASMINOGEN ACTIVATOR ACTIVITIES IN RAT VENTRAL PROSTATE.

25 H Wilson,1,2 C Ludovese,1 A Sinha,1,3 R Estensen,2 VA Medical Center 1 and Dept Lab Med and Path 2 and Genetics and Cell Biol 3 Univ Minn, Minneapolis, MN 55455.

Plasminogen activators (PA) are highly specific, arginine-type proteases which are thought to mediate controlled localized proteolysis in regulation of tissue remodeling. Hence, increased PA activities could be expected during regression of the prostate following castration. However, PA are also secreted by the prostate. Therefore, the effects of castration and testosterone (T) replacement on PA activities in ventral prostate of the rat were studied. Castration resulted in a rise in PA activity: 60-80% at 2 days, 200-300% at 4 days, 85% T 7 days, and no difference at 10 days as compared with intact controls (activities expressed per unit protein or tissu respectively). T treatment of 14 day castrates for 4 days increased PA activity 150% above oil treated controls. SDS-PAGE analysis showed 3 major forms of PA (65, 60, and 38 Kda) which did not appear to change with castration. A minor band of activator at 35 Kda was absent in 4 & 10 day castrates but present in T treated castrates. Three bands of low mol wt proteolytic activity (20,25, and 17 Kda) cleaved gelatin in control gels. These bands were more intense in lanes of 2 & 4 day castrates in gelatin gels containing plasminogen. Three bands of high mol wt PA activities which comigrate with these proteases or these proteases are capable of activating plasminogen to plasmin.
**25 EFFECT OF SEASONAL CHANGES IN LEYOIG CELL (LC) NUMBER ON THE VOLUME OF LC SMOOTH ENDOPLASMIC RETICULUM (SER) AND INTRATESTICULAR TESTOSTERONE CONTENT (IT)**

L.R. Johnson and O.L. Thompson, Jr., University of Texas Health Science Center at Dallas, Dallas, TX 75235 and Louisiana State University, Baton Rouge, LA 70803.

Tests from 47 adult (4-20y) stallions obtained in Nov.-Jan. (nonbreeding) and 41 adult stallions obtained in May-July (breeding) were perfused with glutaraldehyde, placed in osmium and embedded in Epon 812. Percentage LC cytoplasm or LC nuclei in the testis was determined by point counting 0.5µm sections under bright field microscopy. Testes from 6 randomly selected horses per season were processed for electron microscopy. The volume (ml) of SER/testis was calculated from the percentage SER in the cytoplasm measured by point counting of 12 to 20 electron micrographs per stallion), percentage LC cytoplasm, and parenchymal volume. Number of LC was calculated from the percentage LC nuclei, parenchymal volume, histologic correction factor, and volume of single nucleus. It was determined from the contralateral testis by radioimmunooassay. SER/g [81±8 vs 12±9µg], IT/g [75±12 vs 71±15µg], and number of LC/testis (33,000±3,383 pg/ml) were greater in the breeding season. These data emphasize the importance of seasonal changes in the number of Leydig cells on the amount of SER available to produce testosterone and on IT/testis.

**26 INFLUENCE OF 35°C AND MELATONIN (MEL) ON TESTOSTERONE (T), TESTIS AND ACCESSORY SEX ORGANS (ASO) AFTER HCG IN JUVENILE DEER MICE**: Pat Fail, Laboratory for Reproductive Endocrinology, Center for Life Sciences and Toxicology, Research Triangle Institute, P.O. Box 12194, RTP, NC 27709.

Melatonin (mel) capsules suppressed testicular and ASO development in Juvenile deer mice (Whitsett et al., J. Reprod. Fertil. 72:287-293, 1984). Objectives were to determine if: (1) mel or high ambient temperature suppresses T, and (2) the suppressed testis responds to HCG. Ninety 3-wk-old male deer mice, housed in 16 light:8h dark (LD), were randomly assigned to 1 of 3 treatments (T). Treatments were: 1) LD+sham-35°C; 2) LD+mel-35°C; 3) LD+mel+HCG. Temperatures were 27°C (21±2°C) or 35°C. Silentica capsules with 5 µg mel or without (sham) were implanted. At 7 wks, HCG was injected (25 µg/100g body weight) in 1/2 the males, 1/2 received saline. Blood serum collected before and 1 h after HCG was stored at -20°C until RIA for T. One wk after HCG, trunk blood and organ weights were collected. RESULTS: At 7 wks serum T was highest in LD controls (Tr 1: 452±505. Tr 2: 1654±235 and Tr 3: 1250±235 pg/ml; <P<.001). One h after HCG, T was higher than Tr 1: 452±505 (P<.001) in LD controls (33,000±3,383 pg/ml: n=15), and after 35°C (15,123±331 pg/ml) or mel Tr 1 (10,042±2755 pg/ml). T was lowest in males pretreated with mel (P<.001). Testicular weight and ASO were also suppressed by 35°C (P<.001) but only mel suppressed testis weight. Thus, the suppressive influence of mel and 35°C was reversed by HCG for the ASO (P<.01) and T (P<.03) but not for the testis.


We evaluated the CellSoft system (CRYO Resources, Ltd.) for measuring the percentage of progressively motile sperm (>20 um/sec; mot) and velocity (vel) of motile sperm. Measures of linearity, lateral head amplitude and beat-cross frequency were not validated. Video tapes of sperm in filtered (0.2 um) egg-yolk citrate or egg-yolk iso-extenders (8 x 10^6 sperm/ml) were analyzed at 30 frames/sec. With a 10X objective and a 6.7X projection ocular, optimum minimum and maximum settings for SD of a bull sperm head were 70 and 70 pixels. Virtually all motile sperm were detected and debris rarely were classified as immotile sperm if the extender had been filtered. With nonfiltered extender, debris similar in size to a sperm head were classified as immotile sperm. For mot, variation about the mean was greater when only 10 or 20 fields (about 8 sperm/field) were analyzed than 25 fields. With 20 fields, 16,16 and 18%, for freshly collected vs 20% after storage at 4°C for 3 days. With 25 fields, 15% was classified as immotile sperm by the CellSoft system. Variation of mot was similar when 12, 20 or 30 frames/field of view were analyzed (CV = 16, 16 and 18%). To evaluate precision, proportional mixtures of live and killed sperm were evaluated (4 bulls). Mot was correlated (r=0.97) with the ratio of live to killed sperm. The slopes were similar to the theoretical slope. Mean vel of motile sperm was similar for each mixture (P>0.05). To estimate accuracy, mot determined by computer and by "track motility" were compared for 20 samples (0 - 63% motile sperm); the values were correlated (r=0.95). The CellSoft system is precise and reasonably accurate for evaluating swimming parameters of bull sperm if the extender has been filtered and 30 random fields (about 240 sperm) are evaluated using 30 frames/field. Supported by the National Association of Animal Breeders.
29 CORRELATIONS BETWEEN FERTILITY AND COMPUTER-DETERMINED SWIMMING PARAMETERS OF BULL SPERM. P.R. Budworth* and R.P. Aman. Colorado State University, Fort Collins, CO 80523

We determined if any parameter evaluated by the CellSoft system (CRYO Resources, Ltd.) might be useful for predicting the fertility of a semen sample. Trial 1: Semen from 10 bulls was extended in egg-yolk citrate containing 1 of 2 antibiotics (7 to 10 x 10^9 sperm/AI dose) and cryopreserved. Mean (1000 cows/sample) 75-day nonreturn rates ranged from 58.8 to 76.4% for the 20 samples. The percentages of progressively motile (mot) sperm (linearity (lin), linear velocity (vcl), straight-line velocity (vsl), lateral head amplitude (HHA) and lateral head swing frequency (LSF)) of motile sperm at 0 or 1.5 h post-thaw were not significantly correlated with nonreturn rate (r=0.01 to 0.34). Multiple correlations with fertility based on all data for 0 or 1.5 h were 0.39 and 0.64. Trial 2: In a competitive fertilization trial, frozen-thawed sperm from pairs of bulls were mixed in equal numbers to provide 4 or 5 samples per bull of mixed sperm for insemination involving each of 9 bulls. The proportion of calves sired by each bull (260 calves total) was used to calculate a competitive fertility index (CI) which ranged from 46 to 74. Evaluations of mot, vcl, and vsl at 0 h post-thaw were correlated (P<0.05) with the CI (r=0.86, 0.68, 0.79) as were evaluations of mot, vcl, vsl, and lin at 1.5 h (r=0.78, 0.77, 0.73, 0.66). Multiple correlations with CI based on these data for 0 or 1.5 h were (R=0.94 and 0.99). On the latter date, the CellSoft system might enable prediction of fertility (R^2=0.80). We have used one system (American Breeders Service and Dr. R.G. Sacke kindly provided samples and fertility data.) Supported by the National Association of Animal Breeders.

30 AUTOMATED SEMEN ANALYSIS IN LARGE EPIDEMIOLOGIC STUDIES Frank Destefano*, Joseph L. Annest*, Marcie-Jo Kresnow*, Melinda L. Flock* and Steven M. Schrader, Centers for Disease Control, Atlanta, Georgia 30333

Despite concerns that various environmental and occupational exposures may adversely affect male fertility, it has been impractical to perform semen analysis in large population studies because the methods have been labor-intensive and time-consuming. In addition, conventional methods of semen analysis have relied on subjective visual inspection and have been subject to considerable observer variability. Recently, we have developed an automated semen analysis system, the CASA system, in a large cross-sectional study of the health status of Vietnam-era veterans. The CASA system uses digitized image analysis to measure sperm concentration and motility (percent motile cells, velocity, trajectory, lateral head displacement amplitude and frequency), and includes a morphology module for categorizing sperm head shape and measuring individual sperm head dimensions (area, perimeter, length/width ratio, roundness, and major axis length). We present the results of semen measurements on over 500 study participants and evaluate the influence of various demographic and lifestyle factors, such as age, cigarette smoking, alcohol and drug use, on the different measures. Since the participants in the 2088 study were generally healthy men, most of whom had no reported fertility problems, the study data should be useful for establishing guidelines on the expected values for most of the above measures in the general population.

31 ASSESSING HUMAN SPERM VELOCITY IN SEMEN AND DURING THE CAPACITATION PROCESS USING AUTOMATED SEMEN ANALYSIS G. Koukoulis*, D. Vantman, N. Zinaman and R. Sherins Developmental Endocrinology Branch, NICHD, Bethesda, MD.

Sperm motility has previously been correlated with fertility potential. The recent availability of fully automated computer-assisted analysis of sperm motility characteristics (CellSoft) provides an opportunity to assess sperm velocity objectively under experimental conditions. We decided to examine the accuracy of CellSoft when estimating human sperm motility both in semen and in media during the capacitation process. Sperm from 16 men with proven fertility were provided after 36-48 hrs abstinence. Estimates of linear velocity (LV) (µm/sec) were obtained utilizing varying numbers of tracking points during a 0.7 sec interval of observation (Table I). These data show that under the experimental conditions utilized, LV in human sperm did not vary in media during the capacitation process from that measured in seminal plasma (NS). However, our data also suggest that assessing linear velocity by CellSoft may require even more detailed analysis.

32 DISSECTION OF HYPOTHALAMIC TAIL SWELLING PATTERNS IN SPERM FROM FERTILE AND INFERTILE MEN. D. Vantman, N. Zinaman and F. Sherins. Developmental Endocrinology Branch, NICHD. Bethesda, MD.

Recent data suggest that tail swelling on exposure to hypo-osmotic medium (HOS) serves as a marker of sperm function. We questioned whether swelling pattern during capacitation would distinguish between fertile and infertile men. Accordingly, we analyzed the percent of total tail swelling as well as distribution of swelling patterns of seminal sperm and sperm during incubation in capcitated media. Sperm from 15 fertile and 12 infertile men were collected by masturbatory after 36-48 hrs abstinence and aliquots were taken for routine analysis and HOS. The remainder was centrifuged, washed with capcitated medium (Ham-F10 with 3.5% human serum albumin) and the sperm allowed to swim up into medium incubating at 37°C in 5% CO2/95% air. Aliquots were taken at 1, 2, and 3 hrs for HOS which involved incubation of the final mixture at 37°C for 30 min, fixation of the cells with formalin and quantification of swelling patterns. In contrast to recent literature total swelling in semen and redim in both groups was similar (NS), but HOS swelling (type g) and 2 restricted (type b) patterns were significantly different (mean±SD)

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<th>semen 2 hr</th>
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<tbody>
<tr>
<td>Fertile</td>
<td>11.3±2.1</td>
<td>21.1±3.0</td>
<td>35.2±3.3</td>
</tr>
<tr>
<td>Infertile</td>
<td>25.1±4.6</td>
<td>32.0±3.1</td>
<td>37.9±3.0</td>
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<tr>
<td>(type g)</td>
<td>p&lt;0.05</td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
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<tr>
<td>Fertile</td>
<td>49.3±3.7</td>
<td>37.9±3.3</td>
<td>35.2±3.3</td>
</tr>
<tr>
<td>Infertile</td>
<td>62.8±2.6</td>
<td>20.7±3.5</td>
<td>16.3±2.3</td>
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<tr>
<td>(type b)</td>
<td>p&lt;0.01</td>
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We conclude that our modification of HOS may be useful in distinguishing fertile from infertile men.
The hypoosmotic swelling (HOS) test, which measures the functional integrity of sperm membranes, has been shown to be more reliable in predicting the outcome of IVF than the standard semen parameters (J. Androl. 7:190, 1986). Based on these data, 60% or more reactive sperm in the HOS test was tentatively considered "fertile". Although false negative fertility results were minimized by this 60% cut-off level, false positive results were still present. To minimize this, a differential evaluation of HOS test was performed on 31 ejaculates before and after a "swim-up" procedure. Though there was a significant difference (p<0.01) on the HOS test between 24 fertile (mean±SEM; 61±1.8) and 7 infertile (72.1±3.6) ejaculates, both groups had more than 60% HOS-reactive sperm. There was no significant difference on the differential evaluation of HOS between fertile or infertile groups. However, on differential evaluation, percent sperm with tail-tall swelling was significantly decreased (p<0.05) after swim-up (10±2.4; before: 17.6±2.1) in the infertile group. In the fertile group, an increase after swim-up (35.5±3.0; before: 30.7±3.0) was not significant, but an inverse response was observed for whole-tail swelling (fertile: before/after 45.6±3.7/41.6±2.0; infertile: before/after 40/63.3/50.9±3.8). These results indicate that differential evaluation of HOS test before and after swim-up procedure of sperm may minimize false positive fertility results.

A comparison of morphology and volume of human spermatozoa was performed on 29 human ejaculates which were previously analyzed for fertility (IVF), semen volume, sperm concentration, morphology and motility. These parameters were analyzed to determine whether or not they could be used solely to predict fertility.

Slides were used for routine morphology classification were analyzed for volume which includes sperm head area, perimeter, length and width. These morphometric measurements were obtained by tracing the outline of 200 sperm heads from each sample using the Leitz Videoplan computer interfaced with a digitizing board, a Zeiss microscope and video monitor. The enlarged image of each sperm head was projected onto the monitor and traced with a digitizing pen, using hand and eye coordination. Spermatozoan volumes were obtained using an Elmsen Model 112 electronic particle analyzer, and a General Electric 1750 high speed terminal interfaced with a Digital Data System 102 computer and monitor. Samples previously frozen from the IVF procedure were thawed and diluted for volumetric analysis. Volumes on ten thousand cells from each ejaculate were analyzed for population mode, median and mean. Pearson's linear regression correlation coefficients, were used to determine the degree of correlation between morphometry and sperm volume. The best relationships existed between the geometric mean sperm volume and morphometric area (r(2)=0.304, p<0.01), perimeter (r(2)=0.316, p<0.01), and length (r(2)=0.314, p<0.01).

Even though the population size was small (n=29), these parameters were not closely related and probably evaluated different sperm cell characteristics.
A prospective study was planned to evaluate the role of sperm morphology as a predicting parameter in an IVF Program. The male patients had a sperm concentration of > 20 million/ml and a motility of >30% and based on new strict criteria for evaluating normal sperm morphology patients were divided prospectively into 2 groups. In group I (20 pts.) normal sperm morphology was >14% and in group II (71 pts.) normal sperm morphology was >14%, using a threshold that proved to be of value in a previous publication. Multiple regression was used to evaluate different parameters; concentration, motility and morphology against the dependent variables fertilization and cleavage. The only factor which was significantly correlated with fertilization and cleavage was normal sperm morphology (P <0.0001). The fertilization rate (per oocyte) and the cleavage rate were 48.4% and 47.6% respectively in group I and 88.3% and 87% in group II (P <0.0001). The ongoing pregnancy rate/laparoscopy and embryo transfer was 4% and 5.5% respectively in group I and 18.3% and 18.5% in group II. This study demonstrates the value of evaluating normal sperm morphology using the strict criteria recommended. Patients can be better counseled and the probability of fertilization or no fertilization can be more accurately predicted.

A new strict method for evaluation of sperm morphology is currently used in this laboratory. This parameter proved highly predictive for fertilization rate and cleavage rate (p<0.0001) in another IVF program.1 A prospective study evaluated the following seven parameters in samples of all patients referred to the Andrology Laboratory over a set period of time: concentration, motility and normal sperm morphology. These factors were correlated with the SPA. The concentration and motile sperm fraction were evaluated with the aid of computer analysis (Cellsoft Sera Analysis System, Lab-sof Division of Cryo Resources Ltd., New York). Evaluation of 63 patients yielded a concentration ranging from 2 to 219 million/ml, motility ranging from 7% to 87%, and normal sperm morphology ranging from 12 to 39%. The hamster test was performed according to a protocol described in previous publications. The SAS general linear model was used to evaluate the different variables. There was a significant regression relationship between the percent normal sperm morphology and penetration rate in the SPA (p<0.0025).


Sperm from two boars that had shown wide differences in semen quality characteristics after freezing and thawing were tested for fertility and for their ability to penetrate zona-free hamster (ZFH) ova in vitro. Thirteen ejaculates from Boar A (high post-thaw quality) and Boar B (low post-thaw quality) were frozen by the straw method in a lactose-yolk extender. Eggs were recovered from 52 sows and gilts 36-96 hr after insemination and evaluated for fertilization and sperm binding to the zona pellucida. For the sperm penetration assay (SPA), boar spermatozoa were acrosome-reacted in vitro, placed with ZFH ova at 35°C, and evaluated for sperm penetration, sperm binding, and degree of polyspermy after 3 hr incubation. Boar A exhibited a significantly (P<0.05) higher level of sperm penetration and sperm binding (77% and 91 sperm/egg, respectively) than did Boar B (42% and 10 sperm/egg, respectively). With the SPA, sperm from Boar A penetrated a higher (P<0.05) number of ZFH ova than did Boar B (43% and 24%, respectively). However, sperm binding and degree of polyspermy did not differ between boars. Based on these limited data, there may be a relationship between sperm penetration and sperm fertilizing capacity, which would make the SPA useful in predicting potential boar differences in fertilizing capacity of deep frozen sperm. 

42 PENETRATION OF HAMSTER OVA BY DON-HUMAN PRIMATE SPERMATOZOA. Barbara S. Durrant, Ph.D., Zoological Society of San Diego, P.O. Box 551, San Diego, CA 92112

Many zoos worldwide are currently collecting and freezing the semen of exotic animals. Freezing methods employed are similar to those proven successful for the semen of domestic animals. These semen freezing techniques remain virtually untested in exotic animals due to the lack of large numbers of females available for artificial insemination studies. It is, therefore, imperative that laboratory analysis of semen be accurate in predicting the potential fertility of valuable samples. The sperm penetration assay (SPA) is being used as part of a comprehensive semen evaluation program. Semen of six non-human primate species, collected by electro-ejaculation or post-mortem epididymal extraction was frozen for periods of one month to five years. Frozen human sperm was used as an assay control. After a slow thaw to 37°C all semen, regardless of freezing extender, was diluted 1:1 with TES-T and incubated at 37°C for 1 hr. Sperm was heat-shocked by the rapid addition of 12-fold volume of 37°C BWW. Capacitation continued at 37°C for 1 hr. Hamster ova were co-incubated with 2 x 10⁵ spermatozoa for 2-3 hr. Penetration was achieved by the sperm of the pygmy chimpanzee, patas monkey and lion-tailed macaques. No penetration was achieved by the sperm of the gorilla, uakari or lemur. Poor post-thaw motility in all species was correlated with failure to penetrate hamster ova. The SPA may provide important information in addition to traditional semen analyses in determining potential fertility of sperm of exotic species as well as facilitating the development of optimum cryopreservation techniques.


Study of sperm interaction with salt-stored zonae involved 1. Binding of epididymal sperm 2. The rate of capacitated sperm penetration into the perivitelline space (PVS) 3. The affect of uridine diphosphate (UDP) sugars on sperm-zona binding and 4. The requirement of calcium for sperm-zona binding. Mean numbers of sperm attached to ZFH (n>40) for sperm from proximal and distal caput, proximal and distal corpus, cauda and vas deferens were 0.00, 0.13, 0.14, 18.42, 50.41, and 59.27, respectively. In vivo capacitated sperm were attached by 10 min and increasing proportions of ZFH were penetrated up to 100% by 70 min. Increasing numbers of sperm reached the PVS of inseminated ZFH by 270 min. UDPGalactose did not affect the attachment of sperm. UDPGalactose affected a highly significant increase in attachment (P<0.0001). UDPGlucose showed a highly significantly decreased attachment (P<0.001). UDPGalactosamine decreased attachment (P<0.05) when compared to both controls and UDPN-Acetylgalactosamine. In vivo capacitated sperm-ZFH binding was partially inhibited in a calcium-free medium and totally inhibited when EGTA (1 mg/ml) was added. Results indicate significant influences of UDP sugars and of calcium on sperm-zona interaction and emphasize potential use of ZFH. (Supported by NICHD Grant HD 19288, USDA Grant 85-RCR-1-1-1707 and UGA VMES Project 86-225.)

44 VALUE OF ATP ASSAY IN PREDICTING THE OUTCOME OF IN VITRO FERTILIZATION (IVF) TRIALS AND SPERM HAMSTER OVA PENETRATION ASSAY (SPA). M. Morshed, F. M. Hamilton, A. Plabam, A. Acosta, T. Kruger, and R. J. Swanson, Andrology Laboratory, Department of Obstetrics and Gynecology, Eastern Virginia Medical School and Department of Biological Sciences, Old Dominion University, Norfolk, VA 23508.

Seventy samples from 20 IVF patients were used for ATP measurements. ATP levels (both in M/ml semen and in picoM/10⁶ sperm) were compared to human egg penetration, cleavage and embryo transfer rates as well as to basic semen parameters. Using t-test (t=1.83), a p<0.06 was observed for ATP values compared to penetration rate alone. A good correlation between sperm mean velocity and percent penetration (r=0.6, p<0.01) was also observed. In a separate study of 30 patients, ATP levels of spermatozoa subjected to a swim-up separation method (SSM) at the time of insemination, were compared to SPA results. An overall positive predictive value of 90% was calculated. Almost all patients with low ATP had abnormal SPA results. Based on a study of 22 infertile patients, ATP levels dropped an average of 20-30% between measurements in a fresh versus SSM treated, but otherwise identical, specimen. Studies are underway to evaluate a two-step ATP measurement (fresh and SSM treated spermatozoa at time of insemination) in patients with low or no penetration and cleavage in IVF and SPA procedures to determine if their poor performance is due to low ATP levels at time of insemination.
USE OF PENETRAK TO STUDY SPERM PENETRATION PATTERNS.
Richard N. Harrison and Ronald W. Lewis. Delta Regional Primate Research Center, Covington, LA 70433.

The standard Penetrafic test determines the distance traveled (in m) in the cervical mucus in 90 minutes by the vanguard sperm (VAN). More than 50 semen samples were evaluated using the Penetrafic test with the following determinations: the average distances traveled by the lead 20 sperm (N-20), lead 50 sperm (N-50), and lead 100 sperm (N-100); the distance traveled by the 100th sperm (TAIL); and the percent of that distance to the vanguard distance (XVAN). Samples were evaluated for sperm concentration (CONC), X normal forms (NORM), and X motile (MOT). A motility index (MI) was calculated. From these data regression equation analyses showed the fit of VAN to N-20, N-50, N-100, and XVAN were calculated. The data were further analyzed by ranking the semen samples according to VAN values and determining mean XVAN values for various categories of VAN. All the regression equations had coefficients of determination greater than 90%. The mean XVAN value for all specimens was 66.6%. Significant differences were found when the mean XVAN values were compared for samples with VAN values of 30 or less (4.25 ± 3.01) with those having VAN values of 31-40 (72.97 ± 2.89) or VAN values greater than 40 (74.25 ± 2.29). There were no significant differences in mean MI values between these groups but the mean MI values were significantly different. These studies indicate that the distance traveled by a single vanguard sperm is usually indicative of the distance traveled by the following sperm, that can correlate well with the vanaluck value, that grade of motility is important in penetrating cervical mucus, and that the standard Penetrafic kit can be a research tool used for studying characteristics of sperm.

This research supported by RR00164 from N.I.H. to D.R.P.R.C.

47 PREDICTIVE VALUE OF QUANTITATIVE AND QUALITATIVE BACTERIOLOGIC STUDIES IN ZONA-FREE HAMSTER EGG PENETRATION TEST FAILURE. Laszlo Sogor, Hunter Hamail*, Dolina Glavny* and Arlette Coulter*. Department of Reproductive Biology, Case Western Reserve University, Cleveland, OH, 44106.

Twenty-four patients had sperm which was donated for evaluation in zona-free hamster egg penetration assay and for microbiologic colonization in a quantitative and qualitative fashion. Fresh specimens plated on blood-agar plates and brain heart infusion broth (BHI) were grown under aerobic and anaerobic conditions. The specimens had serial dilutions done to 10^6 colony forming units (CFU) per ml, and they were incubated under aerobic and anaerobic conditions. All patients had prior negative cultures for H. hominis and Chlamydia trachomatis, the two major causes of infertility.

Evaluation of the microbiology revealed 10^6 CFUs present in controls that had successful hamster tests. However, of the eleven failures, three of them had greater than 10^6 CFU of Staphylococcus epidermidis and only one patient with counts (greater than 10^6 CFU) of Strep viridans, Enterococcus and Staph had successful penetration with the hamster test. It is possible that quantitative evaluation of bacteria could explain one component in certain cases of the failed hamster test. In this limited series this would give an 80% predictive value when the quantitation of the bacteria is greater than 10^6 colony-forming units and there is a combination for mixed-culture involving Staph epidermidis, and Enterococcus. Further studies will be needed to further clarify this and potential therapy.

45 EFFECTS OF CERVICAL MUCUS SHEARING ON THE BIOMECHANICS OF SPERM PENETRATION. D. Katz, R. Happ*, A. Yudin*, J. Overstreet, F. Hanson*, J. Chang*, University of California Medical School, Davis, CA 95616.

Cervical mucus in vitro is subject to shear forces due to contractions of the female viscera. Given the heterogeneity of the mucus secretion, the result is a mucous column consisting of a mosaic of microdomains whose material properties resist sperm to different extents. This heterogeneity may effectively select which sperm succeed in penetrating the mucus. We are studying the mechanics of this phenomenon, characterizing mucus properties on the scale of individual sperm. Our "sperm probe" technique analyzes the relation between flagellar beat kinematics and swimming velocity to infer mucus resistive properties. We have found that mosaic shearing does reduce microscale resistance to sperm, and that this may be manifest in changes in both viscous and elastic mucus properties. The effect of the latter upon sperm motion, which has not previously been addressed, may be particularly important. We have also employed transmission electron microscopy, using a new freeze-substitution technique, to visualize how shearing affects the mucus microstructure and its interaction with sperm. The micrographs suggest a significant elastic response to sperm, and a "pore" structure which is altered by shearing. (Supported by NIH grant HD12971.)

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48 COMPARISON OF SPERMATID AND EPIDIDYMAL SPERM ANALYSES WITH TESTIS AND EPIDIDYMAL HISTOPATHOLOGY IN COMPOUND EVALUATION. S. L. Wierzycki, L. D. Russell* and J. M. Killiong, Stauffer Chemical Co., Environmental Health Center, Farmington, CT, *University of Southern Illinois, Carbondale, IL

Caput and cauda epididymal sperm and testicular spermatic analyses and reproductive hormone concentrations were compared to epididymal and testicular histopathology during compound evaluation. Male Sprague-Dawley rats were fed 0 and 1000 ppm of R-40244, a male rat specific antifertility agent, for 10 weeks. Animals were sacrificed after 24, 48, and 96 hours and 2,4,6,8 and 10 weeks. Sperm analysis was performed on a caput and cauda epididymis and spermatic analysis was performed on a homogenized testis from one side of the animal. The remaining testis and epididymis were perfused and prepared for histopathology. General toxicity was indicated by decreased body weights and food consumption. Histopathologic changes in the testis due to Sertoli cell damage were indicated by germ cell degeneration, failure of spermatid release and abnormal sperm morphology. Testicular spermatid analyses showed similar increases in abnormal morphology and decreased cell concentration. Changes in the testis resulted in germ cell sloughing into the epididymis and depletion of normal epididymal sperm. Corresponding results were seen in the caput and cauda epididymis with increases in abnormal sperm and decreases in cell concentration and motility. Increases in FSH and LH occurred with changes in tests and epididymal weights, histopathology and sperm parameter analyses. The rise in FSH and LH was secondary to the testicular changes.
49 NEW OBSERVATIONS ON THE MORPHOLOGICAL FEATURES OF THE GUINEA PIG SPERM ACROSOME REACTION. S.P. Flaherty*, V.P. Winfrey* and G.E. Olson, Department of Cell Biology, Vanderbilt University, Nashville, TN 37232.

The membrane fusion events of the guinea pig sperm acrosome reaction (AR) have been examined using routine thin sectioning and negative staining. Sperm were induced to acrosome react either with ionophore A23187 and Ca²⁺ or by preincubation in Ca²⁺ free medium with lyssolecithin followed by the addition of Ca²⁺. Fusion of the outer acrosomal membrane (OAM) and plasma membrane (PM) results in hybrid vesicles, and also membrane tubules which are often oriented in parallel arrays. In rouleaux, the PM over parts of the apical segment are adherent between adjoining sperm; in these zones, the PMs of adjacent sperm are linked by periodic cross-bridges, the PM and OAM are linked by periodic bridging elements, and the luminal face of the OAM exhibits a filamentous substructure. Fusion between the OAM and PM does not occur in these regions, and they remain as sheets after completion of the AR. Hence, in guinea pig sperm there appears to be a degree of directionality in the membrane fusion events of the AR, and stable non-fusigenic domains may be present in both the OAM and PM. Ongoing studies of hybrid membranes isolated on Percoll gradients aim to characterize further their structural and chemical properties. Supported by HD-20419 and HD-05797.

50 ANALYSIS OF SPERM FERTILIZING ABILITY IN THE STALLION. E. Bustos-Obrevec, Héctor Rodriguez* and Sylvia Leiva*, University of Chile Medical School, Santiago, Chile.

Prediction of the sperm fertilizing ability based on classical seminal parameters is limited. Therefore, sperm velocity, objective motility and ATP concentration were analyzed for native and frozen stallion semen during and after the breeding period to broaden our understanding of sperm abilities. Sperm velocity ranges from 10.4 to 12.2 u/sec; ATP concentration from 0.87 to 3.4 x 10⁻¹⁵M and the objective motility from 33 to 38%.

These three parameters were significantly and positively correlated among them and to the subjective motility and sperm vitality. Negative correlation was found with sperm abnormalities. No significant changes could be found between native and frozen samples, though ATP decreased in 50% after freezing.

The new parameters analyzed may constitute valuable tools for fine assessment of stallion semen. (Supported by D18 - U. of Chile 81464-8655).

51 ULTRASTRUCTURAL-PHYSIOLOGIC CORRELATES OF HUMAN SPERM EGG PENETRATING ABILITY. RA Bronson, GW Cooper,*Division of Human Reproduction, North Shore University Hospital, and D Phillips, Population Council, Center for Biomedical Research.

Following incubation in capacitating media, only a small percentage of spermatozae have been found acrosome reacted, as judged by stained preparations of fixed sperm at the light microscope level. Selection of a highly motile population of ultrastructurally uniform sperm has allowed us to categorize the appearance of the acrosome in large numbers of spermatozae by transmission electron microscopy. Following incubation in a modified IVM medium containing human serum albumin at concentrations ranging from 1 to 30 mg/ml, the proportion of acrosome reacted sperm was determined and compared with the number of spermatozae penetrating zona-free hamster eggs. An increased proportion of acrosome reacted sperm was seen at higher concentrations of HSA and correlated with greater egg penetration frequencies. As sperm motility was similar between treatment groups, the varying ability of populations of sperm to penetrate eggs appeared to be on the basis of the observed differences in acrosomal status. Noteworthy was the finding that even at the highest concentration of albumin utilized, only a minority of spermatozae were acrosome reacted. These findings suggest that capacitating conditions in vivo are inefficient, when compared with those within the female reproductive tract. Alternatively, only a small cohort of spermatozae may be capable of acrosome reacting within a larger population of motile, morphologically normal sperm. These results emphasize the need for the establishment of standard conditions under which the fertilizing potential of human spermatozae is judged.

52 REGIONAL DIFFERENCES IN ANDROGEN BINDING BY RAM EPIDIDYMAL TISSUE. P.R. Tekpetey and R.P. Amann, Colorado State University, Ft. Collins, CO 80523.

We determined differences associated with season and epididymal region on binding of dihydrotestosterone (DHT) to tissue from the caput, corpus and cauda (study 1) or 7 regions of the epididymis (study 2). Tissue obtained in Feb-Mar (non-breeding season; NBS) or Aug (breeding season; BS) was used to prepare total low-salt (14 mM) and nuclear high-salt (414 mM) extracts; low-salt extract was stripped of endogenous free steroid with dextran-coated charcoal (DCC). Scatchard analyses used 2-40 mM DHT±100X excess nonradioactive DHT in a DCC assay. Addition of androgen binding protein to extract before DCC stripping did not increase binding of DHT. Relative to DHT (100%), binding of testosterone was 12% and androstenedione, estradiol, or progesterone was <4%. Concentrations of binding sites in low-salt and high-salt extracts were similar. Binding (fmol/mg wet tissue) of DHT was higher (P<0.05) in BS than NBS, but affinity of DHT binding was about 4.7 x 10⁻¹¹ M⁻¹ in all samples. In BS, there were regional differences (P<0.05) in concentration of DHT-binding sites (low- & high-salt extracts) which averaged (N = 6 rams) 11.1, 8.7, and 6.3 (±0.8) for the caput, corpus and cauda in study 1. In study 2, maximum DHT-binding was by tissue from the distal caput epididymis; tissue from the proximal, central, or distal caput; proximal or distal corpus; and proximal or distal cauda averaged (N = 3 rams) 9.4, 10.8, 13.3, 10.3, 9.1, 7.2 and 4.9 (±0.8). For NBS, regional differences were not significant in either study and respective means were 8.1, 5.6 and 3.6 (±0.8) for study 1 and 5.8, 9.6, 7.9, 7.7, 7.5, 7.0 and 7.1 (±1.2) for study 2. These data might be interpreted as evidence that the central caput through proximal corpus regions are most dependent on androgenic stimulation (Supported by EPA CR-8127525-01).
53 THE EFFECT OF VARYING FSH PULSES ON TRANSFERRIN SECRETION IN SUPERSUED SERTOLI CELL CULTURES. Andrzej Jakubowla*, Andrzej Jankowiak*, and Anna Sternberga. Department of OB/GYN and Reproductive Sciences, University of Texas Medical School at Houston, 6431 Fannin, Houston, TX 77030

We have recently demonstrated that pulsatile FSH stimulation of rat Sertoli cells (Sc) in superfused cultures leads to a pulsatile secretion pattern of transferrin (Trf) which differs dramatically from that resulting from continuous superfusion with the same hormone concentration (Jakubowka et al., 1986). In the current study we investigated the secretion of Trf over a 24-hour period in response to FSH pulses which differed in frequency, duration and amplitude. Confluent monolayers of Sc from 18-day-old rats cultured on RPMI matrix were superfused with serum-free, defined culture media and were exposed for 20 min. on longer, intervals to various concentrations of FSH (NIAMDD 0.5-500). Sequential samples of effluent media were collected and radioimmunoassayed for Trf. The total amount and the secretory pattern of Trf varied depending on the frequency and duration of the FSH pulses as well as on the hormone concentration. The response to FSH pulses appeared to be dose-related and biphasic with a rapid increase in Trf secretion followed by a drop, then a smaller more prolonged stimulation. Trf response to continuous FSH superfusion showed a different dose-related and biphasic pattern. These results clearly demonstrate that Sc secretion is influenced not only by the presence of FSH but also by the mode of its delivery.

Supported in part by NIH grant HD 17802.

54 NEUTRAL AMINO ACID ABSORPTION BY RAT EPIDIDYMYS. B. J. Hinton & H. Hernandez. Department of Anatomy & Cell Biology, University of Virginia School of Medicine, Charlottesville, Virginia 22908.

For several years there has been considerable emphasis upon the role of secretion by the epididymys, there have been relatively few studies directed towards understanding its absorptive role. Utilizing the technique of stopped-flow split-drop microperfusion, the absorption of the neutral amino acid, -aminobutyric acid (AIB) from the lumen of different epididymal regions was studied. Absorption from the caput, corpus and cauda regions was found to be via a time dependent and saturable system for each region with similar Apkm values (pmol/ml/hr) but different Vmax values: 20, 26 and 68 pmol AIB absorbed/min/mm² tubular volume for caput, corpus and cauda respectively. The absorption of AIB from the caput lumen was Na⁺-dependent and inhibitable by 2-methyl AIB (2MeAIB), absorption from the corpus lumen was also Na⁺-dependent but not inhibitable by 2MeAIB. Absorption of AIB from the cauda lumen was Na⁺-independent and not inhibitable by 2MeAIB. Results suggest that neutral amino acid absorption along different regions of the epididymis may occur via different transport pathways: that it is also linked to outward Na⁺ movement in proximal epididymal regions. These findings further support our previous observations that there exists a selective permeability barrier from lumen to blood along the epididymal duct.

Supported by N.I.H. grant HD18257.


Current reports remain controversial in regard to the deleterious effect of a unilateral torsion of one testicle to its contralateral counterpart. Histologic as well as immunologic alterations have been demonstrated in several of the studies. In this report the authors investigated the immunologic effect of unilateral torsion both directly on the sperm and indirectly in the plasma. Unlike most of the previous studies which based their immunologic response upon visual examination of fluorescein labelled antibody, we employed quantitative RIA measurement of anti-sperm antibody. Additionally we obtained the ejaculate by electroejaculation while in virtually all past experiments the animals were sacrificed to obtain sperm. Of the 35 adult male Sprague-Dawley rats, 5 had a sham operation for control. The remaining rats were subjected to 360° torsion and divided into 3 groups. Group I: After a 2 hr. torsion, half had the torsed testis detorsed, the other half orchiectomized. Groups II & III had similar events done on the torsed testis save for the duration of torsion of 4 and 6 hrs. respectively. Four and 8 hrs. later, the immunoreactivity was measured by an RIA technique developed by one of the authors (GGH) utilizing goat anti-rat IgG. Electroejaculation was obtained through the use of a rectal probe operating on a 60-cycle alternating current delivering 0-15 volts. Increase immunoreactivity was apparent in the detorsed group compared to the orchiectomy rats. This vasoedematous effect both directly in the peripheral circulation. (P<0.05). This study showed that a testicle testicle left in the scrotum stimulates an immunologic response apparent in 4 hrs. even after only a 2 hr. torsion. Orchiectomy of the testis aneliorates the response.


Gossypol preferentially causes adverse effects to mouse transformed Sertoli TM4 cells. To see if the selective effects to TM4 cells is due to their preferential drug incorporation, [14C]gossypol was, therefore, used for this study. TM4 cells exposed to 25 ug/ml of [14C]gossypol (1.67 aCi/mole) for one hr incorporated the radioactivity at 60,000 cpm/mg protein. The level was twice and ten times as high as that incorporated by mouse kidney epithelial cells (PK2) and mouse BALB/c 3T3 fibroblasts, respectively. The incorporation kinetics of [14C]gossypol at 2.5-25 ug/ml into TM4 cells was biphasic. The level incorporated [14C]gossypol reached the first plateau within 60 min, then rose again and attained the second plateau within 60 min. The level at the second plateau was twice that of the first one. When [14C]gossypol-labelled TM4 cell lysates were dialyzed extensively against H2O, half of the radioactivity still remained in the tubing. The results suggest that gossypol forms conjugates with TM4 cells' macromolecules. To further characterize the nature of gossypol-protein conjugates TM4 cells labelled with [14C]gossypol at 10 ug/ml for 1 hr were solubilized in SDS-sample buffer and electrophoresed on an SDS-polyacrylamide gel. [14C]gossypol-protein conjugates detected by fluorography appeared as prominent bands of Mr's 63, 59, 52.5, 50, 41.5, 38 and 34.5 kD. These proteins may serve as gossypol receptors accumulating the drug from the medium into the cells.

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57 SPECTRAL ANALYSIS OF SHORT-TERM FLUCTUATIONS IN SALIVARY TESTOSTERONE CONCENTRATIONS. Norma F. Besch, James M. Dabbs, Jr.* and Charles H. Hopper, Reproductive Research Laboratory, St. Luke's Episcopal Hospital and Department Ob/Gyn, Baylor College of Medicine, Houston, TX 77030 and Department Psychology, Georgia State University, Atlanta, GA 30303.

Serum free testosterone is believed to represent the biologically active fraction of the total testosterone concentration and is thus probably the more useful determination for clinical purposes. In the male, salivary testosterone concentrations are highly correlated and concordant with serum free testosterone concentrations. Given the non-invasive and stress-free nature of saliva collection, measurement in saliva is particularly suitable to situations where multiple samples are required. In order to determine the degree to which testosterone concentrations in saliva show short-term episodic or cyclic variation, a study was conducted in which 13 men (median age = 19) collected saliva at 2-minute intervals for one hour. Testosterone was measured in 0.250-ml sample volumes using an RIA with an 1-12.5 tracer and a PEG assisted second antibody separation. Each set of 30 determinations was analyzed with the BMDPIT Spectral Analysis Computer program following removal of any linear or first quadratic components by a regression analysis. Cyclicality was demonstrated which was unique for each subject; however, the majority showed cycles in the range of 9-13 per hour, or pulses every 4-7 minutes. The pulses, however, were of low amplitude suggesting that multiple sampling would not significantly increase the diagnostic reliability of the testosterone determination over that obtained for a single sample.

58 DOPPLER EVALUATION OF VARICOCELE. C. S. Dhabuwala and Anil Kumar*, Wayne State University, Hutzel Hospital, Detroit, MI 48201.

Varicocele is a common surgically correctable cause of male infertility. Various modalities such as ultrasonography, thermography, and venography have been used for the evaluation of varicocele. Doppler evaluation of the pampiniform plexus offers a simple noninvasive method of assessing reflux in the venous system of the spermatic cord. 174 patients (348 spermatic cords) were evaluated using a dual frequency directional doppler with a paper recorder. The diagnosis of varicocele was based on shift of baseline during deep breathing (DB), valsalva maneuver (VM), or manual abdominal compression (MC). An audible bruit during these maneuvers helped to confirm the presence of varicocele. Shift of baseline or the presence of bruit lasting two seconds or less were not considered as indicative of varicocele. Of the 174 patients, 74 (42.5%) had left-sided varicocele, 37 patients (21.2%) had bilateral varicoceles, and 4 (2.3%) had right-sided varicocele. No varicocele was detected in 59 (33.9%) patients. The 4 right-sided varicocele patients had left-orchiectomy in the past due to reasons such as testicular torsion or tumor. During VM, closure of the inguinal canal, movement of the testis, and other similar factors may result in a false shifting of the baseline. Various patterns of varicocele records and methods of preventing misinterpretations will be discussed. In conclusion, doppler examination affords a dynamic, inexpensive, noninvasive and sensitive method for the demonstration of duration and intensity of reflux in the pampiniform venous plexus.

59 IS SEXUAL DYSFUNCTION OF HORMONAL ORIGIN IN CHRONIC ALCOHOLIC MALES? Wince, J.P.*, Engle-Friedman, M.*, Hirenberg, T.*, Leilpaan, H.R.*, Brown University Program in Medicine, Providence VA Medical Center, Providence, R.I. 02908.

Inpatients from an alcohol rehabilitation unit volunteered to participate in a prospective study to evaluate effects of alcohol intake on sexual and hormonal function on entry and at 3, 6 and 12 months after entry. Data is reported at baseline in 18 of these patients. The patients were 22-54 years old and had a drinking history of 12-31 years and had scores of 22-106 on the Michigan Alcoholism Screening Test. At entry patients had a drink free period of 2-44 days and had little evidence of liver damage. Ten, (56%) patients reported sexual difficulties and 6 attributed them to alcohol directly. Patients with sexual complaints reported significantly lower subjective arousal while viewing an erotic film (P<.01) when compared to subjects with no complaints; the two groups had similar changes in penile circumference during the erotic stimulus and sleep. Total and free Testosterone, Estradiol, Pro.lactin, LH and FSH levels were normal in the group as a whole and in the two sub groups. In conclusion assessment of 18 male alcoholics showed a high incidence of sexual complaints in a normal hormonal milieu and a decreased cognitive responsiveness to erotic stimulus but intact penile erectile responses.

60 EFFECTS OF CYCLOSPORINE ON TESTICULAR STRUCTURE AND FUNCTION. Seethalakshmi, L., Diamond, D.A. and Menon, M., Division of Urological and Transplantation Surgery, UMass Medical Center, Worcester, MA 01605.

We examined the effects of the immunosuppressive drug, cyclosporine (CsA) on the structure and function of testis in sexually mature rats. The drug was administered subcutaneously in three doses (10, 20 and 40 mg/kg/d) for 14 days. Cyclosporine caused a dose dependent decline in body and reproductive organ weights. Histology of the testis showed definite degenerative changes and spermatid counts declined. A reduction in circulating testosterone and an increase in gonadotropins and creatinine were seen. Determination of CsA levels revealed that both tubular and interstitial compartments of testis take up CsA. Cyclosporine is known to induce a decline in renal function (Whitting et. al., 1985). Renal failure has been reported to affect spermatogenesis and Leydig cell function (Lii and Fang, 1975). A comparison was therefore carried out between the 40 mg/kg/d CsA group and a group subjected to subtotal nephrectomy. Renal function in both groups was comparable in terms of serum creatinine levels, but nephrectomy did not induce the changes in the reproductive system seen with CsA. This suggests that the testicular dysfunction seen in CsA treated rats is due to the direct action of CsA on the testis.
61 The Clinical Features of Hypogonadotropic Hypogonadism

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Hypogonadotropic failure is a common cause of male infertility. A group of 175 men from 100 families in 75 men who were known to be infertile due to hypogonadotropic failure. The presence of hypogonadotropic failure was determined by an elevated serum FSH level in all but the presence on a testicular biopsy.

Of the 175 men with hypogonadotropic failure, 65 (37%) were azoospermic and 110 were oligozoospermic (OS). OS was in at least one region of the testes. A recent study of the incidence of the hypogonadotropic failure was also very similar in both groups, as well as one region of the testes. Testicular tissue was studied in 15 of the azoospermic and in 4 of the oligozoospermic men. The serum FSH was elevated in 56% of the azoospermic and in 80% of the oligozoospermic men. The serum LH was raised in 52% of the azoospermic and in 90% of the oligozoospermic patients. In 5 of the azoospermic and 3 of the oligozoospermic men, the serum LH was also raised. The serum FSH and LH were measured at 1948 of the azoospermic and 10% of the oligozoospermic men and were normal in both groups.

Semen analyses were present in 11 of the azoospermic men, all of which were either, and 1 of the oligozoospermic men. Testicular biopsy was available in 13 of the azoospermic and 18 of the oligozoospermic men. The testicular lesion was not severe in the oligozoospermic men than in the oligozoospermic group. By applying the various criteria, Leydig cell hypoplasia could be demonstrated. A total of 52 of the oligozoospermic patients were subjected either to varicocele tie or to medical therapy. The remainder of treatment was extremely poor and no pregnancies resulted.

This study demonstrates that hypogonadotropic failure may present with either azoospermia or oligospermia. The similarity in etiology and mode of presentation strongly suggest that they are the same disease. The lesion may affect the intra and the extraneuronal compartments of the testes. It can be diagnosed reliably and in an infertility clinic and fails to respond to treatment.

62 PHYSIOLOGICAL ATTRAITS OF EPISODIC FOLLICLE STIMULATING HORMONE SECRETION

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Although the pulsatile attributes of LH release...
65 EFFECTS OF CHRONIC ENDURANCE EXERCISE TRAINING ON MALE REPRODUCTIVE HORMONAL PROFILES. A.C. Hackney* and W.E. Sinning*, Applied Physiology Laboratory, Kent State Univ., Kent, OH, 44242.

This study compares the reproductive hormonal profiles of untrained (UT) n=11) and endurance exercise trained (ET; n=11) males. Testosterone (T), free-T, estradiol (E), luteinizing hormone (LH), prolactin (PRL), and cortisol (C) were measured by RIA in resting blood samples. T, free-T, and C were significantly lower in 1-SCD males compared to UT males. The LH data did not differ between the two groups. Further, the LH data suggest normal hypothalamic-pituitary-testicular function in both groups. PRL and C levels were normal and did not differ between groups. This suggests that chronic endurance exercise training lowers T and free-T levels in males possibly by impairing testicular function.

66 THE ROLE OF SEXUAL STIMULATION AND EJACULATE CHARACTERISTICS DURING THE CLINICAL USE OF A SPERMIAL SEMINAL FLUID COLLECTION DEVICE (1-SCD) FOR SPERM SELECTION. M. Michael, A. E. S. M. Kennedy, University of Kentucky and Andrology Institute of Lex., Lexington, KY 40546.

Recent studies have shown that ejaculates collected at intercourse with the use of a Sattical seminal fluid collection device (1-SCD) were significantly improved when compared to ejaculates collected via masturbation (Fertil. Steril. 43:491 and 44:57, 1985). The routine use of the 1-SCD method in our andrology facilities has provided additional data concerning this device. Of a group of 50 patients that produced specimens via the two methods, 45 patients (90.0%) preferred the 1-SCD method for collection of ejaculates rather than masturbation (83%). Also the same population of patients rated sexual stimulation elicted during production of the ejaculates (1-10) higher when the 1-SCD was used (7.11 versus Mast (2.3)). There was a positive correlation (r=83) between the sexual stimulation rating provided by the patients and the TFSF values of all seminal ejaculates collected. Other features of noted importance were the ease by which the 1-SCD method may be used at the privacy of the patient's home, and the wife or husband may bring the specimen for analysis or sperm wash for artificial insemination purposes (AID) without time loss. The data suggest that increases in seminal characteristics noted when the 1-SCD method was used may be associated with increases in sexual stimulation elicited during the production of the ejaculates. Further, the 1-SCD method, may not only improve specimen characteristics but also may provide other attractive practical aspects making the 1-SCD quite favorable for use during treatment of infertility by the clinician and the infertile couple.


The indications for testicular biopsy in the evaluation of male subfertility have been well categorized. Full scrotal exploration with open biopsy of the testis is a hospital based, expensive, and painful procedure with some morbidity that necessitates a substantial delay in the patient's ability to return to work. Testicular needle biopsies were performed on 65 testes in 35 patients (at the time of submission of this abstract) in the office under local anesthesia using a new all-inclusive biopsy kit that contains a modified Tru-Cut needle for biopsy purposes. The procedure has resulted in no episodes of bleeding or swelling and minimal patient discomfort requiring only acetaminophen for analgesia. Sufficient tissue is obtained for unimpacted gonadotropin management based upon biopsy findings. Results of therapeutic intervention done in this manner will be presented with reference to efficacy of pharmacologic intervention with respect to specific biopsy findings.


We have tested the ability of the human zona pellucida (ZP) to induce acrosome reactions in human sperm. Oocytes were dissected from ovarian tissue and stored at -70°C; they are non-viable. Motile sperm were selected by the swim-up technique, resuspended in 35 mg/ml human serum albumin in modified BWW medium at 30 to 50 x 106 sperm/ml, and incubated at 37°C in 5% CO2, 95% air for 5 hr. Acrosome reactions (AR) were detected as described by Cross et al. J. Androl. 7:28P, 1986. Oocytes were incubated 1 min with 50 x 105 sperm/ml (4 ± 0.4% AR, mean ± SEM, n=6), to allow binding of an average 176 sperm/ZP. One group of oocytes was fixed immediately and another was fixed after a further 1 hr incubation without sperm. Sperm on the ZP were 5 ± 2% AR at 1 min and 44 ± 5% reacted at 1 hr. The increase in AR could be due to induction of AR by the ZP, or to preferential binding by a subset of sperm destined to complete the AR in the next hr. To distinguish these two possibilities, we tested the ability of solubilized ZP to induce AR in a sperm suspension. ZP were removed with a small bore mouth pipette, and 6 ZP were solubilized in 1 µl of 5 mM NaH2PO4, pH 2.5. One µl of 2x medium was added, and then 1 µl of sperm (50 x 106/ml). Controls were acidic buffer alone, or a zona-free vitellus treated with acidic buffer. After 1 hr the sperm were fixed for the AR assay. The results were solubilized ZP: 24 ± 3% AR, n=3; buffer control: 3 ± 1%, vitellus control: 5 ± 1%. We conclude that the human ZP, or material intimately associated with it, can induce AR. Supported by NIH grants HD19587 and HD15149.
Spem microinjection is a valuable tool for obtaining karyotypes of sperm that are otherwise unable to be fertilized in vitro. Microinjection is typically characterized by a high degree of egg mortality and a low rate of egg activation, which reduces the quantity and quality of metaphase spreads obtained from microinjected eggs. A study was conducted to identify critical factors(s) that might improve egg viability and activation after microinjection of sperm nuclei into hamster eggs. In preliminary experiments, sperm nuclei from several mammals (Chinchilla, R.C. hamster, and the creeping vole [Microtus oregoni]) were microinjected into 863 hamster eggs with a gradually tapered needle. After 6 hr incubation at 37°C, 58% of the injected eggs were viable and, of these, 22% were activated. In subsequent experiments, the construction of an injection needle with a longer, more uniform barrel (5-8 μm) substantially increased both egg viability and egg activation (92% and 68%, respectively; n=129). Additionally, while Holmes defined medium was superior to Ham's f-10 in maintaining egg viability and activation after microinjection, pre-treatment of eggs with 5-10% ethanol did not alter viability or activation of microinjected eggs. These data indicate that size and shape of the injection needle and conditions of egg culture are critical factors in maintaining egg viability and activation after microinjection. Optimizing these factors may enhance the quantity and quality of sperm karyotypes obtained from microinjected eggs.


The National Institute for Occupational Safety and Health (NIOSH) has conducted five occupational field studies assessing potential reproductive hazards of male workers, as measured by semen characteristics. Each of these studies was at a different geographic location and each evaluated a concurrent control population. The first study was conducted in May, 1983 in Dillon, CO (N = 19), the second in December, 1983 in Hilo, HI (N = 46), the third in June, 1984 in Portland, OR (N = 36), the fourth in December, 1984 in Groton, CT (N = 40), and the fifth in June, 1985 in Cincinnati, OH (N = 45). This report compares the five control populations for semen volume and pH, and spera motility, velocity, viability (vital stain and hypoosmotic stress assay), morphology and morphometry. The confounding effects of age, smoking and alcohol consumption also were evaluated. The primary conclusion emphasizes the need for evaluating a concurrent control with each study population and not to rely totally on historical controls.

**71.** **INCREASED DEGENERATION OF GERM CELLS LATE IN MEIOSIS AND NO LOSS DURING SPERMIOGENESIS IN AGED MEN.** A. Bagheri, and Larry Johnson, University of Texas Health Science Center at Dallas, Dallas, TX 75235

The amount of germ cell loss late in meiosis was similar between younger (20-48 yr) and older (58-85yr) adult men (Bio1. Reprod. 31:779), and no degeneration occurred during spermiogenesis in younger adults (Bio1. Reprod. 25:217). The objective of this study was to evaluate the incidence of germ cell loss during late meiosis or during spermiogenesis on sperm production in aged men. Potential daily sperm production (DSP/g) based on acrosome primary spermatocytes and daily sperm production (DSP/g) based on early (G0/G1) and cap-phase or maturation-phase spermatids were determined by counting those cells in the homogenates of glutaraldehyde perfused testes. Testes from 14 men (69 to 90, 72±2y) were compared with testes from 13 men (24 to 51, 31±2y). DSP/g or DSP was the number of cells in the homogenate times the theoretical yield of that cell type divided by the weight of tissue homogenized and the life span of that cell type. Percentage loss late in meiosis was calculated by dividing DSP/g into the product of 100 times the difference between DSP/g or DSP. No loss occurred during spermiogenesis as noted by similar (>0.05) DSP/g based on early or maturation-phase spermatids in younger (4.9±0.2 vs 3.9±0.2), or older men (1.5±0.3 vs 2.3±0.5 x106). DSP/g was lower (P<0.01) than DSP/g (10.0±0.6 x106) for younger and 7.4±1.1 x106 for aged). Degeneration late in meiosis was greater (P<0.01) in aged (79.6±3.0%) than younger men (54.8±2.4%). Aged men had an increased loss late in meiosis not previously detected in older adult men. Increased germ cell loss late in meiosis contributes to reduced sperm production in aged men. NIH grant AG02260.

**72.** **EFFECTS OF ETHANOL ON THE ACROSOME REACTION IN HUMAN EJACULATED SPERMATOZOA.** Juan G. Alvarez, J. C. Touchstone, M. Lopez, and Bayard T. Storey. Departments of Physiology and Obstetrics and Gynecology, University of Pennsylvania, Phila, PA. 19104

Although some isolated ethanol effects on mammalian sperm have been documented, its effect on fertility in terms of effects on human spermatozoa is poorly understood. In this study the effects of ethanol on human sperm acrosome reaction (AR), as measured by chlortetracycline fluorescence patterns (CTCF) and indirect immunofluorescence (IFF), were evaluated. Aerobic incubation of human sperm at 37°C in BWW medium (0.3% HSA) in the presence of 0 and 25 mM ethanol (0.11 vol %) for 6 hr resulted in 4±0.5% and 23±3% AR respectively (X±S.D., n=12). No effects were observed at 0 hr. Incubation for 0 and 6 hr in the presence of 250 mM ethanol resulted in 25±3% and 46±5.5% AR, respectively (X±S.D., n=12). AR values for 0.1 mM ethanol were below 4%. Incubation of human sperm in the presence of the calcium ionophore A23187 (10 μM) for 0 and 3 hr. resulted in 3.2±2.2% and 50±1.5% AR respectively (X±S.D., n=5). Interaction of 250 mM ethanol with ionophore for 0 and 3 hr incubation resulted in 35±6.4% and 49±6.5% AR, respectively (X±S.D., n=5). No effects on motility were observed with concentrations of ethanol up to 500 mM. These findings indicate that ethanol ingestion may play a role in unexplained male infertility by inappropriately accelerating the acrosome reaction in sperm. Supported by March of Dimes Birth Defects Foundation, and NIH Grants HD-15842, HL-07027 and HL-19737.
73 EFFECT OF LIVER DAMAGE, ALCOHOL, AND RECREATIONAL DRUGS ON SEMINIFEROUS TUBULES AND LEYDIG CELLS IN MEN. Tho Q. Nguyen*, L. Johnson, Robert E. Wolf*, Ali Bagheri*, Michael E. Bailey*, and W.B. Weavers, University of Texas Health Science Center at Dallas, Dallas, TX 75235.

Tests from 15 control men (31±2y), 9 men (35±3y) with liver damage, 14 men (35±2y) known heavy alcohol users, and 13 men (30±2y) known recreational drug users were perfused with glutaraldehyde within 15 hours of death. Tissues were placed in osmium and embedded in Epon. The percentage of parenchyma occupied by seminiferous tubules and Leydig cells were determined by point counting 0.5µm sections. For the control, liver, alcohol, and drug groups, respectively, the paired testicular weights (45±3, 48±5, 46±3, 49±3), paired parenchymal weights (39±3, 41±4, 40±3, 42±3), daily sperm production per man (164±14, 218±34, 196±24, 206±10), daily sperm counts per man (200±25, 225±35, 24±22, 24±22), seminiferous epithelium volume/ma (15±2, 17±2, 16±1, 18±1ml), tubular diameter (208±4, 225±3, 223±5, 225±5µm), tubular length/ma (108±49, 96±96, 96±96, 102±67µm), Leydig cell volume/ma (1.4±0.1, 1.2±0.1, 1.5±0.1, 1.7±0.1ml), volume of a single Leydig cell (617±1130, 546±480, 546±480, 546±480), and number of Leydig cell is per man (296±45, 25±234, 327±137, 34±164) reveal no significant difference among means on most parameters tested. The liver group had lower (p<0.05) Leydig cell volume/ma than the drug group. Also, the diameter of tubules in the control was less (<P<0.05) than the other groups. Hence, neither moderate liver damage, moderate use of alcohol, nor moderate use of recreational drugs is damaging to seminiferous tubules or Leydig cells. NIH grant AG2260.

74 CNS AND TESTICULAR SENSITIVITY TO ETHANOL AS A FUNCTION OF PUBERTAL DEVELOPMENT. R.A. Anderson, J.F. Phillips*, and S.H. Berryman*, Rush Medical Center, Chicago, IL, 60612

Chronic ethanol ingestion during adolescence delays sexual maturation (Biol Reprod 32:5; 181, 1984). Reproductive impairment is seen in pubertal males consuming ethanol diets with result in minimal effects in adults, suggesting differential sensitivity to ethanol. To examine this, central nervous system (CNS) sensitivity (measured by narcosis) and steroidogenic response to ethanol was measured in Swiss-Webster mice at ages 23, 26, 30, 40, 50 and 60 days. The liver group had lower H-P-T axis suppression at all ages and all doses. Lead exerts its toxicity in a dose response fashion. However, testis suppression of plasma testosterone reached normal levels, and in a further deuterated testosterone, spermatogenesis and ejaculation were completely suppressed by N prior to starting C. By 2 weeks post-treatment (PT) to have spermatogenesis and ejaculation were completely suppressed by N prior to starting C. By 2 weeks post-treatment (PT), plasma testosterone reached normal levels, and in a further 3 weeks ejaculation returned. Animals were hemicastrated at 5-9 weeks PT. Those receiving N only began at 5 weeks PT to have sperm in the ejaculate with normal motility, and normal sperm numbers were reached at 14 weeks PT. Animals receiving CHr had azospermic ejaculates through 65 weeks PT. When the remaining tissue was removed for histology. In contrast, C only animals (see ref) showed a rise in sperm numbers beginning at 10-11 weeks PT which reached 150±10 sperm/ ejaculate at 65 weeks PT. Testicular histology of CHr animals at 5-9 weeks PT showed isolated tubules with apparently complete spermatogenesis. However, at 65 weeks PT no germinal cells were found. In C only animals, spermatogenesis was normal at this time. The addition of H to C treatment exacerbated the deleterious effects of C on the testes, and caution is therefore suggested for use of such a protocol clinically.

The Aladdin Thermo Jar (Aladdin #100 Nashville, TN) has been routinely used as a protective container for semen samples brought to field study sites after home collection. Three temperatures, 40°C, 20°C, and -20°C were used to assess the effectiveness of the containers in protecting semen quality from alterations due to temperature extremes. For each temperature studied five ejaculates were pooled, vortexed and 3 mL were placed into each of three glass jars. Viability estimates (vital stain, hypotonic stress (HOS) assay and percent motile sperm) and sperm velocity characteristics (both path length and end point to end point velocities and progressive ratio) were determined before and after temperature exposures of 90 minutes. A thermocouple was placed into the semen of each glass jar; two jars were placed in the insulated jars with one kept at room temperature and the other placed in the controlled temperature environment along with an unprotected glass jar. An ANOVA with a LSD was conducted on the velocity parameters of nocturnal penile tumescence in 56 men representing five groups: 1) hypogonadotropic hypogonadism (n=7); 2) orchiectomized males with prostate cancer (n=7); 3) prolactin pituitary tumors (n=9); 4) diabetic males with normal sexual function (n=9); 5) normal controls (n=24). Data was analysed utilising both Pearson and Spearman correlation coefficients for the entire group of 56 subjects. Serum testosterone correlated with all parameters of NPT including number of erections (r=+50, p<0.001), maximal penile circumference change (MPCC, r=+71, p<0.0001) and total tumescence time (TTT. r=+62, p<0.0001). In control subjects there was a significant (r=-48, p<0.02) inverse relationship between age and total tumescence time only, Serum prolactin was inversely related to MPCC (r=-76, p<0.02) in young controls (23.9 ± 1.2 y, n=9) but not older men (47.9 ± 2.3 y, n=15). We conclude that: 1) All parameters of NPT have a high direct correlation with serum testosterone. 2) NPT correlates inversely with age in all controls and serum prolactin in only young males.

Hydroflex penile prosthesis: early experience and recommendations. M. David Mitcheson, New England Medical Center, Boston, MA 02111

Fifteen impotence patients have been implanted with the Hydroflex (HFX) penile prosthesis since it became available one year ago. Follow up is necessarily short but useful comments can already be made. The etiology of impotence was as follows: 7 patients had impaired penile arterial supply, 1 had severe diabetes mellitus, 1 had idiopathic hypogonadotropic hypogonadism, 2 were paraplegic, 2 were quadraplegic. 1 had empty sella syndrome and had been previously irradiated for prostate cancer and one became impotent after radical prostatectomy for prostate cancer. Noteworthy is that the man with empty sella syndrome received testosterone shortly before developing prostate cancer. Patient and partners completed questionnaires before implantation and 2 and 12 weeks later. Penile measurements were made in the flacid state, after erectores tunescence and after HFX implantation. The HFX implantation measurements when compared to erectores tunescence were excellent as were tunescence measurements. Deflation characteristics were good except in 1. This was due to kinking of the fluid passage way. Operative recommendations will be made to show how best to determine the exact fit and to avoid complications. Most patients were comfortable after two weeks and learned to use the device in 2 sessions. Apart from the deflation difficulty there were no early mechanical failures. 12 patients considered the device excellent, 2 good and 1 terrible. Partners were equally pleased.

The HFX penile prosthesis provides excellent treatment of impotence regardless of etiology.
Clinical, hormonal, histological, and cytogenetic data from 134 azoospermic men are presented. Medical history, genital examination, semen analysis, and endocrine studies were performed on all patients, except in 8 individuals who were not karyotyped. The patients were divided into the following groups: only Sertoli cells (SCO, n=66); 47,XXY Klinefelter syndrome (K, n=26); obstruction (n=20); spermatogenesis arrest (n=12); and orchitis sequelae (n=10). Besides group K, only two patients (both from group SCO) had an abnormal karyotype: one a de novo del Yq and the other a familial t(X;Y)(q26;q12).

Without considering the results of histological studies, it was observed that clinical data, gonadotropin levels, and karyotype are sufficient for a group diagnosis in over 75% of patients. It is concluded that testicular biopsy should be performed only in selected cases or for other specific studies or investigations.

It is known that white blood cells are present to varying degrees in the semen of both fertile and infertile men, even in the absence of apparent genitourinary tract infection. Little is known about the significance of these leukocytes, or whether certain white blood cell subsets are associated with characteristic abnormalities of semen. Recent studies by our group have provided evidence that some lymphokines and monokines in vitro cause significant impairment of spermatozoal motility. One aim of this study was to evaluate whether in vivo there are associations between the presence of certain white blood cells (as a potential source of the immunologic mediators) and altered sperm motility patterns. Granulocytes, macrophages, and lymphocytes of the T helper/inducer, T cytotoxic/suppressor, and B classes were identified in semen smears by applying a panel of cell-specific monoclonal antibodies in an immunoperoxidase assay. Our results indicate that white blood cells are present in all semen samples to greatly varying degrees. The lowest numbers are recovered from semen of vasectomized men. Studies on a possible relationship between white blood cell subsets and abnormal motility patterns are currently underway and these results will also be presented. Supported in part by the Max Kade Foundation and by the Fearing Research Laboratory Endowment.
These results suggest the potential usefulness of lateral evaluation of larger groups of individuals will be required to validate the correlations presented here.


This study was designed to assess the effects of intracervical (IC) semen deposition at different depths or fertilization rates (FR) and embryonic retardation (BR).

Superovulated Holstein cows were bred at 10.9±2.2 hr after the onset of estrus with a single dose of frozen semen from the same bull. Animals were grouped by site of AI (n=5/group): 1) right uterine horn (UH) 5 cm deep; 2) left UH, 5 cm deep; 3) right UH, 10 cm deep and 4) left UH, 10 cm deep. The number of unfertilized ova, normal and abnormal embryos were recorded for each horn and death at slaughter (5 d post AI). Embryos, recovered from the horn where AI was performed (ipsilateral) IPS were pooled and compared to embryos recovered from the contralateral (CON) horn per depth. FRs were higher in embryos recovered from IPS than that of CON at 5 cm (83.1% vs 43.4%; P<0.002), respectively. FRs were higher for embryos recovered from the CON at 5 cm than those recovered at 10 cm (P<0.05). BR did not differ between IPS and CON at 5 cm deep (5.1% vs 6.4%; P>0.1) or at 10 cm deep (8.3% vs 26.8%; P>0.05), respectively. The results showed no improvement in FR when inseminating into the IPS horn deeper than 5 cm. In contrast, IC-AI into the IPS horn deeper than 5 cm may decrease sperm migration to the CON horn and that may result in lower FR (P<0.002) and/or possible increase in the embryonic retardation ratio.

86 EXPERIENCE WITH INTRAUTERINE INSÉMINATION IN INFERTILE COUPLES. Christine L. Cook, M.D.,* Leigh T. Price*, and Arnold N. Belker, M.D., Dept. of Obstetrics and Gynecology University of Louisville, Kentucky 40292.

Intrauterine insemination is suggested in cases of infertility where cervical, male, combined or unexplained factors are present. One hundred fifty-seven patients underwent intrauterine insemination (IUI) for at least 3 cycles (range 1-18) or until they conceived. Most patients had multiple infertility factors. The overall pregnancy rate was 20%. The pregnancy rate in 41 couples with male factor infertility was 19%, 10% in 10 cases of male anti-sperm antibodies, OX in 8 cases of female antispem antibodies, 53% in 20 cases of poor postcoital test with good nucux, 42% in 12 cases of poor postcoital test with poor nucux, 30% in 10 cases of cervical stenosis and 18% in 11 cases of unexplained infertility. None of the patients with asthenospermia in addition to mild (10-20 x 10⁹/ml) or severe (<10 x 10⁹/ml) oligosperma were evaluated separately, the pregnancy rates were 62% and 40%, respectively. Spontaneous abortion occurred in 6 of the 31 pregnancies (19%). An ectopic pregnancy occurred in one case of poor postcoital test with poor nucux (8%). The average number of cycles to conception was 3.6; no one conceived after the 9th insemination cycle. Intrauterine insemination appears to be particularly valuable in couples with poor postcoital tests and with asthenospermia associated with severe oligosperma.
89 EFFECT OF NEURAMINIDASE ON SPERM SEPARATION FOR SEX SELECTION. Hugh C. Hensleigh* and Denise Trusini*, Department of Obstetrics and Gynecology, University of Minnesota, Minneapolis, MN 55455.

Human sex selection by sperm separation is being done clinically by the albumin swim-down technique that selects for Y-bearing sperm (Ericsson, et al., Nature 246:421, 1973) and the Sephadex column technique that selects for X-bearing sperm (Biochem. Biophys. Res. Com. 124:950-955,1984). To determine if sialic acid residues are important in the sperm separation techniques, sperm samples were incubated with 1 mg sialidase/ml (Type V, neuraminidase, Sigma) for 1 h at 37°C prior to sperm selection. Samples were stained with quinacrine mustard and scored blind for the presence of the Y-fluorescing body. No effect was seen using the Sephadex column technique with an average of 82.2% X-bearing sperm following sialidase incubation (mean ± S.D.; control, 79.3%±5.5%; N=4). However, the sialidase incubation negated separation in the albumin swim-down technique: an average of 44.0% 8.3% Y-bearing sperm was observed (control, 71.0% 5.6%; N=4, P<0.05). These results suggest that a differential presence of sialic acid residues on sperm surface may be of importance in the albumin swim-down technique but not in the Sephadex column technique.

90 INCREASED RATE OF HUMAN SPERM FERTILIZATION ABILITY BY Percoll-GRADIENT CENTRIFICATION. H. Tanghaichitr, A. Agulnick, D. Dias, J. Will, L. Fitzgerald and D. Anderson. Dept. of Obstetrics and Gynecology, Beth Israel and Brigham and Women's Hospitals, Harvard Medical School, Boston, MA 02215.

Human sperm can be capacitated in vitro to achieve fertilization ability. Swim-up and Percoll-gradient centrifugation (PGC) procedures are routinely used for this purpose clinically. This report is to illustrate that the PGC method is more efficient than the swim-up method in producing capacitated sperm as defined by hamster egg penetration. In comparison to the swim-up method, the PGC method yielded non-donor's and non-capacitated sperm with hamster egg penetration. To determine whether the two fold increase in penetration rate was due to enhanced sperm motility, the IVF assay was repeated on five of the above samples. However, progressive motility on the filtered fraction was not adjusted prior to incubation with oocytes to the same level as in the control. Again, higher penetration rate for filtered samples (39.0±24.3) was observed after adjusting motility to the same level as in the unfiltered control (24.2±22.1). Thus, the filtered samples were controlled for concentration and/or motility to minimize the effect of these parameters on the penetration rate. Therefore, it appears that glass wool filtration also effectively isolates a sperm population possessing a greater in vitro penetrating capacity.

91 ABILITY OF GLASS WOOL FILTRATION TO ISOLATE FERTILE SPERM FRACTIONS. W.J. Holmgren, R.S. Jeyendran, N.R. Neff, M. Perez-Felaniz. Mount Sinai Med Ctr, Univ of WI Med School, Milwaukee, WI and Inst of Reprod Med, Chicago, IL.

Semen filtered through a glass wool column yields an enhanced number of motile sperm with chemically active and physically intact membranes (Fertil Steril 45:132, 1986). As fertilization capacity is the ultimate test of sperm quality, the in vitro penetration of zona-free hamster oocyte (IVP) assay was performed to determine if filtered samples also possess greater penetrating capacity. Each sample was washed and divided into two aliquots. One aliquot was filtered through a column containing 15 mg of precleared glass wool microfiber packed to a depth of 3.5 cm. The concentration of the unfiltered control and filtered sample was adjusted to 10 x 10^6/ml and incubated for 18-20 hr at 37°C. The IVP assay was performed using 1 x 10^6 sperm/ml for each aliquot coincubated with oocytes. Of the 10 ejaculates assayed, significantly higher (P<0.01) IVP results were observed for the filtered sample (Mean ± S.D.; 58.6±26.5) than for the control (27.2±20.5). To determine whether the two fold increase in penetration rate was due to enhanced sperm motility, the IVF assay was repeated on five of the above samples. However, progressive motility on the filtered fraction was not adjusted prior to incubation with oocytes to the same level as in the control. A higher penetration rate for filtered samples (39.0±24.3) was observed after adjusting motility to the same level as in the unfiltered control (24.2±22.1). Thus, the filtered samples were controlled for concentration and/or motility to minimize the effect of these parameters on the penetration rate. Therefore, it appears that glass wool filtration also effectively isolates a sperm population possessing a greater in vitro penetrating capacity.
93 ATP DECLINE OF INFERTILE PATIENT SEMEN AFTER SWIM-UP SEPARATION METHOD (SSM). H. Nosheidi, P. A. Acosta, S. P. Pleasants, Jr, M. D. Yoda, and R. J. Swanson, Andrology Laboratory, Department of Obstetrics & Gynecology, Eastern Virginia Medical School & Department of Biological Sciences, Old Dominion University, Norfolk, VA 23508-8502.

In 22 infertile males, ATP content of 4 semen specimens declined up to 95% when processed by SSM using Hae's F10 fetal cord serum medium. The SSM included a 10-minute 200g centrifugation of sample-medium mixture followed by one-hour incubation at 37°C in 5% CO2/gar. ATP values determined by cholineminescence were calculated in picomoles sperms to observe variations in count. The 4 drastic ATP declines were not related to semen count (oligospermia vs. normospermia), motility or SSM recovery rate. All samples demonstrated an ATP decline averaging 20-30%. This finding may represent one possible explanation for the fertilization failure seen in some infertile individuals. Alternatively, low ATP values may represent a damage artifact inflicted upon sperm while processing for AIN, IVF or hamster procedures. Recovery rates in normospermic samples were consistently very low with SSM but very high with discontinuous (40-60-90) percoll gradient separation (PGS). In evaluating these 2 sperm separation techniques in 6 patients, 4 of the 6 semen samples produced a smaller decline in ATP content with PGS. ATP values for the other 2 samples might have been compromised by elevated round cell counts (neutrophils and other types of round cells), which normally have much higher ATP content than sperm. Some round cells and debris from the original sample normally remain after SSM. PGS eliminated round cells and debris. PGS is a method of choice in semen with elevated round cells or debris, or where drastic change in ATP content results from SSM. Intracellular placNAc in human spermatozoa is not accessable in fresh isolated sperm, but is highly dependent on circulating androgens.

94 POLYLACTOSAMINE ON THE MOUSE SPERM SURFACE DURING SATURATION AND CAPACITATION

Charles R. Dwyer, Depts. OBGYN and Biological Structure, University of Washington, Seattle WA 98109.

A polylactosamine-containing glycosyljugate (placNAc), recognized by a monoclonal antibody, first appears on the sperm surface in the mid-to-distal caput epididymis of the mouse. This is the same region in which a high molecular weight, trypsin-sensitive sialylated placNAc appears in the apical cytoplasm of principal epididymal cells and in the luminal fluid. Sial-placNAc in the epithelium is independent of testicular fluid or sperm (as seen after enucleate oviductation), but highly dependent on circulating androgens. PolylacNAc on the sperm surface undetected by immuno-fluorescence overlying the posterior acrosome. Sperm from the proximal caput are negative, and about 90% of sperm from the cauda are positive. During incubation under in vitro fertilization conditions, surface placNAc is slowly lost such that 50% of sperm are positive by 60 min. In contrast, intracellular placNAc in the anterior acrosome is not accessible to freshly isolated sperm, but is exposed progressively at 15,30 and 60 min of incubation. In control conditions, no more than 5% of sperm are exposed with the anterior acrosomal pattern. However, up to 100% of sperm are seen with this pattern after exposure to calcium ionophore A23187. In a dose-dependent fashion. Simultaneously, the posterior acrosomal pattern is lost from ionophore-treated sperm. Intracellular placNAc is also exposed by washing or by extraction with non-ionic detergents. Intracellular placNAc appears in the forming acrosomes of round spermatids and may be a component of the acrosomal matrix. Sperm surface placNAc may serve as a stabilization or zona-recoagnition factor. (Supported by NID Grants HD-16211 and HD-12629)

95 CAPACITATION OF BOVINE SPERM BY REMOVAL OF CHOLESTEROL

Eduardo E. Trujillo*, Robert E. Jones and Stephen R. Plymate, Department of Medicine and Clinical Investigation, KAMC, Texas A&M University System Health Science Center, College Station, TX 77845.

Several reports have indicated that capacitation of bovine sperm includes restoration of the cholesterol (Scho) to phosphatidyl sphingomyelin (PS) ratio of the plasma membrane. In this study, methods were developed to quantitatively reduce the cholesterol/phosphatidyl sphingomyelin ratio of bovine sperm and the effectiveness of this treatment in capacitating sperm was determined. Wasted sperm (2X106) were incubated in 1 ml of modified Tyrode's solution (TS) containing unlabeled liposomes of phosphatidylcholine (PC), cholesterol (Chol) and 150mM CaCl2. After 30 min at 37°C, a 1:10 exchange of 14C-Chol liposomes to sperm was observed (max +80% of sperm motility was regained). After 60 min plus PS were incubated as before with PC/PE liposomes containing no Chol. After 90 min, sperms were separated from liposomes by centrifugation. Measurement of 14C-Chol in the liposomes (supernatant) and parallel gas chromatographic analysis of extracted, saponified liposomes (in) indicated that 30% of sperm cholesterol was removed by this procedure. Chol efflux decreased sperm motility by < 10% but reduced sperm velocity by 50%. Sperm incubated with no liposomes (+Chol), with saponified liposomes (-Chol) were washed and resuspended in 1 ml with 0.2 g/ml PS and 50 g/ml lymphaemia sphingomyelin (CChol). Percentage of sperm undergoing the acrosome reaction (AR) upon incubation with 14C-PC was used as a measure of sperm capacitation. After 60 min at 37°C in 1% PC, percent sperm motility for +Chol and -Chol was 32±4.2, 60±4.7 and 37±6.8, respectively. Corresponding values for percent AR were 14±3.4, 20±3.4 and 37±5.2. These results suggest that loss of Chol from plasma membranes of bovine sperm may be an important step in capacitation in this species. This work was supported in part by NIH grant HD 16628.

96 INTERACTIONS BETWEEN SELECTED FATTY ACIDS AND THE ACTIVATION OF PALMITIC ACID IN SPERMATOZOA

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Human sperm are capable of synthesizing acyl CoA thioesters from nascent fatty acids and coenzyme A. We have extensively characterized the enzyme responsible for this process, fatty acid CoA synthetase (E.C. 6.2.3.1), and have reported that, with the exception of docosahexaenoic acid (~24:6), the saturated and unsaturated acyl substrates for ligase share a common Michaelis constant (Km) of approximately 4mM. Because 22:6 comprises the major fatty acid portion of apical membrane phospholipids, we sought to investigate the effect of 22:6 on the synthesis of palmitoyl CoA. In addition, we studied the influence of another saturated fatty acid, stearic acid (18:0), on palmitic acid (16:0) activation. Human sperm were isolated by centrifugation and washing, and ligase was solubilized from the sperm with 0.1% Triton X-100 (final concentration). The incubation media for the determination of ligase activity consisted of increasing concentrations of tritiated 16:0 with varying levels of unlabelled 18:0 or 22:6 plus saturating levels of coenzyme A. The unreacted 16:0 was extracted into heptane and the aqueous phase counted. Enzymatic rates were calculated as nmol palmitoyl CoA formed/min/mg protein. 16:0 acted as a competitive inhibitor but, surprisingly, 22:6 was a noncompetitive inhibitor with an inhibition constant (Ki) of 9.5mM. We conclude that in contrast to 18:0 (and presumably other fatty acids) which appears to share the same active site, these fatty acids have a different binding site which is distinct from the active site. These data also imply that 22:6 may require a separate enzyme for activation, and that 22:6 may regulate palmitoyl CoA synthesis in sperm.
79 CHANGES IN CYTOSOLIC PROTEIN AND PHOSPHOLIPASE ACTIVITY DURING IN VITRO CAPACITATION OF SPERM FROM FERTILE AND SUBFERTILE BULLS. Margaret B. Rehnert, Gary Killian and David Chapman*. Dairy Breeding Research Center, University Park, PA 16802.

Total protein and phospholipase A2 (PLA2) activity were determined during in vitro capacitiation of fertile (FT) and subfertile (SF) bulls (mean of 30 and 37, respectively). PLA2 activity (µg of fatty acid produced/mg protein) was determined in cytosols by measuring the decrease of fluorescently labeled L-α-phosphatidylcholine and production of labeled fatty acid for a 2 hr period. Activities for both PLA2 and PLA1 increased from 10.5 ± 20 hr to 3.3 ± 7 hr. Although PLA2 activity in FT bulls increased from 8.4 ± 2 to 8 ± 2 /3 ± 1, 13, 2 of 3 SF bulls showed a change in activity (15.2 ± 6 to 12.7 ± 4) during capacitiation. Percent acrosome reaction and PLA2 activity during capacitiation were negatively correlated for the 2 SF bulls (r = -.49) showing no change in PLA2 activity. These data suggest that cytosol protein, PLA2 activity and fertility may be related in bull sperm. (Supported by Sire Power, Inc.)

99 A NEW METHOD FOR OBTAINING A HIGH PERCENTAGE OF VIABLE, CAPACITATED SPERM. Christopher De Jonge, S.R. Heck, L.J.D. Zaneveld, Dept. of Reproductive Physiology, Dept. of Ob/Gyn, Rush University, Chicago, IL 606112.

Capacitation (C) is the period of time during which sperm acquire the ability to undergo the acrosome reaction (AR). Spera can be capacitated using various media in vitro, but require extended periods of incubation. "BMW" (Biggers, Whitten and Whittingham, 1971) medium is often used for incubating sperm. This study determined the effects of incubation of human sperm in hypoosmotic saline on C and AR and developed a system for capacitating human sperm after short incubation periods. Ejaculated sperm were washed in Ficoll and incubated in one of the following solutions: Hypoosmotic (240 mosm) saline (0 or 3.5% HSA), BMW (0 or 3.5% HSA) or isosmotic saline lacking HSA and calcium. To synchronously induce the AR, calcium ionophore (A23187) was added to solutions containing calcium. Calcium chloride was added to calcium-free media. Inducers were added at 0, 1.3, 4, or 5 hr. time periods and sperm were incubated for an additional 15 mins. Spera were then processed for triple staining (I. Exp. Zool. 215:201-208, 1981). Sperm were maximally capacitated by the number of AR spera. After 3 hr. incubation 94% AR spera were seen in hypoosmotic saline (3.5% HSA) and isosmotic BMW (3.5% HSA). Longer incubations did not increase the number of AR spera. Calcium-free, HSA-free saline proved to be ineffective in capacitating sperm (20% AR). Hypoosmotic saline (3.5% HSA) supported AR to the same extent as isosmotic BMW (3.5% HSA) yielding a high viability (90% vs 80%). In conclusion, hypoosmotic saline (3.5% HSA) capactiates a high percentage of spera in a short period of time and yields a highly viable population. Supported by NIH HD 19555.

98 GLUCOSIDASE ACTIVITIES IN BOVINE SPERM DURING IN VITRO CAPACITATION AND THE ACROSOME REACTION. Tamara McNutt*, Gary Killian and David Chapman*. Dairy Breeding Research Center, University Park, PA 16802.

Levels of 6-nanogalactosidase (MAN), 6-α-galactosidase (GAL), 6-α-1-acetylgalactosaminidase (AGAL) and 6-α-1-acetylgalactosaminidase (AGAL) activity associated with sperm during in vitro capacitiation and the acrosome reaction (AR) were determined fluorometrically (unit = nmoles 4-methylumbelliferone liberated from substrate/hr•mg protein). Washed ejaculated sperm (50 x 10^6 cells/ml) from 3 bulls were incubated up to 7 hr at 37°C in 5% CO2 in air in modified Tyrodes medium (Parrish, J. J. et al., 1985, Theriogenology, 23:216). For whole cell sonicates, defined as periods of 100 ± 836 hr but decreased gradually to 90 ± 131 at 7 hr. GAL and GLU increased from 100 ± 66 and 112 ± 62, respectively at 0 hr, to 525 ± 206 and 589 ± 1 ± 1 and 2 hr, respectively. Activities for both GAL and GLU then dropped rapidly to 100-200 units for the rest of the incubation. MAN and GLU activities were negatively correlated (r = -.44 and -.36) with the AR rate (ARR), as defined as the percent sperm undergoing the AR each hourly interval of the incubation. AGAL showed peak activity (488 ± 290) at 3 hr, while AGLU alternately fluctuated between 50 and 230 units over the incubation. GAL, AGAL and AGLU lacked correlation with ARR (r = -.07, .02 and -.1). We conclude that changes in glycosidase activities occur during capacitiation. These may be important in modifying carbohydrate moieties of the sperm and egg in preparation for fertilization.

100 EPIDEMIOLOGICAL COMPARISONS BETWEEN THE BOAR AND HUMAN PROACROSIN-ACROSIN PROTEINASE SYSTEMS. Mark S. Siegel*, Dana S. Bohm*, Janet L. Willand* and Kenneth L. Polakowski*, Washington University Medical School, St. Louis, MO 63110.

Proacrosin is believed to be a critical component of nature sperm that is required for fertilization. Proacrosin derived from boar spermatozoa has been utilized as a model for the majority of detailed studies involving this important sperm antigen. We have purified boar and human proacrosin and have now developed polyclonal antibodies to these antigens. The present studies were undertaken to compare the immunological properties of the proacrosin from these two species. Following western-blotting, it was shown that the antibodies produced against boar proacrosin were specific only to the known components of the proacrosin-acrosin system. These antibodies reacted with boar proacrosin, alpha-acrosin and to a lesser extent beta-acrosin. However, the boar proacrosin antibodies did not cross-react with any antigens in the human sperm extracts. The antibodies to human proacrosin reacted with human proacrosin and alpha-acrosin but apparently not with the beta-acrosin. Human proacrosin antibodies also cross-reacted with the boar proacrosin and to a lesser extent with the boar alpha-acrosin but not with the boar beta-acrosin. Thus, in spite of biochemical similarities (molecular weights and activation kinetics) between the human and boar proacrosin-acrosin systems distinct immunological differences exist. (Supported by NIH grant HD 12863.)
101 INHIBITORY EFFECT OF ZINC ON PROTEIN PHOSPHORYLATION IN THE SPERM HEAD MEMBRANES IN SPISULA CODIEDISSINA.
Balwant Ahluwalia and George Holan*, Department of Obstetrics and Gynecology, Howard University College of Medicine, Washington, D.C. 20059

Zn$^{2+}$ when added in the media as low as one micromolar decreased protein phosphorylation from 70 to 75% in the isolated sperm head membranes. Other divalent cations (calcium, cobalt, barium and copper) in the similar concentration were ineffective. Sodium fluoride, a phosphatase inhibitor, did not reverse Zn$^{2+}$ induced effect on protein phosphorylation, and when Zn$^{2+}$ was added to the media after the phosphorylation was started, the inhibitory effect was lost suggesting that phosphatase are not involved. The autoradiogram of $^{32}$P labelled sperm head membranes separated on SDS-polyacrylamide gel electrophoresis demonstrated lack of protein phosphorylation in the presence of Zn$^{2+}$. The inhibitory effect of Zn$^{2+}$ on protein phosphorylation in the sperm head membranes suggest physiological role of this cation in the membrane functions.

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102 OXYGEN CONSUMPTION RATE VARIES WITH THE CONCENTRATION OF BULL SPERMATOZOA. Carole Wegner*, Gary Killian and David Chapman*, Dairy Breeding Research Center, University Park, PA 16802.

The effect of sperm concentration on the rate of oxygen consumption (ml O$_2$/hr·10$^8$ cells) was evaluated. Suspensions of 2X washed ejaculated sperm were prepared over a concentration range of 70-575 x 10$^6$ sperm/ml by sequential dilution of the highest concentration with 305 mOsm buffer (Brackett & Oliphant 1975, Biol. Reprod. 25:985). O$_2$ consumption was measured with a Clark electrode at 36° C on 3 ml of suspension. Although initial values for O$_2$ consumption were variable among ejaculates (10.6-22.0), O$_2$ consumption and sperm concentration were highly correlated ($r=0.83$) for all 3 replicates. Similar effects of sperm concentration on O$_2$ consumption were observed for rabbit and bull sperm incubated at 27° C. Another experiment was performed to determine the possibility that the time required to dilute sperm, negatively affected the O$_2$ consumption of sperm at low concentrations. When sperm oxygen consumption was measured for sperm at lower concentrations first, O$_2$ consumption increased with increasing sperm concentration ($r=0.87$), as was observed in the original study. A third study was conducted to determine if a factor released into the supernatant by sperm at high concentration stimulated oxygen consumption. When supernatants of sperm at high concentration were used to dilute sperm to lower concentrations, oxygen consumption rates remained high. For 6 replicates, oxygen consumption and sperm concentration were negatively or not correlated ($r=-0.64$ to $r=0.26$). These studies suggest that sperm at high concentration release a factor into the environment which stimulates O$_2$ consumption. (Supported by HD20272)


We are studying sperm transport in Nasacaca fasciolaris, using both surgical and non-surgical collection techniques. The menstrual cycle and mucus-laden cervix of this species suggest its use in modeling events in gamete biology in the human. Sperm numbers and motion are quantitated using videomicrography. Initially, we compared sperm collected 5 hr and 30 hr after mating (pc). Large numbers of vigorous, motile sperm are retained by the cervix (similar to the human), although there are twice as many total and motile sperm at the earlier time. Sperm motility is also nearly twice as vigorous, and is more homogeneous at 5 hr pc. At 30 hr pc over half the cervical sperm exhibit intermittent velocities, with increased trajectory curvature and lateral motions of the sperm head. Over 90% of uterine sperm swim rapidly along straight trajectories at both time points. A small fraction exhibit alternating episodes of straight and tortuous, non-progressive swimming; this pattern resembles the hyperactivated motility of other mammals which is associated with capacitation. Thus, capacitation in the cynomolgus monkey may begin in the lower female tract. (Supported by NIH grants HD012791 and RRO0169.)

104 PERSISTENT LUTEINIZING HORMONE-TREATMENT IN MALE PATIENTS WITH SEVERE OLGOSPERMIA AND SELECTIVELY ELEVATED FSH.
M. Aulitzky, J. Frick, F. Hadziselimovic*, Department of Urology, General Hospital, Salzburg, Austria and Children's Hospital, Basel, Switzerland.

Evidence was presented, that severe oligospermia with high FSH and normal LH-levels may be caused by hypothalamic dysregulation. It was further demonstrated that restoration of physiological LH-pulse frequency by long term pulsatile LHRH-application led to a marked decrease of elevated FSH-levels. The aim of this study was to confirm these preliminary data and to investigate the effect on the spermatogenesis. 15 male patients with severe oligospermia, high FSH-levels and normal LH-levels were treated by pulsatile LHRH for 6 months. All showed disturbed LH-pulse pattern (<10 peaks/24hrs.). According to semen analysis and spermatogonia/tubule ratio (SPT-ratio) 3 groups of patients were created. Group I (n=5), sperm density >1 mil, SPT-ratio <0.1, Group II (n=5), 1-10 mil sperm/ml, SPT-ratio 0.2-0.6, Group III (n=5), >10 mil sperm/ml, SPT-ratio >1.0. Normalisation of LH/FSH secretion was seen in all pat. of group II and III and in 3/5 pat. of group I during LHRH therapy. The transient effect on LH/FSH secretion supports the hypothesis that the hormonal dysregulation is of hypothalamic origin. Improvement of sperm density was observed in 9/10 pat. of groups II and III, whereas no changes were found in group I. These findings correlate with the SPT-ratio. Therefore we conclude that SPT-ratio is a useful method for patients selection for pulsatile LHRH therapy and can be used as prognostic parameter.

Treatment with DES causes the development of prolactin (PRL)-producing adenomas, and hyperprolactinemia is known to have dramatic effects on testicular function. However, there is also evidence for independent effects of estrogens on Leydig cell function. Therefore, the present study was undertaken to analyze the different effects of DES on the testes during and after treatment with the steroid. Fisher 344 rats (8 weeks old) were implanted 14 weeks with DES or empty silastic capsules, which were removed 7 weeks before sacrifice. A second group of rats received DES capsules 7 weeks before sacrifice. DES caused a dramatic decrease in body, testes and seminal vesicle weights. Removal of DES capsules allowed a partial recovery of these weights. Treatment with DES also produced a great decrease in plasma LH. DES treatment caused a decrease in LH receptor content without affecting LH receptor concentration. However, removal of DES capsules allowed LH receptor concentration and content to increase well above the levels measured in untreated rats. The present results thus indicate that DES by regulating LH receptors independently from PRL and LH, can counteract the hyperprolactinemia-induced increase in LH receptor levels.

107 Leydig Cell Function in the Cryptorchid Rat. Tom O. Abney, Department of Physiology and Endocrinology, Medical College of GA, Augusta, GA 30912-3395.

Adult male rats were rendered bilaterally (bi) and unilaterally (uni) cryptorchid. At 14 and 28 days post-surgery, purified Leydig cells (LC), designated populations I and II LC were isolated from extrascrotal gradients. Gonadotropin binding and steroidogenic capacity were assessed in vitro.

Testis interstitial cell numbers (10^6/testis) were decreased at 14 and 28 days in the bi and uni groups, compared to intact and eutopic (uni-acrotal) controls. Cell numbers (10^6/testis) within each LC pop were statistically equivalent for all groups at 14 days; LC pop II from the 28 day uni group was decreased (p<0.05). Binding of 125I-E2 (pmol/testis) was equivalent in pop I and II LC for all groups at 14 days; LC pop II from the 28 day bi and uni groups exhibited decreased (p<0.05) binding.

Testosterone (T) production in response to hCG (0-200 mU) and dexamethasone (0-5 mg) was decreased (p<0.05) in bi and uni pop I and II LC at 14 days, compared to controls. At 28 days, uni pop I and II exhibited decreased (p<0.05) in T production, however, eutopic and bi LC exhibited increased T production above controls. These data suggest that LC from the uni group exhibited the most dramatic crypt-induced alterations in cell function, particularly at 28 days. Supported by NIH grant #18983.
109 GnRH INCREASES SPECIFIC PRECOPULATORY RESPONSES IN TESTOSTERONE-TREATED PONY GELDINGS.

Nancy Diehl,1 Sue McDonnell,2 and Marolo Garcia1

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The effects of exogenous GnRH were studied in 12 long-term castrated male ponies (geldings) with and without androgen replacement. Based on performance in pre-treatment sexual behavior tests, ponies were assigned as rank matched sets to four treatment groups. GnRH alone (G), testosterone and GnRH (TG), testosterone alone (T), and no treatment (C). Testosterone treatment consisted of 200 μg testosterone propionate in sesame oil SQ every 48 hr for three weeks. GnRH treatment consisted of 25 μg Cystorelin (CEVA laboratories) SQ every 3 hr for three weeks. All animals received treatments or equivalent volume vehicle on the same injection schedule. Sexual behavior tests consisted of a 4-minute exposure to an ovariec tomized estrogen-treated pony mare restrained behind a teasing rail on one side of a small (3.05m X 3.05m) outdoor enclosure. Tests were conducted three times weekly between 0730 and 0930 for two weeks before the start of treatment, three weeks during treatment, and for two weeks after treatments had stopped. The following behavioral measures were recorded: sniff mare, sniff ground, jejunum response, lick, bite, strike, vocalization, and roll frequencies; penis drop latency and duration; erection latency, frequency and duration; mount latency, frequency, and duration; and attention latency and duration. During treatment TG ponies exhibited greater (p<0.05) jejunum response and sniff ground frequencies. Attention duration and bite frequency were also greater (p<0.05) among TG ponies. These findings are consistent with our clinical observation of jejunum response in stallions following intravenous injection of GnRH. These results suggest a testosterone-dependent effect of GnRH on certain precopulatory investigatory responses in stallions, and support the hypothesis that GnRH is involved in the regulation of sexual behavior.


Male Lewis rats were randomly divided into seven groups (n=5). Using microdissection techniques, accessory glands were removed from each group as follows: seminal vesicles, coagulating glands, dorsal prostate, ventral prostate, entire prostate, all accessory glands and sham-operated controls.

After a minimum of four weeks, each male was placed for one week in a separate cage with two females of proven fertility. Two weeks after the mating study the females were killed and the uterine implantation sites were counted. Animals whose seminal vesicles, dorsal prostate, entire prostate and all accessory glands were ablated failed to fertilize any ova in every case. Most of those whose coagulating glands and ventral prostates were ablated did fertilize ova as did the shams.

To prove this "selective infertility" was not secondary to impotence caused by the surgery, four rats from the seminal vesicle, dorsal prostate and entire prostate ablation groups were placed with two females for one week. Intravaginal washings were performed on each female every day for seven days. Sperm were found in at least one female from each individual male during the study, thus proving that each male was potent. It is suggested that each accessory gland makes a contribution to the fertility of the subject and that future studies attempt to identify these factors.

111 PROTEIN CONTRIBUTIONS OF THE ACCESSORY ORGANS TO THE COMPOSITION OF HUMAN SEMINAL PLASMA AS DETERMINED BY HIGH RESOLUTION TWO-DIMENSIONAL ELECTROPHORESIS. Edward E. Gaunt, Anibal A. Acosta, Steven B. Ackerman, Patricia A. Pleban1, John F. Stecker, and James H. Yuan. Department of Chemical Sciences, Old Dominion University, Norfolk, Va. 23508.

Human Seminal Plasma (HSP) was evaluated using high resolution two-dimensional electrophoresis (HR2DE). HSP from healthy volunteers with recently proven fertility were characterized using this technique to establish "normal" protein distribution patterns in the 2-D slab gel. Time course studies were performed on selected specimens to determine the effects of liquefaction on HSP protein composition within the first several hours after collection. A series of "coagulation" proteins were identified which disappeared with time. Few changes in protein composition were seen after 60-90 minutes post ejaculation.

Split ejaculates were collected (up to 6 fractions) to identify which protein patterns were contributed by the various accessory glands. Initial fractions correlated well with pure seminal vesicle fluid.Terminal fractions represented pure seminal vesicle fluid. HR2DE of macroscopic gel globules (globoid bodies) separated from whole semen gave a protein pattern similar to terminal fractions of the split ejaculate. Pre-ejaculatory fluids (urethral gland secretions) were evaluated by this technique as well.

112 CHARACTERISTICS OF THE ACCESSORY GLANDS OF THE MALE REPRODUCTIVE TRACT OF SOME NATIVE CALIFORNIA RODENTS. B. Peitz and S. Hembren*, Biology Department, California State University at Los Angeles, LA, CA 90032.

The reproductive tracts of males from the genus Peromyscus and Dipodomys were examined and compared to those of several laboratory rodents. Animals were collected by overnight trapping at several field sites, sexed in the field and males were brought to the laboratory. Weights of the accessory sex glands (ASG), their fructose content (Foreman, et al., 1973 Anal. Biochen. 56: 584), and ability of extracts of ASG to cross coagulate when mixed with extracts of other ASG (Peitz, et al., 1979 J Reprod. Fert. 57:183) were determined. Weights of the ASG are higher in P. maniculatus than in P. truei. In both species the coagulating glands (CG) had the highest fructose concentration. D. agilis had heavier ASG than D. merriami, but they contained less fructose. In D. agilis, fructose concentration was highest in the CG, whereas in D. merriami, the highest concentration was found in the ampulla glands. When ASG extracts of a single species were tested for coagulation with each other the P. maniculatus extracts of all ASG coagulated to some extent when mixed with extracts of the CG, whereas, in D. merriami CV extracts reacted with the CG much better than any of the other glands. Coagulation tests were performed between extracts of ASG of P. maniculatus and D. merriami and the rat and house mouse to determine which glands are involved in the formation of the vaginal plug. In these tests between species the CG of P. maniculatus and D. merriami coagulated more quickly than their SV. These studies indicate that the ASG of Peromyscus species are more similar to the Murid rodents than Dipodomys species.
A RAT MODEL OF FETAL EPIDIDYMAL AND GONADAL DEVELOPMENT AND ITS SIMILARITY TO CLINICAL ABNORMALITIES. J. L. Pourcroy, UMoshi-RIJK, New Brunswick, NJ 08903; G. C. Shen, Naval Hospital, Portsmouth, VA 23708; G. P. Riordan, Nava l Hospital, Bethesda, MD 20884 and D. W. Warren, University of Southern California, Los Angeles, CA 90033.

Testicular cell populations are of multiple origins; normal gonadal development and function depends on the interactions of these populations some thought to be of neomorph-cesenchymal origin. The ACI rat (August x Copenhagen) provides an interesting model of the dependency on the neomorph anlage for normal development with a 10-15% incidence of agenesis of the neomorph duct and its derivatives. The agenesis is noted predominantly on the right side with occasional contralateral renal abnormalities. The testes on the affected side are markedly smaller by puberty.

We have reviewed the light and electron microscopy of normal and abnormal late-gestation fetuses. The defect is seen prior to androgen secretion (15 days) and appears to coincide with vulnerable periods of definitive umbilical vascular reorganization. Altered intratesticular components are observed in the affected gonad.

Clinically, patients with agenesis of vas deferens display similarity to the rat model but unlike the rat, maintain normal spermatogenesis. The atrophy in the rat may reflect an earlier developmental insult blocking migration or mitosis of critical cellular elements.

114 CLONING AND SEQUENCE ANALYSIS OF HUMAN TESTIS-SPECIFIC LACTATE DEHYDROGENASE C4.

Erwin Goldberg, Catherine E. Driscoll*, and Jose L. Millan*, Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60201; La Jolla Cancer Research Foundation, La Jolla, CA 92037.

The testis specific lactate dehydrogenase isozyme of *Homo sapiens* constitutes one of the most dramatic examples of the temporal and spatial specificity of gene function. Of special interest is the close association of LDH-C4 with spermatogenesis. This isozyme first appears in mid-pachytenic primary spermatocytes and increases in concentration until the spermatid stage. As a first step towards investigating regulation of gene function in the germinal epithelium we have constructed and probed a human testes cDNA expression library. A complementary DNA (cDNA) clone comprising the complete coding region of human testis-specific LDH-C4 (LDH-X) has been isolated and sequenced. After nick translation the cDNA detected a single RNA species of 1.5 kb from testes but not placenta. The cDNA begins with 66 bp of 5' untranslated region and ends with 117 bp of 3' flanking sequence. The coding region comprises 987 bp that code for 329 amino acids. The complete amino acid sequence deduced from the nucleotide sequence has 74% homology with the somatic lactate dehydrogenases. The cDNA probe will be useful to identify and isolate the genomic DNA in order to investigate regulation of the LDH-C4 gene. In view of the relationship between LDH-C4 and spermatogenesis these studies may contribute to our understanding of this process at the molecular level. Supported by grants NIH CA40947, NIH CA42595, NIH HD05893, and by the Program for Applied Research on Fertility Regulation, Northwestern University (AID/DPE-0546-A-00-1003).

115 EVIDENCE FOR POLYMORPHIC SPERM ANTIGEN EXPRESSION IN INBRED MICE. Chong Xu*, Deborah J. Anderson, Fearing Research Laboratory, Department of Obstetrics and Gynecology, Harvard Medical School, Boston, MA 02115.

It has been known for some time that T-1 complex gene products affect fertility in male mice. Furthermore, it was recently reported that allogeneic mouse sperm is more immunogenic than syngeneic sperm, and that certain infertile men have sperm that is more immunogenic and antigenic than sperm of fertile donors. These data suggest that there may be genetic differences in sperm antigen expression that affect fertility. To map sperm antigenic differences in mice of different inbred strains, 8-week old virgin females Balb/c mice were immunized with epididymal sperm from the following inbred strains: Balb/c, DBA/2, C3H/6 AJ and 129. Immunizations consisted of 2 x 10^6 sperm in Complete Freund's Adjuvant injected subcutaneously, followed at 21 day intervals by injection of 2 x 10^7 sperm in Incomplete Adjuvant. Sera obtained seven days following final injection were applied to epididymal sperm extracts from the various inbred strains using the Western Blot procedure. Forty-seven major protein bands and no differences were revealed by Coomasie Blue stain following electrophoretic separation of each of the sperm extracts. However, Western Blot analysis indicated that certain major sperm membrane components show strain-dependent differences in immunogenicity and antigenicity. These data provide evidence for the existence of polymorphic antigens on sperm.


A new reversible male contraceptive device (SHUG) made primarily of silicone is being developed. Previous studies demonstrated its ability to block the flow of spermatooza when placed in the monkey vas deferens. The silicone material must be tested for chronic target organ toxicity prior to clinical trials. Two hundred rats were divided into two groups: one group had sham operations and the other group had silicone noodles implanted in the vas deferens and secured by a suture. At six months and one year after implantation animals were sacrificed and the vas deferens removed, fixed, and histologically evaluated. Changes in the vas deferens attributed to the presence of the silicone were minimal-to-mild reactive hyperplasia in the proximal, surgical and distal segments of a few animals. An increased incidence of spermatocytes, aspermatic granulomas and chronic inflammatory changes within the inner walls of the vas deferens were also observed. These changes in the vas deferens are considered of minor toxicological importance. Supported by the Program for Applied Research on Fertility Regulation (Agency for International Development, PARFR 339).
117 Androgen movement into the rat seminiferous and epididymal tubules perfused in vivo. T. T. Turner, Univ. of Virginia School of Medicine, Charlottesville, VA 22901.

Prolinal, net transport of 3H-testosterone (3H-T) from peritubular to intratubular fluids of the adult rat testis and epididymis was studied by in vivo perfusion and subsequent micropuncture of seminiferous tubules (Sertoli's) and caput and cauda epididymal tubules (CPT's and CDT's, respectively). Tubules were perfused with KRP containing 13 uCi/ml 3H-T (30 Ci/mM). 14C-polycholene glycol (14C-PEG) was included as a dilution marker (0.7 uCi/ml) and a control for contamination of intraluminal fluid. Some experiments involved 15N-3H NPN or unlabelled T at 10x or 100x the concentration of 3H-T which was included in the perfusion fluid. Radioactivity of 3H-T and 14C-PEG in these fluids was determined over 1 and 2 hrs and the percentage of peritubular 3H-T appearing in intraluminal fluid was determined. Net entry of isotope into the Sertoli's was low (<15%), but in the epididymis there was a large prostatic movement of isotope against a concentration gradient. The 1 and 2 hr values for the CPT's were 303.9±6.2% and 222.0±6.1%, respectively, and for CDT's were 180.7±1.8 and 183.4±5.0, respectively. The uptake rate into CPT's was significantly and progressively inhibited by 10x and 100x increases of unlabelled T in the perfusion fluid, but not by NPN. Also, in vitro perfusion of epididymal tubules with NPN removed native human fluid and significantly reduced 3H-T uptake. Steady-state binding of 3H-T to electrophoresed epididymal proteins demonstrated a single androgen binding peak in CPT and CDT, but in the caput, a second binding site was identified. PAGE analysis of radiolabeled proteins in apical and basal chambers revealed that the difference in apical-basal secretion in the 30-55kD range were secreted only into the apical chamber. There was also qualitative as well as quantitative. Proteins of 25kD and 68kD were secreted apically and basally from monolayers of both caput-corpus and cauda cells. There were also differences between cells from the two regions of the epididymis with a protein of 35kD being secreted only by cells from the caput-corpus and a protein of 40kD secreted predominantly by cells from the cauda. (Supported by NIH HD 09490.)

118 Protein secretion by rat epididymal epithelial cells growing in bicaleral culture chambers. S.W. Byers. Department of Anatomy and Cell Biology, Georgetown University Medical-Dental School, Washington D.C.

Epididymal epithelial cells growing on extracellular matrix coated filters in bicaleral culture chambers form confluent polarized monolayers (Byers et al., J.Androl. 7, 59-1986). In the present study 3H-leucine incorporation into cellular and secreted proteins was investigated. Epithelial cells isolated from 30 day old rats were grown for 3 days in serum free defined medium (SDFM). After an overnight incubation in leucine-free medium the cells were exposed to 3H-leucine (30uCi/ml) in SDFM for 6 hours. Total TCA precipitable material from the apical medium, the basal medium, and the cell layer was collected. Cells isolated from both caput-corpus and cauda epididymis secreted 50% of total synthesized protein with an apical:basal ratio of 2.1 and 3.1 respectively. Sub-confluent monolayers or monolayers disrupted by scraping did not secrete proteins in a polarized fashion. SDS-PAGE analysis of radiolabeled proteins in apical and basal medium revealed that the difference in apical-basal secretion was qualitative as well as quantitative. Proteins of 25kD and 68kD were secreted apically and basally from monolayers of both caput-corpus and cauda cells. In contrast, a number of proteins in the 30-55kD range were secreted only into the apical chamber. In particular, a prominent protein of 32kD was secreted apically from both caput-corpus and cauda cells. There were also differences between cells from the two regions of the epididymis with a protein of 35kD being secreted only by cells from the caput-corpus and a protein of 40kD secreted predominantly by cells from the cauda. (Supported by NIH HD 09490.)

119 Nucleation-related glycoproteins in the mouse epididymis. S.W. Byers. Department of Anatomy and Cell Biology, Georgetown University Medical-Dental School, Washington D.C.

Steady-state binding of 3H-T to electrophoresed epididymal proteins demonstrated a single androgen binding peak in CPT and CDT, but in the caput, a second binding site was identified. PAGE analysis of radiolabeled proteins in apical and basal chambers revealed that the difference in apical-basal secretion in the 30-55kD range were secreted only into the apical chamber. There was also qualitative as well as quantitative. Proteins of 25kD and 68kD were secreted apically and basally from monolayers of both caput-corpus and cauda cells. There were also differences between cells from the two regions of the epididymis with a protein of 35kD being secreted only by cells from the caput-corpus and a protein of 40kD secreted predominantly by cells from the cauda. (Supported by NIH HD 09490.)
121 THE ROLE OF SUPEROXIDE DISMUTASE IN PREVENTING LIPID PEROXIDATION AND LOSS OF MOTILITY IN HUMAN EJACULATED SPERMATOZOA. Juan G. Alavés,* J.C. Touchstone,* Luis Blasco, and Bayard T. Storey, Departments of Physiology and Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA 19104.

Superoxide dismutase (SOD) and glutathione peroxidase/reductase (GP/R) are most cells' major enzymatic defenses against lipid peroxidation. The function of these defenses in human sperm was examined. Five replicate samples from each of 8 normal donors were assayed for SOD and GP/R activity and for spontaneous lipid peroxidation, as measured by malondialdehyde (MA) production and loss of motility during aerobic incubation at 37°C. SOD activity in fresh sperm ranged between 1 and 10 units/10⁸ cells with a mean value of 5.5 ± 2.2 (X ± SD, n = 40). The concentration of MA at the point of complete loss of motility, defined as the lipoperoxidative lethal endpoint (LLE), was 0.10 ± 0.03 nmol/M cells (X ± SD, n = 40). Despite the variability in the time (tₜ) for complete loss of motility (range: 1-10 h), the LLE remained constant. When the rates of MA production for each individual sample, as defined by (LLE)/tₜ, were compared to the activity of SOD in the fresh samples, a linear correlation was obtained (r = 0.97). GP/R activities were 21 ± 3 and 44 ± 3 nmol/min·10⁸ cells respectively (X ± SD, h = 40) and showed no correlation with the rate of MA production in individual samples. These findings suggest that SOD rather than GP/R plays the major role in protecting human sperm from O₂ toxicity due to lipid peroxidation. Supported by NIH grants HD-15842, HL-07027, and HL-19737.

122 PATENCY AND PREGNANCY RATES FOLLOWING MICROSURGICAL EPIDIDYMOSPOSOSTOMY FOR EPIDIDYMOS- VASAL OBSTRUCTION. Charles O. Turk*, and Lawrence S. Ross, Michael Reese Hospital and Medical Center, Chicago, IL 60616

Fourteen azoospermic males with epididymal obstruction underwent microsurgical epididymosposostomy. The surgical technique used was approximation of a single tubule of epididymis to the proximal vas. End to side anastomosis was performed with single layer 10-0 monofilament suture. Patency and pregnancy rates for the entire group were 71% and 29% respectively. An additional 20% had a normal postoperative semen analysis with no pregnancy. The surgical procedure is described in detail and results are subgrouped by etiology of obstruction as either primary congenital or secondary to previous infection. All patients having epididymosposostomy for previous vasectomy were excluded.

123 RESULTS OF SPECIFIC TUBULE MICROSURGICAL VASOEPIDIDYMOS­ TOMY. Sherman J. Silber, M.D., St. Luke's Hospital & St. John’s Mercy Medical Center, St. Louis, MO 63107.

A total of 341 patients underwent bilateral microsurgical vasoepididymostomy with the specific tubule method originally described by the author. Twelve percent (77) required an anastomosis to the caput region because of extensive disease distal to that site. Reliable followup for greater than three years was obtainable on 2/3 of those patients. "Patency" of the anastomosis was achieved in 81% of the patients undergoing anastomosis to the caput. Forty-five percent of the patients with anastomosis to the caput had a sperm count over 20,000,000 per cc with directional motility over 50%. Fifty-two percent had good directional motility but none of those had low total numbers of sperm. The wives of 26% of men with anastomosis to the caput epididymis achieved a normal full-term pregnancy (approximately 1/2 of those with normal semen). Patients undergoing anastomosis to the corpus epididymis had a 91% "patency" rate. Seventy-eight percent had good motility, and 61% achieved pregnancy. Patients undergoing vasovasostomy (epididymis presumably intact) had a 98% patency rate, and an 83% pregnancy rate. In most of the non-pregnancies with vasovasostomy (or vasopseudomethod to the corpus) and normal semen parameters, the wife was found to have either ovarioly dysfunction or tubal disease. This was not the case with caput epididymis anastomosis. In conclusion, good success rates were achievable with microsurgical vasoepididymostomy. The site of obstruction influenced the outcome. Metabolic microsurgical technique was important for achieving a high success rate.

124 PREGNANCY AFTER VASOVASOSTOMY. Sherman J. Silber, M.D., St. Luke's Hospital & St. John's Mercy Medical Center, St. Louis, MO 63107.

This paper represents a 10 year study on our first 199 patients who underwent vasovasostomy for reversal of vasectomy. Fifteen (8%) patients developed early marital difficulties, became separated or divorced, and were excluded. One hundred fifty-three (83%) of the remaining 184 eventually impregnated their wives. Patients with no sperm in vas are excluded, the adjusted pregnancy rate in a group of patients requiring simply vasovasostomy (i.e. epididymis intact) would be 8%. Twenty-five of the 184 patients had varicoceles (14%). There was no significant difference in pregnancy rate in patients who had varicoceles versus those who did not. Sperm antibody titers were not associated with success or failure. Serum immunoassay antibody titers were elevated in 25% of those who impregnated their wives, and 22% of the overall group. Serum agglutinating antibody titers were elevated in 40% of those who impregnated their wives and in 42% of the overall group. Detailed quantitative testicular biopsy analysis showed no testicular defect either in successful or unsuccessful patients. We conclude that microsurgical vasovasostomy has a high rate of anatomic success and also a high pregnancy rate. Pregnancy is not influenced by sperm antibody titers, varicoceles, or previous failure at surgery, and is only moderately affected by sperm count, except at very low levels. However epididymal blockout with secondary epididymal obstruction is a major cause of failure if vasovasostomy is not performed in appropriate cases.
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