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INNOVATIONS TO RELY ON.
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<tr>
<td>8:00 A.M.</td>
<td>POSTGRADUATE COURSE (Le Galeria 4 and 5) Mammalian Fertilization</td>
<td>8:00 A.M.</td>
<td>Opening and Welcome (Rooms E-H)</td>
<td>6:00 A.M.</td>
<td>Past Presidents' Breakfast (The Napolean Suite)</td>
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<td>Serono Lecture Frank S. French (Rooms E-H)</td>
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<td>Basic Science Symposium</td>
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<td>11:30</td>
<td>DISCUSSION</td>
<td>12:00</td>
<td>LUNCH (The Bissonet)</td>
<td>10:00</td>
<td>The Conceptual Basis of Prospective Male Contraceptives (Rooms E-H)</td>
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<td>12:00</td>
<td>LUNCH NOON</td>
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<td>Treatment of Male Infertility</td>
<td>10:30</td>
<td>COFFEE BREAK (The Bissonet)</td>
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<td>DISCUSSION</td>
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<td>6:00</td>
<td>STUDENT SOIREE (Le Galeria 3)</td>
<td>9:15</td>
<td>COFFEE AND TEA (The Bissonet)</td>
<td>4:00</td>
<td>Workshop on Sperm Cryopreservation (Rooms E-H)</td>
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<tr>
<td>7:00</td>
<td>OPENING RECEPTION (Le Galeria 1 and 2)</td>
<td>12:00</td>
<td>LUNCH (The Bissonet)</td>
<td>12:30 P.M.</td>
<td>Scientific Papers Male Infertility</td>
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<td>3:30</td>
<td>COFFEE BREAK (The Bissonet)</td>
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<td>Scientific Papers #19-58 (The Bissonet)</td>
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<td>3:30</td>
<td>Scientific Posters #73-135 (The Bissonet)</td>
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<td>Scientific Posters #19-58 (The Bissonet)</td>
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<td>4:00</td>
<td>COFFEE BREAK (The Bissonet)</td>
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PRESIDENTIAL MESSAGE

This has been a busy and productive year for our active and growing society. Robert A. Schmidt has completed his first year as Business Manager; he has had a significant impact on the efficiency with which Society business is executed. The Publication Committee selected a new editor and negotiated a new contract with J.B. Lippincott for publication of the Journal of Andrology. The Finance Committee is recommending change in several bylaws; you will be asked to vote on these changes at this meeting. We offer special commendations to Marie-Claire Orgebin-Crist, Editor-in-Chief of the Journal, her Assistant Editor, Benjamin Danzo, and Associate Clinical Editor, Spyros Pavlou. As they turn over their jobs to the new editorial staff, we acknowledge their tremendous influence on improving the quality and recognition of the journal. The outstanding postgraduate course, scientific program, and local arrangements were possible because of the efforts of Stuart S. Howards, Johannes Veldhuis, and Richard Harrison, who respectively chaired the committees arranging these activities. Welcome to New Orleans!

C. Wayne Bardin
President, American Society of Andrology

AMERICAN SOCIETY OF ANDROLOGY
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Constitution and Bylaws ............................... Terry R. Brown
Finance .................................................. Ronald S. Swerdloff
Future Meetings ........................................ Juraj Osterman
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Program .................................................. Johannes D. Veldhuis
Publication ............................................. David W. Hamilton
Student Affairs ......................................... Gail Prins
Editor, Journal of Andrology ........................ Marie-Claire Orgebin-Crist
PROGRAM CHAIRMAN

Welcome all members, supporters, and guests of the American Society of Andrology! The 14th Annual Meeting embraces an exciting range and depth of scientific presentations. Plenary speakers of international reputation will review both clinical and basic topics in male reproduction, contraception, cell biology, and in vitro fertilization. This year’s program is selected from a large number of abstracts and offers excellent quality in free communications in the form of slide and poster presentations. The Postgraduate course will focus on mammalian fertilization and male infertility. The local arrangements committee has provided hospitable surroundings for all activities. Consequently, we anticipate a rewarding, genial and intellectually stimulating 1989 Annual Meeting.

Johannes D. Veldhuis, M.D.
Program Chairman
GENERAL INFORMATION

Headquarters
Marriott Medical Center
(504) 581-1000
Canal and Chartres Streets
New Orleans, LA 70140

On-site Registration
Acadia Foyer
7:00 A.M. - 8:00 P.M.
April 13-15
7:00 A.M. to noon
April 16

Annual Business Meeting
Sunday, April 16 (12:00 noon)

Transportation
Local taxi and limousine service available to hotels

Registration Fees
Postgraduate Course
Without CME credit $125
With CME credit 150
Students (w/o lunch) 30

Annual Meeting
Non-member 95*
Regular member 80*
Student member 40*
Student non-member 50*


The Press Room—Galvez 3 Room
The Slide Preview Room—Jackson Room

Sponsors and Supporters of the 1989 Annual Meeting
Delta Regional Primate Research Center
Tulane University Medical School
Serono Laboratories, Inc.
University of Virginia Health Sciences Center

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 and Andrology, Inc.
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West Michigan Reproductive Institute
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Sponsors and Supporters of the Workshop on Sperm Cryopreservation
California Cryobank
Los Angeles, CA
Fertility Institute of New Orleans
New Orleans, LA

I.M.V. International Corporation
Minneapolis, MN
T.S. Scientific
Perkasie, PA
Newton Andrology & Cryopresentation Center
Newton, MA
C. Alvin Paulsen, M.D., Professor of Medicine at the University of Washington School of Medicine (Seattle), received his B.A. and M.D. (1952) at the University of Oregon. His house staff training and subsequent USPHS Research Fellowship (1953-55) was at Wayne State University, where he later joined the faculty. He moved to the University of Washington in 1958. Throughout his research career Dr. Paulsen has concentrated on studies of the male reproductive system. His major research contributions include the recognition of sex chromosomal mosaicism in patients with Klinefelter's Syndrome and the appreciation that mosaicism can modify clinical and pathologic features in these patients; the delineation of the clinical characteristics of patients with hypogonadotropic eunuchoidism and description of the conditions of their treatment, and the documentation of the cause and effect relationship between varicocele and abnormal spermatogenesis with infertility. Dr. Paulsen has published over 140 articles, book chapters, and review articles. He has served on advisory boards for NIH and on the Male Task Force for the WHO. He is Past President of the Pacific Coast Fertility Society, the American Society of Andrology, and the American Fertility Society. Dr. Paulsen has also devoted himself to training new investigators; a total of 44 fellows have worked in his laboratories and clinics.

Distinguished Andrologists

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

Award

Barry T. Hinton, Ph.D., Associate Professor of Anatomy & Cell Biology, University of Virginia, was educated in England. He was trained in micropuncture and microanalytic techniques at the Max Planck Institute for Biophysics, Frankfurt, West Germany, and then received his Ph.D. at the Institute of Animal Physiology, Cambridge, U.K. under the supervision of Prof. B. P. Setchell. Dr. Hinton has made a significant contribution towards understanding the mechanisms that control the testicular and epididymal luminal microenvironment, and the role played by this microenvironment in sperm maturation. Dr. Hinton has also been an active participant in the American Society of Andrology, where he has served on a number of committees. He was Chairperson of the Student Affairs Committee and was recently elected to the Executive Council. For these significant contributions, Dr. Hinton has been selected to receive the 1989 Young Andrologist Award.

YOUNG ANDROLOGISTS

1982 L.J.D. Zaneveld, D.V.M., Ph.D.
1983 William B. Neaves, Ph.D.
1984 Lonnie D. Russell, Ph.D.
1985 Bruce D. Schanbacher, Ph.D.
1986 Stephen J. Winters, M.D.
1987 Ilpo T. Huhtaniemi, M.D., Ph.D.
1988 Larry Johnson, Ph.D.

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

NEW INVESTIGATOR AWARD

Previous Recipients
1983 Thomas T. Tarter
1984 Peter S. Albertson
1985 Randall S. Zane
1986 Mark A. Hadley
1987 Peter Grosser
1988 Stuart E. Ravnik
1989 Will be announced at the Awards Ceremony

Sponsor: West Michigan Reproductive Institute

STUDENT INFORMATION

Student Mixer ...............Thursday, April 13, 6:00 to 7:00 P.M.
Guest of Honor .............Stuart E. Ravnik, 1988 New Investigator Award Winner
Sponsor .................Student Affairs Committee

Student Colloquium ..........Friday, April 14, 12:00-1:30 P.M.
Speaker ..................Florence Haseltine, M.D., Ph.D.
Director, Center for Population Research, NIH
Topic .....................Prospects for Contraceptive Research in the 1990's
Sponsor .................Student Affairs Committee

Placement Service ..........A placement service for candidates and employers is sponsored by the Student Affairs Committee. The Placement Service Board will be near the Registration Desk. Contact: Dr. Don Cameron (813-974-2843).

Student Merit Awards ........Five awards of $100 will be presented to students on the basis of their presentations at the Annual Meeting. The winners will be selected by the Awards Committee and announced at the Awards Ceremony.
SPECIAL EVENTS

FRIDAY
8:15 A.M. **THE SERONO AWARD LECTURE** (Rooms E-H)
Molecular Biology of the Androgen Receptor
Frank S. French
University of North Carolina, Chapel Hill

Noon **STUDENT COLLOQUIUM** (La Galeria 5 and 6)

4:00 P.M. **WORKSHOP ON SPERM CRYOPRESERVATION** (Rooms E-H)
to Rupert P. Amann, Chairman
7:00 P.M. Cryobiology of Mammalian Spermatozoa—Roy H. Hammerstedt
Evaluation of Cryopreserved Spermatozoa—Paul Watson
Cryopreservation of Human Sperm and Current Success with Artificial Insemination using Cryopreserved Human Spermatozoa—John Critser
Design of Experiments Evaluating Frozen Semen—James Rosenberger
Ethics of Research using Frozen Semen—Harvey Green
Discussion—Rupert P. Amann

SATURDAY
8:00 A.M. **BASIC SCIENCE SYMPOSIUM** (Rooms E-H)
to The Conceptual Basis of Prospective Male Contraceptives
10:00 A.M. Ronald Swerdloff, Chairman
Molecular Mechanisms of GnRH Action—P. Michael Conn
Antagonadial Activity of Novel GnRH Antagonists—Spyros N. Pavlou
Role of Epididymal Factors in Male Fertility Regulation—Terry T. Turner
Regulation of Leydig Cell Function by Specific Growth Factors—Mario Ascoli

10:30 A.M. **STATE OF THE ART LECTURE** (Rooms E-H)
to *In Vitro* Fertilization: Recent Advances
11:30 A.M. Allen Trounson
Melbourne, Australia

SUNDAY
8:00 A.M. **CLINICAL ANDROLOGY SYMPOSIUM** (Rooms E-H)
to Clinical Pathophysiology of Male Hypogonadism
10:00 A.M. Philip Troen, Chairman
Assessment of The Aging Male—Howard R. Nankin
Management of Impotence—Thomas Lue
The Gonadotropin-Deficient Male—Richard J. Sherins
Germ Cell Maturation in Man—Alvin M. Matsumoto

**Opening Reception** (Le Galeria 1 and 2)
Thursday, April 13, 7:00 P.M.
This event is free to all registrants at the Annual Meeting and their guests. Hot and cold hors d’oeuvres will be served along with beverages.

**Wine and Cheese Party** (The Bissonet)
Friday, April 14, 7:00 P.M.
Scientific poster session accompanied by wine and cheese.

**Steamboat Cruise Banquet**
Saturday, April 15, 6:00 P.M. (Boarding)
Departure at 6:45. Jazz band and regional food. Limit 300 persons.

**Awards Ceremony**
Sunday, April 16, 12:00 Noon (Rooms E-H)
FOURTEENTH ANNUAL MEETING

POSTGRADUATE COURSE IN ANDROLOGY

Thursday, April 13, 1989
Le Galeria 4 and 5

MAMMALIAN FERTILIZATION

8:00 A.M.  David M. Phillips (New York)—Morphology of Fertilization
          B. Jane Rogers (Nashville)—Physiology of Fertilization
          Umbert A. Urch (Davis, CA)—Molecular Biology of Fertilization

10:00-10:20  Coffee Break

10:20  Pat Saling (Durham)—Relevance of Animal Studies to Human Fertilization
       Jon W. Gordon (New York)—Gamete Micromanipulation in Treatment of Infertility

11:30-12:00 noon  Discussion

12:00-1:15 P.M.  Luncheon

TREATMENT OF MALE INFERTILITY

1:15 P.M.  Allen Trounson (Melbourne, Australia)—Use of IVF and GIFT for the Treatment of Male Infertility
          James W. Overstreet (Davis, CA)—AID, AIH, and Sperm Processing
          Arnold M. Belker (Louisville)—Surgical Treatment of Infertility

3:10  Break

3:30  Rebecca Z. Sokol (Los Angeles)—Medical Treatment of Oligozoospermia
     Richard J. Sherins (Bethesda)—Controversies in the Treatment of Hypogonadotropic Hypogonadism

4:45-5:15  Discussion

Sponsored by the University of Virginia Health Science Center, which is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing education for physicians, and by Tulane University.

The University of Virginia Health Science Center designates the Postgraduate Course as a continuing medical education activity for 8 hours in Category 1 of the Physicians Recognition Award of the American Medical Association.
THURSDAY, April 13, 1989

8:00 A.M.- 5:00 P.M.  Postgraduate Course
6:00-7:00 P.M.  Student Soiree, Le Galeria 3
7:00-8:00 P.M.  Opening Reception for all registrants and their guests, Le Galeria 1 and 2
(Open Bar) Hors d'oeuvres will be served with beverages

FRIDAY, April 14

8:00 A.M.  Opening and Welcome (Rooms E-H)
Johannes D. Veldhuis, Program Chairman
C. Wayne Bardin, President
8:15 A.M.  The Serono Lecture (Rooms E-H)
Frank S. French
MOLECULAR BIOLOGY OF THE ANDROGEN RECEPTOR
9:15 A.M.  BREAK—Coffee and Tea (Exhibitor Area in The Bissonet)
9:30 A.M.  Scientific Papers (Slide Session) (Rooms E-H): CLINICAL ANDROLOGY AND ENDOCRINOLOGY
Chairpersons: Stephen J. Winters and Johannes D. Veldhuis
2 Alterations in the pulsatile properties of luteinizing hormone (LH) in diabetic men with organic impotence. Frederick T. Murray, James Rountree,* Marcus Sciadini,* Hedwig U. Wyss,* and Ronald G. Thomas.*
5 Regulation of glycoprotein alpha subunit secretion by a LHRH agonist and antagonist in man. Jill Lindner,* and Spyros N. Pavlou.
6 Altered pulsatile and circadian patterns of cortisol secretion in abstinent alcoholic men. Ali Iranmanesh, Johannes D. Veldhuis, Michael L. Johnson, and German Lizarraide.
7 Effects of inhibin on gonadotropin secretion by superfused pituitary cell cultures in response to different GnRH exposures. A. Jakubowiski, A. Janecki, and A. Steinberger.
8 Improvement of the Leydig cell function in adolescent males after varicocelectomy. Mariano Castro-Magana, Moris A. Angulo,* J. Atilio Canas,* and John Uy.*
9 Testosterone replacement therapy and nocturnal penile tumescence in hypogonadal men. G.R. Cunningham, M. Hirshkowitz,* and I. Karacan.*

12:00-1:30 P.M.  Lunch

12:00-1:30 P.M.  Student Colloquium (Le Galeria 5 and 6) (Box lunch)
Chairperson: Gail Prins
Speaker—Florence Haseltine, Director, Center for Population Research, NIH, PROSPECTS FOR CONTRACEPTIVE RESEARCH IN THE 1990's

*Not a member of the American Society of Andrology.
1:30 to 3:30 P.M. SCIENTIFIC PAPERS (Slide Session, Rooms E-H): SPERM PATHOPHYSIOLOGY

Chairmen: Thomas Chang and Matthew B. Wheeler

11 Relationship between support of human sperm capacitation and lipid transfer activity among different albumin preparations. Stuart E. Ravnik and Charles H. Muller.


13 Physiology of cryopreserved sperm in the human cervix. P.R. Clisham, E.Z. Drobnis, P.M. Morales, M. Zinaman, F.W. Hanson,* and J.W. Overstreet.


15 Effect of bovine oviduct fluid (ODF) on sperm capacitation in vitro. Tamara McNutt and Gary Killian.

16 Comparison of tight sperm binding to the zona pellucida in the hemizona assay (HZA) utilizing fresh versus frozen sperm from the same male. C. Coddington,* W. Oosthuizen, D. Franken, L. Burkman, G.D. Hodgen.*

17 The molecular mechanism for the human sperm acrosome reaction involves a second messenger system. Christopher De Jonge, Stephen R. Mack,* and Lourens J.D. Zaneveld.

18 Lectin binding by uterine sperm in the House Mouse. B. Peitz, W. Jackson,* A. Gell,* and S. Toohey.*

3:30-4:00 P.M. Coffee Break (The Bissonet)

4:00-7:00 P.M. WORKSHOP ON SPERM CRYOPRESERVATION (Rooms E-H)

Chairman: Rupert P. Amann

4:00 P.M. Roy H. Hammerstedt: Cryobiology of Mammalian Sperm

4:30 P.M. Paul F. Watson: Evaluation of Cryopreserved Sperm

5:00 P.M. John J. Critser: Cryopreservation of Human Sperm and Current Success with Artificial Insemination Using Cryopreserved Human Sperm

5:30 P.M. James L. Rosenberger: Design of Experiments Evaluating Frozen Sperm

6:00 P.M. Harvey Green: Ethics of Research using Frozen Semen

6:30 P.M. Rupert P. Amann: Discussion

7:00-9:00 P.M. Scientific Poster Session† (The Bissonet)

Posters 19-58 and wine and cheese.

19 Effects of castration on FSH and LH secretion by rat pituitary cells perfused with pulses of GnRH. Satoshi Kitihara,* Stephen J. Winters, Hiroyuki Oshima,* and Philip Troen.


22 Estradiol concentration in seminal plasma is negatively correlated with sperm concentration in Holstein bulls. Sharon R. Henney,* Gary J. Killian, and Daniel R. Deaver.*


*Not a member of the American Society of Andrology.
†Posters should be installed by noon the day of the session and removed by 10:00 A.M. the next day.

10-P
28 Variability of sperm parameters in infertile males. Christine L. Cook and William Diebold.*
30 Impaired adrenal androgen production in men after non-burn trauma. C. Richard Parker, Jr.,* and Daniel M. Drake.*
31 A solution to the "Dilemma of Silber" in cases of vasoepididymostomy. V. Krylov,* A. Borovikov, M. Wilson,* and S. Silber.
32 The epididymal Golgi complex. Carlos A. Suarez-Quian and Nicole Jelesoff.*
33 Alterations in the epididymis of the spontaneously diabetic (DB/DB) mouse. Eric L. Sun, Chad W. Schultheis,* and Jun Tao.*
35 Endocytosis of oxytocin in the ductuli efferentes and caput epididymidis of the ram. D.N. Rao Veeramachaneni and Rupert P. Amann.
42 Motility characteristics of mouse sperm with abnormal heads or no heads. P. Olds-Clarke and M.L. Meistrich.
44 Observations on hamster ovum penetration and chromosome conversion of cold-stored sperm of the cynomolgus monkey, Macaca fascicularis. T. Fogle,* B. Bean, S. Schrader,* T. Turner,* and L. Zotter.*
46 The survival of washed and swim-up sperm based on the prewash and swim-up characteristics. Martha Beck and Robert Shelden.
47 ESR determination of human sperm cell volume. F.W. Kleinhans,* V.J. Spitzer,* P. Villines,* K.E. Colvin, and J.K. Critser.
50 Effects of polymorphonuclear leukocytes on mouse sperm motility. Sopit Mongkolsirikieat,* Nongnuj Tanphaichitr, and Deborah Anderson.

*Not a member of the American Society of Andrology.


The relationship between human semen parameters, follicular fluid (hFF), and the hamster egg penetration test (HEPT). Mark S. Siegel, Richard J. Paulson,* and Mark Sauer.*

Past Presidents' Breakfast (The Napoleon Suite)
Basic Science Symposium (Rooms E-H): THE CONCEPTUAL BASIS OF PROSPECTIVE MALE CONTRACEPTIVES
Chairman: Ronald Swerdloff
P. Michael Conn: Molecular Mechanisms of GnRH Action
Spyros Pavlou: Antigonadal Activity of Novel GnRH Antagonists
Terry Turner: Role of Epididymal Factors in Male Fertility Regulation
Mario Ascoli: Regulation of Leydig Cell Function by Specific Growth Factors

Coffee Break (The Bissonet)

State of the Art Lecture (Rooms E-H)
Allen Trounson
IN VITRO FERTILIZATION: RECENT ADVANCES

Scientific Papers (Slide Session) (Rooms E-H): EPIDIDY MAL FUNCTION AND PHYSIOLOGY
Chairmen: Barry Hinton and Michael Holland

Obstruction of sperm transport through the epididymis by ablation of the inferior mesenteric plexus. Kevin Billups, Shelly Tillman,* and T.S.K. Chang.


The effect of hypophysectomy on proluminal transport of 3H-testosterone across the epididymal epithelium in the rat. Masanori Yamamoto and Terry T. Turner.

Morphometric analysis of the ovine epididymal epithelium. S.R. Marnigo, R.P. Amann, and H.R. Sawyer.*

Lunch

*Not a member of the American Society of Andrology.
1:30-4:00  Scientific Papers (Slide Session) (Rooms E-H): MALE INFERTILITY
Chairmen: Joel Marmar and Philip Troen

63 Documentation of human antisperm antibodies reactive with evolutionarily conserved antigens of rhesus spermatozoa. R.A. Bronson, G.W. Cooper, and D.P. Wolff.


65 Elevated levels of white blood cells in semen are associated with poor semen quality. Hans Wolff, Adriana Martinez, Joseph A. Politch, Florina Haimovici, Joseph A. Hill, and Deborah J. Anderson.


67 Comparison of flow cytometry to routine testicular biopsy in male infertility. Wayne J.G. Hellstrom, Henry Tesluk, Arline D. Deitch, and Ralph de Vere White.

68 Sperm capacitation time differs among fertile men, men from infertile couples, and men with abnormal semen analyses. Charles H. Muller, Susan C. Jeffay, Susan M. Harvey, and Paul W. Zaretzkie.


4:00-4:30  Coffee Break (The Bissonet)

3:30-5:30 P.M.  Scientific Posters Exhibit† (The Bissonet)
Posters 73-135.

73 Template activity of human sperm nuclei after microinjection into hamster oocytes. Sally D. Perreault.


75 Intraoperative post-ligation venography for prevention of failure of varicocelectomy. Arnold M. Belker.


77 Routine semen analysis versus computer automated semen analysis to predict male fertility potential. J.H. Check, C.A. Winkel, A. Bollendorf.

78 The hypo-osmotic swelling test as an adjunct to the semen analysis as a prediction of fertility potential. J.H. Check, C.A. Winkel, C.H. Wu, B.S. Shanis, and A. Bollendorf.

79 Chromosomal anomalies and their clinical features among 1210 infertile men. Anne M. Jequier and N. Pandiyan.

80 Monthly variations in seminal parameters in the human. P.M. Zavos.

81 Effect of length of sexual abstinence period on quality and quantity of human ejaculates. P.M. Zavos.

*Not a member of the American Society of Andrology.
†Posters should be installed by noon the day of the session and removed by 10:00 A.M. the next day.
Comparison of two devices for semen collection during intercourse. P.M. Zavos.

Relationship of pregnancy in a GIFT program to in vitro fertilization of extra oocytes in couples with male factor infertility. Sherman Silber, Robert Cohen, M. Wilson,* J. Hicks,* M. Deters.*

Use of immunobeads for antisperm antibody (ASA) testing. M. Schroeder-Jenkins, L. Henrich,* and M.B. Ellison.*


Quality of cryopreserved inseminates used for donor insemination. Susan A. Rothmann, MaryBeth Ellison,* Linda Henrich,* and Mari Schroeder-Jenkins.


Differences in sperm motion patterns for fertile men and infertility patients. Peter M. Fetterolf and B. Jane Rogers.

Interspecimen variation in sperm morphology in a donor population. Richard Scott,* Sergio Oehninger, Mary Mahoney, Mahmood Morshedi, and Anibal Acosta.


Isolation and staging of equine seminiferous tubules by transillumination. Larry Johnson and Vince B. Hardy.*


Calmodulin (CaM) and S-100-binding proteins in rat Leydig cells. William H. Moger.

Interaction of photoperiod and vitamin D on FSH-induced lactate secretion in Sertoli cell cultures isolated from Djungarian hamsters (Phodopus sungorus). S.C. Newton, A. Mayerhofer, and A. Bartke.

Production of antibodies against synthetic peptide from inhibin α and β-subunits. Seiichi Saito,* Patrick C. Roche,* Daniel J. McCormick,* and Robert J. Ryan.*


Effects of histamine on the function of Leydig cells and Sertoli cells in the immature golden hamster. A. Mayerhofer,* S. Newton, A. Bartke, and T. Began.*


Sperm ATP concentrations and ATP/ADP ratios in normospermic (NS) and oligospermic (OS) specimens. C. Vigue,* L. Vigue,* and G. Huszar.

CPK activity and isoform ratios in stallion sperm. Gabor Huszar, Lynne Vigue,* and Melanie Willetts.*

Relationship between heparin binding characteristics and hamster ova penetrating capacity of human spermatozoa. G.S. Prins, S. Vedantham,* C. Wagner,* and R.A. Lalich.

Docosahexaenoic acid regulates fatty acid: CoASH ligase activity in human spermatozoa through negative cooperativity. R.E. Jones and S.R. Plymate.


Both flutamide and RU-486 reduce the effects of cortisol on prostatein C3-mRNA in rat prostate explants. T.C. Shao,* A. Kong,* C.Y.F. Young,* D.J. Tindall, and G.R. Cunningham.*

*Not a member of the American Society of Andrology.
107  Sensitivity of mammalian spermatozoa to H$_2$O$_2$: Role of glutathione peroxidase. Bayard T. Storey and Juan G. Alvarez.


110  Abstract withdrawn.

111  Evaluation of the co-mutagenicity of ethanol and delta-9-tetrahydrocannabinol (THC) with Trenimon. S. Berryman,* A. Bartke, J. Weis,* R. Anderson.


113  Argument against phospholipase A$_2$ (PLA$_2$) as regulatory in the human acrosome reaction. R.A. Anderson Jr., P. Bielfeld,* L.J.D. Zaneveld.

114  Absence of histocompatibility (HLA) antigens on the surface of spermatozoa. Allan R. Glass.


117  Acrosomal integrity of fresh and cryopreserved (CP) human sperm. William C. Baird, Grant E. Schmidt,* Steven R. Williams,* Karen L. Leonhart.*

118  Sources of variation of sperm head measurements. Steven M. Schrader, Terry W. Turner, and Stephen D. Simon.


120  Movement characteristics of progressively motile human spermatozoa involved in collisions. Sharon T. Mortimer,* and David Mortimer.


122  Comparison of sperm select and swim-up for sperm processing. G.M. Centola, A. Partridge,* C. Calalang,* and J.H. Mattox.*

123  Quality control procedures for semen analysis using a computer-assisted (CASA) system. Joseph L. Annest,* Frank DeStefano,* Marcie-jo Kresnow,* Steven M. Schrader, and David F. Katz.


125  Fractal dimensions and the classification of sperm swimming trajectories. R.O. Davis and D.F. Katz.

126  Analysis of rat sperm movement characteristics by computer-assisted image analysis. R.Z. Sokol and H. Okuda.*

127  Abstract withdrawn.


129  The effect of actinomycin D and hydrocortisone treatment on the castration-induced increase in plasminogen activator activity in the rat ventral prostate. Michael Wilson, Mary Vogel,* and Akhouri Sinha.*


*Not a member of the American Society of Andrology.
VIP does not have a direct relaxing effect on human cavernosal smooth muscle. Jacob Rajfer, Andrew Freedman,* David Coy,* and Suresh C. Sikka.*


Effect of ketoconazole on fertility in male rats. Donald P. Waller, Annamarie Martin,* and Lourens J.D. Zaneveld.


6:00 P.M. Steamboat Cruise Banquet
6:00 P.M. boarding, 6:45 P.M. departure
Jazz band and regional food; limited to 300 persons

SUNDAY, April 16

8:00-10:00 A.M. Clinical Andrology Symposium (Rooms E-H):
CLINICAL PATHOPHYSIOLOGY OF MALE HYPOGONADISM
Chairman: Philip Troen
Howard Nankin: Assessment of the Aging Male
Thomas Lue: Management of Impotence
Richard Sherins: The Gonadotropin-Deficient Male
Alvin Matsumoto: Germ Cell Maturation in Man

10:00-10:30 Coffee Break (The Bissonet)

10:30-11:30 Scientific Papers (Slide Session) (Rooms E-H)
SERTOLI AND LEYDIG CELL PHYSIOLOGY
Chairpersons: Sally Perreault and Tu Lin


In vitro responsiveness to LH of Leydig cells from the testis surviving unilateral orchidectomy: Failure of propranolol to block increased responsiveness. David K. Pomerantz.

Up-regulation of insulin-like growth factor-I receptors of Leydig cells in primary culture by human chorionic gonadotropin, 8-bromo cyclic AMP and forskolin. Tu Lin.

11:30 Awards Ceremony (Rooms E-H)
Richard A. Bronson, Presiding
New Investigator Award
Student Travel Award
Young Andrologist Award: Barry T. Hinton, Charlottesville, VA
Distinguished Andrologist Award: C. Alvin Paulsen, Seattle, WA

12:00 Business Meeting (Rooms E-H)
C. Wayne Bardin, Presiding

ADJOURN

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ABSTRACTS

1  MOLECULAR EVIDENCE OF ANDROGEN RECEPTOR GENE MUTATIONS IN THE COMPLETE FORM OF HUMAN ANDROGEN INSENSITIVITY SYNDROMES. Terry R. Brown, Dennis B. Lubahn*, Regina R.E. Kinter*, Elizabeth N. Vilot, David R. Joseph*, Frank S. French and Claude J. Nison. Johns Hopkins University School of Medicine, Baltimore, MD 21205 and University of North Carolina School of Medicine, Chapel Hill, NC 27599.

A 3.6-kbp human androgen receptor (hAR) cDNA has been cloned and sequenced. It contains an open reading frame of 2537 bp encoding a protein of 919 amino acids. hAR cDNA fragments have been used as probes to screen for mutations on Southern blots of genomic DNA and on Northern blots of poly-(A)^+RNA from human subjects with complete androgen insensitivity (AIS). Affected subjects from nine families with complete AIS (female external genitalia and body habitus, 46 XX karyotype, breast development at puberty but scant pubic and axillary hair, elevated LH and testosterone) and absence of intracellular androgen ligand binding were studied. In 8 of 9 patients, hAR restriction fragment patterns were as in normal subjects. In two patients from one family, a partial deletion of the hAR gene involving the steroid binding domain was detected. In these same patients, the normal 11.1-kb hAR of the hAR gene was not detectable. In one family with complete AIS but normal AR binding activity, hAR restriction fragment patterns and the normal sized 11.1-kb hAR were seen. In conclusion, our data provide direct proof that complete AIS in some families can result from a deletion of the hAR structural gene. However, other families do not demonstrate such deletion, suggesting that point mutations or small undetectable deletions may also result in complete AIS, receptor (-) or receptor (+), adding further to the genetic heterogeneity of this syndrome.


Previous studies in diabetic men with organic impotence have demonstrated low free testosterone and increased urinary LH excretion. Morphological findings in the testes of these impotent diabetic men also suggest a primary gonadal disorder. In contrast, several studies measuring single samples or pools of multiple samples for luteinizing hormone have demonstrated normal values. The present study examined serum LH at 10 minute intervals for 12 hours in 10 patients with diabetes mellitus and organic impotence. An aged and weight matched non-diabetic group of 8 healthy men with normal sexual function served as controls. Both groups were evaluated using a cluster analysis program for the p.c. computer developed by Yehuda, Johnson and Thomas. Parametric (two-tailed Student's t test) and nonparametric (Wilcoxon) statistics were applied where appropriate. Results showed that the number of LH pulses per 12 hours were normal in these impotent men with diabetes co-varied with controls. However, we found increased mean 12-hour concentrations of luteinizing hormone (10.1 ± 2.31 vs. 6.46 ± 2.87 ng/ml, p=0.09), increased peak heights for LH (p=0.02), increased interpulse interval for LH (p=0.01), decreased interpulse intervals for LH area (p=0.05), and increased interpulse valley gap LH (p=0.04). Serum levels of total testosterone were normal (p=0.07). Pituitary release of LH was normal in response to 10 ug of GnRH given intravenously. The present observations indicate that in chronically diabetic men with organic impotence elevated luteinizing hormone are associated with intact gonadotropin pulsatility, and preserved pituitary responsiveness to exogenous GnRH. These findings are consistent with a primary testicular defect.

3 ASSESSING THE NATURE OF THE GNRH STIMULUS-LH SECRETORY RESPONSE IN HUMAN GONADOTROPHS IN VIVO. Johannes D. Velupillai, Louis St.L., O'Dea, Michael T. Johnson. Dept's of Int Med and Pharmacol, University of Virginia School of Medicine, Charlottesville, VA 22908; and Massachusetts General Hospital, Boston, MA 02114.

To examine the stimulus-secretion response of human pituitary gonadotrophs in vivo, we applied a new multiple-parameter deconvolution technique to analyze: (i) exogenous GnRH-stimulated LH secretory bursts in 10 men with Kallman's syndrome; and (ii) endogenous GnRH-stimulated LH release in 8 normal men. GnRH-deficient subjects were given 4 bolus doses of synthetic GnRH (7.5, 25, 75, and 250 ng/kg) intravenously at 2-hr intervals. Exogenous GnRH-induced LH secretory episodes could be modeled as Gaussian distributions of instantaneous LH secretory rates having a mean (±SD) half-duration of 14±1.7 min and a simultaneously resolved endogenous LH half-life of 71±5.1 min. Analysis of the log dose-response relationship for GnRH dose versus maximal LH secretory rate or vs. calculated mass of LH released per secretory GnRH disclosed linear responses (p<0.001). Varying GnRH doses did not alter duration of LH secretory bursts, half-life or latency of LH secretory bursts following GnRH. Deconvolution analysis of spontaneous (endogenous GnRH-stimulated) LH peaks in normal men revealed half-durations of secretory bursts of 9.2±1.3 min, and resolved half-lives of LH disappearance of 76±5.2 min (P<0.05 vs. GnRH-treated subjects). We conclude that GnRH exerts dose-dependent effects on specific attributes of human male gonadotropes secretory responses in vivo.

4 3620 OPEN-ENDED VASECTOMIES: ION FAILURE RATE AND HIGH REVERSIBILITY. Sherman J. Silver, St. Luke's Hospital, St. Louis, MO, U.S.A.; Edward T. Shapiro, Riverside Medical Center, Ottawa, Canada.

We first suggested "open-ended" vasectomy in 1977 as a means of making male sterilization more easily reversible by allowing the formation of a sperm granuloma and thereby preventing pressure build-up (Fertil Steril 32:546, 1979). This suggestion stirred up heated controversy. Legitimate concerns were: (a) possibly high failure rate due to spontaneous recanalization, (b) possible pain at the site of sperm granuloma, and (c) would it really increase the ease and success of reversibility? Between August 1977 and December 1987, 3620 open-ended vasectomies were performed by us with an overall failure rate of 0.82%. In the last 2 years, since instituting the combined use of cautery and fascial interposition to the proximal vas, 1200 "open-ended" vasectomies have resulted in a 0.67% failure rate. We recommend "open-ended" vasectomy as an easily reversible form of male sterilization.
REGULATION OF GLYCOPROTEIN ALPHA-SUBUNIT SECRETION BY A LH-RH ANTAGONIST AND ANTAGONIST IN MAN. Jill Ludick* and Sonya I. Pevsner, Vanderbilt University, Nashville, TN 37232

Serum α-subunit levels are elevated in patients with primary gonadal failure, pituitary tumors, and after administration of LHRH. We studied the effects of chronic administration of the [D-Tyr6, Pro7] LH-RH antagonist (LH-R) and the [Ac-D2Nal, D4Ciphe, D3PAl, Arg9, DGM(A)A, DAla8] LH-RH antagonist (Nal-Glu) on α-subunit secretion in normal men. LH-R was given sc as 100 mg every 10 days to 7 men and then testosterone enanthate, 100 mg im every 2 weeks, was added to the agonist regimen for 10 more weeks. Nal-Glu was given as a twice daily sc injection of 5 mg to 7 men for 7 days. Following administration of LH-R, serum α levels increased rapidly from 1.6±0.2 (mIU/ml) to a peak value of 87±10 at 2 weeks and remained elevated (P < 0.001) until TE was added. Then, levels of α decreased (P < 0.05) to a lower level, but still remained above baseline. Following administration of the Nal-Glu antagonist, α levels gradually decreased by (P < 0.001) from 29.9 ± 0.5 ng/ml to a nadir of 14.4 ± 0.3 ng/ml on day 8. This decrease occurred slower than that of serum gonadotropins or testosterone levels. These results demonstrate that LHRH plays an important role in the regulation of pituitary α-subunit. Consequently, LH-RH synthetic analogs significantly upregulate or downregulate α-subunit secretion.

Pulsatile and circadian patterns of cortisol secretion during acute (3–16 days) and chronic (29–39 days) abstinence were examined in 10 alcoholic men without hepatic dysfunction or nutritional deficiencies. Serum cortisol concentrations (assessed by blood sampling q 20 min/24 hr) increased in 6 subjects during acute abstinence, Chronic abstinence in 7 subjects significantly decreased mean maximal cortisol peak amplitude (13±1.0 SD ng/ml at 4 days) to 0.01 ng/ml. 24-hr serum cortisol (10.9±1.6 ng/ml) decreased and valley nadir (1.9±1.6 ng/ml) decreased to 1.3 ng/ml on day 8. This decrease occurred slower than that of serum gonadotropins or testosterone levels. These results demonstrate that LHRH plays an important role in the regulation of pituitary α-subunit. Consequently, LH-RH synthetic analogs significantly upregulate or downregulate α-subunit secretion.

EFFECTS OF INHIBIN ON GONADOTROPIN SECRETION BY SUPERFUSED PITUITARY CELL CULTURES IN RESPONSE TO DIFFERENT GNRH EXPOSURES. A. Jakubowlak, A. Janecki and A. Steinberger, University of Texas Health Science Center at Houston, Houston, TX 77030

Recently we described inhibin effects on gonadotropin secretion by superfused pituitary cell cultures stimulated intermittently (6 min/h) with GNRH (A. Jakubowlak et al., J. Andrology, 1988: 9: P-19). In the present study, using the same system, we examined the influence of different exposure times to GNRH on the time-course of inhibin effects. Pituitary cells isolated from 18- to 20-day-old male rats were plated in Matrigel-coated superfusion chambers. After initial 4 days of static culture in GNRH-free, chemically defined medium, the pituitary cells were superfused (0.25 ml/min) with inhibin (NIH standard, mL-4-117, 1 µg/ml) in the absence or presence of GNRH (10°-100) delivered intermittently (1 min/0.5 hr or 6 min/h) or continuously. Effluent media were collected at 6 min intervals and radiolmmunoassayed for FSH and LH. Significant suppression of FSH and LH responses to either GNRH pulses was first observed after 1–2 h of inhibin exposure. In cultures stimulated continuously with GNRH, significant inhibition of FSH and LH secretion was detected after 3–h of inhibin exposure. If cultures were not stimulated with GNRH, only FSH secretion was suppressed (after ~6 h of inhibin exposure), whereas LH secretion was not affected during the entire superfusion period (9 h). We conclude that time-course of inhibin action on gonadotropin secretion by superfused rat pituitary cells is influenced by the presence of GNRH and varies depending on the mode of GNRH exposure.

IMPROVEMENT OF THE LEYDIG CELL FUNCTION IN ADOLESCENT MALES AFTER VARICOCECTOMY. Mariano Castro-Hagana, MD; Moris A. Arguino, MD; J. Ceviél Casas, MD; and John Uy, MD. Dept. of Pediatrics, Nassau County Medical Center, East Meadow, NY 11554 and State University of New York, Health Sciences Center at Stony Brook, Stony Brook, NY 11790

The incidence of varicoceles in adolescent boys has been reported to range from 9 to 16%. However, there have been no studies concerning the endocrine function of the testes in this age group. We have studied five adolescent males, ages 17 to 20 years, with visible left-sided varicoceles. All the boys had achieved pubic hair, Stage V (Tanner), and had testicular volumes of between 25 to 35 ml. LH and FSH response to the IV administration of 100 µg GnRH as well as testosterone (T) response to the administration of 200 µg HCG/43 days, prior to and 3 months after varicocelectomy, were determined.

Response of Gonadotropins (mIU/ml±SD) to GnRH

<table>
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<th>Basal</th>
<th>Peak</th>
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<tr>
<td>LH/R</td>
<td>12.0±5.0</td>
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<tr>
<td>FSH/R</td>
<td>14.3±6.0</td>
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Response of Testosterone (ng/dl±SD) to hCG

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<tr>
<td>LH/R</td>
<td>408±110</td>
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<tr>
<td>FSH/R</td>
<td>610±58</td>
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Our findings suggest that endocrine evaluation in adolescent males with varicoceles may yield early Leydig cell dysfunction which could be corrected by varicocelectomy.
from these three men

TESTOSTERONE REPLACEMENT THERAPY AND NOCTURNAL PENILE TUMESCENCE IN HYPOGONADAL MEN. G.R. Gunnishan, M. Hirschkowitz, and I. Kracen. Depts. of Medicine and Psychiatry, Veterans Administration Medical Center and Baylor College of Medicine, Houston, Texas 77030.

Studies show that testosterone replacement in hypogonadal men increases sexual activity, erotic thoughts, and nocturnal erections. Nocturnal penile tumescence (NPT) is an objective index of erectile capability and is useful for differentiating psychogenic from organic erectile dysfunction. In this study we evaluated NPT in six hypogonadal men during androgen replacement and after withdrawal. Multi-night, laboratory sleep studies were conducted 1-4 days and 7-8 weeks after patients received a 200 mg IM injection of testosterone cypionate. EEG-EOG-EMG measures were used to identify sleep stages, and penile strain gauges were used to evaluate NPT. Penile rigidity was assessed using a typical NPT episode. The mean serum testosterone level during the week following injection was 1035±99 ng/dl, and it fell to 672±351 ng/dl after 7-8 weeks. Significant declines (p<0.05) in the number of NPT episodes (3.7 to 2.0), the maximum increase in penile circumference (12.9 to 10.2 cm), and the total tumescence time (10.6 to 5.2 min) accompanied the drop in testosterone level. No androgen-related changes in the amount or integrity of REM sleep were found. Finally, penile rigidity decreased from a mean of 710±97.8 to 590±81.4 grams (p<0.05). Comparison of these data with laboratory adrenarche and pubertal groups suggests that none of these men met diagnostic criteria for organic impotence, even though androgen deficiency may present with a wide variety of symptoms. Penile rigidity also was assessed during a typical NPT episode. These men met diagnostic criteria for organic impotence, the female partners of all three men achieved pregnancy by intracytoplasmic sperm injection. The female partners of all three men achieved pregnancy by intracytoplasmic sperm injection.


Recent work on lipid transfer activity (LTA) in human follicular fluid (HFF) (Ramik and Muller, Biol. Reprod., 38) has suggested that LTA may play a role in capacitation by transferring cholesterol out of the sperm membrane. In preliminary work comparing the effects of HFF with albumin in the ultra-short sperm capacitation assay (SPA), Fraction V bovine serum albumin (FV BSA) had residual LTA and supported capacitation but not as effectively as HFF. We now report that albumins which have LTA support capacitation, but albumins that have no LTA do not support capacitation. Two different preparations of FV BSA (Sigma #4503 and #6607), FV HSA (Sigma #2158), and fatty acid ester (FAE) BSA and HSA (Sigma #600 and #1878 respectively) were made up to 105, total protein in BSA was diluted to various concentrations and incubated with 125I-chlorohydrin-labeled HDL and unlabeld LDL for 18 hours in the LTA assay. The albumins were also tested in the ultra-shortSPA (1.2 hour capacitation time). Sperm were washed from semen using each albumin at a concentration of 0.5%, then incubated at 37°C, 5% CO2 for 2 hours. Sperm (5*10^6/ml) were incubated with zona-free hamster oocytes for 2.5 hours. The eggs were scored for the # of sperm penetrated per egg (penetration index, PI). All results are the average of 4 experiments. Only FV BSA (Sigma #4503) and FV HSA had any LTA (8.1 ± 1.17 ± 1.1% transfer, respectively, at 25% total protein) and only FV HSA showed a significant dose response (54.25 ± 6.6% of total protein). FV BSA #4503, FV HSA, and FV HSA had no LTA even at 10% total protein. The results for the ultra-shortSPA were similar. FV BSA #4503 and FV HSA both supported penetration (P1.0 ± 0.25 ± 0.11 ± 0.11, respectively). FV BSA #6607 had no LTA, and FV HSA had a lower LTA activity compared to FV BSA #4503. FV BSA #4503 at 0.02%, p=0.01; FV BSA 0.06 ± 0.02, p=0.04. These results may suggest that some commercial preparations of albumin are contaminated with serum-derived LTA and that more purified preparations lack the activity. The presence of contaminating LTA may be responsible for the support of capacitation. [Supported by NIH HD14279, NRSA R2 T3 HD10183, and University of Washington Graduate School Research Fund]

ADDITION OF SPERM FREE DONOR SEMINAL PLASMA TO LOW VOLUME ANTEGRADE EJACULATE IN MEN WITH RETROGRADE EJACULATION. Check J.L., Michael CA*, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology, The Jefferson Medical College of Thomas Jefferson University, Philadelphia, PA.

Three men with infertility ranging from 2 to 10 years had asthenospermia and low semen volume as the result of retrograde ejaculation. All three men were treated with Omnadrozol to increase antegrade ejaculation. In the antegrade semen specimens obtained, no sperm were progressively motile 40 minutes after collection. Sperm counts in the antegrade semen specimen ranged from occasional to 137 million/ml. Values ranged from less than 0.005 to 4.1 million/ml. Motility ranged from 50% (between 5 and 15 minutes). Sperm counts in the retrograde portion of the ejaculate ranged from occasional to 63 million/ml in retrograde ejaculate. Sperm-free seminal plasma was obtained from normal males by centrifugation of donor semen in a microcentrifuge at 16,000 rpm. Supernatant seminal plasma thus obtained was checked microscopically for the absence of sperm. Sperm-free donor seminal plasma from whole semen specimens with demonstrated sperm motility greater than 70% was added to the antegrade portion of ejaculate obtained from men suffering retrograde ejaculation in a quantity sufficient to bring the total semen volume of 0.5 to 1.0 ml. The addition of donor seminal plasma resulted in an increase in motility in the antegrade ejaculate. Semen specimens thus prepared were employed for intrauterine insemination. The female partners of all three men achieved pregnancy in 1, 2, and 5 cycles respectively. Sperm motility and quality in specimens prepared during the conception cycles were 25-35%, grade 2 to 2.5; 15-35%, grade 2 to 2.5; and 0-43%, grade 2 to 3.0, respectively (quality grade scale 0-4 based on progressive forward motion). We conclude that in men with retrograde ejaculation, semen volume is a key factor in the poor fertilization rates observed and that addition of sperm-free donor seminal plasma to the antegrade semen specimen provided the necessary sperm supply to achieve pregnancy.


Bovine oviducal secretions were collected and analyzed throughout the estrous cycle, and the capacity for their protein and lipoprotein components to support cholesterol efflux from bovine sperm was evaluated. Protein and lipoprotein separation was achieved by density gradient centrifugation. Two major bands were identified. The first (1% w/v + 0.145 g/ml) corresponded to bovine and rabbit plasma high density (approx. HDL) based on the density gradient and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the protein components for each fraction. The second band (1% w/v + 0.145 g/ml + 0.05 m/l) was composed predominantly of bovine seminal plasma albumin (BSA, Oviductal fluid) (OF) protein concentrations increased as sperm progesterone (P4) decreased. Mean OF protein concentration was 21.3 µg/ml when sperm P4 was below 0.5 µg/ml and 6.5 µg/ml when sperm P4 was greater than 0.5 µg/ml. To determine the ability of OF components to serve as sperm binding lipoproteins, OF samples were incubated with and without bovine sperm (5 x 10^6 in 1.1 mg/ml of Tyrode's solution and OF/SP) for 2 hr at 37°C. After incubation, HDL and BSA fractions were isolated and analyzed for changes in protein and lipid content. An inverse log relationship was found between HDL protein concentration and sperm P4. Unesterified cholesterol (UC), cholesterol ester (CE) and phospholipid (PL) concentrations for HDL protein concentrations of 3 to 56.1 µg/ml were 13.5 to 48.2 µ/ml, 191 to 44.8 µ/ml and 1.69 to 59.8 µ/ml, respectively. Phosphatidylcholine (PC) and ethanolamine (PE) were the major PL species in the HDL fraction and in the molar ratio (41.1 mol/ mol) was relatively constant throughout the estrous cycle. The BSA fraction of the samples accounted for more than 50% of total protein and for most of the variation in OF protein. After OF samples were incubated with sperm an increase in UC was found in the HDL fractions. UC in HDL decreased by 52% to 9.95 µg/ml (k = 0.5%/h) when sperm P4 was 0.5 µg/ml. For samples corresponding to higher P4, the increase in UC was 35% to 38.9% µg/ml. Values for UC in HDL were corrected for the UC contribution from OF of OF samples. Cholesterol efflux from sperm has been implicated in the process of sperm capacitation. These results indicate that HDL from OF is essential during the latter phase of the estrous cycle and can serve as an acceptor for bovine sperm CHOL.
PHYSIOLOGY OF CRYOPRESERVED SPERM IN THE HUMAN CERVIX

M. O. Opper, E. E. Daniel, P. M. Stobbe, M. Zhang, M. W. Hansen, and J. W. Overstreet, Departments of Obstetrics and Gynecology and Physiology, University of California, Davis, CA, and Georgetown University, Washington D.C.

Cryopreservation (CP) causes acrosomal damage, but previous studies on human sperm have not restricted analysis to motile sperm capable of evoking cervical mucus (CM) following artificial insemination (AI). To test if the acrosomal condition of this subpopulation is altered by CP, CM was collected after 1 and 24 hr following AI. Percent acrosome reaction (AR) of sperm in CM was evaluated on viable sperm (those excluding supravital dye) with FITC-PSA lectin. Sperm were allowed to swim out of CM into BIVF medium (3 mg/ml BSA). Following 3 hr (swim period) and 6 hr (final incubation) AR was assessed after treatment with 20% (v/v) human follicular fluid (FF) or BW5 (control). The data were compared with those obtained after AI with non-frozen (NF) sperm, as shown below (mean NF P + SEM):

<table>
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<th>NF</th>
<th>Hour</th>
<th>Spem recovered from CM</th>
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<tr>
<td></td>
<td>1 hr Incubation</td>
<td>6 hr Incubation</td>
</tr>
<tr>
<td>CP</td>
<td>1 (5)</td>
<td>1, 1 ± 5.8 ± 2</td>
</tr>
<tr>
<td>CP</td>
<td>24 (5)</td>
<td>0 4 ± 2 7 14 ± 11 17 ± 14 16 ± 6</td>
</tr>
<tr>
<td>NF</td>
<td>24 (5)</td>
<td>1, 4 1, 4 2 ± 6 3 ± 6 11 ± 3</td>
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These data indicate that NF sperm and CP sperm which enter the CM following AI remain acrosome intact in the CM for 24 hr, but after swimming out in vitro spontaneous AR is higher for CP than for NF sperm. Also, CP sperm AR in response to FF after a shorter incubation than that required for NF sperm. Thus, the physiology of CP sperm stored in the cervix is different than that of NF sperm. This increased acrosome lability may result in premature AR which may be responsible, at least in part, for the decreased fertility of CP sperm.

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EFFECT OF BOVINE OVIDUCT FLUID (ODF) ON SPERM CAPACITATION IN VITRO.

T. L. Overstreet and G. W. Overstreet, Dairy Breeding Research Unit, Center, Penn State University, State College, Pa. 16802.

This study evaluated the effect of hCG (5.0 ng/ml) and non-luteal (NL: progesterone<1.5 ng/ml ODF) on sperm capacitation in vitro. ODF was collected daily from cows with oviduct cannulas. Washed sperm (50 x 10^6 sperm/ml) were incubated up to 6 hr at 39°C in 5% CO2 in a protein-free modified Tyrode's medium (Partham, et al., F. R. 53:1171) plus 1 mg PVA/ml (MTM), MTM with 10 mg/ml heparin sulphate (MTM HS) or MTM supplemented with 5, 20, 40, 60 or 80% NL or L-ODF (v/v). Capacitation was assessed by induction of the acrosome reaction (AR) with 60 µg/ml saponin/bovine serum/ml. NL-ODF had no effect on sperm motility; sperm motility tended to be higher in NL than MTM:H (65% vs 50% at 4 hr). Increasing concentrations of L-ODF were (+) correlated with sperm motility (r = 0.87, 0.86, 0.82, 0.80 and 0.75 at 2, 5, 10, 15 and 20% respectively). Sperm incubated in MTM did not AR and 5% NL-ODF had little effect on sperm AR. Total proteins and glycosaminoglycan (GAG) concentration were determined for NL- and L-ODF. L-ODF had 35% more proteins than NL-ODF. GAG concentrations were similar for NL- and L-ODF samples. NL-ODF protein or GAG contents were correlated with AR (r = 0.85, 0.84, 0.87, 0.89, 0.90). These were higher than with L-ODF (r = 0.1, 0.4, 0.5, 0.2, 0.5). Increased agglutination of sperm occurred with higher ODF in the medium. This study suggests that both NL- and L-ODF capacitate sperm, but the dynamics of capacitation and the effects on motility differ, with type of ODF. (USDA Grant 85–CIRC–1–1841)

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There are scant data on cryopreservation (CP) methods for non-human primate sperm. In efforts to CP Cynomolgus monkey semen (CSM), three egg-yolk diluents, which have been used successfully in CP of human sperm, were compared for their ability to protect CSM against cryodamage. TEST (Tes + Tris), TEST with 20% skim milk (TSM), and egg-yolk citrate (EYC) were compared. A single ejaculation was collected from each of five males. Three diluents and two glycerol levels (0% and 5%) were evaluated in a 3 x 2 factorial design using six aliquots of each ejaculate. Following stepwise glycerolization, diluted CSM was cooled to 5°C over 2 hr, packaged in 5 ml cryosticks and frozen in liquid nitrogen vapor. Straws were thawed in a 37°C water bath. The percent motility (NM), percent viability (NV, exclusion of supravital dye), percent acrosome intact (NAI, percent of viable sperm having uniform staining of the acrosome with FITC-PSA lectin), and the curvilinear velocity (Vc) were determined after thawing. TEST and TSM were better preserved (NM < 0.001) than did EYC (mean ± SEM: 62 ± 3, 39 ± 7 and 14 ± 3 for TEST, TSM and EYC respectively). The Vc (72 ± 1, 0.9 ± 3 41 ± 5) followed the same pattern (p < 0.001). TSM was a better preservative of stable acrosomes than was EYC (p < 0.05), while TEST was intermediate and not significantly different from TSM or EYC in this ability (NAI = 39 ± 5, 28 ± 4, 15 ± 3). TSM, and TEST produced equivalent Vc while EYC had slightly reduced Vc (p < 0.001, 57 ± 3, 55 ± 3, 42 ± 2 um/sec). There were no interactions between diluent and glycerol level. The 3% glycerol level gave superior results to 5% glycerol for NM (47 ± 2.39 ± 0.6% and 5% vs 8% respectively), NV (64 ± 5, 54 ± 6, 8% vs 5 and 2%, respectively), and Vc (32 ± 3.51 ± 3), though this reached statistical significance (p < 0.05) for only Vc. TSM with 3% glycerol offered the best protection to CSM during CP. We wish to emphasize that EYC, which is widely used for CP of human sperm, is not suitable for CP of CSM. In artificial insemination trials, an ongoing pregnancy has been achieved with CSM cryopreserved in TSM.

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COMPARISON OF TIGHT SPERM BINDING TO THE ZONAR PELLICUDA IN THE HENIZONA ASSAY (HZA) UTILIZING FRESH VERSUS FROZEN SPERM FROM THE SAME MALE.

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The HZA has demonstrated a difference in tight binding of the sperm from fertile vs infertile males. From 5 males, we compared the zona binding capacity of fresh vs frozen (-190°C) sperm. The HZMA oocytes were obtained from human ovaries recovered during surgery. These non-living eggs were stored in a 1.5 M MgCl2 solution at 270°C, rinsed before use and then microsurgically bisected. Each henizona (HZ) was incubated in a 100 µl drop with 500,000 cattle sperm/ml for 1.24 hrs. Each HZ was assessed for the number of tightly bound sperm at 1, 2, 3, 4, 5, 24 hrs. The henizona index (HZI) is the number of tightly bound fresh sperm over the tightly bound sperm from the frozen specimen x 100. For the 7 eggs tested, the mean HZI per experiment varied between 3.46 ± 0.01. Based on the HZI values, the frozen-thawed sperm from 3 ren exhibited a 50-70% decrease in zona binding capacity as compared to fresh. The remaining 2 cases reflect an essentially no change in binding. The binding HZI... were equivalent for fresh vs frozen sperm assay on each of the 7 cases. The peak binding at 3 to 4 hrs. The decrease in binding of fresh-thawed sperm may contribute to the decreased fertilizing potential of frozen sperm.
The molecular mechanism for the human sperm acrosome reaction involves a second messenger system. Christopher De Jonge, Stephen R. Mack, Lourens J.D. Janeveld, Dept. of Obstet & Gynecology, Rush University, Chicago, 11 60612.

The acrosome reaction (AR) of spermatozoa may be analogous to various serosal cell exocytotic events which involve cascade reactions, i.e. transmission of an external signal across the cell membrane resulting in activation of an "amplifier" enzyme and the generation of a second messenger. Previously, we have shown, using the acrosome reaction system, that calcium ionophore A23187 and analogues of the second messenger CAMP, dibutyryl CAMP (dbcAMP) and 8-bromo CAMP, are able to induce the AR of capacitated human spermatozoa (De Jonge et al., J. Androl. 9:46-P, 1988). In order to further substantiate the role of adenylate cyclase (AC) as an "amplifier" enzyme in the AR, various modulators were tested: Forskolin (1M), an AC stimulator, caused a significant (p<0.001) increase in AR (39±3% AR; mean ± SD). Inhibitors of AC inhibition (1nM), 2'-O-methyladenosine (1nM) and 2',3'-dideoxyadenosine (1nM) significantly (p<0.05) inhibited the forskolin-induced AR (respectively: 17±5%, 12±4%, 14±3% AR). Protein kinase A (PKA) is activated by CAMP. PKA inhibitor (10nM) caused a significant (p<0.05) inhibition of the forskolin-induced AR (11±2% AR), tentatively identifying PKA as part of the AR cascade. Finally, even in the absence of extracellular calcium, dbcAMP (1nM) induced a significant (p<0.005) AR (33±4%) in contrast to A23187 (7±1%). These results indicate that 1) activation of AC occurs after the influx of extracellular calcium, and 2) the molecular mechanism for the human sperm AR involves the CAMP second messenger system. Supported by NIH HD 05555.

Effects of castration on FSH and LH secretion by rat pituitary cells perfused with media of GnRH, Tamotsu Kitahama, Stephen J. Winters, Hiroaki Oshira and Philip Teper, Department of Medicine, Kintool Hospital, University of Pittsburgh, Pittsburgh, PA, 15213.

LH and FSH secretion increases when the testes are removed or severely damaged. Multiple factors are responsible for this effect. To examine castration-induced changes in the pituitary in the absence of increased GnRH stimulation or decreased gonadal steroid and inhibin exposure, we studied FSH and LH release from pituitary cultures perfused with pulses of GnRH. Pituitary cells from 7-8-old intact rats and rats castrated 14 days before were harvested and cultured with cycled beads. After 4 days in culture, cells were washed into perfusion chambers, and stimulated with 2.5 nM GnRH for 2 min every h for 8 h. Fractions of the column effluent were collected every 10 min for FSH and LH RIA. Cytosol protein (mean ± SE) of LH levels (10-100) (6.2±0.3 vs 0.3±0.1 ng/ml; 10-100 nM) and FSH levels 2.5 fold (27.5±1.0 vs 11.7±0.8 ng/ml). Pituitary LH content rose 2.8 fold from 4,600.3 to 13,444.9 ng/10 cells (6±0.8 ng). Conversely, FSH content was unchanged by castration (3.7±0.5 vs 3.5±0.07 ng/10 cells). With GnRH stimulation, pituitary cells from castrated rats released 70±6% as much LH and 20±2% as much FSH as did normal rats. Internuclein FSH and LH secretion also increased to 200±20% and 4±0±9% of the values for intact rats. In summary, 1) castration increases the responsiveness of pituitary cells to GnRH. This change may be partly explained by the previous findings that GnRH receptors are increased in the pituitaries of castrated rats. 2) castration increases intercyclic gonadotropin secretion. The factors responsible for this change may be independent of GnRH stimulation and remain to be clarified.

Estrogen regulation of the priming effect of GnRH on LH release in the rat. Lee N. Sanford, McGill University Centre for the Study of Reproduction, Departments of Animal Science and of Obstetrics and Gynecology, St Anne de Bellevue, Quebec H9X 1C4.

The effectiveness of estradiol (E2) in maintaining the priming effect of GnRH on LH release in the adult rat was investigated. Experiment 1 assessed the usefulness of 5- and 2-μg doses of GnRH in producing the priming effect when administered (i.v. in 1 ml saline) 80 min apart. LH pulse amplitude over four .5 μg injections remained unchanged for both intact and castrate rats. With the 2-μg dose, the peak 2 (P2);peak 1 (P1) amplitude ratio was 1.8±2 for intact rats and 1.6±1 for castrate rats. In Experiment 2, long-term E2-treated castrated rats (s.c. Silastic implants maintained blood E2 at breeding season concentration) were paired with two 2-μg doses (inh1) 80 min apart. Although the P2: P1 ratio was the same for intact (1.9±5.5) and E2-treated castrate (1.8±3.5) rats, the amplitude of both peaks was increased ±2.5 fold in the latter group (P1, 61±8 ng/ml; P2, 118±20 ng/ml). Pulse-amplitude values were comparable but reversed for the control castrate rats (P2: P1, 4.51) versus the E2-treated castrate rats. Experiment 3 involved inserting E2-filled implants into long-term castrate rats and examining reestablishment of the priming effect. P2: P1 ratios increased progressively from day -1.5 (4.1) through day +5 (1.9±3) and day +15 (3.1±5). P1 and P2 amplitudes on these 3 days were 120±13 and 57±6 ng/ml, 131±16 and 25±43 ng/ml, and 67±12 and 190±32 ng/ml, respectively. In conclusion, the priming effect of GnRH on LH release in the rat is maintained by E2, and reestablishment in the castrate rats involves both accelerated priming and diminished initial release. Supported by the MRC of Canada.
DOSE-DEPENDENT HORMONAL INDUCTION OF BENIGN PROSTATIC HYPERPLASIA (BPH) IN CASTRATED DOGS. PF Junkwitz, EH Long, TA Barbol, TA Lardie, PH Barid, and JF Millard, Sterling Winthrop Research Institute, Rensselaer, NY 12144

Dose-dependent hormonal induction of BPH in castrated beagles has been achieved using subcutaneous diethyl implants containing 5α-androstan-3β,17β-diol (andro) and estradiol-17β (E2). Estimated in vitro release of 5α-diol and E2 implants averaged 122±24 and 23±1 µg/day, respectively. Implantation of castrated dogs with either 10 or 20 5α-diol implants and 1 E2 implant or the intramuscular injection of 3α-diol (25mg x 3 per wk) and E2 (0.25mg x 3 per wk) for 99 days increased (P < .01) prostate weights (2717; 384; 47±10 g, respectively; X ± S.E.; n=4) and total prostatic volume (59±25; 12±51; 145±50 mg, respectively) compared to intact controls (10±2g; 32±11mg; DIA; n=4). These treatments resulted in a histomorphological pattern similar to that observed in dogs with the glandular form of spontaneous BPH. Implantation of 5α-diol and 1 E2 capsules into castrates (n=4) resulted in prostatic weights (8±1g) and total DIA (26±5mg) that were similar (F<.05) to intact controls; however, these prostates were characterized by glandular atrophy and squamous metaplasia. One week prior to necropsy, prostates were scanned in both the transaxial and sagittal planes using transrectal ultrasound (Model 1049 Bruel and Kjaer). Prostatic volume (length X width X depth) was converted into weight using the equation: estimated weight = volume (cm3) X 0.602 ± 1.16. A significant correlation (r=0.79; P < .001) was obtained between true prostatic weight and that estimated by ultrasound. This study has led to the development of a model which will facilitate evaluation of competitive androgen antagonists and a method of monitoring prostatic response over time.

Estradiol Concentration in Seminal Plasma Is Negatively Correlated With Sperm Concentration in Holstein Bulls. JH Henn, MD. Killion, and Daniel R. Deaver*, Dairy Breeding Research Center, Pennsylvania State University, University Park, PA 16802

The relationships between sperm concentration, semen volume and estradiol concentration in seminal plasma were studied in 18 mature Holstein bulls. The 5-wk experimental trial of each bull consisted of a 4-wk acclimation period followed by a 1-wk data collection period. Two ejaculates were collected from each bull 3 times per wk. Bulls were not sexually prepared prior to semen collection. Seminal plasma samples from first ejaculates collected during the data collection wk were radioimmunoassayed for E2. The average ± SE E2 concentration in seminal plasma was 25±5.8 pg/ml, which compares with 10±2.1 pg/ml for blood plasma. Average seminal plasma E2 concentration was negatively correlated (r = 0.70, P = 0.002) with the average sperm concentration among the 18 bulls. However, average semen volume was not correlated with E2 concentration in seminal plasma. Likewise, sperm concentration and semen volume were not correlated with E2 concentration in blood plasma. The major source of E2 in seminal plasma is prostatic fluid (150 ± 9 pg/ml), and epididymal sperm bind relatively high amounts of estrogens (112 ± 15 pg/1010 sperm) at ejaculation (Ganjam & Amann, 1976, Endo. 66:1616-1620). The non-saturating binding of steroids by sperm (Amann & Hammerstedt, 1976, Biol. Reprod. 15:670-677) probably accounts for the negative correlation found between sperm concentration and E2 concentration in seminal plasma of dairy bulls. (Supported in part by Atlantic Breeders Cooperative)

FALF In Testicular and Adrenal Androgens After Adrenal Nudility Transplant For Parkinson's Disease. EY Clark, HB Watts*, R L Watts*, S D Graham*, and R A Dalby*, Emory University School of Medicine, Atlanta, Georgia, 30322.

Autologous adrenal nudility transplantation is being tested as a treatment for Parkinson's disease. We hypothesized that placement of this tissue near the hypothalamus would alter hypothalamic-pituitary regulation. We studied 6 men before, and 1 week, 3, 6, and 12 months after surgery with combined insulin tolerance test (ITT), thyrotropin-releasing hormone, and gonadotropin-releasing hormone (GnRH), stimulation tests to evaluate adrenal, thyroid, and gonadal axes as well as growth hormone and prolactin responses. Adrenal function was studied with renin, aldosterone, dopamine, epinephrine, and norepinephrine, supine and upright. Androgens, both testosterone and dehydroepiandrosterone-sulfate (DHAES), were suppressed to 32% and 24% respectively one week post-operatively despite normal basal gonadotropins and normal GnRH tests. Subsequently, testosterone levels normalized, however DHAES levels remained suppressed to 44% of baseline. Cortisol levels were unchanged basally and after ITT, and other adrenal hormone levels were unchanged. No long-term effects on hypothalamic-pituitary function were observed. We conclude that autologous adrenal nudility transplantation is associated with a transient suppression of the testicular axis consistent with a transient rise in prolactin, which may be related to surgical stress or the elevated prolactin, and that there is a prolonged suppression of DHAES production by the remaining adrenal gland disproportionate to other adrenal steroids.
25.

ANABOLIC STEROID INDUCED HYPOGONADOTROPIC HYPOGONADISM
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Anabolic steroid abuse continues in epidemic proportion amongst athletes of all ages despite universal condemnation by professional organizations. Side-effects of these drugs, including hepatic disorders and azoospermia, are considered rare or self-limited. In particular, previous reports have demonstrated recovery from anabolic steroid induced hypogonadotropic hypogonadism within 16 weeks in all men studied.

Herein, we report four men who presented to a male infertility clinic for evaluation of either infertility or decreased libido and had a history of anabolic steroid use. Evaluation included history, physical examination, baseline endocrine studies, semen analyses and a GnRH stimulation test. The results of this investigation confirm in part previously described anabolic steroid induced hypogonadotropic hypogonadism with recovery in both serum hormonal values and semen parameters within 16 weeks. However, 2 patients demonstrated persistent hypogonadotropic hypogonadism for 1 and 3 years as evidenced by low baseline serum luteinizing hormone and testosterone as well as a blunted response to a GnRH stimulation test.

This study suggests that anabolic steroids may exert permanent deleterious effects upon the hypothalamic-pituitary-gonadal axis.

26.

SEMIN CHARACTERISTICS OF VIETNAMESE VETERANS
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As part of an epidemiologic study of the health status of a randomly selected group of Vietnam veterans, we measured the semen characteristics of 324 Vietnam veterans and compared them with a similar group of 247 veterans who did not serve in Vietnam. Measurements of sperm concentration, motility, morphology, and head dimensions were performed using the Cellsoft computer-assisted semen analysis system. We found that Vietnam veterans had significantly decreased mean sperm concentrations (4.8 x 10^9 sperms/ml for Vietnam veterans versus 79.8 x 10^9 sperms/ml for non-Vietnam veterans) and Vietnam veterans were twice as likely to have sperm concentrations ≤ 20 million/ml (15.9% versus 8.3%). Vietnam veterans also had a significantly decreased mean proportion of morphologically normal sperm heads (57.9% versus 60.8%), with significantly longer mean major axis length and head circumference. The proportion of motile cells, velocity, linearity, amplitude of lateral head displacement, and beat frequency were not different between the two groups. Despite differences in sperm characteristics Vietnam and non-Vietnam veterans reported fathering similar numbers of children.

27.

THE DETECTION OF ANTI-SPERM ANTIBODIES IN MEN WITH CARCINOMA OF THE TESTIS. Leslie R. Rubin, Richard S. Foster*, and Ann McNulty*, Indiana University School of Medicine, Dept. of OB/GYN Indianapolis, IN. 46223

The most common solid tumor in men aged 18-36 years is carcinoma of the testicle. Men who present with this type of carcinoma are reported to have a low fertility potential which has recently become important in light of their ability to continue to ejaculate after modified nerve-sparing retroperitoneal lymph node dissection.

Reduced fertility could result from an autoimmune response through a breakdown of the blood-testis barrier as a result of disease or surgery. Precedence for antisperm antibody production under these conditions has been noted. Antisperm antibody incidence is as high as 40% in patients who have undergone a vasectomy reversal while the rate for fertile adult males is 6X.

Research was undertaken in our lab to determine whether antisperm antibodies are present in patients who fit the criteria of unilateral testicular carcinoma, post orchectomy, and without previous lymph node dissection or chemotherapy. Serum samples were obtained from fifty such patients. Formalin fixed semen from patients exhibiting no antisperm antibodies served as the antigenic source to which serum antibodies could bind.

The presence of antibodies was qualitatively established using fluorescent conjugated goat anti-human antisera. All the samples were initially screened using a 1:2 dilution and compared against known positive and negative samples. Subsequently, a 1:8 dilution was used to eliminate borderline determinations. Thirty-four patients had positive samples from testicular cancer patients were positive in the preliminary experiments; positive specimens titrated 1:32 before becoming negative.

Further work is ongoing to quantitatively substantiate our findings, to increase the patient population studied, to obtain same-day semen and serum.

28.

VARIABILITY OF SPERM PARAMETERS IN INFERTILE MALES.
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Sperm concentration and motility have been reported to be highly variable for any given individual. Efforts to predict fertility based on one or more semen analyses are therefore unlikely to succeed. Using an automated semen analyzer (Hamilton-Thorn), variability in sperm movement parameters was assessed for 24 male partners of infertile couples. Each man produced at least five semen samples at approximately one month intervals. Each sample was evaluated both before and after a two step wash preparation for intruterine insemination. The coefficient of variation (CV) was determined pre and post wash for each individual for each parameter.

Rut Sperm Washed Sperm
% Motile 21.2±1.85 25.5±2.95
Mean path velocity 27.3±2.19 30.3±1.85
Mean progressive velocity 30.6±2.33 32.7±2.33
Mean linear index 8.05±0.71 9.5±0.84
Lateral head displacement 32.0±3.73 30.4±3.53

The mean linear index, which measures the efficiency of forward movement of sperm, is demonstrated to have low variability. If this particular movement parameter can be correlated with the probability of pregnancy, the ability to predict male fertility based on an automated analysis will be improved.

*CV:standard error of the mean

Seminal samples from 28 HIV-seropositive men have been analyzed for HIV by p24-antigen ELISA, virus culture, immunohistology and transmission electron microscopy (TEM). 200-µl aliquots of cell-free seminal plasma (SP) were consistently HIV-negative by p24 ELISA (0/28 positive), but when 1-ml SP-aliquots were centrifuged at 10,000 x g, 2/13 pellets were p24-positive. By virus culture, 10/17 SP were HIV-positive. Significant numbers of HIV-infected WBC were not detected in seminal smears by immunohistology or TEM, but HIV was cultured from 3/6 WBC-enriched fractions of semen. In contrast, motile sperm, isolated by Percoll centrifugation from 6 samples (2 SP positive), were HIV negative by virus culture. Furthermore, no association between sperm and HIV was observed in 10 samples by TEM analysis. Thus, seminal samples from seropositive men can contain cell-free infectious virus as well as HIV-infected WBC, and provide preliminary evidence that HIV does not bind to sperm. (Funded by grants from the NIH (AI25305) and U.S.A.I.D. (CONRAD 88020))

30 IMPAIRED ADRENAL ANDROGEN PRODUCTION IN MEN AFTER NON-BURN TRAUMA. C. Richard Parker, Jr.* and Daniel M. Drake*. University of Alabama at Birmingham, Birmingham, AL, 35294

Gonadal androgen production is reduced in severely stressed men. The effect of stress on androgen production in the adrenals, a quantitatively important source of anabolic steroids, is, however, ill defined. To address this issue, we quantified dehydroepiandrosterone sulfate (DHEA-S), the major C19-stereoid produced by the adrenal cortex, and cortisol (C) in 12 men within 60 min. of trauma that required emergency surgery and during convalescence. Compared to those in non-traumatized, healthy adult men (D: 229 ± 117 µg/ml, mean ± SE; C: 13.3 ± 1.7 µg/dl), serum levels (% of normal) of P increased significantly upon entry to the ER (774 ± 761), whereas DS was normal (103 ± 84%). Immediately post-operatively, P levels remained increased (376 ± 84%) while DS levels were normal (106 ± 16%). P levels remained increased on post-op days 1 (298 ± 64%), 2 (176 ± 36%), 3-4 (124 ± 17%) and 5-6 (215 ± 17%). In contrast, DS levels declined progressively to subnormal values on post-op days 1 (89 ± 6%), 2 (74 ± 5%), 3-4 (50 ± 13%) and 5-6 (44 ± 8%). Thus, these data indicate an escape in secretion of adrenal glucocorticoids versus androgens in traumatized men. Common mechanisms may exist for the recognition impairment in testicular function and the decline in adrenal androgen secretion in severely stressed men.

31 A SOLUTION TO THE "DILEMMA OF SILVER" IN CASES OF VASEPIDIDYMOANASTOMOSIS. V. Khulan, A. Brezinka*, All Union Surgery Research Center, Soviet Union, M. Wilson, S. Silber, St. Luke's Hospital, St. Louis, MO

In the USSR the decision of where to perform the vasoepididymal anastomosis has been termed the "Dilemma of Silver." If you perform the anastomosis too far proximally, sperm may not be able to mature. If you perform the anastomosis distally you may not have bypassed all of the obstructions. To solve this dilemma, 100 cases of vasoepididymal anastomosis in the USSR and the US were prospectively studied for sperm motility and morphology at all levels of the epididymis. In this study we did not stop at the most distal level where sperm were found but in every case studied the entire length of obstructed epididymis. In 76% of cases, sperm with progressive linear motility were found. In all 76 cases with progressive motility, non-motile sperm were found in the distal epididymis and motile sperm in the proximal epididymis. In 64 of the 76 cases motile sperm were found only in the caput. This "inversion" of motility in the obstructed epididymis (best motility in caput and poorest in caudal) supports Young's findings in rodents in 1931, with these important physiological consequences: 1) Sperm gain motility and mature as a function of time alone and don't require passage through the epididymis. 2) In the obstructed epididymis distal sperm are senescent and less likely to be motile; proximal sperm are more recently produced and more likely to be motile. 3) To be certain all obstructions have been bypassed, when in doubt, vasoepididymostomy should be performed more proximally without fear of compromising sperm maturation.

32 THE EPIDIDYMAL GOLGI COMPLEX. Carlos A. Suarez* and Richard Quinn and Nicole Jelesoff* Georgetown University, Dept. of Anatomy & Cell Biology, 3000 Reservoir Rd NW, Washington, D.C. 20007

The epididymis participate in the post-testicular maturation and storage of spermatozoa by actively engaging in secretion into, and resorption from, the tubule lumen, events that occur along disparate tubule regions. The underlying molecular mechanisms leading to biogenesis of these differences, however, are not known, although components of the Golgi complex membrane container must undoubtedly be intimately involved. Two monoclonal antibodies raised against Golgi integral membrane proteins, recognizing either the cis (GIMPc) or trans (GIMPt) membranes, were used as molecular probes to begin the characterization of the Golgi complex of in vivo and in vitro epididymal cells. Immunolocalization of GIMPs was performed on frozen, 5 µm sections and in cultured cells using biotin-streptavidin-peroxidase immunocytochemistry. Immunodetection of GIMPs and GIMPt in epididymal cells in vivo revealed a reticular, perinuclear pattern, and NH4Cl treatment preferentially disrupted the GIMPt immunocytochemistry. In tissue sections, immunostaining with both antibodies was extremely robust in supranuclear areas throughout the epididymis. The only major regional difference discerned was that immunostaining of the proximal caput with both antibodies appeared significantly more intense than in other regions of the epididymis. These results characterize the molecular components of the Golgi complex and form the basis of additional studies to gain further insight into mechanisms leading to generation of regional differences in epididymal function.

Supported by NH Grant HD23484 to C.A.Q.
33 ALTERATIONS IN THE EPIDIDYMS OF THE SPONTANEOUSLY DIABETIC (DB/DB) MOUSE. Eric L. Sun, Chad W. Schlotter* and Jim Tao*, Anatomy Section, Medical Sciences Program, Indiana School of Medicine, Bloomington, In., 47403

It is well established that sexual dysfunction is one of the secondary effects of diabetes mellitus. The male spontaneously diabetic (db/db) mouse has been shown to be infertile and the testes of other diabetic animal models have been reported to be below normal. The present study was performed to determine the effect of diabetes on the epididymis, an androgen target organ. The initial (EI) and terminal (ET) segments from the epididymides of 5 db/db mice were examined. Corresponding samples from 5 non-diabetic animals carrying the diabetic gene (db/y) and 3 non-diabetic non-carriers (y/y) were used as controls. The tissues were routinely processed for light and electron microscopic evaluation. The following observations were made: 1) The principal cells in the EI of diabetic animals appeared to have more lysosomes than those in control mice. 2) Large empty intracellular vacuoles were present in the ET of diabetic animals. These vacuoles were surrounded by epithelial cells and lined with short microvilli. 3) Sperm were sparse in the lumen of the ET of diabetic animals. 4) The proportion of basal cells was higher and that of the principal cells lower in the ET of diabetic animals when compared with the controls. 5) The epithelial cell height was significantly lower in the EI but higher in the ET of db/db animals. 6) Tubular diameter in the EI and ET of diabetic mice was also much less than that of controls. These changes indicate that diabetes has a profound effect on the epididymis, and these alterations may contribute to the infertility of the db/db mouse.

34 THE RESPONSE OF THE HAMSTER EPIDIDYMIS TO CHANGES IN PHOTOPERIOD. M. K. Holland, L. A. Gregory*, and H.-C. Orphelin-Crist, Center for Reproductive Biology Research, Vanderbilt University, Nashville, Tn., 37232-2633

Epididymal function was investigated in hamsters exposed to short (8L:16D) photoperiod for 11 weeks (recession phase) before transfer to a long (16L:8D) photoperiod for a further 11 weeks (recrudescence). Plasma testosterone (T) levels declined to undetectable levels within 5 weeks in a short photoperiod while epididymal weight declined 23%. In the subsequent 3 weeks, the epididymis showed precipitous weight loss and at transfer was 16% of initial weight. In a similar period, weight of a castrate epididymis declined to 19%. The epididymis was devoid of spermatotrophs and the epithelium was decreased in height with little evidence of active secretion. However, it was less regressed than in 6-week-castrate animals. Serum T was first detected 4 weeks after return to a long photoperiod and rose up to 7 weeks, then returned to normal values. Epididymal weight did not change significantly for the first 7 weeks after transfer and then rapidly rose to 75% of initial weights at 11 weeks. The epididymal epithelium was similar to that of an intact animal, although the tubules were less distended by spermatocysts, possibly accounting for difference in weight. T-administered at full regression was incapable of stimulating growth of the epididymis, whereas castrated animals responded. Responsiveness to T started 3-6 weeks into recrudescence. We conclude that the photoperiod-regressed epididymis and the castrate respond differently to fluctuations in T.


In the ram, about 22 ng oxytocin enters the ductuli efferentes (DE) via rete tests fluid daily. It may be removed by epithelia lining the excurrent ducts since immunohistochemical localization of oxytocin is conspicuous in the caput epididymis (Biol Reprod 39:391, 1988). To determine if luteinizing hormone is taken up by a receptor-mediated process in specific regions, 10 µl oxytocin conjugated to colloidal gold (OG), 100 ng oxytocin/10 µl conjugate, a 20-fold excess of oxytocin followed by OG, or colloidal gold alone (GA, 9-10 nm) was microinjected over a period of 2-3 min into the lumen of the DE, and initial (IS), proximal (PC), central (CC) and distal (DC) segments of the caput epididymis of rams under general anesthesia. The duct at the injection site was sampled 20 min later, and tissue was processed for electron microscopy. Gold particles present in different organelles found in a constant surface area (4000 µm²) of epithelial cells cut longitudinally were counted. Data were analyzed using one-way ANOVA and Duncan's multiple range test.


Sperm maturation is dependent on both androgens and de novo protein synthesis by the epididymis. To determine which epididymal proteins are important in this process, mouse epididymal proteins were characterized. Two epididymal proteins of 29 kD and 25 kD were purified and polyclonal antibodies raised against them. In vitro incorporation of [35S]methionine into epididymal tissue slices revealed that the 29 kD protein is present in the media from distal caput, corpus, and cauda and that the 25 kD protein is present in the media of caput and corpus. Incorporations performed using tissue isolated from 4-week castrate and 4-week castrate/4-week testosterone-replaced animals demonstrated that the synthesis of both proteins is dependent on androgens. Both the 29 kD and the 25 kD proteins are glycoproteins present in murine caudal fluid and proteins of similar mobility are present in Trion X-100 extracts of spermatocysts. Polyclonal antisera raised against the 29 kD protein bound a single 29 kD band on Western blots of caudal epididymal fluid. The 25 kD protein was localized by immunohistochemistry in the epididymal lumen beginning at the mid-proximal corpus, consistent with the incorporation data. These data show that both the 29 and 25 kD proteins are secreted in regions of the epididymis important in the acquisition of sperm fertilizing ability, and that they are androgen-dependent. The antibodies will be used to confirm whether they are indeed spern associated and determine if they play a role in sperm-oog interaction.
37 COMPOSITION AND SECRETION OF AMINO ACIDS IN RAT EPIDIDYMIS. Barry T. Hinton, Dept Anatomy & Cell Biology, University of Virginia Health Sciences Center, School of Medicine, Charlottesville, Va. 22908.

The epididymal amino acid microenvironment is a product of testicular and epididymal secretion, absorption and sperm metabolism. The goal of this study was to determine the contribution of epididymal secretion of amino acids towards this microenvironment. To collect sufficient volume of luminal fluid for amino acid analysis, segments of rat caput, corpus and cauda epididymis were microperfused with mineral oil (rate 0.3-0.5µl/min) and the luminal contents collected. The split-drop stopped-flow micropervfusion technique was used to determine the secretion of amino acids into the lumen of each epididymal region. Free amino acid content measured using a D-300 ion exchange analyzer or a Gilson HPLC with a C18 reverse phase column. 

38 VALIDATION OF A COMPUTERIZED SYSTEM FOR EVALUATION OF EQUINE SPERMATOID MOTILITY. Dickson D. Warner. Larry Johnson, and Scott C. Vaughan, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843.

Three ejaculates from each of 3 stallions were used to test the feasibility of a computerized system (Hannon-Thorn Motility Analyzer; HMA) for evaluation of equine spermatozoal motility. Al ejaculates were diluted in 25 x 10^6 spermatozoa/ml using a skim milk-glucose seminal extender. Varioce components (ejaculate within stallion, sample within ejaculate, and microscopic field within sample) were determined for each stallion using HMA. HMA was compared with frame-by-frame playback videomicroscopy (VIDEO) for estimation of spermatozoal number per field (SN) and percentage of total sperm motility (TSM) in seven samples, and estimation of curvilinear velocity (VCL) and straight-line velocity (VSL) of individual spermatozoa. The effect of spermatozoal concentration on incidence and outcome of intersecting spermatozoa was also evaluated. The greatest variability was attributed to the microscopic field-within-sample component. HMA yielded higher (p<0.05) SN and TSM than VIDEO (54.7±4.6 vs 44.9±3.4 and 77.9±2.2 vs 70.7±2.8, respectively). Mean VCL and max VSL were higher (p<0.05) with HMA than VIDEO for 3 of 5 characteristic spermatozoal motility tracks (straight tracks, bending tracks, zig-zag tracks), but repeatability was always better with HMA (p<0.05). Using HMA, the number of intersecting spermatozoa was highly correlated with SN (r = 0.97) and incidence of spermatozoal interactions (r = 0.99). HMA appears to be a valid method for determining TSM, VCL and VSL of equine spermatozoa provided ejaculates are diluted appropriately prior to evaluation. (TARU Research Enhancement Fund)


There are differences among men in the ability of their sperm to survive cryopreservation, with few men having sperm survival suitable for cryobanking. Our hypothesis is that variation in cryodamage results from differences in phase transition (PT) behavior of sperm membrane lipids. Membrane lipid PT is thought to be a major damaging event in cryopreservation. Until recently, this PT could not be non-invasively measured in living cells. We have applied Fourier transform infrared spectroscopy (FTIR) to measure the transition temperature (Tm) of sperm membrane lipids (Crowe et al., 1986, Cryobiology, in press). To test the hypothesis that sperm lipid PT behavior varies among men, motile sperm from five donors were selected by swim-up into BWW medium. The swim-up fraction was centrifuged, and the resulting pellet was loaded into a temperature-controlled chamber. FTIR scans were made every 1°C as the sample was cooled from 35°C to -12°C. A shift in the frequency of the symmetrical CH2-stretching peak occurs indicates the Tm of membrane lipid. There were significant differences in PT behavior of sperm membrane lipids for the five donors tested. Tm values were 7, 15, 16, 21 and 22°C. 

40 EFFECT OF TEST-YOLK BUFFER ON THE HUMAN SPERM ACROSOME REACTION. P. Biefield, R.S. Joyendran and L.J.O. Zanewold, Department of Ob/Gyn, Rush University, Chicago, IL 60612, Department of Ob/Gyn, University of Dusseldorf, FRG.

Human spermatozoa show enhanced penetration into zona-free hamster oocytes after incubation in TEST-Yolk buffer (TYB) for 18 hr at 3°C followed by washing with BWW (containing 0.35% HSA) as compared to incubation for 18 hr in BWW at 37°C and washing. In order to determine the effect of TYB on the acrosome reaction, aliquots of ejaculates were incubated for 3 and 18 hr either in TYB at 5°C or in BWW at 37°C. Percent acrosome reacted (AR) spermatozoa were evaluated after washing with BWW and fixation, using the double stain technique (De Jonge et al. J. Androl. 9:46F, 1988). At each time point, TYB-treated spermatozoa showed a significantly (p<0.001) higher AR than those treated with BWW (3 hr: 30.9±2.0% vs 17.9±2.1%, n=11; 18 hr: 42.0±5.2% vs 19.9±1.6%, n=8; mean±SD). The AR obtained with TYB at 3 hr was essentially identical to that of 3 hr BWW-incubated spermatozoa treated with ionophore A23187 (30.9±2.0% vs 30.2±2.0%; n=1). Washing the TYB-treated sperm with BWW at 37°C tended to give higher AR (31.3±8.4%) than washing at 5°C (24.6±7.5%). If the TYB-treated sperm were fixed before washing, no increase in AR was observed (15.3±4.0%). These results indicate that TYB treatment enhances AR but that this only occurs during or after washing the spermatozoa, possibly due to the removal of a lipid component from the sperm membrane. Supported by NIH HD 19555.
FACTORS GOVERNING HUMAN SPERM-CERVICAL MUCUS INTERACTION

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We have evaluated the influence of semen characteristics, immunobead-binding and cervical mucous quality upon the outcome of standardized Kr€öner-type tests using discriminant function analysis. CellSoft was used to determine sperm movement characteristics but, because of their documented unreliability, CellSoft sperm count and motility results were excluded. We used both v3.6 CellSoft and a new “Forward Progression” (vFP) version which only uses progressive cells for movement analysis. The most significant predictor variable was progressive sperm concentration (17% of variance), followed by % immunobead-positive spermatozoa and the amplitude of lateral head displacement (ALH), with CellSoft v3.6, mucous pH, normal sperm morphology and the Inler score were also accepted into the analysis giving a Wilks’ lambda of 0.597 (i.e. 40.3% of all variance accounted for) and a 79.2% correct result classification. Using CellSoft vFP values, straightness (ratio of straight line and average path velocities) and beta/cross frequency were also significant predictor variables which the analysis accounted for 43.7% of all variance (Wilks’ lambda = 0.563) with a 79.8% correct classification. Without CellSoft results the analysis was only based on 32.8% of the variance and produced a less reliable prediction. Clearly the concentration of progressively motile spermatozoa is an extremely important semen characteristic. Furthermore, because ALH is a significant predictor of nucus penetrating ability, we conclude that automated sperm movement analysis (preferably using a system similar to CellSoft vFP) is a valuable addition to diagnostic andrology.

LOGLITUDINAL STABILITY OF SPERM KINETIC MEASUREMENTS DETERMINED BY COMPUTERIZED VIDEOMICROGRAPHIC ANALYSIS.
K.A. Ginsburg, M.L. Poland* and K.S. Hoppinger, Wayne State University School of Medicine, Detroit, MI 48201.
Spern count, semen volume and normal morphology determined manually have been reported to be stable over a 6 month period in normal males (Androl 7, 1986). Using computer-aided semen analysis (CASA), we examined the longitudinal stability of motility as well as concentration characteristics. Six semen samples from 6 healthy men were obtained every 2 weeks for 3 months and the following were determined: sperm concentration (CCUC), percent motility (PMT), average curvilinear velocity (VEL), average linearity (LIN), maximum amplitude of lateral head deflection (MALH), mean amplitude of lateral head deflection (MALH) and head beat frequency (HRF). For each measurement, the coefficient of variation (CV) was computed for the 6 samples, then averaged for the 6 subjects. Using ANOVA, average CVs were significantly (p > 0.05) higher for CCUC and HRF compared to the other measurements.

NOTILITY CHARACTERISTICS OF MOUSE SPERM WITH ABNORMAL HEADS OR NO HEADS. F. Olds-Clarke and H.L. Heisterich, Temple University School of Medicine, Philadelphia, PA and M.D. Anderson Cancer Center, Houston, TX.

The relationship between sperm motility and sperm morphology is uncertain. Theoretically, because differences in sperm head size and shape affect physical characteristics such as mass and drag, they would be expected to alter swimming speed and/or path shape. To study the effect of the sperm head on motility, we took advantage of mice nonagous for the az mutation, which markedly affects sperm head shape. About 80% of the motile sperm have normal-size, but abnormally-shaped heads (headed sperm), while the remainder of the motile sperm have no visible head (headless sperm). If head size affects the movements of sperm, headless sperm should differ from headed sperm. Sperm from 4 asl/asz mice were isolated in medium which will support HFP videography were made at 30, 90 and 270 min of incubation. Motile sperm were selected at random for tracing; a mean of 36 headed sperm and 8 headless sperm were traced per line per nile. Sperm were traced for 1.0 sec of real time at 30 frames/sec. The VCL,0 (an estimate of instantaneous swimming speed in um/sec) and LIN (an estimate of the straightness of the path) were calculated (Neil and Olds-Clarke, Gam. Res. 18: 121, 1988). There were no significant differences between headed and headless sperm in the VCL,0 or LIN at any time. These data suggest that gross differences in head size do not necessarily have significant effects on swimming speed or path shape. Thus motility and head size appear to be independent sperm characteristics.

OBSERVATIONS ON BACMR AMBER OVUM PENETRATION AND CHROMOSOME CONVERSION OF COLD-STORRED SPERM FROM THE CYMNOIGUS MONKEY. Macaca fascicularis. T. Foulis**, A. Skalnik**, T. Turner*, R. S. Schader*, T. Schindler** and I. Loeser*, Department of Biology, St. Mary’s College, South Bend, IN; I. Lehigh Univ., Bethlehem, PA; & Experimental Toxicology Branch, NIOSH, Cincinnati, OH.

We have evaluated conditions that may enable experimental haploid chromosome analysis of monkey sperm following toxic exposures. Fresh whole ejaculates (0.1 to 0.5 ml) were collected by penile electrostimulation, mixed with test-yolk buffer (TYB), then chilled and shipped on ice by overnight air express. Traditional methods of sperm washing using Percoll or BWW plus 0.3% BSA, particularly at ambient temperatures, resulted in loss of motility during processing. Similarly, monkey sperm in TYB at room temperature lost activity over a 3 hr period in contrast with an identically treated human specimen. However, sperm diluted directly from cold TYB into various media, warmed and under oil, maintained activity for 2-3 hrs. These media included: BWW + 0.3% BSA, TALP, Banks + 10% fetal calf serum, or CAMEL + 0.3% BSA. When incubated with zona-free hamster ova, strong sperm-egg interactions were observed. Sperm penetrated about 4% of the eggs (11 of 289 ova), and monkey chromosomes were evident in two nuclei following Cleaves staining. Thus, cymnoigus monkey sperm are able to function within the hamster ooplasm, preliminary conditions are available for examination of haploid chromosome transmission by this species. These methods may be useful for analyzing effects of toxic agents on the structure and transmission of chromosomes of primates.
Simplified Method for Evaluating Sperm Hyperactivation: A Test Based on Dilution Effect versus Washed Sperm Membrane

Philip Chan, Israel Kenigs*, Donald Treadway and Ken Salley

The latest method for evaluating sperm hyperactivation, known as hyperactivation (MSA) when the cells are washed and resuspended in synthetic media such as Ham's F-10 supplemented with serum. The purpose of this study was to develop a simple method for determining the semen in order to quantitate hyperactivation. Liquefied semen containing sperm cells was diluted 1:10 with Ham's F-10 supplemented with 10% cultured cell and snap-top centrifuge tubes and placed in a 37°C heated block. Aliquots of the same semen were washed by centrifugation and the resuspended sperm samples placed at room temperature (identical to temperature of samples evaluated by cellsoft hyperactivation modules). The experiment was repeated 3 times. The results were 78.8 ± 2.6, 96.5 ± 2.5, 84.5 ± 3.7 and 84.3 ± 0.8% (diluted sperm) versus 79.0 ± 4.1, 83.5 ± 2.5, 57.5 ± 3.4, and 73.2 ± 2.5% (washed sperm) for hours 1, 2, and 3 respectively. There was no statistical difference (P> 0.05) between the procedures up to 2 hours. Analyses of different samples collected at different times indicated the same result. The data suggest that sperm cells diluted free from seminal plasma materials exhibit better levels of hyperactivation comparable to washed sperm for up to 2 hours. The dilution method has the added advantage of generating a range of sperm cells under actual conditions that do not involve damaged or artificially altered sperm membrane characteristics through centrifugation. It is not known whether the results observed after 2 hours is a manifestation of an artifact of the washing procedure itself.

Survival of Washed and Swim-Up Sperm: Based on the Predawn and Swim-Up Characteristics

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Semen samples that have undergone washing and swim-up have improved characteristics such as motility, velocity, and morphology. Using survival as a means to determine the quality of washed and swim-up sperm, a comparison was made between the per cent of sperm and swim-up parameters to the survival of the specimen after it had undergone washing and swim-up.

Specimens were considered normal if they exhibited the following parameters before washing and swim-up:

1. 300,000 sperm/ml
2. 50% motile sperm
3. 10 microns/sec velocity
4. 50% normal morphology

To determine survival, live postwash and swim-up specimens were analyzed daily until the sperm were no longer motile.

Specimens that exhibited normal parameters before washing and swim-up survived a mean of 5.8 days postwash and swim-up. Specimens that exhibited abnormal motility prior to swim-up survived a mean of 4.1 days postwash and swim-up; abnormal motility and morphology, 3.5 ± 1 days; abnormal motility and concentration, 2.8 days; and abnormal motility, concentration and morphology, 2.6 days.

These data suggest that although specimens may improve postwash and swim-up then the in vivo survival ability and postwash quality may be based on their wash and swim-up characteristics.

ESR Determination of Human Sperm Cell Volume

F. Kleinbahn, WY Spiteri, P. Wittman, KE Colvin, and JK Critser

Foer the Fourteenth Annual Meeting of the American Society for Reproduction and Transplantation Immunology, 1987. (Abstract)

Electron spin resonance (ESR) was used to determine the volume and osmotic properties of normal human spermatozoa. Sperm were isolated using a swim-up procedure, suspended in TALP, labeled with 20 mT eq. terpene, and the extracellular signal broadened with 35 to 50 kEq chro-mium oxalate and measured at 35°C using a Varian E109 spectrometer. The average intracellular aqueous volume was found by measuring the cell water signal strength against a standard and dividing by the number of cells in the sample (hexazostterex count). The results of this experiment indicate an intracellular volume of 30 ± 34% (4540 ± 22). This is significantly larger than literature values derived from Coulter Counter techniques. This may be due to the assumptions regarding the volume density of the sperm and their overall length which is largely relative to the Coulter orifice. ESR calibration experiments with human immature RBCs yielded a net volume of 1.1 ± 0.4 microns which is 1.1% globular volume with a literature value. Preliminary osmotic response data for human sperm, as measured by intracellular aqueous volume, have been obtained in the range of 15% to 25% collapse (6.5% ± 3.0%). The results fall below that expected for a linear response and may reflect the presence of an osmotically fragile subpopulation. These data seem to support the usefulness of ESR in evaluating osmotic events of human sperm.

Screening Technique for the Detection of Sperm Antibodies Using the Dot Immunobead Method

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A simplified method for screening for sperm antibodies was described by Clarke (AJRM 13:1-3, 1987) using rabbit anti-human immunoglobulin immunobeads (GAM bead, Biorad). The present study was conducted to evaluate the use of GAM bead for initial screening of samples for sperm antibodies. Immunobead binding tests were performed on 98 undiluted sera using rabbit antihuman IgG, IgA, IgM, and GAM immunobeads. The percent binding of antibody immunobeads was recorded for each of the four bead types. The highest percent binding occurred in the IgM bead and a moderate level of binding was observed in the IgA and IgM bead. The percent binding of the GAM bead is highly correlated (r=0.96; p=0.002) with the percent binding of the GAM bead and the percent binding of the GAM bead is highly correlated (r=0.96; p=0.002). Using our criteria of 25% binding for positive, 7% (81%) of the test samples resulted in the same interpretation. False positive results were obtained in 31% (13%) of the samples which had binding of 25% binding of the GAM bead and 35% binding of the highest individual class beads. In contrast to previous studies which did not report false positive results (BMC Med Sci 14:503-506, 1986; AJRM 13:1-3, 1987). False positive results would have been detected when the individual class bead binding was performed. A false negative result was observed in 6 (6%) of the samples as indicated by the use of GAM bead and 25% binding of the highest individual class beads. Three of these false negative results were IgG positive with binding indicate that the use of the binding of 25% and may be of questionable clinical significance. Thus, initial GAM bead screening using GAM bead could increase laboratory efficiency.
In the present study the Hamilton-Thorn Motility Analyzer (HT-M2030) was used to investigate the effects of relaxin on cryopreserved spermatozoa from 6 stallions. Ejaculates were extended 2 to 1 in an extender containing sterile H2O, 100 ml; glucose, 3.0 g; sucrose, 5.0 g; BSA, 1.5 g; pH 6.7 for centrifugation at 200 to 400 g for 4 to 7 minutes, depending on individual seminal characteristics. Following aspiration of the supernatant a freezing extender containing sterile H2O, 100 ml; sucrose, 7 g; glucose, 0.9 g; Nanalac, 2.4 g; egg yolk, 4 ml; and glycerol, 3.5 ml was added slowly to give a concentration of 125 x 10⁶ sperm/ml. Sperm were frozen in 5 x 280 ml plastic straws (5 ml) in static N2 vapor for 15 min. 1 cm above the N2. After storage in N2 for at least 60 days, straws were thawed and diluted 5 fold in a skim milk, glucose extender containing 400 ng purified porcine relaxin (HKN-PI NIDDK & NPP) or extender only and incubated 1 hr. at room temp. before evaluation. Parameters studied were: Motility, % (M); Velocity, microns/second (V); Progression Velocity, microns/second (Vp); Linear Index, % (LI).

Our results indicate that relaxin significantly increased M, V, and Vp in frozen thawed stallion semen.

EFFECTS OF POLYMORPHONUCLEAR LEUKOCYTES ON HOUSE SPERM MOTILITY. Sopit Mongkolrigit-Kleat*, Jompat Tongphalee*, and Deborah Anderson*, Pearing Laboratory, Dept of OB/Gyn, Brigham & Women's Hospital, Harvard Medical School, Boston, MA 02115; * Reproductive Biology Unit, Dept of OB/Gyn, Ottawa Civic Hospital, Ottawa, Ontario, Canada.

This study was designed to examine whether polymorphonuclear leukocytes (PMNs) and/or their secretory products affect sperm motility. PMNs were obtained from mouse peritoneal exudates after 6 hours of casein induction. Motile epididymal sperm were obtained by Percoll 45%/90% discontinuous gradient centrifugation. Marked agglutination was observed when sperm were incubated with PMNs or fresh PMN supernatant. The degree of agglutination was increased with the number of PMNs in the culture medium. Immobilization of sperm by agglutination resulted in a significant reduction in the number of free sperm in the incubation mixture. In the nonagglutinated sperm population, motility was significantly decreased in the presence of PMN supernatant after 2 hours of culture. These results suggest that PMNs could adversely affect fertility by decreasing the number of motile sperm through agglutination and inhibition of motility.

TRI-FUNCTIONAL FLUORESCENT STAINING OF CRYOPRESERVED BOVINE SPERMATOZOA. Scott A. Ericsson*, Babuna Y. Kramer†, Duane L. Garner‡, and Doug Redelman*, Departments of Biology, Animal Science and Microbiology, University of Nevada-Reno, Reno, NV 89557.

The functional capabilities of individual spermatozoa were estimated using three fluorescent stains to examine specific organelles. Two replicates of cryopreserved spermatozoa from six bulls were incubated with the tri-functional fluorescent staining system consisting of Rhodamine 123 (R123), Hydroethidine (HED) and fluorescein Isothiocyanate-labeled lectin from Pisum sativum (PSA). Plasma membrane integrity was assessed with HED, the presence of acrosomal components with PSA and mitochondrial function with R123. Stained spermatozoa were examined flow cytometrically for levels of red (HED) and green (R123/PSA) fluorescence and microscopically for specific organelle staining. Three major fluorescent cellular populations were identified. The major population of spermatozoa represented cells with intact membranes and functional mitochondria that were identified by the combination of high red (HED) and moderate green (R123) fluorescence. Moribund cells had decreased red (HED) and high green (R123/PSA) fluorescence representative of cells with reduced membrane integrity and functioning mitochondria. Dead cells emitted the least amount of both red (HED) and green (R123/PSA) fluorescence which is reflective of a lack of membrane integrity and degenerative mitochondria. The tri-functional staining system provided meaningful information on specific spermatozal organelles. Supported by USDA grant 85-CRCR-1-1852 and NSF Grant DMB 8518021.


Semen samples were collected from 13 cynomolgus monkeys by electroejaculation and by miming the epididymis (at sacrifice), and motion analysis was performed using a Makler chamber and a computer assisted semen analysis system (Cellsoft, Cryo Resources, New York). Two ejaculates from each animal were compared and the means were: M - 18, 181 um/sec, V - 181, 173 um/sec, LIN - 9.4, 9.3, LHA - 24, 2.2 um, BFR - 15.1, 15.0 um. The means for the epididymal samples were: M - 133 um/sec, V - 123 um/sec, LIN - 9.0, LHA - 23 um, BFR - 18.7 um. Tukey's studentized range test found V, Vp and BFR for the ejaculated samples to be similar to each other but different (p <0.05) than the epididymal samples. LHA was similar in all three cases. LIN showed the two ejaculates to be significantly larger than the epididymal sample.

Little is known about the seminal pH and its relationship to other semen constituents. In order to obtain more information, ejaculates were obtained from donors and partners of infertile couples after 3-5 days of sexual abstinence. Along with a number of semen characteristics, the pH was measured using a Corning pH meter (Model 125). The pH of 373 ejaculates with normal semen parameters (WHO guidelines) was 7.84 ± 0.2 (mean ± SD). The ejaculate pH decreased with time: from 7.75 ± 0.2 after an average of 51 minutes to 7.63 ± 0.2 after an average of 109 minutes (n=69). A significant (p<0.01) correlation was present between the pH and the sodium, potassium, calcium, magnesium, iron, zinc, chloride, carnitine and GPC content of semen as well as with the volume and sperm concentration, but not with the phosphorus, carnitine and GPC content or with sperm motility and morphology. The initial portion of the ejaculate (mostly prostatic fluid) was lower in pH (7.77 ± 0.5) than the remaining ejaculate (mostly seminal vesicle fluid) (8.02 ± 0.3) (n=16). In addition, ejaculates with a pH of 7.4 or less had a significantly higher zinc (prostate indicator) and lower fructose (seminal vesicle indicator) content than the other ejaculates. The ejaculates of men diagnosed as having asymptomatic infection of the accessory glands possessed a higher (p<0.01) pH (7.91 ± 0.2; n=191) than those without infection (7.82 ± 0.2; n=183). Essentially no differences were noted in the pH of fertile (7.84 ± 0.3, n=81) and infertile (7.83 ± 0.3, n=38) ejaculates as established by IVF.

QUALITATIVE TESICULAR BIOPSY AS A PREDICTOR OF SPERM CONCENTRATION IN ELECTROSTIMULATED EJACULATES OF SPINAL CORD INJURED (SCI) MEN


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A program of male reproductive rehabilitation following SCI has been established utilizing electroejaculation as a semen procurement method. As part of a comprehensive evaluation of their fertility potential, quantitative testicular biopsy was carried out in 14 men with a mean age of 28 years and a mean duration of the SCI of 6.5 years. Quantitative analysis of the germinal epithelium was performed on photomicrographic prints taken at 400X. Randomly selected round seminiferous tubules (SNT) were evaluated and corresponding micrometric measurements were made using an AO micrometer. Acrosomal late spermatid formation (>10/SNT) was noted in 11/14 men. In 8 men undergoing electroejaculation, no significant correlation between the sperm concentration of the electrostimulated ejaculates and the mean number of spermatids/SNT was noted (r=0.44). However those with <10 spermatids/SNT showed either azoospermia or severe oligospermia. From these preliminary results testicular biopsy may be used for the appropriate exclusion of unsuitable SCI men from assisted ejaculatory techniques.

SEMIN CULTURE: ITS IMPORTANCE IN EJACULATES WITH QUESTIONABLE SEMEN QUALITY.


Bacteriological cultures were performed in midstream collected urine and in semen obtained by masturbation from patients exhibiting more than 5 WBC/HPF in semen. Of the 129 ejaculates with negative urine cultures, 102 (79.1%) showed bacterial growth. Aerobic bacteria were isolated from 31 (30.4%) cultures and anaerobic bacteria from 28 (27.5%) cultures while the remaining 43 (62.8%) had both. Moreover, 78 (76.5%) ejaculates with bacterial growth had two or more different types of bacteria present in their semen. Antibiotic susceptibility revealed that the use of chloramphenicol and tetracycline were effective against 80% or more of the bacteria cultured. Penicillin and ampicillin were less than 50 effective while gentamicin or ampicillin was intermediate against the bacteria tested. Although the various semen parameters were different between ejaculates with positive and negative cultures, only the semen volume (2.6 ± 1.8 vs 3.9 ± 1.3 ml) and sperm drive (1.6 ± 0.4 vs 1.2 ± 0.4, respectively) were significantly (p<0.05) different between these two groups. The high incidence of positive culture results of ejaculate and relative resistance of these bacteria to common antibiotics suggest the importance of routine cultures of ejaculate with questionable semen quality, and appropriate antibiotic therapy whenever necessary, prior to intrauterine insemination.

FEMALE INFERTILITY: A GROWING PROBLEM, A PUBLIC HEALTH ISSUE.


To address the consistent finding of atherosclerosis in anejaculatory men, we compared the biochemical constituents of the anejaculatory men to groups of age and race-matched donor semen. Ten groups of normal semen samples were analyzed for 19 biochemical parameters, pH, and osmolality. Organic components included triglycerides, glucose, fructose, uric acid, creatinine, BUN, total protein, albumin, and cholesterol. Metabolic enzymes including glutamic oxaloacetic transaminase (GOT), glutamic pyruvate transaminase (GPT), lactate dehydrogenase, and alkaline phosphatase were measured. Inorganic constituents included chloride, sodium, potassium, zinc, and phosphorous. Though not significant, higher levels of BUN and creatinine were demonstrated in most ejaculates suggesting urinary contamination of the antegrade samples. In the electroejaculates we also noted significantly lower levels (p<0.05) of fructose, albumin, GOT, and alkaline phosphatase as well as significantly higher levels (p<0.05) of chloride. No significant difference in osmolality or pH was found. Moreover, in the electroejaculates the levels of glucose, uric acid, and all inorganic constituents approached their corresponding levels in serum. This may suggest a partial equilibration of such components between the blood serum to the seminal compartments and possible atrophy of the accessory glandular epithelium in men with neurogenic ejaculatory failure.

Our objective was to study the role of the sympathetic nervous system in regulating sperm transport through the epididymis. Sprague-Dawley rats were: 1) denervated by surgical ablation of the inferior mesenteric plexus (IMP); 2) vasoconstricted by ligation of the proximal or distal vas deferens; or, 3) sham-operated (controls). One, 2, 4, and 8 weeks following surgery, the tests, epididymides and vas deferens were examined and weighed, and the number of sperm present in the epididymides was determined. Testicular weight, histology and sperm content did not differ between control and treated rats 1-8 weeks following surgery. In contrast, at 1, 2, 4, and 8 weeks, caudal epididymal weights in denervated rats were, respectively, 37, 29, 32, 38% and 22% greater (P<0.01) than in sham-operated rats. More strikingly, the number of sperm accumulated in the cauda epididymides of denervated rats was 606, 624, 644, 424% and 424% greater (P<0.01) than in control rats at 1, 2, 4, 6 and 8 weeks, respectively. Although not present earlier, sperm granulomas, located bilaterally at the cauda-vas deferens junction, were observed in 24% (1/12) of the denervated rats 8 weeks following surgery. Bilateral sperm granulomas at the cauda-vas deferens junction were also observed, after 1 wk, in all rats in which the vas deferens were ligated proximally. However, sperm granulomas were not observed at the cauda-vas deferens junction in rats ligated at the distal vas deferens. These results show clearly that ablation of the IMP inhibits sperm transport through the epididymis and suggest that the primary lesion is an obstruction at the junction of the cauda epididymis and vas deferens.
THE EFFECT OF HYPOPHYSECTOMY ON PROLUMINAL TRANSPORT OF \( \text{H} \)-TESTOSTERONE ACROSS THE EPIDIDYMAL EPITHELIUM IN THE RAT. Yasuo Tanamoto and Terry C. Turner, University of Virginia School of Medicine, Charlottesville, VA 22908.

Net proluminal movement of \( \text{H} \)-testosterone (\( \text{H} \)-T) across the rat epididymal epithelium was studied by in vivo perfusion and subsequent microculture of caput (CPT) and proximal (PCP) tubules. Experiments were performed on animals without hypo-physectomy (Hypox) or on day 5-6 after Hypox and 9-10 after Hypox. Tubules were perfused with KRPF containing 26.7 uc/ml \( \text{H} \)-T, \( \text{C} \)-polyethylene glycol (\( \text{C} \)-PEG) was included in perfusion fluid as a marker (1.3 uc/ml) for contamination of intraluminal fluid by peritubular fluid. Radioactivity of \( \text{H} \)-T and \( \text{C} \)-PEG in perfusion and intraluminal fluids was determined at 1 and 2 hrs after perfusion and the percentage of peritubular \( \text{H} \)-T and \( \text{C} \)-PEG appearing in intraluminal fluid was determined. A sperm concentration microassay was performed on microculture samples from CPT tubules to assess testicular contribution to the luminal content. Net entry of \( \text{H} \)-T into the CPT tubules of rats 5-6 days after Hypox was similar to that of normal animals. In contrast, net proluminal movement of \( \text{H} \)-T into the CPT tubules of rats 9-10 days after Hypox significantly decreased at both 1 and 2 hrs (p<0.05). Sperm counts in the CPT tubules of rats 5-6 days after Hypox were also consistent with normal values, but those in the CPT tubules of rats 9-10 days after Hypox were significantly reduced (p<0.025). These results imply that proluminal androgen movement is regulated by the presence of some testicular product in the epididymal luminal. Subsequent experiments will determine whether the factor is replaced by FSH or LH administration to Hypox animals and whether proluminal movement occurs in the absence of spermatozoa. Supported by NIH HD 09490.


Ovine sperm acquire fertilizing potential after sequential exposure to different factors secreted by principal cells (PC) lining the proximal epididymis. To determine if different functions of PC are reflected ultrastructurally, we analyzed PC from the initial segment (IS), proximal and central caput epididymis (PCAP, CCAP) and proximal and central corpus epididymis (PCPR, CCPR). One epididymal segment (n=4) was fixed by vascular perfusion and processed for LM, NPC, and nuclear/cytoplasm ratio (N/C) and TEM. Histochemical (VD) of organs in the cytosol of the organellar (CYP) were assessed. For each region (am), 4 cross sections with complete epithelial profiles were analyzed. To determine % PC, 100 nuclei were classified as PC or other. An AloQuat System IV was used to characterize 1700.0 mm sq of epithelial area/tubule to calculate N/C. The VD of organelles was determined on 4800-2790 mum' tubule using the point-count method at a magnification of 14,000. If regional differences were detected, ANOVA (p<0.05), the SNK test was performed.

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>IS</th>
<th>PCAP</th>
<th>CCAP</th>
<th>PCPR</th>
<th>CCPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>%PC</td>
<td>72±0</td>
<td>73±0</td>
<td>74±0</td>
<td>72±0</td>
<td>68±0</td>
</tr>
<tr>
<td>N/C (PC)</td>
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<td>3</td>
<td>5</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Golgi (VD)</td>
<td>10</td>
<td>12</td>
<td>12</td>
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<tr>
<td>Endo RNC (V/D)</td>
<td>14</td>
<td>15</td>
<td>14</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Mitochondria (V/D)</td>
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<td>4</td>
</tr>
<tr>
<td>Vat (V/D)</td>
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<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
</tr>
<tr>
<td>Lysosomes (V/D)</td>
<td>0</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
</tr>
</tbody>
</table>

Means in a row with different superscripts differ (p<0.05) at SEM. 2±4%.

While quantitative differences in %VD were small, qualitative differences in percentage of each region may be associated with the different functions of PC in those regions responsible for creating the proper environment for sperm maturation (Grant HD 14509).

CRITICAL ASSESSMENT OF SPERM MORPHOLOGY BEFORE AND AFTER DOUBLE SWIM UP PREPARATION FOR IN VITRO FERTILIZATION (IVF). Richard Scott*, Roelf Menkeveld, Sergio Gehniger, Lucinda Veeco*, AHABT ACSTA, Jones Institute for Reproductive Medicine, Norfolk, VA. 23507.

Critical assessment of sperm morphology using specific and stringent criteria is predictive of the subsequent ability of those sperm to fertilize oocytes in IVF. Previous studies have evaluated sperm morphology prior to sperm preparation, thus not assessing the actual sperm used for insemination. We studied the impact of the double swim up technique used for IVF on sperm morphology using the strict criteria of Kruger in 63 consecutive patients undergoing IVF. Pre- and post-swim up morphologic assessments were done in a prospective, randomized, and blinded fashion. The mean percent normal forms pre- and post-swim up were 19.8% and 23.4%, respectively, an improvement of 18% (p<0.05) with 62 of 73 patients showing improvement. Significantly, analysis of the 27 patients with abnormal morphology upon initial assessment (normal forms 14%) revealed an improvement in percent normal forms from 8.2 to 20.3, a mean increase of 243.9% (p<0.005). Eighteen of these 27 patients showed improvements in sperm morphology, 8 were unchanged, and only one patient had a decline in percent normal forms. We conclude that the double swim up preparation used for IVF substantially improves the percentage of sperm with normal morphology and that the benefit is most substantial in those cases where the percent normal morphology is abnormal.
ELEVATED LEVELS OF WHITE BLOOD CELLS IN SEMEN ARE ASSOCIATED WITH POOR SEMEN QUALITY. 
Hans-Willi Wolff, Adriana Martinis, Joseph A. Pollitch, 
Florina Halmovici, Joseph A. Hill, and Deborah J. Anderson. 
Fertility Research Laboratory, Department of Obstetrics, Gynecology and 
Reproductive Biology, Harvard Medical School, 
Boston, MA 02115.

White blood cells (WBC) in semen from 179 
infertility patients were quantified by the 
monoclonal antibody Hein to an immunohistochemical 
technique (Fertl Steril 49:497, 1988). Routine semen 
analysis was performed by 
CellSoft automated semen analysis. One 
hundred thirty-nine semen samples showed less 
than 10 WBC per ml semen (Group A) and 41 
semen samples had more than 10 WBC/ml semen (Group B). Applying the 
unpaired student’s t-test or the Kolmogorov- 
Smirnov test for non-parametric samples, the 
means of the following semen parameters were 
significantly (P < 0.05) lower in Group B: 
percent motility (40 vs 53%); velocity (32 vs 35 um/sec); motility index 
(1365 vs 1863); and total motile sperm number 
(121 vs 258 x 10^6).

These data, in combination with previous 
findings from our laboratory on adverse 
effects of soluble products from WBC, indicate 
diagnostic and therapeutic relevance of WBC in semen.

REMOVAL OF THE HOUSE VITELLIUS BY MICRO-
MANIPULATION: EFFECTS ON SPERM BINDING TO 
THE ZONA PELLUCIDA. 
Farid Ahmed and Ralph B.L. 
Gwatkin, Reproductive and Developmental 
Biology & Gynecology, The Cleveland Clinic 
Foundation, Cleveland, OH, 44106.

Micro-manipulation was employed to investigate 
the effect of removing the mouse egg-cell (vitellus) 
from the zona pellucida (z.p.). A fine glass 
needle was passed through the z.p. of Het II 
oocytes and their contents were aspirated. 
Intact oocytes (CD-1) and empty z.p. were then 
added to microdrops of sperm (CD-1), 
which were incubated for 90 min. in Whittingham's 
medium (30 mg bovine serum albumin per ml). 
At 5-10 min. intervals, the oocytes and empty 
z.p. were washed and examined for bound sperm. 
The intact oocytes each bound 70-100 sperm 
after only 10 min., followed by a marked 
decline in binding after 15-20 min. that 
reached background (2-5 sperm/oocyte) in 30-40 min. 
Sperm binding to the empty z.p. began 
later, at 15-20 min. and rose steadily to > 
100 sperm/z.p. by 60 min. These observations 
support a role for the vitellus, or peri-
vitelline material, in accelerating sperm binding 
in addition to its recognized role in 
producing the zona reaction (loss of sperm receptors). Attempts are underway to identify 
the mode of action of the vitelline material.

COMPARISON OF FLOW CYTOMETRY TO ROUTINE TESTICULAR 
SURGERY IN MALE INFERTILITY. 
D. L. H. Pollitt, 
Heathy & Rose, 
Arline D. Edel and Ralph D. 
Lutes, Tulane University School of Medicine, New 
Orleans, LA. 70112 and University of California, 
Davis, Sacramento, CA. 95817.

We compared the use of FACS flow cytometry 
of testicular tissue to routine biopsies to assess 
semenograms for a group of 19 infertile male 
patients. The findings for both studies were 
divided into three groups: normal, moderately 
abnormal and markedly abnormal. Correlations 
between the findings for routine biopsies and 
flow cytometry were good. For nine patients 
having normal testicular morphology, seven had normal 
ploidy classes by FACS flow cytometry while two had 
moderately abnormal histograms. For five cases with 
moderately abnormal morphology, one had moderately 
abnormal and three had markedly abnormal ploidy 
distribution. For five cases described as Sertoli 
cell only, all the FACS histograms were markedly 
abnormal, consisting almost exclusively of diploid 
cells. FACS flow cytometry of testicular aspirates 
and biopsies was found to be rapid, reproducible 
and an objective approach to evaluate the infertile man. 
It can be used in an outpatient clinic in lieu of 
formal testis biopsy.

SPERM CAPACITATION TIME DIFFERS AMONG FERTILE 
MEN, M FERTILE COUPLES AND MEN WITH ABNORMAL 
SEMINA ANALYSIS. 
Charis H. Miller, Susan C. Cisse*, Susan H. Harris* and 
David Zuckerman, Departments of 
Obstetrics and Gynecological Surgery, 
Population Research Institute in 
Reproduction, University of 
Washington, Seattle WA 98195.

Human spermatozoa are able to capacitate spontaneously across semen, fuse with the acrosome 
membrane, and capacitate in the acrosome, under the simple condition of air 
conditions of the zona free human oocyte sperm penetration essay (SFA). A factor known 
significantly influence SFA results are sperm phosphatidylcholine (pseudoplatelets) late and 
abnormal concentrations in the capacitation medium. Although both are essential in 
nullifying spermatogenesis for normal donors as for small groups of patients, we have been routinely 
performing these two types of 
SFA on every patient entering the male infertility workup for SFA (3). A factor known 
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performing these two types of
Cryopreserved semen yields lower conception rates than fresh semen in artificial insemination. In IVF, frozen/thawed and fresh semen samples from donors seem to produce similar results. We compared IVF outcome in cases using cryopreserved semen from fertile donors (26 patients, 37 cycles) and infertile patients (18 patients, 19 cycles). Semen was cryopreserved by controlled vapor cooling with glycerol (8.5%) as cryoprotectant and TEA/Tris-sodium citrate-egg yolk as an extender. Viats were stored in liquid nitrogen at -196°C. After thawing, a swim-up separation was performed during 1 hour of incubation at 37°C in 5% CO2 in air. Results:

<table>
<thead>
<tr>
<th>Donors</th>
<th>Post-thaw motility %</th>
<th>Fertil rate</th>
<th>preg rate/transfer %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40.3±12.2a</td>
<td>89.0</td>
<td>32.4</td>
</tr>
<tr>
<td>Patients</td>
<td>25.8±12.2b</td>
<td>78.2</td>
<td>36.8</td>
</tr>
</tbody>
</table>

*p<0.01

In the patient group, 8 male factors (4 oligo-, 3 astheno-, 2 teratozoospermic patients) produced 4 pregnancies. Overall results suggest that the fertilizing ability of spermatozoa after freezing/thawing is satisfactory under standard IVF conditions (for donor samples and some subfertile patients), yielding pregnancy rates comparable to those obtained by fresh semen in non-male-factor patients.


Multiple media have been suggested for semen washing and concentration of motile spermatozoa before intra-uterine insemination (AIH). The purpose of this study is to determine if the B1 medium (JNRM Menexo B1 Medium - API System S.A. - La Balme les Grottes - Montalieu Vercieu 38390 - France), which is a ready-for-use preparation containing bovine serum albumin (BSA), commonly used for embryonic culture, can be also used to improve subfertile semen.

AIH was performed in 111 cycles divided into 2 groups:

1) A control group with 10 AIH cycles for cervical factor only (with normal semen). Two pregnancies (20% per cycle) were obtained near the natural conception rate.

2) One hundred and one cycles with normal or corrected female factors and abnormal semen (inflammatory, oligo-astheno- and/or teratoastrokinia), couples with an 8 year average of infertility. AIH resulted in 5 pregnancies (5% per cycle).

We conclude that the B1 medium can be used efficiently in selected cases of male infertility since our success rate is close to the one generally obtained in donor insemination with a normal cryopreserved semen (5 to 8%).

Hyperactivation conditions for human sperm. Daniel L. Houlihan, Susan A. Rothmann, Linda Henrich, Karl Schroeder-Jenkinson, and Anthony J. Theaoz, Andrology Laboratory and Section of Male Infertility, Dept of Urology, Cleveland Clinic Fdn., Cleveland, OH 44195

Computer assisted methods for quantifying hyperactivated (HA) motility have recently become commercially available but optimal conditions for analysis have not been determined. Twenty-five freshly ejaculated semen samples were obtained from 7 proven fertile donors and divided into 3 portions. Each was washed twice and the pellets overlayed in either Ham's F-10, BBM, or synthetic human tubal fluid (HTF). All media were supplemented with 0.3% or 1% human serum albumin (HSA). NTIle sperm were isolated using a swim-up procedure. Aliquots were incubated at 37°C in 5% CO2 in air for one hour. HA cells were measured at 0, 90, 180, and 270 min, using a digital image analyzer (CellSoft, CryoResources). To test for differences across media and time, a general mixed model of analysis of variance was used. Few HA cells were observed with 3% HSA, thus all experiments were performed using 0.3% HSA. Media influenced % HA with highest values occurring in HTF (24%) and BBM (23.1%). Ham's F10 was associated with significantly lower (p<0.001) HA at 14%. Time of analysis did not influence results during the experiments suggesting that sperm became HA immediately upon removal from semen. A significant inter and intra donor variation was observed; however, differences among media and times were the same across donors. The experiments define laboratory conditions for HA which may be used to reveal abnormalities in infertile men.

Artificial insemination using cryopreserved donor sperm. Susan A. Rothmann, Jeffrey Seiler, Marybeth Collins, Linda Henrich, Rita P. Gudman, Delbert L. Booher, and Robert L. Collins, Andrology Laboratory and Sperm Bank, and Dept. of Gynecology, Cleveland Clinic Fdn., Cleveland, OH 44195

830 insemination (insen) cycles using cryopreserved donor sperm were performed in 176 patients. Semen was frozen in 7% glycerol (1/- egg yolk buffer) using a staged nitrogen vapor procedure. Sperm were stored in liquid nitrogen for an average of 12 months including a 3 month quarantine period. Insems were performed 13-24 hours after the natural urinary luteinizing hormone surge as determined by patient-performed testing; ovulation was induced with hCG in 4 patients. A significant effect of insems route was noted; one pregnancy occurred in 107 cycles of intracervical insems contrasted with 83 pregnancies in 723 cycles of intrauterine insems with washed semen (average dose was 27 million motile sperm). 66 of 83 pregnancies (80%) occurred within the first 4 cycles of intrauterine insems; 77 of 83 (93%) occurred in 6 cycles. A significant difference in fecundity rates was noted between women with 1 insen/cycle (560 cycles) and 1 or 2 insen/cycle (163 cycles) of 13% vs 5%, suggesting that the latter group had ovulatory abnormalities or problems with interpretation of LH tests. 11 spontaneous abortions and 1 ectopic pregnancy occurred. The program's previous fecundity rate using fresh donor semen was 10%. The results indicate that cryopreserved sperm was as effective as fresh sperm when timed, intrauterine insems were used.
Favorable in vivo influences on fertility of rabbit sperm populations with cytoplasmic droplets. R.A. Fryzer-Husken, R.G. Brackett and A.B. Sanders, College of Veterinary Medicine, The University of Georgia, Athens, GA. 30602

The fertility of sperm cells with cytoplasmic droplets (proximal or distal) is unclear. Normally cytoplasmic droplets indicate immaturity. A proven, fertile rabbit was castrated more than once a week, and sperm were used for in vivo fertilization. The sperm were divided into three samples: A, B, and C. The sperm from group A was used for in vivo fertilization. The sperm from group B was used for intraluminal fertilization. The sperm from group C was used for in vitro fertilization. The results showed that the sperm from group A had a higher fertilization rate than the sperm from group B. The fertilization rate of the sperm from group C was lower than the sperm from group A.

Successful treatment of oligoasthenospermia with Depot Pudatin. Abdulwahid, N.A., El-Hag, J.M., Al-Ajouj, B.T., Kalaian, Y., Endocrine Unit, Obstetrics & Gynecology Department and Semiology Laboratory, Al Sabah Hospital, Kuwait.

We have treated 28 male patients with infertility due to oligoasthenospermia using Depot Pudatin (Kalikrin 40KU) as 3 injections weekly given intramuscularly for 2 courses, each course for 3 months. There was a 3-month gap with no treatment between the course of therapy. Those 28 patients have been divided into 3 groups: Group 1 is having testicular biopsy of germ cell hypoplasia which needs longer course of therapy. Group 2 is having varicocele or idiopathic and their sperm count were never azoospermic but do not exceed 10 million/ml (N = 13). Group 3 were either due to varicocele or idiopathic and their sperm count were never azoospermic but do not exceed 10 million/ml (N = 5). Group 3 were either due to varicocele or idiopathic and their sperm counts were always above 20 million/ml but the sperm motility did not exceed 15% (N = 10).

After treatment there were 4 pregnancies, 2 in each of group 2 & 3. However, the semen analysis (count and motility) showed dramatic improvement in all these groups. There was an early improvement even after few injections in Group 3 and there was promising late improvement in Group 1 which reached 2 million/ml with 40% activity. In conclusion, Depot Pudatin is a useful drug in the treatment of oligoasthenospermia and we think that it can be of great value if combined with 17F or GIFT specially for germ cell hypoplasia which needs longer course of therapy.
Pregnancies have been achieved by men with subnormal semen parameters (WHO criteria). The assumption is made, however, that this is the exception rather than the rule. Frequently, the male is evaluated and aggressive therapy is commenced before evaluation and treatment of the female counterpart has been undertaken. On the other hand, quite often the female is treated concurrently. The question is raised whether some of the success attributed to treatment of the male might be related solely to the correction of a problem in the female partner. Pregnancy rate over six months was evaluated in 155 couples in which a female factor was discovered and corrected. Routine semen analysis (RSA) employing the Makler Chamber was performed on all males, but no therapy was undertaken; 84% of men with normal RSA achieved a pregnancy compared with 77% of men with subnormal RSA (not statistically different). An additional 81 couples were evaluated similarly by computer automated semen analysis (CSA). Semen parameters recorded by CSA were divided into norm, quality, morphology, velocity, motility, ALH mean, ALH maximum, and beat frequency. Eight percent of men with abnormality in CSA were able to achieve pregnancy in their female partners. In the group of couples evaluated by RSA and CSA, only 8 men were found to have normal RSA and CSA versus 15 men with normal RSA but abnormal CSA. Female partners of 75% of males with normal RSA and CSA and 75% of female partners of men with normal RSA alone. Inter became pregnant. Interestingly, 75% of the patients with abnormal RSA and 75% of the patients with abnormal CSA also achieved pregnancy. These data are suggestive that neither the RSA nor CSA accurately predict fertility potential in the male. For this purpose, there does not appear to be an additional advantage of the CSA over RSA. Until we are able to predict the fertilizing capacity of sperm, the best therapy for a "subfertile male" is aggressive therapy of the female partner.

This study describes the occurrence of testly variations occurring during August. Calculated total functional sperm fraction, which is an inclusive parameter (TPS% = volume x sperm/ml x motility x x normal morphology /1.5 x 10^9 sperms), revealed a more evident biphase pattern with the greatest values occurring during May and December (5.2 x 10^9 sperms) and the lowest values (P<0.05%) during the January and August (1.4 and 10.9 x 10^9 sperms). This study describes the occurrence of monthly variations in the seminal characteristics of 8 human males in Lexington, KY during 1987, in support of studies performed in other geographic locations.
EFFECT OF LENGTH OF SEXUAL ABSTINENCE PERIOD ON QUALITY AND QUANTITY OF HUMAN EJACULATES. P.M. Zavos, Department of Animal Science, University of Kentucky and Andrology Institute of Lexington, Lexington, KY 40546.

Seminal characteristics in various animal species can be influenced by the frequency of collection, degree of stabilization of epididymal sperm reserves (sexual abstinence), sexual stimulation, maturity, season and nutrition. The objective of this study was to assess the effects of two different lengths of sexual abstinence on semen parameters. Fifteen patients (36.4 ± 1.8 years old) produced 2 specimens via the use of a seminal collection device at intercourse, within approximately 5 days, one with 4.3 ± 0.1 days of abstinence (IA) and a second specimen with 1.5 ± 0.0 days of abstinence (SA). Specimens were assessed for volume, concentration, motility, drive range, morphology, calculated total functional sperm fraction (TFSF = total sperm (×10⁶) × % motility × % normal morphology/1.0 × 10⁹) and compared between the two abstinence periods. SA specimens showed lower volume (39.3 ± P<0.05), lower sperm concentration (27.5 ± P<0.05) and lower TFSF values (45.7 ± P<0.01), but improved percent normal morphology (20.6 ± P<0.05) and non-significant improvement (P<0.05) in % motility and grade of motility (6.9%). Therefore, as the sexual abstinence period is reduced, qualitative and quantitative changes take place, influencing the overall profiles (TFSF) of these ejaculates quite significantly (45.7 ± P<0.01). If necessary, specimens that are used for AHI, AID or seminal evaluation should be produced under the IA rather than the SA conditions, unless sperm morphology is the major concern.

COMPARISON OF TWO DEVICES FOR SEMEN COLLECTION DURING INTERCOURSE. P.M. Zavos, Department Animal Science, University of Kentucky and Andrology Institute of Lexington, Lexington, KY 40546.

It has been reported previously that ejaculates collected at intercourse via a condom-like device are superior to ejaculates collected via masturbation (MAS) for the same patients (Fertil. Steril. 4:6 1987). The objectives of this study were to 1) compare 2 such devices to each other for ejaculate characteristics and 2) to determine if sperm motility (MO) or velocity (VE) are affected by incubation of semen in the devices. The devices compared were the SCD (SDC Corp., Mountain View, CA) and the Male Factor Pak (MFP; Apex Medical Technologies, Inc., San Diego, CA). In the ejaculate comparison study, men (n=15) produced 2 specimens within approximately 8 days and with 4 days of abstinence each time. Each man rated his sexual stimulation (scale of 0-10 with 10 being natural intercourse) elicited during production of specimens using the 2 methods and post MAS experience. For sperm viability assays each ejaculate was collected into either an SCD (n=10) or an MFP (n=10) and aliquoted into both an SCD and an MFP, and assessed for 8 hours (37°C). The results showed no differences (P>0.05) in seminal parameters including sperm MO and VE measurements between the 2 collection devices. Also, the sexual stimulation ratings (SSR) were not different (P>0.05) between the SCD and MFP (5.8 ± 0.8) but both were higher (P<0.05) than those of MAS (3.5). However, the SSR did not differ (P>0.05) between intercourse and MFP but differed (P<0.05) with SCD. Both devices used for semen collection at intercourse therefore performed similarly with regard to semen characteristics, sperm viability and sexual stimulation.

RELATIONSHIP OF PREGNANCY IN A GIFT PROGRAM TO IN VITRO FERTILIZATION OF EXPIRED OOCYTES IN COUPLES WITH MALE FACTOR INFERTILITY. Sherman Silver, Robert Cohen, M. Wilson*, J. Hicks*, M. Deters*, St. Luke's Hospital, St. Louis, MO.

37 couples with infertility greater than 5 years underwent GIFT procedures. Less than 10 million sperm per cc or less than 20% sperm with progressive linear motility were defined as "male factor." The wives were all stimulated with Lupron/Durafert regimes. Sperm was prepared with a two step wash and swim up technique using heparin buffered HEPES with 10% maternal serum. Oocytes were cultured in HEPES with 10% serum in 5.4% CO₂ at 37°C. Four mature oocytes were replaced in fallopian tubes along with 100,000 to 1 million motile sperm (more sperm in male factor cases when possible). Extra oocytes were cultured with similar numbers of sperm. The overall ongoing pregnancy rate was 45%. In couples without male factor, 5% became pregnant. In those with male factor, 15% became pregnant. Couples with sperm counts as low as 150,000 per cc were able to conceive. In couples without male factor, fertilization occurred in 92% of cases. With male factor, fertilization occurred in 5%. We conclude that with a Pergonal/Lupron regime GIFT is an appropriate treatment for male factor infertility.

USE OF IMMUNOBEADS FOR ANTISPERM ANTIBODY (ASA) TESTING. M. Schroeder-Jenkins, L. Henrich *, and M.M. Ellison *. Andrology Laboratory and Sperm Bank, Cleveland Clinic Foundation, Cleveland, OH 44195

The lack of specificity using traditional methods for ASA testing has led to new methods for detecting actual immunoglobulin (Ig). Reported here is the use of Ig-coated polycrylanide beads (Immunobeads (IB), BioRad) for qualitative screening and an adaptation for quantitation of ASA. Sera used to supplement wash medium from 154 women undergoing intrauterine insemination, were heat-inactivated, and incubated with normal donor sperm. The sperm were washed and incubated with IB coated with all classes of human Ig, and examined microscopically for a IB binding. Less than 10% binding was considered negative; greater than 20% binding was positive. Positive sera were typed using IgG, IgA or IgM coated IB and titrated against the specific IB at serum dilutions of 1:10, 1:100 and 1:1000. Any specimen with 10-20% binding was repeated using different donor sperm and typed and titrated if results were positive or consistently borderline. The incidence of ASA positive sperm wash sera using this method was 24% (3/154). Other sera had borderline screening results, but titered negative or < 1:10. In previous studies using agglutination or immobilization methods, 16% of sperm wash sera were positive on initial screen, but only 2% had positive titer. Thus, the false positive rate appears to be lower with this new methodology. ASA detection using IB is a rapid and reliable screening method, IB can be used to obtain a titer sufficient for most clinical applications.

134 ejaculates by 26 sperm penetration assay positive donors were evaluated before and after cryopreservation in human sperm preservation medium (15% Glycerol) and modified tyrode-albumin-lactate-purivate medium, as the diluent. Using the 'cell' track computer assisted digitized image analysis system we plotted the curvilinear velocity (VC) microns/sec, mean linearity (LIN), straight line velocity (VSL) microns/sec, and lateral head displacement (ALH) microns, sperm concentration and percent motile sperm samples above 10 microns/sec.

The mean percent motility and sperm concentration was significantly lower as compared to fresh samples (p<0.0001, p<0.001) respectively. The mean frozen VC (26.21, SD 4.31) was higher than mean fresh VC (23.48, SD 6.54) p=0.02. Mean fresh LIN, however, decreased (16.31, SD 8.62) compared to mean fresh LIN (19.08, SD 3.84) p<0.004. However, VSL and ALH showed no significant change after freezing (p<0.01, p<0.049 resp). Statistical calculations were made with both the paired-T test and sign rank sum test. The scatter plots with distribution around the line of identity will also be presented.


86 QUALITY OF CRYOPRESERVED INSEMINATIONS USED FOR DONOR INSEMINATION IN THE CINCINNATI HOSPITAL. G. A. Rothnann, R. H. Schroeder-Jenkins, Andrology Lab and Sperm Bank, Cincinnati General Hospital, Cincinnati, OH 45219

To determine whether insemination outcome was linked to insemination quality in an artificial insemination program, motility parameters were measured in 12 donor semen preparates for insemination using CASA (CellSoft I). Values used for analysis were % motility (HOT), mean curvilinear velocity (VC), linearity (LIN), mean amplitude of lateral head displacement (ALH), and inseminate dose. Inseminates were grouped by relative fertility (in PREG) and in 212 pregnancy did not. No differences between inseminate groups were apparent for any parameter. No significant changes between pre and post freeze parameters was observed except for % HOT which declined 5% post thaw. Post wash % HOT was slightly (5%) lower than post thaw. In both groups, VC and ALH decreased slightly post thaw but were higher in post wash samples than original prefreeze values (VC 45 vs 46.2 u/sec in prep vs 46.2 u/sec in nonpreg: ALH 5.0 in prep vs 5.1 in nonpreg); in both groups, LIN was slightly less in post thaw and post wash. Mean dose for PREG was 25.7 million MOT, for NONPREG 29.2 million MOT. All donors achieved pregnancies. The results suggest that pregnancy outcome was not related to insemination quality but was linked to recipient fertility. In addition, the data delineate normal seminal sperm values for fertile men of 73% HOT, VCL 43 u/sec, LIN 5.6, and ALH 2.4.u.

87 QUANTITATIVE EVALUATION OF 132 TESTICULAR BIOPSY SPECIMENS: DETERMINATION OF THE INCIDENCE OF TOTAL AND PARTIAL OBSTRUCTION.


The incidence of obstructive causes of azoospermia and oligozoospermia is still uncertain. We studied 132 bilateral testicular biopsies from the same number of infertile patients by the quantitative method previously described by Silber and Rodríguez-Rigau (Fertil. Steril. 36: 472-480, 1981) based on the count of mature spermatids per tubule. These data, in our hands, closely correlated with the sperm count (r=0.78 P < 0.0001).

In azoospermic patients (n=40) we found 7.5 ± 0.3% with total obstruction whereas in oligozoospermic patients with sperm count below 10 million/ml (n=87) the incidence of partial obstruction was 14.9 ± 4.0.


We have previously demonstrated that active immobilization of testes with FSH leads to oligospermia, reduction in sperm UWW, hypotestes in normal plasma testosterone levels. Further, the ability of sperm from immobilized animals to attach to matrix was impaired. In this report a follow up study over a 5 year period and results of breeding experiments are presented. The antitestis titers of immobilized animals ranged from 1:131-1:10,200 sperm counts in ejaculates from immobilized testes demonstrated oligospermia for up to 3 years (controls 132 ± 12.41 u/ml, n=8; immobilized 32.62 ± 13.41, n=9). All the immobilized testes proved to be infertile in breeding studies (controls, 8 pregnancies in 10 evaluatory cycles; immobilized, 0 pregnancies in 9 evaluatory cycles). However by the end of 3.5 years, 3 of 9 immobilized testes started to show increase in sperm counts (tails: 106-402 × 10⁶) in spite of high antitestis antibodies (1:131-1:10,200). Breeding during this period resulted in 1 pregnancy, altering the overall pregnancy rate to 1 pregnancy in 10 evaluatory cycles in the immobilized group. Active immobilization of testes with some OA absorbed to albumin gel, led to low titer antitestis antibodies (testis, 1:131-1:200) and oligospermia (0.001-1.6 × 10⁶, n=2); immobilization of two testes with a combination of FSH and OA absorbed to albumin gel also resulted in low titer antitestis low titer antitestis antibodies to FSH. From these observations we conclude that: 1. Immunization of FSH results in oligospermia and severe impairment of fertility. 2. Neither OA alone nor FSH/OA combination given in a mild form as albumin gel, leading to low titer antitestis antibodies leading to azoospermia. Possible causes for the rebound of spermatogenesis in the 3 FSH untreated testes is currently being investigated. Supported by grant NIH HD 15277 and HR-KI 12417 Proj.
Differences in sperm motion patterns for fertile men and infertility patients. Peter M. Fetterolf and B. Jane Rogers, Department of Obstetrics and Gynecology, C-YAKR, Vanderbilt University School of Medicine, Nashville, TN.

Objective sperm motion values have been determined for seven samples by numerous investigators using computer assisted technology in an effort to develop prognostic parameters for fertility diagnosis. Threshold values which clearly differentiate between fertile and infertile samples have not been ascertained by any investigators. In an effort to establish more useful values for motion parameters, we have evaluated two groups of clearly defined fertility status: fertile men from our AI, IUI and IVF programs and infertility patients categorized as nonpenetrators (ON) in a TEST-yolk buffered sperm penetration assay. Motion parameters were determined on fresh semen samples using the CELL SOFT system. In a group of 4) samples from donors and 21 samples from patients, significant differences (P < 0.01) were found for motility % (62.1 vs 33.8) velocity (75.7 vs 53.3 microns/sec) and beat/cross frequency (18.0 vs 15.5 Hz). In addition, correlations (P < 0.05) between motion parameters were evaluated and significance was found for A H vs ALH mean (-0.42), velocity vs linearity (-0.42) and linearity vs ALH mean (-0.86) for the fertile men. In contrast, no significant correlations were found for these comparisons for the nonpenetrators. In conclusion, computerized motion analysis generates parameters which show distinctly different correlations for fertile men and nonpenetrators.

Interspecies variation in sperm morphology in a donor population. Richard Scott*, Sergio Oehninger, Mary Mahoney, Mahmood Marhedi, Anibal Acosta. The Jones Institute for Reproductive Medicine, Norfolk, VA, 23507.

Sperm morphology as assessed by the strict criteria described by Kruger is a good predictor of in vitro fertilization outcome. The degree of interspecies variation in morphology using these criteria has not been studied. We evaluated sperm morphology using the Diff-Quick staining technique after semen liquefaction. Replicate specimens were collected over a 6 month period from 6 individuals participating in our artificial insemination donor program. The number of specimens per donor ranged from 5 to 8 (6.0 ± 1.2SEM ± 50). All samples had concentrations > 45 x 10^6 sperm/ml and >50% motility. The mean of the interspecies variations were 4.0% for normal forms, 5.1% for slightly abnormal forms, 6.7% for tail defects, and 2.9% for neck defects. Significantly, the patient’s morphology classification regarding a good or poor prognosis for fertilization, changed in only 5.5% of the specimens. The results indicate that there is not a significant interspecies (intra/individual) variation in sperm morphology over a period of 6 months in our donor population.
Protein-kinase C activation may modulate protein kinase A activity, often in a Ca⁺⁺-dependent manner, to certain intracellular proteins. This study compared the binding of CaM and S-100 to Leydig cell proteins. Leydig cells were isolated from adult Sprague-Dawley rats by discontinuous Percoll gradient centrifugation. The cells were disrupted by sonication in Sample Buffer and the proteins separated by SDS-PAGE using Laemmli buffers. The gels were incubated with ¹²⁵I labelled CaM or S-100 in buffers containing either 1 mM Ca⁺⁺ or 1 mM EGTA, washed, and autoradiographed. In the presence or absence of Ca⁺⁺, [¹²⁵I]CaM and [¹²⁵I]S-100 binding was found in bands of 20, 20, and 32.34 kDa. A band at 43 kDa bound both CaM and S-100 in the presence of Ca⁺⁺, but only S-100 in the absence of Ca⁺⁺. Ca⁺⁺ dependent CaM binding was observed in bands of 54, 134, and 240 kDa. The 240 kDa CaM binding protein has been tentatively identified as the α-subunit of fodrin by immunoblotting with anti-chicken spectrin (highly homologous to mammalian fodrin). These results suggest that rat Leydig cells contain at least four proteins ranging between 40 and 240 kDa that bind CaM, but not S-100, in a Ca⁺⁺ dependent manner. One of these proteins is likely the α-subunit of the CaM and actin-binding cytoskeletal protein fodrin. Supported by the NIH.
PRODUCTION OF ANTIBODIES AGAINST SYNTHETIC PEPTIDE FROM INHIBIN a AND b-SUBUNITS. Seiichi Saito,* Patrick C. Roche, Daniel J. McNicholl*, and Robert J. Ryan*, Mayo Clinic, Rochester, MN 55905

The a-amino acid sequences of inhibin a- and b-subunits were analyzed for hydrophilicity and chain flexibility to predict regions that are on the surface of the subunits and therefore potential antigenic sites. Based on this analysis, peptides for each subunit (a[1-16]YLL, bA CGG(9-105), bB CGG(93-101)) were synthesized, and rabbit antisera against each peptide were prepared.

Immunoblotting of inhibin (follistatin: NIH #119-6-4) with anti a-antiserum revealed a 32X band (inhibin) and an 18X band (a-subunit). Immunoblotting with anti b-antiserum detected a 32X band (inhibin), a 24X band (activin) and 11–14X (b-subunits). These antisera when injected iv (5 mL/kg) into immature rats (15 days), induced dramatic elevation of serum FSH in 6–12 hrs. This suggests immunoneutralization of endogenous inhibin. Radioimmunoassay (RIA) for each subunit were developed using radiiodinated peptides as tracers. Competition binding assays indicated cross-reactivity with porcine and human follicular fluids, human semen, serum and plasma. Parallel dilution curves were obtained. Antisera against each peptide were prepared.

In these studies suggest that antibodies against synthetic inhibin peptides are useful in elucidating the role of inhibin in the reproductive system.

LOCALIZATION OF INHIBIN IN THE GONADS BY IMMUNOCOLD STAINING. Seiichi Saito*, Patrick C. Roche, Daniel J. McNicholl*, and Robert J. Ryan*, Mayo Clinic, Rochester, MN 55905

Inhibin is a glycoprotein hormone made up of two disulfide bonded subunits termed a and b. Using specific rabbit antisera directed against synthesized inhibin peptide (a[1-16]YLL, bA CGG[9-105]), bB CGG[93-101]), we have established the cellular localization of each subunit of inhibin in the gonad. The specificity of these antibodies has been established by immunoabsorption and immunoneutralization assays.

Paraformaldehyde-fixed paraffin embedded tissues from rat testis, porcine ovary, human testis and ovary were subjected to immunohistochemical staining. The primary antisera were used in conjunction with gold labeled goat anti-rabbit IgG to localize inhibin-reactive cells in various tissues. Specific inhibin immunoreactivity was localized within the Sertoli cells of some tubules in the testes, and granulosa cells of the ovaries. Positive staining in gonadal tissues could be blocked by preabsorbing the serum with the synthetic peptides.

The present study demonstrates the localization of inhibin in the gonads by immunogold staining, and demonstrates the utility of these antisera in the study of reproduction.

EFFECTS OF HISTAMINE ON THE FUNCTION OF LEPIDIC CELLS AND SERTOLI CELLS IN THE IMATURE GOLDEN HAMSTER. A. Myhrer,* S. Newton, A. Batke, and J. Regan, Department of Physiology, School of Medicine, Southern Illinois University, Carbondale, IL 62901-6512.

In the testis of the immature rat histamine (H) levels are considerably higher than in the adult (Seeher et al., Endocrinology 88:151, 1973); and in human testis, the numbers of test cells, which are the source of H, are increasing during puberty (Hidal et al., Acta Anat. 119:155, 1984). However, the physiological role of H is unclear. We have examined the effects of H on testicular testosterone (T) production in vivo and on testosterone production by cultured Sertoli cells in vitro [30:20 days old] golden hamsters (Mesocricetus auratus). Fractions of decapsulated testes were prelabelled for 30 min in Krebs-Ringer bicarbonate buffer containing 15 glucose and subsequently exposed to 6 h to the same media containing a phosphodiesterase-inhibitor MIX (10 M) and various combinations of HCG (1 UI/ml), H (10 M), H1-receptor blocker Pyrilline and H2-receptor blocker Cetrizin (5 x 10 M). Histamine stimulated T production but did not augment the stimulatory action of HCG. Pyrilline, but not Cetrizin prevented the stimulatory effects of H. Sertoli cells were isolated (Welsh & Wiebe, Endocrinology 96:618, 1975) and plated with DMSO-121050 for 2 days. They were subsequently treated with H (10 M) and/or FSH (ovine 5-17 NIH; I.U./ml). The accumulation of lactate, a preferred substrate for developing germ cells, in the media after 8 h was increased by FSH and H. Moreover, H augmented the stimulatory effect of FSH on lactate secretion. We conclude that H can affect both Leydig and Sertoli cell function and that its action on Leydig cells is mediated via H1-receptors. Thus, a modulating role of H in the testis during maturation can be suspected.

(Supported by DFT PA 102017+1 and NO 0053.)

INCORPORATION OF EXOGENOUS FATTY ACIDS INTO HUMAN SPERM PHOSPHOLIPIDS. Juan G. Alvarez and Bayerd T. Storey, Dept. of Obstetrics & Gynecology, University of Pennsylvania, Phila. PA 19104

Human sperm have been shown to synthesize acyl-CoA thioesters from free fatty acids. In this study we report the ability of intact human sperm to incorporate exogenous fatty acids into the phospholipid pool. Samples from healthy donors were pooled and washed twice in 10 vol RPMI-1640 medium and the final pellet resuspended in 2 ml of the same medium (cell conc: 4.8 x 106 cells/ml). Each sample was incubated at 37°C for 2 hr, centrifuged, and lipid extracted. The extract was redissolved in CHCl3/CH3OH (1:1), and 10 µl aliquots streaked on Whatman HPTLC plates. The plates were developed in C-M for 1 hr. The phospholipid bands were visualized with spraying CHCl3/EtOH-H2O (30:40:30:8), dried and the radiolabelled bands scanned.

INCORPORATION (µCi/106 Cells)

<table>
<thead>
<tr>
<th>phosphatidylethanolamine (PC)</th>
<th>PE</th>
<th>PI</th>
<th>PS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1C:PA</td>
<td>0.60 ± 0.08</td>
<td>0.50 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>1C:OA</td>
<td>0.95 ± 0.08</td>
<td>0.23 ± 0.03</td>
<td>0.20 ± 0.04</td>
</tr>
<tr>
<td>1C:AA</td>
<td>0.97 ± 0.20</td>
<td>0.18 ± 0.02</td>
<td>0.15 ± 0.05</td>
</tr>
<tr>
<td>1C:DHA</td>
<td>0.40 ± 0.05</td>
<td>0.60 ± 0.08</td>
<td></td>
</tr>
</tbody>
</table>

Fatty acids were preferentially incorporated into phosphatidylethanolamine (PC), except for DHA which was preferentially found in the phosphatidylcholamine (PE) OA and AA and the other fatty acids were absent in phosphatidylethanolamine (PE) OA and AA, while PS and PI could not be detected in PS and PI. These results indicate that human spermatozoa have the required acylating enzymes necessary to esterify fatty acyl-CoA derivatives into membrane phospholipids. Supported by NIH Grants HD 15482 and HL 59737.
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CPK (creatine-N-phosphotransferase) is a key enzyme in the synthesis and utilization of sperm energy. We have demonstrated that CPK activity predicts sperm quality (Huszar et al., Gamete Res., 19:67, 1988 and Biol. of Reprod., 38:1061, 1988). Also, the M-type CPK isoform appears to be a marker of sperm cellular maturity. In the present work we examined CPK activity and CPK-isofrom ratios in stallion sperm. Extracts of 5 stallions (sperm conc.: 2151±92 million sperm/ml, all data expressed as SD) were assayed as raw (R) or as milk-extended (E) semen on ice. After 3 hours the R sperm showed motility of ∼50% whereas the E the respective value was >90%. The R and E sperm were extracted with 30 mM imidazole, pH 7.2 and 0.15 Tris. The CPK activity and CPK-isofrom ratios were measured in the extracts by spectrophotometry and by electrophoresis on agarose gels, respectively. The CPK activities of stallion sperm (n=5, expressed as U/100 million sperm) in the R and E were 0.03±0.003 and 0.016±0.01. These data are lower than the CPK activity of normospermic men (0.106±0.080). The relative concentrations of the M-type and B-type CPK isoforms were 76.2±11.3% vs 23.8±11.3% in the R and 62.8±24.8% vs 34.1±26.2 in the E samples, indicating that the cytoplasmic content of immobilized sperm is not altered. We conclude that stallion sperm contains CPK and that both the M-type and B-type CPK isoforms are present. In further studies we will examine a possible correlation among sperm quality, CPK activity parameters and CPK isoform ratios in older and in fertile stallions (Supported by HD-19505).

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RELATIONSHIP BETWEEN HEPARIN BINDING CHARACTERISTICS AND HAMSTER OVA PENETRATING CAPACITY OF HUMAN SPERMATOZOA. OS Price, S. Vedanthan, S. Winstead, PA Latch, Dept Obst/Gyn, Michael Reese Hospital, University of Chicago, Chicago, IL 60616.

Heparin binding sites on spermatozoa have been described for many species including humans. Although glycosaminoglycans have been associated with increased capacitation and/or acrosome reaction, the physiologic role of specific binding sites has not been defined. The zona-free hamster ova-sperm penetration assay (SPA) is a biosay which monitors the ability of a sperm population to adequately capacitate/acrosome react prior to egg penetration. In order to determine whether heparin binding sites on human sperm may be biologically important molecules, binding site density and affinity were compared between fertile and infertile men with normal or abnormal SPA results. A portion of fresh semen from 10 fertile donors and 26 infertile men was processed through the SPA while the remainder of the ejaculate was used to quantitate heparin binding. Saturation binding assays with 3H-heparin (15-375 nM) were analysed for 1) infertile men with abnormal SPA results (4% ± 1); 2) infertile men with normal SPA results (36% ± 3) whose semen parameters matched group 1; and 3) fertile donors [SPA: 40% ± 5]. Results revealed that the heparin binding site density was significantly higher in fertile men [29.6 ± 3 x 10⁴ sites/spinosperm] and fertile men with normal SPA results [27.2 ± 2.6 x 10⁴] compared to infertile men with abnormal SPA scores [19.4 ± 1.5 x 10⁴]. There were no differences in binding affinity between the groups. This is the first report to link reduced heparin binding site quantity with capacitation/acrosome reaction dysfunction. These findings support the hypothesis that heparin binding site density may be related to the ability of human sperm to successfully acrosome react. In order to further investigate the apparent regulatory role of docosahexaenoic acid (22:6) in spermatozoan fatty acid utilization, we conducted the following experiments to characterize the synthesis of docosahexaenoyl coenzyme A (22:6-CoA). Fatty acid:CoASH ligase (AMP) (EC 6.2.3.1) activity was isolated from human sperm and initial velocities were measured as previously described (Jones, Pymat, Biol Repro. 39:76-80, 1988). Authentication of the 22:6-CoA product was performed by comparing it to an organically synthesized compound using thin layer chromatography. The pH optimum for 22:6 activation was 8.4 which was identical to the value obtained with palmitic acid (16:0). The Kₐ for ATP was 0.5 mM when 22:6 was the acyl substrate; however, when 16:0 was incubated in this system, the Kₐ was 2.9 mM. When CoASH was varied and 22:6 was the fatty acyl acceptor, a pattern of negative cooperativity was observed. This was confirmed by a downwardly concave double reciprocal plot, a Hill coefficient of 0.61, and an Rₐ in excess of 150. The Hill coefficient with 16:0 and GASH was 0.94. Palmitic acid was demonstrated to be a competitive inhibitor of 22:6-CoA synthesis with a Kᵢ of 7.4 µM. Based upon these data, we conclude that the kinetics of spermatozoan fatty acid:CoASH ligase are such that there is likely a single ligase enzyme in human sperm. In addition, these data support the hypothesis that 22:6 may regulate ligase activity, and therefore free fatty acid utilization, in sperm.
The precise mechanism will require additional studies.

Recent evidence in our laboratory has demonstrated the presence of a unique α-D-mannosidase on the plasma membrane of rodent spermatozoa. This enzyme is different from the previously studied acrosomal acidic α-D-mannosidase, in that it exhibits a high pH optimum (6.2) and is active only towards mannose-rich oligosaccharides. Incubation of sperm with sugars such as α-D-mannose (α-MAN) and α-D-glucosylglucose (α-Glc), and mannose resulted in a dose-dependent inhibition of the enzyme activity. At 50 nM concentration, the enzyme activity was 15% of control in the presence of α-MAN, 5% with α-Glc, and 36% with mannose. Galactose, which did not inhibit sperm surface mannosidase, had no effect on sperm-egg binding. Preliminary studies in the hamster showed that both α-MAN and α-Glc inhibited enzyme activity and sperm-egg binding in a dose-dependent manner. These studies suggest that the sperm surface α-D-mannosidase may be important for sperm-egg interaction.

Both flutamide and RU-486 reduce the effects of cortisol on prostate tissues in rat prostate explants. In vivo studies also indicate that flutamide and RU-486 inhibit the effects of cortisol on prostate tissue cultures (Exp Cell Res 77:111, 1973). Recently, we have observed that either cortisol or DHT in a dose-dependent manner maintains high concentrations of the α-D-MAN for prostate in rat prostate cultures. We sought to determine if this effect is mediated by the androgen receptor, the glucocorticoid receptor, or both. Explants were cultured for 4 days in serum-free media + Cortisol, OHT, flutamide (F), cyproterone acetate (CA), or RU-486 (a glucocorticoid antagonist). Cytoplastic levels of the α-D-MAN were assessed by the dithiothreitol assay and expressed as pmol/g DNA.

Control Cortisol Cortisol Cortisol Cortisol Cortisol
(250 nm) (250 nm) (250 nm) (250 nm) (250 nm)

Reduction

1792 5434 2432 1927 1946

p<0.001 p<0.003 p<0.005 p<0.01

Control OHT OHT OHT OHT OHT
(50nM) (50nM) (50nM) (50nM) (50nM)

Reduction

975 4241 2939 2429 5441

p<0.01 p<0.001 p<0.005 p<0.005 p<0.005

Mean50%, p<0.05

The effects of cortisol on prostate α-D-MAN levels in extracts of rat ventral prostate appear to be mediated through both a glucocorticoid and an androgen receptor. The precise mechanism(s) will require additional studies.


Recent evidence in our laboratory has demonstrated the presence of a unique α-D-mannosidase on the plasma membrane of rodent spermatozoa. This enzyme is different from the previously studied acrosomal acidic α-D-mannosidase, in that it exhibits a high pH optimum (6.2) and is active only towards mannose-rich oligosaccharides. Incubation of sperm with sugars such as α-D-mannose (α-MAN) and α-D-glucosylglucose (α-Glc), and mannose resulted in a dose-dependent inhibition of the enzyme activity. At 50 nM concentration, the enzyme activity was 15% of control in the presence of α-MAN, 5% with α-Glc, and 36% with mannose. Galactose, which did not inhibit sperm surface mannosidase, had no effect on sperm-egg binding. Preliminary studies in the hamster showed that both α-MAN and α-Glc inhibited enzyme activity and sperm-egg binding in a dose-dependent manner. These studies suggest that the sperm surface α-D-mannosidase may be important for sperm-egg interaction.
Studie's are now underway to measure secreted cytokines in human semen and to elucidate their effects on sperm function. A monoclonal antibodies (MAbs) specific for various human white blood cell cytokines were applied to two groups of human semen samples: 39 semen samples with less than 10³ white blood cells (WBC) per ml, and 37 semen samples with more than 10³ WBC/ml (leukocytospermia). Positive reactivity was defined by staining of more than 20 cells per mm² glass slide area. Of the 37 leukocytospermic semen smears, 39 non-leukocytospermic semen samples were reactive with one cytokine, granulocyte-macrophage colony-stimulating factor, 6 with tumor necrosis factor alpha, 4 with interleukin-1 alpha, and 3 with MAb against interleukin-2. In contrast, only one of 39 non-leukocytospermic semen samples was reactive with one cytokine, granulocyte-macrophage colony stimulating factor. Our data indicate the presence of cytokine-producing white blood cells in inflammatory semen. Studies are now underway to measure secreted cytokines in human semen to elucidate their effects on sperm function.

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Our recent work demonstrated that pachytyne spermatocytes (PS) synthesize and secrete proteins that stimulate secretion of novel Sertoli cell proteins (Djakiew and Dym, Biol. of Reprod. In Press). We initiated a morphological and immunocytochemical study of the Golgi apparatus in PS during the cycle of the seminiferous epithelium in an effort to correlate structure and function in the meiotic prophase. In stages I-II of the cycle, the Golgi of PS is spherical, 0.4-0.5um, and composed mainly of numerous vesicles interspersed with a few classically described Golgi cisternae. During subsequent PS maturation (stages IV-XIII), the size of the Golgi complex increased significantly to 1-2um. However, unlike PS of stages I-II, the majority of the Golgi complex of more mature PS was characterized by an abundance of cisternae stacks, containing few associated vesicles. The identity of specific proteins of the Golgi complex was studied by using two monoclonal antibodies that recognize either the cist or the trans Golgi cisternae and employing biotin-streptavidin-peroxidase immunocytochemistry in 5 um frozen sections of testes. Immunodetection of the distinct cisternae revealed that the increase in size of the Golgi complex during PS maturation was due predominantly to an accumulation of trans Golgi; the amount of cis Golgi remained unchanged. It is not known whether the very large Golgi apparatus in PS is present in preparation for the two maturation divisions yielding spermatids, or whether the molecular heterogeneity is, in part, the underlying basis for complex mechanisms incurred by PS during meiosis leading to their interaction with Sertoli cells. Supported by NIH Grants HD16260 (MD) & HD3484 (CASQ).
ARGUMENT AGAINST PHOSPHOLIPASE A2 (PLA2) AS REGULATORY IN THE HUMAN ACROSOME REACTION. R.A. Anderson Jr., P. Biefield* and L.J.O. Janeyal, Rush Medical Center, Chicago, IL

Studies (115 and Meizel, J Exp Zool 207: 173,1979) have suggested involvement of PLA2 in the acrosome reaction (AR), based upon inhibition of the AR by relatively nonspecific PLA2 inhibitors (e.g., quinacrine). The purpose of the present study was to critically evaluate the role of PLA2 in the human AR through correlational analysis of the effects of PLA2 inhibitors on the AR with their effects on the human sperm enzyme. Washed sperm were capacitated (3 hr., 37°C) in modified BWW medium, after which, ARs were induced with 10 µM A23187 and visualized after differential staining with Bis­

mark Brown and Rose Bengal (J Androl 9: P39, 1988). A23187 increased the AR of cells in control incubations from a baseline value of 14.4±0.0% confidence limits=13.3±1.5%) to 33.2 (28.9-37.6%). Inhibition of the A23187-induced increase in AR in the presence of 20 µM trifluoroperazine (TFP; 4.8%; 0.2 mM p-bromophenacyl bromide (pBPA; 16.9%) was not significant, whereas inhibition was noted for the presence of 0.4 mM quinacrine (31.7%) and 5 µM McIl (31.4%: p < 0.01). Since A23187 has high affinity for McIl, McIl could compete with AR for cell entry. ARs, induced by 10 µM tomycin (a Cyt C type affinity) were 33.4 (31.7-46.5%) and 38.0 (31.4-40.9%) in the presence and absence of McIl respectively. Studies showed that 20 µM TFP, 0.4 mM pBPA and 5 µM McIl inhibited PLA2 activity by 24.5%, 47%, 70% and 77%, respectively. Correlation between the effects of these agents on the AR and their efficacy as PLA2 inhibitors was poor (Kendall's tau = 0.105; p=0.1). The data indicate that if PLA2 is involved in the human AR, its role is neither regulatory nor rate limiting. Supported by NIH grant HD 10555.

ASSENCE OF HISTOCOMPATIBILITY (HLA) ANTIGENS ON THE SURFACE OF SPERMATOZOA. Allan R. Glass, Endocrinology Service, Walter Reed Army Medical Center, Washington, DC 20307

The major histocompatibility antigens (HLA antigens) are found on the surface of cost nucleated cells, but there is controversy about their presence on the surface of germ cells and spermatozoa. Previous studies of this question may have been hampered by the use of non-specific anti-HLA antibodies to detect surface HLA antigens and imprecise methods have been hampered by the use of non-specific anti-HLA antibodies to detect surface HLA antigens and imprecise methods. To obviate some of these difficulties, washed spermatozoa were unchloroform-stained for surface HLA antigens by incubation with two highly specific monoclonal anti-HLA antibodies. One of these monoclonal antibodies was directed against a determinant common to HLA-A, B, and C antigens (Class I antigens), while the other monoclonal anti­

body was directed against a determinant common to HLA-B8 antigens (Class II antigens). Monoclonal anti-HLA antibody bound to the sperm surface was then detected using second antibody and a highly sensitive peroxidase-antiperoxidase staining method. Using this technique, both peripheral blood lymphocytes and EB virus-transformed lymphocytes showed detectable Class I and Class II antigens on their surface when the monoclonal anti-HLA antibodies were stained at 1:1000. In contrast, spermatozoa from several donors uniformly fail­

to express Class I and II HLA antigens on their surface. Since the cytokine gamma-interferon has been shown to stimulate the expression of HLA antigens on the surface of various cell types, we incubated spermatozoa for 72h with gamma-interferon (1000U/ml), but this preincubated spermatozoa still showed no detectable HLA antigens on their surface. In conclusion, using currently available detection techniques, spermatozoa do not express surface HLA antigens.

DNA METHYLATION SWITCHING DURING SPERMATOGENESIS IN THE HOUSE. J.H. Traeger*, D.A. Johnson*, L.E. Hake*, A.A. Alticar*, C.F. Milette* and R.B. Mecht, Dept. of Biology, Tufts University, 02155 and LHRR1, Harvard University, Bos­

ton, MA.

Deoxycytosine methylation of DNA has been linked to the transcriptional activation of genes, chromatin structure and genetic imprinting. We have studied the potential role of DNA methylation in the expression of testis-specific genes by restriction endonuclease analysis of somatic and testi­

tical DNA. The testis-specific genes cytochrome c (cyt c), transition protein 1 (TP1), protamine 1 (mP1) and protamine 2 (mP2) showed different methylation patterns in somatic as compared to testicular tissue; in contrast, genes that are expressed in both somatic and testicular tissue, i.e. β-actin and genes encoded by the mitochondriod genome had identical methylation patterns in the spleen and testis. Data from prepubertal mice indicated that CpG sites in the coding and flanking regions of mP1, mP2 and cyt c became progressively more methylated, sites in TP1 became progressively less methylated and sites in β-actin and genes en­
coded by the mitochondriod genome remained unmethylated at the testis developed. Analysis of isolated testicular cells revealed that the methylation of mP1, mP2 and cyt c and the demethylation of TP1 occurred in germ cells between type B spermatogonia and pachytene spermatocytes. The results sug­
gest that methylation patterns for at least some testis­
specific genes switch during spermatogenesis.

AN IMPROVED ASSAY FOR PHOSPHOLIPASE A2 ACTIVITY IN SFEMM. Anna A. Golap*, David A. Chapman* and Gary J. Hillig*, Dairy Breeding Research Center, Penn State University, University Park, PA.

The enzyme phospholipase A2 (PLA2) hydrolyzes the second acyl group from phosphoglyceride, yielding equimolar amounts of lysophospholipid and free acid. This ubiquitous enzyme is found in sperm, where it is membrane-associated and may serve a regulatory function in sperm capacitation and induction of the acrosome reaction. Existing assays for PLA2 activity include repeated partitioning of lipids and thin layer chromatography, or utilize radiolabelled precursors, with varied methods for separation of substrate and product. These assays are time consuming and insensitive, or require the use of expensive radioactive compounds. The new assay utilizes a fluorescent-labelled phosphatidylethanolamine substrate, which is cleaved by PLA2 to yield fluorescent-labelled fatty acid. Substrate and product are separated by HPLC equipped for fluorescence detection. The assay is sensitive (to 50 nmole substrate), rapid and inexpensive. After validating the results of the new technique by comparing them with those of existing methods, the fluorometric assay was used in preliminary studies to test the effect of glycerophosphocholine (GPC) on PLA2 activity. GPC is found in high levels in the epididymis, where its function is unknown. We determined that PLA2 activity was significantly inhibited by GPC. These results sug­gested that in the epididymis, GPC may inhibit sperm·
membrane bound PLA2 from hydrolyzing phosphoglyceride to lysophospholipid and free fatty acid. This could serve to stabilize sperm membranes, and may play a role in preventing capacitation and the acrosome reaction during sperm storage in the cauda epididymis.
ACROSOMAL INTEGRITY OF FRESH AND CRYOPRESERVED (CP) HUMAN SPERM. William C. Baird, Grant E. Schmidt, Steven R. Williams, and Karen L. Leonhart, Riverside Methodist Hospitals, Columbus, Ohio, 43215.

Fresh and CP sperm from 31 patients attending an IVF program were evaluated for viability and acrosomal integrity using combined Hoechst 33525 stain and FITC-labeled Plasmon sativum agglutinin as described by Cross et al., Gamete Res. 15:213, 1985. Fresh or thawed sperm were washed twice and resuspended in BWW containing 0.3% BSA and incubated at 37°C in 5% CO₂. The percent of total and live acrosome reacted cells was evaluated at 0, 18, and 24 hr for fresh sperm and at 0, 18, and 24 hr for frozen sperm and the following results were obtained.

<table>
<thead>
<tr>
<th></th>
<th>% Total AR</th>
<th>% AK of live sperm</th>
</tr>
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<tbody>
<tr>
<td>Fresh</td>
<td>4.4 (±1.6)</td>
<td>7.1 (±2.7)</td>
</tr>
<tr>
<td>Frozen</td>
<td>5.2 (±1.7)</td>
<td>5.8 (±0.8)</td>
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</table>

*Mean (range)

Human sperm cells must undergo the acrosome reaction to penetrate the zona-free hamster egg or fertilize a human egg, either In vitro or In vivo. However, premature acrosome reaction may decrease or inhibit the ability of CP sperm to traverse the reproductive tract, cumulus complex, and/or zona pellucida. The acrosome reaction assay may be useful for determining improved CP media and methodologies.

SOURCES OF VARIATION OF SPERM HEAD MEASUREMENTS. Steven N. Schrader, Terry W. Turner, and Stephen D. Simon, Experimental Toxicology Branch, National Institute for Occupational Safety and Health, Cincinnati, OH 45226

As part of a longitudinal study of human semen characteristics of unexposed workers, sperm head measurements were made using image analysis (Image Technologies Corp.) Morphometry was conducted on monthly samples from 46 men for nine months. Measurements of area, perimeter, length, width, the length-width ratio, and the oval factor (4π area/π perimeter²) of 100 sperm heads per sample were obtained. The variability within a sample, between samples from the same individual (between monthly samples), and between individuals were calculated. Tolerance intervals were calculated which were expected to contain 75% of all individual measurements. Similar intervals were calculated for a mean and for a standard deviation of a semen sample. The large individual cell variation is reflected by the fact that 75% of individual cells can be expected to have a length between 3.6 and 3.5 micrometers and the width between 2.3 and 3.4 micrometers. The ejaculate mean is much more stable; 75% of all semen samples can be expected to have standard deviations from 0.48 to 0.80 and 0.25 to 0.48, respectively.

EFFECTS OF COOLING RATE AND REPEATED FREEZING ON HUMAN SPERM CRYOSURVIVAL. Stephen D. Simon, Experimental Toxicology Branch, National Institute for Occupational Safety and Health, Cincinnati, OH 45226.

Two experiments were conducted to examine the effects of cooling rate (Exp 1) and repeated freezing-thawing (Exp 2) on human sperm cryosurvival. In Exp 1 (n=6) semen was diluted 1:1 with HSPN (7.5% glycerol final concentration) and frozen using cooling rates of 0.1, 1.0, 1.75, and 800°C/min. In Exp 2 semen samples from 10 individuals (n=10) were diluted 1:1 with HSPN, divided into aliquots, and exposed to 1 to 4 freeze-thaw cycles (10°C/min between -5 and -80°C). In both experiments, samples were subsequently warmed rapidly (400°C/min), and survival was estimated by measuring post-thaw motility using a computer-assisted semen analysis system (CellSoft, CRYO Resources, ITH, NY). Data were analyzed using ANOVA and regression analyses. The results of Exp 1 indicate that human sperm produce an inverted U-shaped survival curve. Maximum survival using these conditions (45°C) was at 10°C/min which was significantly (p<0.05) different than all other rates. Minimum survival was observed at the slowest (0.1°C/min; 8.8%) and the fastest (800°C/min; 21.3%) cooling rates. Survival rates at 1 and 1.75°C/min were intermediate (31.3 and 33.9%, respectively) and not different from each other (p>0.10). The results of Exp 2 produced a plot of the log of motility vs number of freeze-thaw cycles with two linear components. The slope of the first line was -1.0 (r = 0.90) and the slope of the second line was -0.4 (r = 0.41), suggesting subpopulations of freeze resistant and freeze susceptible cells.

MOVEMENT CHARACTERISTICS OF PROGRESSIVELY MOTILE HUMAN SPERMATOZOA INVOLVED IN COLLISIONS. Sharon Mortimer* and David Mortimer, Gamete Biology Division, Reproductive Medicine Research Group, Univ. of Calgary, Alberta, Canada.

Aliquots of liquefied semen were videotaped in 20 µm deep preparations at 37°C under a 40x phase contrast objective. Tracks of 40 cells involved in collisions were plotted manually at 30 Hz (final mag = 2650x) and analyzed using a semi-automated digitizer/computer system. Each track was analyzed in three segments of 0.67 to 1.0 s (before, during and after the collision) for curvilinear, average path and straight line velocities (VCL, VAP and VSL), mean amplitude of lateral head displacement (ALH), and beat/cross frequency (BCF). Linearity (LIN-VSL/VCL), straightness (STR-VSL/VAP) and wobble (WOB-VAP/VSL) were also calculated.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before</th>
<th>During</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCL (µm/s)</td>
<td>59.5 ± 18.4</td>
<td>63.2 ± 17.3</td>
<td>66.4 ± 19.2</td>
</tr>
<tr>
<td>VAP (µm/s)</td>
<td>35.8 ± 13.8</td>
<td>41.4 ± 13.8</td>
<td>44.7 ± 15.8</td>
</tr>
<tr>
<td>VSL (µm/s)</td>
<td>36.8 ± 14.7</td>
<td>36.0 ± 14.8</td>
<td>41.6 ± 16.7</td>
</tr>
<tr>
<td>LIN (%)</td>
<td>62 ± 20</td>
<td>58 ± 21</td>
<td>63 ± 20</td>
</tr>
<tr>
<td>STR (%)</td>
<td>91 ± 16</td>
<td>91 ± 16</td>
<td>91 ± 9</td>
</tr>
<tr>
<td>WOB (%)</td>
<td>60 ± 17</td>
<td>60 ± 17</td>
<td>60 ± 17</td>
</tr>
<tr>
<td>ALH (µm)</td>
<td>2.8 ± 1.4</td>
<td>3.2 ± 1.4</td>
<td>2.9 ± 1.3</td>
</tr>
<tr>
<td>BCF (Hz)</td>
<td>14.7 ± 4.1</td>
<td>13.8 ± 3.2</td>
<td>14.7 ± 3.7</td>
</tr>
</tbody>
</table>

Although there were some small changes in several tracks, there were no significant, large or systematic differences in any mean movement characteristic for this population of cells either during or after the collisions. These results do not support the need for the exclusion of cells involved in collisions during automated motility analysis for populations where only a few cells will actually collide.

This study was conducted to determine the suitability of computer-assisted digitized image analysis as a replacement for conventional semen analysis procedures (hemocytometer for sperm density; objective analysis for motility). We compared sperm density and percent motility from computerized semen analysis (CSA) against manual semen analysis (MSA) on 68 fresh samples with sperm concentrations between 3.836 x 10^6/mL. For this study, a Motion Analysis System (with a threshold setting of 330) coupled to a Nikon photomicroscope (set at 4x) with 20 x phase objectives was used. Centroid parameter settings for the Cell Track System were 3, 3, 3, and 7 for neighborhood width, height, and minimum and maximum number of pixels, respectively. Particle parameter settings were 6, 0, 40, and 40 for search mask size, average minimum movement, maximum interpolation, and prediction percentage, respectively. Minimum path duration was 7 for high density samples and 15 for low density samples. Results of both methods were analyzed with correlation and linear regression, and means were compared with paired t test.

Sperm density values for CSA (13.4 ± 8.1) and MSA (122.8 ± 19.4) (mean ± SE, x 10^6) were similar (p>0.08), and the correlation was high (r=0.96, p<0.00). Additionally, motility values for CSA (59.9 ± 2.3%) and MSA (59.3 ± 3.2%) were similar (p=0.06) and exhibited good correlation (r=0.94, p<0.00). These data support the hypothesis that a computerized system is reasonable replacement for more traditional, but cumbersome techniques.


Capacitation, the acquisition of fertilizability, is initiated as the sperm pass through the endocervix. Sperm Select (SS; Pharmacia), a hyaluronate-based medium, is reputed to be similar to cervical mucus (Wilkand et al., Human Reprod. 2: 191-193, 1987). The purpose of this project was to determine if there are any differences in motion characteristics and fertility potential of sperm treated by SS and ejaculated processed by a standard swim-up procedure (SW). The specimens (N = 12) were evaluated 0.5 h post-emission using a Hamilton-Thorne 2030 motility analyzer. The specimens were divided into NS (x20 million/ml, <50% motile; N = 9), AS (≥50% motile; N = 3); OA (≥20 million/ml, ≥50% motile; N = 1) AS ≥≥20 million/ml, ≥50%), and 1. After the specimens were divided into two equal aliquots, SW from washed pellet and SS, the SS was layered directly onto the semen prior to a 1 h incubation at 37°C, 5% CO2. Both groups were then subjected to motion analysis, and a hyposmotic swelling test (HOS). In a separate experiment, semen samples (N = 8) after motion analysis were frozen in liquid N2 using 3.5% glycerol as the cryoprotectant.

After a minimum of 48 h storage, the specimens were thawed at room temperature, then prepared for SW from pellet and SS. The % motility and HOS were not significantly different between the groups. For NS, the SW group exhibited a significantly greater % recovery than the SS group. In the total group (15), the recovery, progressive velocity, and lateral head displacement was significantly better in the SW than SS group. Thawed semen separated by SS exhibited a lower motility than the initial specimen and the SW group. Although SS appears to be similar to cervical mucus and a centrifugation step can be avoided, using this costly medium presents no advantages over standard laboratory processing procedure.


To examine whether the rapid loss of sperm motility in stallion semen is related to diminished sperm membrane integrity in the tail and head, we utilized the hypotonic swelling test (HOS, Jeyendran et al., J. Reprod. Fert. 70:219, 1984) and the fluorescent DNA-binding H-33258 stain (ST, Cross et al., Gamete Res. 15:213, 1986). Ejaculated sperm of 5 stallions were placed on ice either in the raw form (R) or extended with 3 volumes of skim milk (E). After 3 hours the sperm motility in the R and E samples was <5% and >70%, respectively. After ejaculation and at the 3 hour time point seven semen were taken for HOS and ST. Typically, 0.1 ml semen was diluted in 1.0 ml of HOS-medium and was incubated at 36°C for 35 minutes. In the last 5 minutes 2 µg/ml H-33258 dye was added. The sperm was fixed with absolute ethanol on glass slides, and 3 x 100 sperm in each sample were evaluated with light- and fluorescent-microscopy for HOS and ST. In the R and E samples all values are expressed as %, mean±SD of the rate of curled sperm were 37.3±8.0% and 52±11.9% (p<0.16). The respective values in the 3 hours R and E samples were 27.6±2.5 and 53.6±4.1 (p<0.02). The rates of dye positive ST sperm at 0 time in the R and E samples were 56.5±13.2 and 61±19.2 (p<0.08) whereas after the 3 h time period the R and E showed ST rates of 37.2±6.5 and 39±7.0 (p<0.01). Thus, the preservation of the motility in the E samples there was a significant improvement in the head and tail membrane properties. We can conclude that the membrane integrity of stallion sperm exposed to unextended seminal fluid deteriorates very rapidly (Supported by HO-19505).
Based on repeated measure ANOVA, we conclude that incubation of sperm samples under oil decreases variability of sperm motility parameters over time.

Plasminogen activators (PA), proteases specific for the activation of plasminogen to plasmin, are considered important in biological events requiring tissue remodeling. We have measured PA activities in castration induced atrophy of the rat ventral prostate. PA activities increased nearly 5 fold at 4 days postcastration and returned to the intact level by 7 days. There is a 60% decrease in prostate gland protein (presumably due to supranuclear cytoplasm loss) at 4 days castration with little change in DNA content. Administration of hydrocortisone (5 ng/day) beginning with castration, reduced the increase in PA activity at 4 days by 55% but did not significantly affect the decrease in gland protein content. Actinomycin D (50 ug/day) treatment, began with castration, resulted in an increase of PA activity above that brought about by castration alone (fat 2 days castration PA activity of Act D treated rats was 67% saline controls and 96% saline controls at 4 days castration). This observation after castration could encode for an inhibitor of PA or an enzyme/system which can degrade PA. The former possibility is supported by the inhibition of PA activity by hydrocortisone, an agent known to stimulate PA inhibitor production in other cells/tissues. There are several other possible explanations of this phenomenon and they are being explored. (Supported in part by General Research Fund of the VA)
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ALBUMEN COLUMN SEMEN PREPARATION REDUCES CYTOTOXIC ACTIVITY OF ASA. Jack H. Ming, Department of Biomedics, Faculty of Medicine, Airlangga University, Surabaya, Indonesia, Lisniail Versteelen, Jees Goethals, Frank Goushaire, Department of Internal Medicine, State University Hospital, Ghent, Belgium, and Marc Dhont, Department of Gynecology, State University Hospital Ghent, Belgium.

In vitro manipulation of semen followed by IUI or IVF has been advocated for the treatment of male infertility. We studied whether particular procedures of semen preparation can reduce the proportion of spermatozoa coated with antibodies, or the agglutinating or cytotoxic activities of ASA.

Investigation of 10 semen samples with positive direct MAR demonstrates that neither vashing nor supplementation with cord serum reduces the proportion of antibodies bound to spermatozoa.

Further investigation also demonstrates that high concentrations of cord serum or human serum albumin do not abolish the agglutinating activity. A tendency towards reduction of agglutinating activity and spermatozoa prepared in medium supplemented with 50% cord serum or a column with 7.5% human serum albumin is observed in 3 out of 10 preparations.

The cytotoxic effect of ASA is not reduced against spermatozoa prepared in medium supplemented with cord serum. However, it was significantly decreased against spermatozoa prepared on a column with 7.5% human serum albumin. This may be due to the fact that a high concentration of albumin has stabilized the sperm membrane protecting it against complement activity.

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EFFECT OF KETOCONAZOLE ON FERTILITY IN MALE RATS. Donald P. Waller, Annamarie Martin * and Loren J.D. Zaneveld, Dept. Pharmacodyn., Univ. of Illinois at Chicago; and Dept. Ob/Gyn, Rush Univ., Chicago, IL 60612

A number of recently studied imidazoles, including ketoconazole, are potent spermicides in vitro. Oral administration of ketoconazole causes immobilization of ejaculated spermatozoa (Vickery et al., Adv. Contracept. 1:34, 1985). However, the antifertility effect of orally administered imidazoles has never been established. Proven male Sprague Dawley rats were treated orally with 0 (vehicle), 200 mg/kg or 400 mg/kg ketoconazole once daily for three days and then mated. Vaginal smears were obtained to assure the presence of spermatozoa. On day ten of gestation, the mated females were euthanized, the reproductive tracts excised, and the number of corpora lutea, fetuses, and implantation sites were recorded. The males were euthanized on the morning following pairing and the testes and epididymides weighed. Cauda epididymal sperm were evaluated for motility, number and forward progression. In the control, 200 mg/kg and 400 mg/kg groups, respectively, 14/15, 15/16 and 16/16 females became pregnant. There were no differences between the groups in the epididymal and testicular weights or the concentration of sperm in the epididymis. However, a dose related decrease in caudal epididymal sperm motility and forward progression was observed. These data demonstrate the ability of ketoconazole to inhibit fertility in the male rat. Contact of spermaozoal with ketoconazole may occur while they reside in the epididymis or when they are mixed with accessory sex gland secretions during ejaculation.

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PREGNANCIES IN THE DOMESTIC FERRET AFTER LAPAROSCOPIC ARTIFICIAL INSEMINATION. J. Zaneveld1, L.G. Hommert1, S.M. Hubbell1, C. Morton2, F. Nazario2, M. Bush3 and D.E. Wild4, 1National Zoological Park, Smithsonian Institution, Washington, DC 20008 and Path Valley Farm2, Willow HIll, PA 15271.

The domestic ferret (Mustela putorius f. domestica) serves as a model for the critically endangered black-footed ferret (Mustela nigripes), a species considered extinct until rediscovery in Wyoming in 1981. A founder population of 18 black-footed ferrets has been established and artificial breeding strategies applicable to this rare species are being explored. This study was conducted to determine the feasibility of freeze-thawing domestic ferret spermatozoa. Three egg yolk diluents (TEST, PDI, BSF), 2 freezing methods (straws, pellets) and 2 thawing temperatures (37°C, 60°C) were assayed. A total of 36 ejaculates was evaluated for sperm motility and progressive status (scale; 0, 5, 10, 15), and a spermatids mobility index (SMI) was calculated (spermatids progressive status X 20 + % spermatids motility divided by 2). Seminal aliquots were diluted in each cryodiluent cooled for 30 min at 5°C and pelleted on dry ice or frozen in 0.35 ml straws (20°C/min to -100°C).

Following thawing, a SMI was calculated and acrosomal integrity was categorized into 4 classes: normal acrosomal ridge (NAR), damaged acrosomal ridge (DAR), missing acrosomal ridge (MAR) and loose acrosomal cap (LAC). Ten females with maximal value swelling were given 50 IU human chorionic gonadotropin and laparoscopically inseminated in vivo with spermatozoa previously frozen using the optimum diluent and freezing method. Mean per estrus SMI and NAR were similar among TEST (50.3 ± 6.6), PDI (60.9 ± 8.3) and BSF (74.3 ± 6.8), respectively. Post-thaw sperm viability was determined (P0.05) by acrosome reaction, freezing method and thawing temperature. A wide variation in post-thaw SMI (218 ± 56.9) and NAR (59.3 ± 41.0) was detected between mice. Maximum spermatids motility and acrosomal protection was achieved with the PDI diluent and pelleting method using 37°C as thawing temperature (SMI: 59.6 ± 4.4; NAR: 41.0 ± 5.2; D:AR: 31.3 ± 5.4%; T:FAR: 11.2 ± 3.4%; L:AC: 16.5 ± 3.0%). Seven of the 10 ferrets (70%) inseminated with sperm frozen by this approach became pregnant and produced 31 kits (mean litter size, 4.4; range 1-9 kits). The results demonstrate the feasibility of freeze-thawing domestic ferret spermatozoa and suggest that germ plasm cryopreservation could be useful in captive breeding of the endangered black-footed ferret. (Supported by the U.S. Fish & Wildlife Service)

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OVARIAN HORMONE GENE EXPRESSION USING SEXUAL MATURATION IN THE CORDOBLAN HUFFY TESTIS. Hugh S. Keeling, Barbara Atwood *, Stephen J. Winterman, and Philip Troen, Department of Pathology, Westmoreland Hospital and the University of Pittsburgh, Pittsburgh, PA 15213

We have used specific cDNA probes for the m, f, g, f and f subunits of human inhibin to measure testicular mRNA levels during sexual maturation. Polyadenylated (poly A)-enriched RNA was isolated from testes obtained at the following stages of sexual development: early prepubertal (1.5-2.5 yrs), late prepubertal (2.5-3.5 yrs), postpubertal (3.5-4.0 yrs), and adult (6-8 yrs). Poly A+(poly A) mRNA was electrophoresed on 1.2% agarose-glycophorin gels before transfer to nylon membranes and hybridized to antipoly A+(poly A) probes made from subunits of murine inhibin. Northern blot mRNA bands were quantitated by densitometric analysis. The f subunit precursor mRNA was confirmed as a major component in addition to the mature hormone. The information was categorized among different postpubertal stages (f at 3.5, g at 4.0) and adult (f at 5.0) with the exception of castrated f subunit which was not observed. The f subunit precursor mRNA was increased by 10-15% above the early prepubertal stage, was maximal during puberty, and then declined in the adult stage. The f subunit precursor mRNA was competed out of a major band approximately 5.5 kD, and a minor band of approximately 4.8 kD. The concentration of f subunit mRNA was maximal during the late prepubertal stage, and progressively decreased during the pubertal and adult stages. The f subunit precursor RNA was a major band of approximately 3.5 kD, which could only be observed in early pubertal testes. The f subunit mRNA was present in large excess at all stages of development, relative to the f and g subunits.

In summary, our results demonstrate the f, g, f, and f subunit mRNA steady state RNA concentrations vary during sexual maturation. Furthermore, the changes in relative expression of mRNAs for these subunits are consistent with the hypothesis that the expression of each subunit gene is independent of the other. (Murine inhibin cDNAs were kindly provided by Biotechnology Australia Pty Ltd)
STIMULATORY EFFECTS OF TUMOR NECROSIS FACTOR AND INTERLEUKIN-1 ON ADULT RAT LEYDIG CELLS IN CULTURE. D.W. Warren, V. Pavlopoulou, T. Xu, B. Plakter, and R. Horton. Department of Physiology and Biophysics and Endocrine Section, Department of Medicine. USC School of Medicine, L.A., CA 90033.

Tumor necrosis factor (TNF) and interleukin-1 (IL1) inhibit many functions in malignant tissue. However, these compounds are mitogenic in many normal cells. Both compounds can be produced by macrophages and these cells are a normal interstitial component of the adult testis. Thus, these compounds could influence regulation of steroidogenesis through a paracrine mechanism. To determine the effect of TNF and IL1 on the Leydig cell, adult rat testes were dispersed with collagenase, washed and 2 × 10^6 cells per dish cultured. After 24 h, the medium was replaced with fresh medium containing HCG (1 ng/ml) or varying doses of TNF, IL1, HCG + TNF or HCG + IL1. Samples were collected at 4, 48 hrs and 5 days and testosterone (T) measured. Control cells produced 7.4 and 9.0 ± 0.8 pg/ml/hr for the first 4 and 48 hours, respectively. Between 2 and 5 days of culture, T production fell to 5.66 ± 0.43 pg/ml/hr. HCG stimulated T production 4.5-fold at 4 and 48 hours and 3-fold at 5 days. 100 units TNF stimulated T as much as HCG. IL1 unit of IL1 was equivalent to the TNF or HCG in stimulating T at all time periods. Both TNF and IL1 enhanced HCG-stimulated T secretion. These results show that the cytokines, TNF and IL1, may play a role in the normal regulation of T production in the adult testis. They are both capable of increasing T production in the absence of gonadotropins and may augment HCG-stimulated T secretion by adult Leydig cells in culture.

IN VITRO RESPONSIVENESS TO LH OF LEYDIG CELLS FROM THE TESTIS SURVIVING UNILATERAL ORCHEDECTOMY: FAILURE OF PROPRANOLOL TO BLOCK INCREASED RESPONSIVENESS. David Y. Powersanz, MRC Group in Reproductive Biology, Dept. of Physiology and Obstetrics & Gynaecology, Univ. Western Ontario, London, Ont. Canada, N6A 5C1.

Within 8 h of unilateral orchidectomy (UO) circulating androgen (A) returns to normal. This "compensatory response" occurs with no significant change in gonadotropin secretion. The doubled A output by the remaining gonad may be dependent on testicular nerves because the compensatory response is blocked by intra-testicular (IT) injection of DL-propranolol (PROP). The behavior of the surviving gonad's Leydig cells (LC) in this situation has not been directly examined. Percoll purified LC were used to determine if the LC of the surviving testis double their androgen (A) output for a given dose of LH and if β-adrenergic receptors mediate such a response. Four groups of 5 animals were used: Sham operated (S), right side UO without treatment (U), UO with IT saline vehicle, and UO with 1 mg IT PROP. Injections occurred at 0 h and 19 h later. Animals were killed 24 h after initial surgery. LC were incubated for 2 h with varying amounts of LH and A release was estimated by RIA. Cells from U animals released nearly twice as much A as controls. Saline and PROP injection of the surviving testis was not different from U. The failure of β-blockade to reduce the compensatory response suggests that the role of the sympathetic nervous system proposed by others may be: 1) not demonstrable 2 h after LC collection, 2) due to an effect on testicular blood flow or 3) mediated by another testicular cell which in turn modulates LC function.

UP-REGULATION OF INSULIN-LIKE GROWTH FACTOR-I RECEPTORS OF LEYDIG CELLS IN PRIMARY CULTURE BY HUMAN CHORIONIC GONADOTROPIN, 8-BROXO CYCLIC AMP AND FORSKOLIN. Tu Lin. WJB Dorn Veterans' Hospital and University of South Carolina School of Medicine, Columbia, SC 29201. Insulin-like growth factors (IGF-I and II) are a group of low molecular weight growth factors with extensive structure homology to pro-insulin. Previously we have reported that IGF-I can enhance Leydig cell steroidogenesis and that Leydig cells contain high affinity IGF-I receptors. Furthermore, IGF-I receptors of Leydig cells can be up-regulated in vivo administration of HCG. This may be one mechanism by which HCG and IGF-I interact to enhance Leydig cell androgen biosynthesis. In the present study, we have further evaluated the mechanism responsible for HCG-induced up-regulation of IGF-I receptors. Purified Leydig cells were prepared from adult Sprague-Dawley rats (55-65 days old) and cultured under 95% air-5% CO2 for 24 h. Cells were treated with HCG (0 - 1000 ng/ml), β-bromo cAMP (0 - 1 mM) or forskolin (10 µM). Binding of 125I-IGF-I to Leydig cells was determined after 24 h. HCG caused a dose- and time-dependent increase in testosterone and cyclic AMP production. Binding of 125I-IGF-I to Leydig cells was increased as early as 6 h after the addition of HCG and peaked at 16 h. The effects of HCG were inhibited by the addition of either β-bromo cAMP or forskolin. β-bromo cAMP (0.1 µM) and forskolin (10 µM) increased IGF-I receptors about two-fold without changing in binding affinity. In conclusion, IGF-I receptors of Leydig cells can be up-regulated in the HCG treated testes by β-bromo cAMP and forskolin in vitro. Induction of IGF-I receptors by HCG is mediated by increased cyclic AMP formation.
MEMBERSHIP APPLICATION FOR
(Please type or print)

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   (first)   (initials)   (last)

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Phone to List In Directory: __________________________________________________

Education (undergraduate, graduate, postdoctoral - in chronological order)

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<tr>
<th>Institution</th>
<th>Degree</th>
<th>Year</th>
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</tbody>
</table>

Present Position: ____________________________________________________________

Speciality or Area of Research Interest: ______________________________________

Membership Requested: □ Active   □ Student

EACH APPLICATION MUST INCLUDE:

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   Residents of Canada, U.S. & Mexico:
      Active Members - $50/yr
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Harvard Medical School
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Boston, MA 02115 (Tel: 617-732-0841)

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

National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Polypeptide Hormones, Subunits, and Antisera Available to Qualified Investigators

The materials listed below are procured through programs supported by the National Institute of Diabetes and Digestive and Kidney Diseases, Center for Population Research of the National Institute of Child Health and Human Development, and the Agricultural Research Service of the U.S. Department of Agriculture. These materials are distributed to qualified investigators for research purposes, not for therapeutic, diagnostic or commercial uses.

APPLICATION: Application forms must be submitted before an award is made. Letters of request unaccompanied by an application form are not acceptable. Forms are available from and should be submitted to: Dr. Salvatore Raiti, Director, National Hormone and Pituitary Program, 210 West Fayette Street, Suite 505, Baltimore, MD 21201, USA. Phone: (301) 837-2552, Electronic Mail: BITNET NHPP@UMAB: Fax Phone: (301) 837-9566.

AWARDS: Only one ampoule or one RIA kit of each material will be awarded at a time. Requests for larger amounts of materials may be granted, subject to review by the Hormone Distribution Program Advisory Committee.

NEW MATERIALS AVAILABLE:

1. Rat LH, FSH, and TSH Subunits
2. Antiserum to Synthetic Human GH
3. Frozen Human Pituitary Glands
4. 20K Variant of hGH
5. Porcine FSH for iodination

FOR RIA: PITUITARY & PLACENTAL HORMONES & SUBUNITS, ANTISERA, & REFERENCE PREPARATIONS

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<thead>
<tr>
<th>Human</th>
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<th>Porcine</th>
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<tr>
<td>GH</td>
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H = Hormone for iodination; AS = diluted antiserum for 10,000 RIAs or as indicated; RP = reference preparation.

NOTE: RIA's for Ovine pituitary hormones cross react strongly with, and may be used for the measurement of, the corresponding Bovine pituitary hormones, with the exception of FSH. RIAs for Rat pituitary hormones cross react strongly with, and may be used for the measurement of, all corresponding Mouse pituitary hormones, with the exception of Prolactin. For mouse Prolactin the available, homologous RIA should be used. RIAs for Human pituitary GH, PRL, and TSH cross react strongly with, and may be used for the measurement of, the corresponding Monkey pituitary hormones. All antisera are distributed in liquid form containing merthiolate, which can be dialyzed, if desired.

These materials may be obtained from Dr. A.F. Parlow, Harbor-UCLA Medical Center, 1000 West Carson Street, Torrance, CA 90509.

†50 µg, ‡100 µg, §200 µg, ¶300 µg, ||400 µg, *500 µg, ††1 mg, ‡‡2 mg, §§5 mg, †††Antisera for 2,500 RIAs, ¶¶Antisera for 3,750 RIAs, *Antisera for 5,000 RIAs, †††Antisera for 9,750 RIAs.

†††These materials will be shipped frozen by air express. Overseas investigators must supply the following additional information for shipping purposes: 1) Telex number, 2) Point or city of entry of materials into that country.

P-55
FOR BIOLOGIC STUDIES: POLYPEPTIDE HORMONES & OTHER MATERIALS

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<thead>
<tr>
<th>Material</th>
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**Antisera for Immunocytochemistry**
(undiluted or in low dilution)

- hGH natural, hGH synthetic, hPRL, hFSHβ, hLHβ, hTSHβ, and hACTH
- rHG, and rPRL
- oLHβ, and oPRL

**Other Antisera**

- Aldosterone antiserum
- Somatomedin-C antiserum (polyclonal)
- Somatomedin-C antiserum (monoclonal)

**Pituitary Glands, Frozen, Human**

U.S. investigators only. Not for clinical investigation.

**Hypothalami, Frozen, Rat**

---

FOR RIA: OTHER PITUITARY HORMONES AND ANTISERA

The materials listed below are obtained by writing directly to Dr. A.F. Parlow, Director, Pituitary Hormones and Antisera Center, Harbor-UCLA Medical Center, 1000 West Carson Street, Torrance, CA 90509, USA. Phone: (213) 533-3537; Fax Phone: (213) 533-3432.

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<td>RP</td>
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</tbody>
</table>

1 = kit; RP = reference preparation.

FOR MATERIALS OTHER THAN THOSE LISTED ABOVE, INQUIRE OF DR. SALVATORE RAITI OR DR. A.F. PARLOW.
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