FOURTH ANNUAL MEETING
and
POSTGRADUATE COURSE

March 13-16, 1979
Houston, Texas

PROGRAM
AND
ABSTRACTS

"Please bring to the meeting"
PROGRAM COMMITTEE

R. P. Amann, Ph.D.
A. Bartke, Ph.D.
A. T. K. Cockett, M.D.
F. C. Derrick, Jr., M.D.
M. Dym, Ph.D.
T. J. Lobl, Ph. D.
D. J. Mehan, M.D.
R. C. Northcutt, M.D.
M. -C. Orgebin-Crist, Ph.D.
L. J. Rodriguez-Rigau, M.D.
A. Sandberg, M.D.
R. K. Tcholakian, Ph.D.

Anna Steinberger, Ph.D., Chairman

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J. A. Lloyd, Ph.D.
J. P. Preslock, Ph.D.
K. D. Smith, M.D.
B. M. Sanborn, Ph.D.

Anna Steinberger, Ph.D., Chairman
AMERICAN SOCIETY OF ANDROLOGY

Fourth Annual Meeting

and

Postgraduate Course

March 13-16, 1979
Shamrock Hilton Hotel
Houston, Texas

SPONSOR

The University of Texas
Medical School at Houston

This program has been supported in part by The University of Texas Medical School at Houston continuing education fund and grants from Ayerst Laboratory and Organon, Inc.
AMERICAN SOCIETY OF ANDROLOGY
1978 - 1979

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A. Steinberger, Ph.D.
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P. Troen, M.D.
P. C. Walsh, M.D.

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Membership - R. C. Northcutt, M.D.
Nominating - S. S. Howards, M.D.
Publications - E. Rosemberg, M.D.
### AMERICAN SOCIETY OF ANDROLOGY - 4th Annual Meeting
Shamrock Hilton Hotel-Houston
March 13-16, 1979

For Registration see General Information

<table>
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<th>Tuesday</th>
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<tr>
<td><strong>March 13</strong></td>
<td><strong>March 14</strong></td>
</tr>
<tr>
<td><strong>8:00 to 12:00 noon</strong></td>
<td><strong>7:50 to 8:00 a.m.</strong></td>
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<tr>
<td>POSTGRADUATE COURSE</td>
<td>WELCOME</td>
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<td><strong>8:00 to 10:45 a.m.</strong></td>
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<tr>
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<td>BRIEF COMMUNICATIONS</td>
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<td><strong>11:00 to 12:00</strong></td>
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<td></td>
<td>&quot;THE FUNCTIONAL SIGNIFICANCE OF THE BLOOD-TESTIS BARRIER&quot;</td>
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<td>B. P. Setchell, Ph.D.</td>
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<td><strong>1:30 to 3:00 p.m.</strong></td>
<td><strong>1:30 to 4:15 p.m.</strong></td>
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<tr>
<td>POSTGRADUATE COURSE</td>
<td>BRIEF COMMUNICATIONS</td>
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<tr>
<td><strong>3:30 to 5:00 p.m.</strong></td>
<td><strong>4:30 to 7:00 p.m.</strong></td>
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<tr>
<td>EDUCATIONAL FILMS</td>
<td>WORKSHOP ON SEMEN EVALUATION</td>
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<td><strong>6:00 to 9:00 p.m.</strong></td>
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<td>COCKTAIL RECEPTION</td>
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| Thursday  
March 15 | Friday  
March 16 |
|-----------------|-----------------|
| **8:00 to 10:30 a.m.**  
BRIEF COMMUNICATIONS | **8:00 to 10:45 a.m.**  
BRIEF COMMUNICATIONS |
| **11:00 to 12:00**  
"EXPERIMENTAL APPROACH TO  
THE ETIOLOGY OF BENIGN  
PROSTATIC HYPERTROPHY"  
P. C. Walsh, M.D. | **11:00 to 12:00**  
"THE ROLE OF NUTRITION AND  
OBESITY IN MALE  
REPRODUCTIVE FUNCTION"  
R. S. Swerdloff, M.D. |
| **1:30 to 5:00 p.m.**  
BRIEF COMMUNICATIONS | **1:30 to 5:00 p.m.**  
BRIEF COMMUNICATIONS |
| **5:00 to 6:00 p.m.**  
BUSINESS MEETING | |
AMERICAN SOCIETY OF ANDROLOGY

PAST PRESIDENTS
1975-1976 ........................................ Emil Steinberger, M.D.
1976-1977 ........................................ Emil Steinberger, M.D.
1977-1978 ........................................ Don W. Fawcett, M.D.

HONORARY MEMBERS
M. C. Chang, M.D. ........................................ 1976
R. O. Greep, M.D. ........................................ 1976
R. E. Mancini, M.D. ........................................ 1977
T. Mann, M.D., Sc.D., Ph.D., F.R.S. .......................... 1978
R. S. Hotchkiss, M.D. ........................................ 1978
MEMBERSHIP INFORMATION

This is an invitation to join the American Society of Andrology. The ASA is composed of scientists and clinicians interested in the male reproductive system. Our membership has grown to 400 and includes anatomists, animal scientists, biochemists, endocrinologists, gynecologists, internists, pathologists, physiologists, psychiatrists, urologists, veterinarians, and others, including many students.

This Society was founded in 1975 to meet the need of concentrating attention and exchanging scientific information in the area of male reproduction in its broadest sense. Interests of members range from fundamental morphological, biochemical, endocrine, neuroendocrine, and physiological studies to clinical, diagnostic, therapeutic, surgical, and behavioral studies.

We invite you to become an active member of the Society and take part in its annual meetings. The next annual meeting (1980) will be held in Chicago, Illinois. Your contribution to the Society's growth will be most welcome.

Application forms are available at the Registration Desk, or can be requested from:

Robert C. Northcutt, M.D.
Membership Chairman, ASA
Mayo Clinic
Rochester, MN 55901
GENERAL INFORMATION

Headquarters for the 1979 Meeting of the American Society of Andrology will be at the Shamrock Hilton Hotel, Houston, Texas where all scientific sessions and social events will be held. A block of rooms has been set aside at the Shamrock Hilton Hotel for the registrants and their companions. Requests for room reservations should be mailed directly to the hotel specifying Annual Meeting, American Society of Andrology. To assure accommodations, the requests should be made several weeks in advance. The hotel telephone is (713) 668-9211.

Registration and Information Desk will be open Monday, March 12, from 6:00 p.m. to 8:00 p.m. and Tuesday, March 13 from 7:00 a.m. to 6:00 p.m. in the Lobby North. On March 14-16, the Registration and Information Desk will be open from 7:30 a.m. to 4:00 p.m. and will be located in front of the Cabaret Room.

Preregistered participants should pick up their badges at the Registration Desk.

POSTGRADUATE COURSE

The Postgraduate Course on “Recent Advances in Andrology” will be held on Tuesday, March 13, from 8:00 a.m. to 12:00 noon and from 1:00 p.m. to 3:00 p.m. in the Columbia Room. Those who have not registered for this course in advance may do so at the Registration Desk.

As an organization accredited for continuing medical education, The University of Texas Health Science Center, Office of Continuing Education for the Medical School certifies that this continuing medical education offering meets the criteria for (6) credit hours in Category 1 of the Physician’s Recognition Award of the American Medical Association, provided it is used and completed as designed.

EDUCATIONAL FILMS

Several educational films of interest to Andrologists will be shown Tuesday, March 13, 3:30 p.m. to 5:00 p.m. in the Columbia Room. All registrants are invited to attend.
COCKTAIL RECEPTION
A cocktail reception (no-host bar) for the registrants, accompanying members and guests has been planned for Tuesday, March 13 from 6:00 p.m. to 9:00 p.m. at the Poolside (weather permitting) or the Grand Ballroom. Come to meet old friends and acquire new ones.

WORKSHOP ON SEMEN EVALUATION
A Workshop on Semen Evaluation will be held Wednesday, March 14 from 4:30 p.m. to 7:00 p.m. in the Columbia Room. This workshop will include a group discussion of methods, results, and the importance of various commonly used parameters in semen analysis. Other topics to be covered include: the constituents of seminal plasma and their affects on sperm; abstinence; collection techniques; terminology and definitions of infertility. All interested registrants are invited to participate.

BUSINESS MEETING
The Annual Business Meeting of the American Society of Andrology will be held Thursday, March 15, 5:00-6:00 p.m. in the Cabaret Room, immediately following the scientific session. Committee reports, unfinished and new business will be presented. Attendance is limited to members of the Society. All members are urged to attend and participate in the affairs of the Society.

BANQUET
The Annual Banquet will take place in the Embassy Room on Thursday, March 15 beginning 7:30 p.m. All registrants, accompanying members and guests are cordially invited. Tickets for the Banquet can be purchased at the Registration Desk prior to Wednesday evening.

COMMERCIAL EXHIBITS
Approximately twenty commercial companies will exhibit their products, (equipment, supplies, drugs, publications, etc.) on Thursday and Friday, March 15-16, in the Columbia Room. Plan on seeing the “latest” and enjoy complimentary refreshments.

SITE-SEEING TOURS
Tours to various points of interest in the Houston area (NASA, Astroworld, Astrodome) can be arranged through the hotel information desk.
STATE-OF-ART LECTURES
(Cabaret Room)

WEDNESDAY, MARCH 14 - 11: a.m.

"THE FUNCTIONAL SIGNIFICANCE OF THE BLOOD-TESTIS BARRIER"

B. P. Setchell, Ph.D.
Department of Biochemistry
ARCH Institute of Animal Physiology
Cambridge, England

THURSDAY, MARCH 15 - 11:00 a.m.

"EXPERIMENTAL APPROACH TO THE ETIOLOGY OF BENIGN PROSTATIC HYPER trophy"

P. C. Walsh, M.D.
Brady Urological Institute,
Johns Hopkins Hospital
Baltimore, Maryland

FRIDAY, MARCH 16 - 11:00 a.m.

"THE ROLE OF NUTRITION AND OBESITY IN MALE REPRODUCTIVE FUNCTION"

R. S. Swerdloff, M.D.
Harbor General Hospital,
UCLA School of Medicine
Torrance, California
POSTGRADUATE COURSE

RECENT ADVANCES IN ANDROLOGY
TUESDAY, MARCH 13
(Columbia Room)

Director:  Emil Steinberger, M.D.

8:00 a.m.  "Basic Biology of Vas Deferens"
           Dr. David Hamilton

9:00 a.m.  "Microsurgery of the Male Reproductive Tract"
           Dr. Sherman Silber

10:00 a.m. "Surgical Therapy of Male Infertility"
          Dr. Larry Dubin

11:00 a.m. "Micro- vs Macrosurgery"
           Dr. Arnold Belker

12:00      LUNCH

1:00 p.m.  "Medical Therapy of Male Infertility"
           Dr. Philip Troen
            to
            3:00 p.m.  Dr. Stephen J. Winters
Wednesday, March 14 - A.M.

7:50 a.m.  WELCOME (Cabaret Room)
           Anna Steinberger, Ph.D.
           Program Chairman

           Robert L. Tuttle, M.D., Dean
           The University of Texas
           Medical School at Houston

Session 1: SEX ACCESSORIES (Cabaret Room)

Chairpersons: C. B. Metz, Ph.D. and S. Silber, M.D.


8:30 a.m.  3. Reinnervation of the ductus deferens after vasectomy and subsequent vasovasostomy in rabbits. N.J. Alexander*, D.L. Fulgham, D.L. Toyooka, and H. Uno, Oregon Regional Primate Research Center, Beaverton, Oregon.

8:45 a.m.  4. Biological model for ciliary propulsion in the ductuli efferentes. H. Winet*, Southern Illinois University, Carbondale, Illinois.

9:00 a.m.  5. Sperm transport through the vas deferens. G.S. Prins, and L.J.D. Zaneveld*, Univ. Illinois Medical Center, Chicago, Illinois.

9:15 a.m.  6. Sperm production and epididymal sperm reserves in humans. R.P. Amann* and S.S. Howards, The Pennsylvania State University, University Park, Pennsylvania and University of Virginia Medical School Charlottesville, Virginia.

9:30 a.m.  7. Intralumenal proteins of the male rat reproductive tract. T.T. Turner*, Univ. of Virginia School of Medicine, Charlottesville, Virginia.
9:45 a.m. 8. Effects of vasectomy in rhesus monkeys. N.J. Alexander*, T.B. Clarkson, J. Hren, and B. Mixon, Oregon Regional Primate Research Center, Beaverton, Oregon, and Bowman Gray School of Medicine, Winston-Salem, North Carolina.

10:00 a.m. 9. Epididymal extravasation following vasectomy as cause for failure of vasectomy reversal. S.J. Silber*, St. Louis, Missouri.

10:15 a.m. 10. Developmental study of epididymal Δ5-3α-reductase and 3α-hydroxysteroid dehydrogenase. H. Scheer* and B. Robaire, McGill University, Montreal, Quebec, Canada.

10:30 a.m. 11. Plasma prostaglandin F2α level following sexual stimulation of dairy bulls. P.S. Weathersbee* and J.R. Lodge, University of Illinois, Urbana-Champaign, Illinois.

10:45 a.m. REFRESHMENTS

11:00 a.m. State-of-Art Lecture: "The Functional Significance of the Blood-Testis Barrier", B.P. Setchell, Ph.D.

12:00 LUNCH

Wednesday, March 14 - P.M.

Session 2: SEMEN (Cabaret Room)

Chairpersons: A.T.K. Cockett, M.D. and K.S. Moghissi, M.D.

1:30 p.m. 12. Lipids and prostaglandins in human seminal fluid. H.T. Jonsson, Jr.*, D.L. Morris, F.C. Derrick, Jr., M.N. Berkaw and R.E. Powers, Medical University of South Carolina, Charleston, South Carolina.


2:00 p.m. 14. Evidence for the usefulness of vasocystostomy to measure sperm output. R.L. Urry* and R. Hill, Brigham Young University, Provo, Utah.
<table>
<thead>
<tr>
<th>Time</th>
<th>Presentation</th>
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<tbody>
<tr>
<td>2:15 p.m.</td>
<td>15. The statistical evaluation of sperm analysis: definition of subgroups by linear transform analysis. G.S. Bernstein, R. Dozono-Takano, R.M. Nakamura*, LAC/USC Medical Center, Los Angeles, California.</td>
</tr>
<tr>
<td>2:30 p.m.</td>
<td>16. The effect of creation of an experimental varicocele in the dog on semen quality. A.A. Caldamone*, A. Al-Juburi, V. Altebarmakian and A.T.K. Cockett, University of Rochester School of Medicine and Dentistry, Rochester, New York.</td>
</tr>
<tr>
<td>2:45 p.m.</td>
<td>17. Immunosuppressive effects of seminal plasma in vivo. D.J. Anderson* and H.C. Hensleigh, Oregon Regional Primate Research Center, Beaverton, Oregon and University of Oregon Health Sciences Center, Portland, Oregon.</td>
</tr>
<tr>
<td>3:00 p.m.</td>
<td>18. Correlation between human sperm motility scores and semen carnitine levels. R.L. Urry* and D. Puckett. Brigham Young University, Provo, Utah and University of Utah Medical Center, Salt Lake City, Utah.</td>
</tr>
<tr>
<td>3:45 p.m.</td>
<td>21. The contraceptive action of 6-chloro-6-deoxysugars in the male rat. G.M.H. Waite*, W.C.L. Ford and E.B. Rathbone, The University, Whiteknights, Reading, United Kingdom.</td>
</tr>
<tr>
<td>4:00 p.m.</td>
<td>22. Synthetic guanidino compounds as most active human acrosin inhibitors. A.K. Bhattacharyya*. Calcutta University, Calcutta, India.</td>
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<tr>
<td>4:15 p.m.</td>
<td>REFRESHMENTS</td>
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4:30 p.m. Workshop on Semen Evaluation
(Columbia Room)

Thursday, March 15 - A.M.

Session 3: Molecular Endocrinology
(Cabaret Room)

Chairpersons: P. Troen, M.D. and J.L. Vaitukaitis, M.D.

8:00 a.m. 23. Nonspecific age-related decline in the cyclic AMP response to hormones in isolated rat Sertoli cells. J.J. Heindel*, S.J. Strada and A. Steinberger, The University of Texas Medical School at Houston, Houston, Texas.

8:15 a.m. 24. Characterization of β-adrenergic receptors in rat Sertoli cells. J.J. Heindel*, A. Steinberger and S.J. Strada, The University of Texas Medical School at Houston, Houston, Texas.

8:30 a.m. 25. Desensitization at both the gonadal and pituitary levels after treatment with LHRH agonists. L. Cusan*, C. Seguin, C. Auclair, and F. Labrie, MRC Group in Molecular Endocrinology, CHUL, Quebec, Canada.

8:45 a.m. 26. Androgen binding protein (ABP) and fertility in 5-thio-D-glucose (5TDG) treated male rats. Y.C. Lin*, D.W. Fawcett and M. Dym, Harvard Medical School, Boston, Massachusetts.


9:15 a.m. 28. Localization of immunoreactive androgen binding protein in caput epididymis of the rat. L.J. Pelliniemi*, M. Dym, M. Durand, G.L. Gunsalus, N.A. Musto, C.W. Bardin

9:30 a.m. 29. Androgen responses and binding in seminal vesicle epithelium. M.J. Weinberger* and C.M. Veneziale, Mayo Clinic, Rochester, Minnesota.

9:45 a.m. 30. The effect of ionic strength on the interaction of the androgen-receptor complex with Sertoli cell nuclei. B.M. Sanborn* and A. Steinberger, The University of Texas Medical School at Houston, Houston, Texas.

10:00 a.m. 31. Characterization of androgen receptors in female rat tissues. C.H. Chang* and D.J. Tindall, Baylor College of Medicine, Houston, Texas.

10:15 a.m. 32. Androgen receptors of the normal and neoplastic human prostate and lymph node metastases of prostate adenocarcinoma. S.A. Shain*, R.W. Boesel, D.L. Lamm and H.M. Radwin, Southwest Foundation for Research and Education, The University of Texas Health Science Center and Audie L. Murphy Memorial Veterans Administration Hospital, San Antonio, Texas.

10:30 a.m. REFRESHMENTS

11:00 a.m. State-of-Art Lecture: "Experimental Approach to the Etiology of Benign Prostatic Hypertrophy", P.C. Walsh, Ph.D.

12:00 LUNCH

Thursday, March 15 - P.M.

Session 4: TESTIS (Cabaret Room)

Chairpersons: B. Gondos, M.D. and J.M. Leonard, M.D.

1:30 p.m. 33. The effect of irradiation on testicular histology and serum levels of LH, FSH and testosterone in the rhesus monkey (Macacus Mulatta). D.G. deRooij*, State University, Utrecht, The Netherlands.
1:45 p.m. 34. Recovery of spermatogenesis following irradiation and cyclophosphamide. J.M. Leonard* and J.E. Sanders, USPHS Hospital and University of Washington, Seattle, Washington.

2:00 p.m. 35. Time course of the effects of tetrahydrocannabinol on testosterone levels in the male rhesus monkey. C.G. Smith*, N.F. Besch and N.J. Makela, Baylor College of Medicine, Houston, Texas.

2:15 p.m. 36. Testicular morphology of rats with protein-calorie malnutrition. D.C. Herbert* and F.J. Weaker, The University of Texas Health Science Center at San Antonio, San Antonio, Texas.

2:30 p.m. 37. Inhibition of spermatogenesis in the rat by treatment with human chorionic gonadotropin (hCG). G. Pelletier*, L. Cusan and F. Labrie, MRC Group in Molecular Endocrinology, CHUL, Quebec, Canada.

2:45 p.m. 38. The role of the chromatoid body in spermatogenesis. M. Parvinen* and L.-M. Parvinen, University of Turku, Finland.

3:00 p.m. REFRESHMENTS


3:30 p.m. 40. Preleptotene DNA synthesis in germ cells of the neonatal rabbit testis. B. Gondos*, A.G. Byskov, J. Larsen and J. Grinsted, University of Connecticut Health Center, Farmington, CT, and Finsen Laboratory, Finsen Institute, Copenhagen, Denmark.

3:45 p.m. 41. Gamma-glutamyl transpeptidase activity in transsexual, varicocele, prostatic carcinoma and cryptorchid conditions in man. C.C. Lu* and A. Steinberger, The University of Texas Medical School at Houston, Houston, Texas.
4:00 p.m.  42. Ultrastructural cytodifferentiation of the rat Sertoli cells. A.S. Ramos, Jr.*, University of Nebraska, Lincoln, Nebraska.

4:15 p.m.  43. Immunocytochemical localization of testosterone in the rat testis: the effectiveness of fixation. M.P. Leuschen*, B.A. Hultman, and P.J. Gardner, University of Nebraska Medical Center, Omaha, Nebraska.


4:45 p.m.  45. Blood velocity waveform characteristics in the evaluation of internal spermatic vein reflux in the infertile male. A.V. Hirsh* and J.P. Pryor, King's College Hospital and St. Peter's Hospital, London, England.

5:00 p.m.  BUSINESS MEETING (Cabaret Room)

5:00 p.m. to 6:00 p.m.

6:00 p.m.  ANNUAL BANQUET (Embassy Room)

Friday, March 16 - A.M.

Session 5: STEROIDS (Cabaret Room)

Chairpersons: A.K. Christensen, Ph.D. and M.J. Free, Ph.D.

8:00 a.m.  46. Dihydrotestosterone (DHT), adrostenedione (A) and testosterone (T) values of healthy men and men exposed to 1,2-dibromo-3-chloropropene (DBCP). G. Castañeda*, H. Arellano, R. Alonso, N. Bedolla, and V. Cortes-Gallegos, Division deBiologia de la Reproduction and Hospital de Convalecencia, Mexico City, Mexico.

8:15 a.m.  47. Estradiol-induced inhibition of testosterone production by testicular interstitial cells incubated in vitro. H.E. Grotjan, Jr.* and E. Steinberger, The University of Texas Medical School at Houston, Houston, Texas.
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Authors</th>
<th>Institution</th>
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<tr>
<td>8:30 a.m.</td>
<td>48</td>
<td>Effect of estrogens and of prolactin on testosterone metabolism in the brain and in the anterior pituitary.</td>
<td>L. Martini*, F. Celotti and R. Massa.</td>
<td>University of Milano, Italy</td>
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<tr>
<td>8:45 a.m.</td>
<td>49</td>
<td>In vitro metabolism of testosterone by cultured Sertoli cells of 18-day-old rats with and without FSH stimulation.</td>
<td>R.K. Tcholakian* and A. Steinberger.</td>
<td>The University of Texas Medical School at Houston, Houston, Texas.</td>
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<tr>
<td>9:15 a.m.</td>
<td>51</td>
<td>Effects of bilateral cryptorchidism on testosterone secretion in bulls.</td>
<td>B.D. Schanbacher*.</td>
<td>Agricultural Research, Science and Education Administration, USDA, Clay Center, Nebraska.</td>
</tr>
<tr>
<td>9:30 a.m.</td>
<td>52</td>
<td>Action of gonadal steroids in affecting lipogenesis in cultured liver cells.</td>
<td>R.B. Tulloch* and S. Lee.</td>
<td>The University of Texas Medical School at Houston, Houston, Texas.</td>
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<td>9:45 a.m.</td>
<td>53</td>
<td>Development of testicular 3α-hydroxy-steroid dehydrogenase (3α-HSD) activity in the male rat.</td>
<td>W. Peng*, J. Wisner and D. Warren.</td>
<td>University of Southern California, Los Angeles, California.</td>
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<tr>
<td>10:00 a.m.</td>
<td>54</td>
<td>Changes in testicular venous blood testosterone concentration with time in anesthetized rats.</td>
<td>M.J. Free*, R.A. Jaffe and H.-C. Cheng.</td>
<td>University of Kansas Medical Center, Kansas City, Kansas.</td>
</tr>
<tr>
<td>10:15 a.m.</td>
<td>55</td>
<td>The relationship of average testosterone (T) levels and the pattern of T in plasma to mating behavior in mice.</td>
<td>S. Dalterio* and A. Bartke.</td>
<td>The University of Texas Health Science Center at San Antonio, San Antonio, Texas.</td>
</tr>
<tr>
<td>10:30 a.m.</td>
<td>56</td>
<td>Changes of testicular steroidogenesis induced by treatment with [D-ALA₆,DES-</td>
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10:45 a.m. REFRESHMENTS

11:00 a.m. State-of-Art Lecture: "The Role of Nutrition and Obesity in Male Reproductive Function", R.S. Swerdloff, M.D.

12:00 p.m. LUNCH

Friday, March 16 - P.M.

Session 6: PITUITARY-TESTIS (Cabaret Room)

Chairpersons: L. Martini, M.D. and K.D. Smith, M.D.

1:30 p.m. 57. Clinical trial on reversible male contraceptive with long-acting sex hormones. H.Y. Lee* and S.I. Kim. Seoul National University Hospital, Seoul, Korea.

1:45 p.m. 58. FSH levels in adult male rats following passive immunization with LH antiserum. M. Dym*, Y.C. Lin and H.G. Madhwa Raj. Harvard Medical School, Boston, Massachusetts.

2:00 p.m. 59. Effects of growth hormone and thyroxin in two strains of dwarf mice: differences from prolactin. P.C. Doherty*, S. Dalterio and A. Bartke. The University of Texas Health Science Center at San Antonio, San Antonio, Texas.

2:15 p.m. 60. Monotropic FSH elevation and germ cell changes in rats exposed to marihuana (Mh) smoke. H.P.S. Huang*, G.G. Nahas and W.C. Hembree, Columbia University, New York, New York.

2:30 p.m. 61. Acute decreases in blood prolactin (PRL) concentrations caused by delta-nine-tetrahydrocannabinol (THC). R.H. Asch*, C.G. Smith, T.M. Siler-Khodr, and C.J. Pauerstein. The University of Texas Health Science Center at San Antonio, San Antonio, Texas.
2:45 p.m. 62. Anti-serotonin therapy for male infertility.
S. Segal*, M. Ron, H. Yaffe, N. Laufer and Z. Palti. Hadassah-Hebrew University Medical Center, Jerusalem, Israel.

3:00 p.m. REFRESHMENTS


3:30 p.m. 64. Alterations in the feedback regulation of gonadotropin secretion by nonaromatizable androgen in men with primary hypogonadism. S.J. Winters*, R.J. Sherins and D.L. Loriaux, University of Pittsburgh and ERRB, NICHD, Bethesda, Maryland.


4:00 p.m. 66. Possible relation between FSH levels and Leydig cell function in azoospermic and oligospermic men. L.J. Rodriguez-Rigau*, K.D. Smith and E. Steinberger. The University of Texas Medical School at Houston, Houston, Texas.

4:15 p.m. 67. Effect of subcutaneous implants of testosterone and/or melatonin on reproduction in male hamsters. L.J. Petterborg* and R.J. Reiter. The University of Texas Health Science Center at San Antonio, Texas.

4:30 p.m. 68. Effects of chronic hyperprolactinemia on the pituitary-testicular axis and on mating behavior in the golden hamster. A. Bartke*, P.C. Doherty and B.D. Goldman. The University of Texas Health Science Center at San Antonio, San Antonio, Texas.

4:45 p.m. 69. Clomiphene treatment of idiopathic male infertility. J.C. Emperaire*, A. Audebert and J. Riviere, USN Haut-Leveque, Bordeaux, France.
A METHOD FOR THE ISOLATION AND IDENTIFICATION OF EXFOLIATED PROSTATIC CELLS IN HUMAN SEMEN. Margaret L. Couture and Matthew Freund. Department of Physiology, Southern Illinois University, Carbondale, Illinois.

Human semen contains white blood cells, mature and immature germ cells, and exfoliated genitourinary tract cells. The exfoliated cells consist of four distinct populations: 1) transitional epithelium of the bladder or prostatic or membranous urethra; 2) squamous epithelium of the Fossa Navicularis of the penis; 3) prostate cells; 4) seminal vesicle cells. The present study was undertaken to determine if the Burstone method, which is currently used for specifically staining acid phosphatase in sections of prostate tissue, could be applied to exfoliated prostate cells. The cell suspension, free of seminal plasma, was left in 10% neutral buffered formalin for 80-83 hours after the method of Stonington. This formalin solution leaches out acid phosphatase contained within the cells. Since the prostate cells contain many times the concentration of acid phosphatase as do the other cells, after 80-83 hours they are the only cells that retain stainable quantities of acid phosphatase. This formalin solution was then filtered (Nuclepore, 8 micron) to remove the spermatozoa and white blood cells; retaining most of the exfoliated genitourinary tract cells on the filter. The filter with attached cells was stained by the Burstone method for acid phosphatase localization. This method permits the specific identification of exfoliated prostate cells in the presence of other exfoliated cells of similar appearance.

AN ANDROGEN BINDING PROTEIN IN THE PROSTATE OF FEMALE PRAOMYS (MASTOMYS) NATALENSIS: EFFECT OF ESTROGEN.

S. C. Hung, C. Lee and J. M. Holland, Department of Urology, Northwestern University Medical School, Chicago, Illinois 60611

Female Praomys (Mastomys) Natalensis has a well-developed prostate which, in an androgen-poor milieu, responds readily to androgen. In vitro labelling of tissue slices or tissue cytosol with tritiated 5α-dihydrotestosterone followed by analyzing on Sephadex gel chromatography, an androgen binding protein could be identified from soluble cytoplasmic fraction of the prostate of female Mastomys. When this androgen-protein complex was incubated with isolated prostatic nuclei, the retention of radioactivity by nuclei was 100% higher at 25 C than at 2 C. The formation of this complex was 80% inhibited by 1000 fold of unlabelled androgen and 60% by unlabelled estradiol. The inhibition by estradiol was time-dependent and reached its maximum in 2 hr of incubation. This observation suggests that the antiandrogenic effect of estrogen might reside directly at the level of androgen binding protein in this tissue.

(Supported by a grant from the Grainger Foundation and by PHS Grant HD 11411)
REINNervation of the ductus deferens after vasectomy and subsequent vasovasostomy in rabbits. N.J. Alexander, D.L. Fulgham, D.L. Toyooka, and H. Uno. Reproductive Physiology and Laboratory of Pathology, Oregon Regional Primate Research Center, Beaverton, ORE.

Sperm transport through the ductus deferens depends on excitatory neurons derived from short adrenergic fibers in the hypogastric plexus. Ten sexually mature New Zealand white rabbits were given bilateral vasectomies and (30 days later) vasovasostomies. Subsequently, samples of tissue were collected at 7-day intervals. The urethral end of the ductus deferens after vasectomy retained a normal adrenergic innervation density as evaluated with formalin-induced fluorescence, whereas innervation of the testicular end of the ductus deferens and the caudal epididymis was severely compromised. The site of the vasectomy may have affected the degree of denervation. Inguinal vasectomies destroyed most, but not all innervation, but vasectomies performed adjacent to the caudal epididymis caused total denervation. With vasovasostomy 30 days after vasectomy, significant reinnervation of the ductus deferens was observed 28 days post operation. Reinnervation of the caudal epididymis, however, was slower than that of the ductus deferens; by day 49 post vasovasostomy, only 20% of the normal innervation density was found. Less innervation of the caudal epididymis post vasovasostomy may result in reduced sperm transport. At the vasovasostomy site, the nerve fibers were tortuously swirled, and innervation often appeared to be much less than in the adjacent urethral and testicular ductus deferens. Such a zone may impede the flow of sperm during sexual excitement while not significantly affecting slow filling. Although sperm were present in the urethral ductus deferens after vasovasostomy, their density remained low for about 1 month, the same time interval required for significant reinnervation to occur. (Supported by NIH grants HD05959 and RR00163).
BIOLOGICAL MODEL FOR CILIARY PROPULSION IN THE DUCTULI EFFERENTES. H. Winet. Physiology Department, Southern Illinois University, Carbondale, IL; Engineering Science, California Institute of Technology, Pasadena, CA.

The relative roles of smooth muscle contraction, secretion and ciliary beat in moving sperm from the testis to the epididymis have not yet been clarified. While there is increasing evidence that smooth muscle in the tunica albuginea or lamina propria are major factors in the propulsion of ductal fluid into the rete testis (Ellis et al., 1978; Hargrove et al., 1977), the relative roles of secretion, smooth muscle and cilia in fluid propulsion from the rete to the epididymis has not been determined.

We have developed a ciliated channel model utilizing frog palate epithelium to assess the ability of cilia to contribute to sperm transit in the ductuli efferentes. This experimental model is free of the local wall drag effects of previous cilia-in-thin slide preparations and is qualitatively equivalent to ciliated tubes (Liron, 1978). By placing fluid tracers in the channel and photographing them with medium speed cine, we have found from analysis of the films that volumetric flow rates $Q$ in the absence of unci liated wall drag are at least 3 times that of estimates from the previous model (Winet, 1977) and at least 10% of the $Q = 6 \times 10^{-3}$ ml/hr (Waites & Setchell, 1969) flow of fluid into the rat rete testis. This 10% contribution is a valid estimate of the in situ role of ductal cilia if 1) the fluid entering the rete is the same as that entering the epididymis (fluid continuity) and 2) there is a large pressure drop from the rete to the caput epididymis (pressure gradient continuity). Since the secretory and absorptive contribution of the ductuli as compared to the caput (Hamilton, 1975) has not been determined; and since the hydrostatic pressure between the seminiferous tubules and the caput epididymis appears to rise (Johnson & Howards, 1976), one cannot accept the flow measurements from the rete as applicable to the ductuli without more direct evidence from the ductuli. Only then can the true significance of the model $Q$ values be assessed.

Supported in part by NSF Grant ENG 77-21236.

To study sperm transport mechanisms through the vas deferens, the number of spermatozoa (sperm) in certain regions of the rabbit vas and in the cauda epididymis (epid) was determined after 0 h, 48 h and 1 wk of sexual rest and after sexual stimulation. The rabbits were standardized by 48 hr ejaculations for 2-3 wks. After euthanasia, their vasa, divided into ampullary, abdominal and scrotal portions, and the epid were removed and the sperm collected. The number of sperm in the vas increased from 0 h to 48 h and 1 wk sexual rest although not significantly (p>0.05). While the epid count increased significantly (p<0.05) during that time, the amount was not large enough to account for all the sperm produced by the testis. A sperm distribution gradient was observed: the greatest number of sperm were present in the scrotal vas, less in the abdominal vas and few in the ampulla. The difference in number between the 3 portions was significant (p<0.05) in the 48 h and 1 wk sexually rested groups whose gradients were identical. Following ejaculation (0 hr sexual rest), the abdominal and scrotal portions contained similar amounts of sperm. Only after sexual stimulation when the sperm number in the vas increased markedly (p<0.05), the amount was large enough to account for the number of sperm found in the ejaculates. The scrotal portion then still contained the largest amount of sperm but a greater percentage was in the abdominal portion as compared to sexual rest. The epid sperm count decreased significantly (p<0.05) following sexual stimulation. These results indicate that during sexual rest, the sperm which are not stored in the epid are transported into and through the vas which maintains a decreasing sperm gradient towards the urethral end of the tract. During sexual stimulation, sperm are moved from the epid into the vas. At ejaculation, these vesical contents are then rapidly removed from the vas into the ejaculate. The vas then reestablishes the sperm gradient maintained during sexual rest.
SPERM PRODUCTION AND EPIDIDYMAL SPERM RESERVES IN HUMANS. R. P. Amann and S. S. Howards. Dairy Breeding Research Center, The Pennsylvania State University, University Park, PA and Department of Urology, University of Virginia Medical School, Charlottesville, VA.

Sperm reserves were determined (Biol Reprod 15:586-592, 1976) for 12 males 19-49 yr old. Seven died instantaneously and five died 1-8 days after a myocardial infarction, an automobile accident or a gun shot wound. None was an alcoholic or user of drugs; necropsies revealed no evidence of chronic illness. Testis weight averaged (N=23) 16.2 ± 1.0 g. Assuming that the elongated spermatids enumerated in testicular homogenates represented those in the last 8 days of spermiogenesis, daily sperm production averaged (N=23) 63 ± 5 x 10⁶/testis or 4.4 ± 0.3 x 10⁶/g of testis parenchyma. Compared to values for other species, both daily sperm production/g testis parenchyma and epididymal reserves of sperm were surprisingly small.

<table>
<thead>
<tr>
<th>Portion of epididymis</th>
<th>10⁶ spermᵃ</th>
<th>% of total</th>
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<tbody>
<tr>
<td>Caput</td>
<td>38.4 ± 6.6</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>Corpus</td>
<td>39.4 ± 7.0</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>Cauda</td>
<td>110.9 ± 21.6</td>
<td>52 ± 3</td>
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<tr>
<td>Total</td>
<td>182.0 ± 31.7</td>
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ᵃ Mean per epididymis ± SEM (N=19). Excludes data for one individual for whom values for the caput, corpus and cauda epididymidis averaged 954, 397 and 1382 x 10⁶ sperm.

Correlations between daily sperm production and testicular weight or total epididymal sperm reserves were 0.77 and 0.50. Although the interval of abstinence prior to death of these individuals and the number of sperm typically contained in the ductus deferens and ampulla are unknown, it is evident that for humans the productivity of the testis and the storage capacity of the epididymis are very limited.
Characterization of the intraluminal fluids of the male reproductive tract is a necessary step in understanding the final processes of sperm maturation. The present study was undertaken to examine the protein component of the intraluminal fluids of the male rat reproductive tract. Fluids were collected from the seminiferous tubule (SNF), rete testis (RTF), caput epididymidis (CPF), and cauda epididymidis (CDF) in vivo micropuncture. Blood was collected by cardiac puncture. Cell-free fluids were obtained by centrifugation at 35,000 g for 15 min. at 0°C. Total protein concentrations were determined by a micro-Lowery method in five 1 μl samples of each fluid. Five 1 μl samples of each fluid were subjected to conventional polyacrylamide gel electrophoresis in micro-gel columns (1.8 x 7.5 mm). Electrophoresis was carried out at .5 mA/gel column for 2 hours. Gels were stained in .5% Amido-Schwartz and destained in 7% acetic acid. Total protein concentrations in rat blood serum (79.9 ± 5.3 μg/μl) were twice the concentrations in SNF (38.6 ± 4.0), but 20 times those found in RTF (4.1 ± 1.1). Epididymal fluid protein concentrations increased toward SNF levels. The micro-electrophoresis system allowed visualization of protein concentration < .06 μg/μl. SNF demonstrated 4 protein bands not appearing in blood serum. CPF contains another newly detectable protein plus the increased concentration of some of the SNF proteins. CDF has at least two new proteins. This preliminary work presents new information about intraluminal protein concentrations and indicates sites of synthesis and reabsorption of reproductive tract-specific proteins. This work is supported by NIH Grant #HD09490-02 and Contract #NIH-NICHD72-2770.
EFFECTS OF VASECTOMY IN RHESUS MONKEYS. N.J. Alexander, T.B. Clarkson, J. Hren, and B. Mixon. Reproductive Physiology, Oregon Regional Primate Res. Ctr., Beaverton, ORE., and Dept. of Comparative Medicine, Bowman Gray School of Medicine, Winston-Salem, NC.

Results of biochemical, hormonal, pathological, and immunologic analyses in rhesus monkeys vasectomized up to 14 years earlier, were compared with age-matched control animals. No significant differences were observed in LH, FSH, E₁, E₂, T or DHT levels. Serum electrolyte, cholesterol, blood urea nitrogen, creatinine, and albumin levels were similar in both groups. The gamma globulin levels were significantly higher in the vasectomy group; there were no differences in the albumin to globulin ratios. Alkaline phosphatase, serum glutamic oxaloacetic transaminase, and lactic dehydrogenase levels were similar in both groups. Circulating antisperm antibodies (measured by agglutination, immobilization, and immunofluorescence) developed in vasectomized monkeys and remained elevated in 40% of the group. Acrosomal fluorescence was the most common pattern (90%), although many sera (48%) had antibodies to multiple sites on the spermatozoa. In newly vasectomized monkeys, IgM antibodies developed first; subsequently, both IgM and IgG were commonly found. Adsorption of vasectomy sera with sperm removed the antibody activity, whereas adsorption with liver powder did not, demonstrating sperm-specific effect. Possible immunopathologic changes were evaluated on frozen sections of liver, kidney, testis, epididymis, and aorta. Granular deposition of C3 was common in the basement membrane of the ductuli efferentes. No immune deposition was found in the testes. The majority of vasectomized and control monkeys exhibited some immunoglobulin deposition in the renal glomerulus, but C3 deposition was more commonly associated with the vasectomy group (40%). The most significant findings in these studies were in the cardiovascular system. Evaluation of the major arteries in these monkeys (fed Monkey Chow, i.e., rations low in fat, devoid of cholesterol, and high in fiber) revealed that monkeys with long-term vasectomies had more frequent, more extensive, and more severe arteriosclerosis than their age-matched controls. (Supported by NIH grants HD4-2866 and RR00163).
EPIDIDYMAL EXTRAVASATION FOLLOWING VASECTOMY AS CAUSE FOR FAILURE OF VASECTOMY REVERSAL. S.J. Silber, St. Louis, Mo.

We wished to determine the cause of sperm absence in the vas fluid of 5% of men undergoing vasovasostomy. Twenty-eight such men undergoing vasectomy reversal who were found to have no sperm in the proximal vas fluid on one or both sides underwent microscopic epididymal exploration. In 33 of 39 testes so explored, normal sperm was found in the epididymal fluid of the corpus despite the absence of sperm in the vas fluid. Epididymal histology distal to this site revealed extensive interstitial sperm granuloma from rupture of the epididymal duct. Testicular biopsy revealed normal spermatogenesis. It is concluded that the cause of persistent azoospermia after an accurate microscopic vasovasostomy is often the secondary epididymal obstruction induced by rupture of the epididymal duct related to the pressure increase after vasectomy.

Re-reversal of patients with oligospermia after conventional vasovasostomy showed partial obstruction of the vas anastomosis in most cases (demonstrated by vasograms, serial histologic sections, and improved semen analysis in most cases after re-operation). It is possible that oligospermia after an accurate vasovasostomy may also be related to partial epididymal blockage.
DEVELOPMENTAL STUDY OF EPIDIDYMAL \( \Delta^4-5\alpha\)-REDUCTASE AND 3\(\alpha\)-HYDROXYSTEROID DEHYDROGENASE. H. Scheer and B. Robaire

Depts. of Pharmacology and Experimental Therapeutics and of Obstetrics and Gynecology, McGill University, Montreal, Quebec.

Epididymal steroid \( \Delta^4-5\alpha\)-Reductase (5\(\alpha\)-R) activity seems to be controlled by a testicular factor secreted directly into the epididymis, whereas 3\(\alpha\)-Hydroxysteroid-dehydrogenase (3\(\alpha\)-HSD) activity appears to depend on circulating testosterone levels (Endocrinology 101:1379, 1977). To test whether the presence of spermatozoa or other factors regulate epididymal 5\(\alpha\)-R, a developmental study was undertaken. Epididymal enzymatic activities were correlated with the appearance of spermatozoa in the genital tract and with the weights of testes, epididymides and of sexual accessory tissues. Seven Sprague-Dawley rats were killed at weekly intervals starting at seven days of age. Testes, epididymides, ventral prostate and seminal vesicles were removed, blotted and weighed. Epididymides were assayed for 5\(\alpha\)-R and 3\(\alpha\)-HSD activities. Testicular and epididymal content of spermatozoa were measured hemocytometrically.

Significant 3\(\alpha\)-HSD activity was found at 7 days (0.28±0.06 (±S.E.M.) nmoles 5\(\alpha\)-androstane-3\(\alpha\),17\(\beta\)-diol (3\(\alpha\)-diol) formed/hr/epididymis), and it increased until day 63 (640±80 nmoles 3\(\alpha\)-diol formed/hr/epididymis). The increase in 3\(\alpha\)-HSD activity was coincident with that of the growth curves of the ventral prostate and seminal vesicles, biological markers for circulating testosterone. 5\(\alpha\)-R was undetectable at days 7 and 14 and rose between days 21 (0.37±0.04 nmoles dihydrotestosterone (DHT) formed/hr/epididymis) and 77 (41±4 nmoles DHT formed/hr/epididymis). A greater than 60% decline in activity was observed between days 77 and 105. Spermatozoa were not detectable before 49 days of age, but increased linearly up to day 91 where the content plateaued. From these results we conclude that during development 1) 5\(\alpha\)-R activity appears not to be directly dependent on the presence of spermatozoa in the epididymis and that 2) 3\(\alpha\)-HSD activity appears to reflect circulating androgen levels.

Supported by D.G.E.S. and M.R.C. of Canada
PLASMA PROSTAGLANDIN F2α LEVEL FOLLOWING SEXUAL STIMULATION OF DAIRY BULLS. P. S. Weathersbee and J. R. Lodge. Department of Dairy Science, University of Illinois, Urbana-Champaign, IL.

In the male, prostaglandins (PG's) are present not only in the various secretory products of the reproductive tract, but through their exogeneous administration can be related to increases in both sperm transport (Hafs, et al., 1974. Proc. Soc. Exp. Biol. Med. 145: 1120-1124) and testosteronerelease and synthesis (Haynes, et al., 1975. J. Endocrinol. 66:329-338). The present study was undertaken to measure the release pattern of PGF in dairy bulls following varying forms of sexual stimulation.

Four dairy bulls, three Holstein-Friesan and one Ayrshire, ranging in age from 1½ to 4½ years, were fitted with indwelling jugular cannulas on the day preceding the start of the experiment. Following a two hour adjustment period, three control blood samples were drawn at 15 minute intervals. Semen was then collected with an artificial vagina from two of the bulls and the other two allowed to visually observe the semen collection process. Blood samples were then drawn at 1, 3, 5, 10, 20 and 30 minutes after stimulation, with the treatments being reversed 2½ hours later and the procedures repeated.

Plasma PGF levels at 1 minute (150 ± 61.5 pg/ml) and 3 minutes (147 ± 79) after ejaculation were significantly higher (P < 0.05) than those of the control period (60.3 ± 21.7). After visual stimulation the two older bulls (4½ years) plasma PGF levels increased roughly two-fold over control values and followed a pattern roughly approximating that seen after ejaculation however, the two younger animals (1½ years) displayed no consistent changes in plasma PGF values following visual stimulation. These results suggest that PGF is released in the male following sexual stimulation.

Supported by the Illinois Agricultural Experiment Station
LIPIDS AND PROSTAGLANDINS IN HUMAN SEMINAL FLUID. H.T. Jonsson, Jr.\textsuperscript{1}, D.L. Morris\textsuperscript{2}, F.C. Derrick, Jr.\textsuperscript{3}, M.N. Berkaw\textsuperscript{4}, and R.E. Powers\textsuperscript{5}. Depts. of Biochemistry\textsuperscript{1,4,5}, Urology\textsuperscript{3} and College of Medicine\textsuperscript{2}. Medical University of S.C., Charleston, S.C.

The present study was undertaken to evaluate changes in prostaglandin, and phospholipid content of human seminal fluid, and the possible relation to fertility. Our earlier finding that 19-hydroxy PGE is the major prostaglandin in human seminal fluid (Science 187:1093-4, 1975) focused attention on the relationship between the levels of prostaglandins, and of their precursor lipids in human seminal fluid, and the possible role of these factors in male fertility.

Total lipids were extracted from individual semen samples, and phospholipid, and prostaglandin fractions isolated. Phospholipids were fractionated by thin-layer chromatography and quantitatively measured by colorimetric assay. Comparison of specimens having different sperm counts led us to conclude: 1) the major phospholipid is sphingomyelin, with lesser amounts of phosphatidyl choline, phosphatidyl serine, phosphatidyl ethanolamine and lysophosphatide. 2) Quantitative measurement of total phospholipids, and of several phospholipid fractions confirms that sphingomyelin is the major phospholipid.

Group I contained semen from patients with low sperm counts <20 x 10\textsuperscript{6} sperm/ml. Group II patients had sperm counts >20 x 10\textsuperscript{6} sperm/ml. Semen phospholipid (PL) levels (µg/10\textsuperscript{6} sperm ± SEM) in groups I and II are: total PL 26 ± 6 vs 5 ± 1; sphingomyelin (SM) 12 ± 4 vs 2 ± 1; phosphatidyl choline (PC) 8 ± 2 vs 1 ± 1 with SM/PC ratio being 1.4 vs 3.5 for groups I and II respectively. Prostaglandin E (PGE) levels are reduced in group II (24 µg/ml) compared to group I (43 µg/ml).

The relationship between fertility and sperm count, and phospholipid content can now be directly assessed and could relate to rate of liquification as well as to phospholipase levels in semen.
DETECTION OF SPERM SURFACE ANTIBODY. R.J.T. Hancock and A.T.K. Cockett. Division of Urology, University of Rochester School of Medicine and Dentistry, Rochester, N.Y.

The purpose of this study was to develop procedures for the detection and localization of sperm surface antibody which would use simpler reagents and equipment than immunofluorescent techniques, and would avoid artefacts peculiar to immunofluorescence. It has been shown that sperm can be attached to transparent plastic dishes using poly-L-lysine and that surface antibodies on these immobilized sperm can then be detected by indicator sheep red cells coated with immunoglobulins which bind specifically to the sperm surface antibody (mixed haemadsorption). Populations of sperm which carry surface anti-sperm antibodies can be clearly distinguished from those which do not. Red cell binding can be observed using simple phase contrast microscopy and in addition the plates may be fixed and stained to provide permanent records.

It is concluded that procedures of this type might be valuable in investigations of human infertility thought to be associated with anti-sperm immunity, either using immobilized sperm as targets for sera from such individuals, or detecting antibodies at the surface of sperm obtained from infertile patients.
EVIDENCE FOR THE USEFULNESS OF VASOCYSTOSTOMY TO MEASURE SPERM OUTPUT. R.L. Urry & R. Hill. Dept. of Zoology, Brigham Young University, Provo, Utah.

Previous reports from our laboratory have suggested that by surgically diverting the vas-deferans of rats in the bladder, one can obtain a determination of daily sperm output in each animal. We have named this technique "vasocystostomy" and have previously utilized the model to evaluate the effects of ultra-sound and experimental varicocele creation on testicular function. Several additional studies were undertaken to verify the usefulness of the model and to determine its correlation with several accepted parameters of reproductive function. Two such studies will be presented. The first study utilized 24 animals who had previously had a vasocystostomy. Consecutive urine samples were obtained from each animal at 4 hour intervals each day for 5 consecutive days. This study was designed to determine if sperm output is continuous throughout a day. The results suggest that the concentration of spermatozoa is not dependent upon the urine volume of the sample and is continuous throughout the 24 hour period. Sperm do seem to peak in numbers around 12:00 p.m. and reach a low at 4:00 a.m. and this rhythm precedes by 4-8 hours a circadian rhythm in the urine volume. The second experiment consisted of injecting different doses of estrogen in different groups of both rat and mice and periodically sacrificing various groups of animals to obtain testicular histology, reproductive organ weights and plasma testosterone levels. Other rats and mice that had undergone a vasocystostomy were similarly treated and sperm counts were followed before, during and for prolonged periods following estrogen injections. The results demonstrate the correlation between sperm counts obtained using the model and the histological events taking place in the reproductive organs. The work further demonstrates the usefulness of the vasocystostomy model in male reproductive studies in the rats or mouse.
THE STATISTICAL EVALUATION OF SPERM ANALYSIS: DEFINITION OF SUBGROUPS BY LINEAR TRANSFORM ANALYSIS. G.S. Bernstein, R. Dozono-Takano, R.M. Nakamura, Department of Obstetrics and Gynecology, LAC/USC Medical Center, Los Angeles, CA.

Data from semen analyses have been published since the early 1920's. A confounding factor of many of these statistical surveys was the values associated with the lower 95 per cent confidence interval. These lower confidence interval values calculated by assuming a Gaussian distribution, obtained by subtracting 2 standard deviations from the mean, were very small or sometimes negative. Since such values had no physiological reality, they were generally ignored and only the mean values were used. Clinically useful ranges were subsequently deduced from correlation of sperm count with fertility.

In order to examine the apparent contradiction with clinical reality, results from semen samples were analyzed by classical linear transform methods (probits, rankits etc.) to investigate whether the distributions were Gaussian or log-Gaussian and whether one or more subsets of sperm counts could be defined.

The data were from 2721 semen analyses performed on 1908 male partners of couples attending the USC Infertility Clinic. A number of parameters were evaluated including the total sperm count, number of sperm per ml etc.

The most interesting result was found with the parameter, motile sperm count. When the number of motile sperm per ml was analyzed, two subgroups were defined by the linear transform method. The first subgroup covered the range from 100,000 to 20 million with a median value of about 5 million per ml. The second subgroup ranged from 25 to 180 million with a median value of 60 million per ml. When total motility was considered, we again observed 2 distributions. The first group comprised a range of less than 100,000 to 35 million with a median value of about 5 million. The second group had a range from 35 to 480 million with a median value of 110 million. Therefore, these two subgroups may represent the division between the sub-fertile and fertile men.
THE EFFECT OF CREATION OF AN EXPERIMENTAL VARICOCELE IN THE DOG ON SEMEN QUALITY. A.A. Caldamone, A. Al-Juburi, V. Altebarmakian, and A.T.K. Cockett. Division of Urology, University of Rochester School of Medicine and Dentistry, Rochester, N.Y.

An experimental varicocele was created in 8 dogs by partially ligating the left renal vein proximal to the junction of the spermatic vein. Five paired control dogs underwent sham procedures. Baseline and post-operative semen samples were analyzed along with serum testosterone, serotonin, creatinine and renin. Pre-operative semen samples were obtained twice weekly for 4 weeks; postoperative samples were obtained twice weekly for 24 weeks. Testicular size and temperature were determined. At the time of sacrifice blood was obtained peripherally and from both spermatic veins for comparison; renal, testicular and epididymal weights were obtained; biopsies for histologic evaluation were processed. Sperm count and motility were significantly decreased by creation of the experimental varicocele. Due to the particular adrenal venous drainage in the dog, the reflux of catecholamine theory is excluded. Testosterone levels were unchanged by the experimental varicocele. In the majority of left testes histology was normal, however some demonstrated decreased numbers of advanced spermatocytes and cell layers lining the seminiferous tubules and sloughing of immature cells into the tubule lumens. We suggest that this model is a reproducible means of evaluating the relationship between varicocele and infertility.
IMMUNOSUPPRESSIVE EFFECTS OF SEMINAL PLASMA IN VIVO.
D. J. Anderson and H. C. Hensleigh. Reproductive Physiology, Oregon Regional Primate Research Center, Beaverton, OR, and the University of Oregon Health Sciences Center, Portland, OR.

Several investigators have demonstrated the immunosuppressive effect of human seminal plasma in vitro. The following study was performed to determine if seminal plasma also suppresses immunological reactivity in vivo. In a pilot experiment, we determined that mouse seminal plasma (a combination of seminal vesicle and prostatic fluids) exerted an immunosuppressive effect similar to that of human seminal plasma in vitro. We immunized groups of eight mice with three concentrations of sonicated mouse epididymal sperm or bovine serum albumin (0.01, 0.1, and 1 mg/injection) with or without mouse seminal plasma (1 mg/injection). Two immunization regimens were followed: (1) mice received daily subcutaneous injections of soluble antigen with or without seminal plasma for 2 weeks, were bled and boosted on day 21, and bled again on day 28, and (2) mice received a single subcutaneous injection of antigen mixture incorporated into complete Freund's adjuvant (CFA) on day 1, were bled and boosted on day 21, and bled again on day 28 as above. Seminal plasma almost completely suppressed both the primary and the secondary immune responses in both antigen- and antigen/CFA-treated mice when 0.01- and 0.1-mg doses were given. Primary and secondary antibody titers were also markedly reduced in animals immunized with large amounts (1 mg) of antigen in seminal plasma. These results demonstrate that seminal plasma exerts an immunosuppressive effect in vivo as well as in vitro, and suggest that seminal plasma may suppress immunological reactivity to sperm antigens in females after intercourse. (Supported in part by NIH grant RR00163.)
CORRELATION BETWEEN HUMAN SPERM MOTILITY SCORES AND SEMEN CARNITINE LEVELS. R.L. Urry and D. Puckett. Dept. Zoology, Brigham Young University, Provo, Utah and Div. of Urology, Univ. of Utah Medical Center, Salt Lake City, Utah.

Previous studies have suggested that the epididymis has high levels of carnitine which are reduced by castration and brought to near normal with hormone replacement therapy. The exact function of carnitine in the epididymis is unclear although it may serve as a cofactor in the transfer of fatty acids into the middle piece mitochondria for oxidation. Most of the carnitine in an ejaculated semen sample is thought to come from the epididymis. The relationship between semen quality and semen carnitine levels do not correlate with the onset of sperm motility in the epididymis but the relationship of carnitine levels to sperm motility, once developed, remains unclear.

These studies were undertaken to determine if semen levels of carnitine, measured in semen samples obtained at a fertility evaluation clinic, correlate with the motility of the spermatozoa. Carnitine levels were measured in 300 semen samples. The results indicate that semen carnitine levels are predictive for the motility of the sperm. Motility scores showed a hyperbolic relationship with levels of carnitine in the semen. The higher motility scores correlate with higher levels of carnitine and samples with decreased motility generally had lower levels of carnitine. Five semen samples obtained from two patients with necro-sperma showed a complete absence of measurable carnitine. The results in the semen and the motility of spermatozoa. Carnitine thus may be able to be utilized as a marker of epididymal function and may be an added useful test in selected patients.
CYTOCHEMICAL LOCALIZATION OF ATP PYROPHOSPHOHYDROLASE ACTIVITY IN INTACT CELLS AND PLASMA MEMBRANE VESICLES OF BOAR SPERMATOZOA: RELATION TO ADENYL CYCLASE. L. Russell, R.N. Peterson, and M. Freund. Department of Physiology and School of Medicine, Southern Illinois University, Carbondale, IL.

Plasma membrane vesicles from boar spermatozoa, prepared by nitrogen cavitation and purified by sucrose gradient centrifugation (Gillis et al., 1978), were found to be enriched more than 10-fold compared to the cavitate in the activity of adenyl cyclase (AC). Activity was low in the presence of Mg ATP alone, but was enhanced more than 4-fold when vesicles were solubilized in Triton X 100. This suggests that the vesicles are right side out and not easily penetrated by charged substrates. In the presence of the detergent, enzyme activity was more than doubled by Mn²⁺ but activity was not enhanced by estradiol (10⁻⁵-10⁻⁶M), bovine LDH (10⁻⁷, 10⁻⁶M), FSH (10⁻⁷-10⁻⁶M), porcine follicular fluid, or calcium ions (0.1mM-1mM). Cytochemical localization of the enzyme ATP pyrophosphohydrolase using the electron microscope method described by Cutler et al. (1978) showed enzyme activity to be present along the inner aspect of boar plasma membrane vesicles and fused plasma membrane-outer acrosomal membrane vesicles obtained by an ionophore (A23187) induced acrosome reaction. In intact spermatozoa, enzyme activity was located on the inner aspect of the plasma membrane with some activity near mitochondria of the middle piece. While the relationship of the cytochemical reaction product, which depends on pyrophosphate formation, to AC remains to be definitively established, the formation of pyrophosphate by other membrane ATPases is rarely observed and, therefore, unlikely. These observations suggest the usefulness of this cytochemical method for determining the sidedness and purity of plasma membrane vesicles and support the results of enzyme assay indicating that, in boar spermatozoa, adenyl cyclase is located on the inner aspect of the plasma membrane. Supported by HD11823 and HD10947 NICHD.
FLOW-THROUGH ANALYSIS OF HUMAN SPERM. U. Hacker, F. Otto, J. Schumann, and W. Göhde. M.D. Anderson Hospital and Tumor Institute, Houston, Texas, Institut für Aerobiologie, Grafschaft, W. Germany, and Universität Münster, Münster, W. Germany

Flow-through analysis (flow microfluorometry, FMF) is of great interest to andrology and mutagenesis research because it provides a method to measure the amount of DNA in sperm with a high resolution and at a high speed.

Ejaculated sperm were stored in 10% acetic acid, stained with the fluorescent dye Dapi, and treated with dithiothreitol or dithioerythritol. No enzymatic treatment was necessary. DNA histograms with coefficients of variation of less than 2% were obtained with a pulse cytophotometer 21 (Phywe). The distributions obtained were symmetrical. This method, however, does not allow a discrimination between X- and Y-bearing sperm because the Y-chromosome is A/T-rich and Dapi binds preferentially to A/T-rich regions of chromatin. Staining with ethidium bromide and mithramycin should permit discrimination between X- and Y-bearing sperm; however, in general, the coefficients of variation were broader than after staining with Dapi.

With this very simple and rapid method abnormalities in patients with fertility problems could be detected. One sample from an infertile patient showed a higher frequency of diploid sperm; sperm cells from another patient resulted in a skewing to the right side of the DNA histogram. In addition, this method should be useful in detecting mutational changes after irradiation and/or drug treatment.
THE CONTRACEPTIVE ACTION OF 6-CHLORO-6-DEOXYSUGARS IN THE MALE RAT. G.M.H. Waites, W.C.L. Ford and E.B. Rathbone. Department of Physiology & Biochemistry and the Philip Lyle Memorial Research Laboratory, The University, Whiteknights, Reading, UK.

6,6'-Dichloro-6,6'-dideoxysucrose, 6 and 6' chloro-deoxysucrose and the 6-chlorodeoxy derivatives of fructose, galactose, glucitol, glucose and mannose, have an antifertility activity in the male rat. Low fertility is associated with inhibition of glucose oxidation in spermatozoa from treated animals and more rapid loss of motility compared with sperm from control rats. The minimum effective dose of the most potent compound (6-chloro-6-deoxyfructose) is <18 mg (90 µmol) kg⁻¹.day⁻¹ but effects on the glycolysis of sperm are always obtained with lower doses. These compounds have no effect on fertility when administered to the female rat.
SYNTHETIC GUANIDINO COMPOUNDS AS MOST ACTIVE HUMAN ACROSIN INHIBITORS. A.K. Bhattacharyya.
Department of Biochemistry, Calcutta University, Calcutta, India.

Human spermatozoal acrosomes contain acrosin which helps spermatozoa to penetrate the zona pellucida of ovum. It is well established that if this enzyme is inhibited, both in vitro and in vivo, the process of fertilization is blocked. More than 125 synthetic, low molecular weight inhibitory compounds were tested for their inhibitory power towards partially purified human spermatozoal acrosin. Amongst all the inhibitors evaluated, guanidino benzyloxy phenylacetate methyl derivative (I), p-nitrophenyl p'-guanidino benzoate (II), guanidino hexanoyloxy benzoate derivative (III), ethyl p-guanidino benzoate (IV) and phenyl ε-guanidino caproate p-toluenesulfonate (V) were found to cause 50% inhibition of human acrosin at molar concentrations of $9.0 \times 10^{-9}$, $1.2 \times 10^{-8}$, $3.5 \times 10^{-7}$, $5.3 \times 10^{-7}$ and $4.1 \times 10^{-6}$ respectively. $K_i$ values of these compounds were in the range of $4.0 \times 10^{-5} \text{M}$ to $3.0 \times 10^{-7} \text{M}$, signifying that these inhibitory compounds have very high affinity for human acrosin. Compound II, although was found earlier to be highly effective and specific against human acrosin (Bhattacharyya et al, J. Reprod. Fert., 47 97, 1976), was found to be fairly toxic after oral administration using mice ($LD_{50}$ value : 720 mg/Kg). Compounds I and III were non-toxic ($LD_{50}$ values : 3,100 and 6,000 mg/Kg respectively) at concentrations which were necessary to inhibit all spermatozoal acrosin. These results indicate that these anti-acrosin and non-toxic guanidino compounds may have high potential of becoming contraceptive agents in future.

The work was supported by WHO Grant 76238.
NONSPECIFIC AGE-RELATED DECLINE IN THE CYCLIC AMP RESPONSE TO HORMONES IN ISOLATED RAT SERTOLI CELLS. J.J. Heindel, S.J. Strada and A. Steinberger. Department of Reproductive Medicine and Biology and Department of Pharmacology, The University of Texas Medical School at Houston, TX.

Follicle stimulating hormone (FSH) stimulates cyclic AMP accumulation in isolated cultured Sertoli cells. Prostaglandins (PGE₁, PGA₁, PGB₁ and PGF₂α) and the catecholamines (isoproterenol, epinephrine and dopamine) increase cyclic AMP accumulation in cultures of isolated Sertoli cells. A dramatic decrease in the cyclic AMP response to FSH was observed in Sertoli cells obtained from rats of 18 and 36 days of age. The purpose of this study was to determine if this age-related loss of cyclic AMP response was specific for FSH. In the presence of 1 methyl-3-isobutyl xanthine (1 mM), a potent in vitro inhibitor of phosphodiesterase, the prostaglandins (10⁻⁴M) stimulated cyclic AMP accumulation several fold in 18 day Sertoli cell cultures (pmoles/mg protein) basal 22±3; PGE₁ 786±42; PGA₁ 977±63; PGB₁ 592±42; PGF₂α 419±66. The effects of prostaglandins at 10⁻⁴M were not additive with maximal doses of FSH (100 µg/ml). The stimulatory effects of the prostaglandins on cyclic AMP accumulation in cultured Sertoli cells from 36 day old rats were dramatically reduced: PGE₁ 592±42; PGA₁ 374±20; PGB₁ 151±28; and PGF₂α 114±8 pmoles/mg protein. Similarly, the cyclic AMP accumulation produced by maximal doses of isoproterenol, epinephrine and dopamine (10⁻⁴M) was reduced in Sertoli cells from the 36 day old rats compared to the 18 day old rats. Values calculated as picomoles of cyclic AMP/mg protein were: isoproterenol 1005±55; epinephrine 986±68; and dopamine 630±76; at 18 days and 408±54; 408±21; and 138±20 at 36 days respectively. These results indicate that changes in the cyclic AMP response to FSH in isolated cultured Sertoli cells during sexual maturation are not specific for FSH but are common to several hormones known to increase the accumulation of cyclic AMP.

Supported by UPS grants GM21361 and 5 P05 HD 08338.
CHARACTERIZATION OF β ADRENERGIC RECEPTORS IN RAT SER- 
TOLI CELLS. J.J. Heindel, A. Steinberger and S.J. 
Strada. Department of Reproductive Medicine and Biology 
and Department of Pharmacology, The University of Texas 
Medical School at Houston, TX.

The β-adrenergic receptors on Sertoli cells isolated 
from 18 day old rat testes have been characterized by 
binding of the radioligand [125I]-iodohydrxybenzylpin-
dolol ([125I]-HYP) to monolayer cultures. The Sertoli 
cell cultures were used 3-5 days after initial planting 
and media was changed 24 hours prior to experimentation. 
On the day of the experiment the cultures were rinsed 
twice with media (MEM) and incubated for various times 
at 34°C in 95% air/5% CO₂ in 1 ml MEM containing various 
concentrations of [125I]-HYP. The reaction was terminated 
by decanting the medium and washing the cells with 3 x 
2 ml of cold MEM. The cells were then dissolved in 2 
ml of 0.2 N NaOH and counted in a Beckman gamma counter. 
Specific binding to receptors was defined as total [125I]- 
HYP binding minus nonspecific binding seen in the pre- 
sence of 0.01 µM ± d, l propranolol. Specific binding 
averaged 65-75% of total binding. Specific binding of 
[125I]-HYP to the cultured Sertoli cells was complete af- 
after a 30 minute incubation and was linear with cell 
concentrations of 50,000-400,000 per assay. The bind- 
ing was saturable with half maximal binding at approx- 
imately 300 pM. There appeared to be a single class of 
binding sites with 300-400 fmoles bound/10⁶ cells. The 
binding could be competitively displaced by the β an- 
tagonsists HYP and propranolol and the β agonists 
norepinephrine and isoproterenol. FSH, dopamine and 
the α antagonist phentotamine had no effect. Stereo- 
specificity of [125I]-HYP binding was shown by the fact 
that - propranolol was a 10 fold better competitor than + propranolol. These studies indicate that rat Sertoli 
cells contain specific, saturable β-adrenergic binding sites. 

Supported by UPS grants GM21361 and 5P05HD08338.
DESENSITIZATION AT BOTH THE GONADAL AND PITUITARY LEVELS AFTER TREATMENT WITH LHRH AGONISTS. L. Cusan, C. Séguin, C. Auclair, and F. Labrie, MRC Group in Molecular Endocrinology, CHUL, Quebec, Canada.

Although our previous data clearly indicate that the ovary and the testis are the main sites of the desensitization process observed after treatment with LHRH or its agonists in the rat, it was of interest to study a possible effect of the same treatment at the pituitary level. Chronic treatment of intact or castrated adult male rats with the potent LHRH agonist [D-Ala^6, des-Gly-NH^10]LHRH ethylamide (100 ng every second day for 2 weeks) led to a 50-55% reduction of the pituitary LH response to the s.c. injection of 200 ng LHRH while no significant effect could be detected on the FSH response to the neurohormone. In intact animals, the pituitary gonadotropin content and basal plasma LH levels were unchanged while basal plasma FSH levels were 100% increased. In castrated animals, treatment with the LHRH agonist led to a decrease of both pituitary content and basal plasma circulating levels of LH and FSH. A single injection of 10 µg of the peptide on the morning of diestrus I in normal 4-day cycling rats led to an almost complete suppression of the pituitary LH response to LHRH measured on the afternoon of expected proestrus. A similar inhibitory effect was seen in animals treated with a daily dose of 3 µg of the same agonist between days 7 and 9 of pregnancy. No significant effect was seen on the FSH response. Such treatment in cycling and pregnant rats did however lead to a marked decrease in the pituitary content of both LH and FSH. Basal plasma LH levels were reduced in both groups while a stimulatory effect was seen on plasma FSH. The present data show that acute or chronic treatment with a potent LHRH agonist not only causes a marked inhibitory effect at the gonadal level but can also impair the pituitary LH response to LHRH while FSH secretion is unaffected or even stimulated. The findings of similar effects in castrated animals exclude the role of sex steroids in this LHRH agonist-induced loss of pituitary responsiveness.
ANDROGEN BINDING PROTEIN (ABP) AND FERTILITY IN 5-THIO-D-GLUCOSE (5TDG) TREATED MALE RATS. Y.C. Lin, D.W. Fawcett and M. Dym. Department of Anatomy, Harvard Medical School, Boston, MA.

Adult rats were treated with 40 mg/kg body weight of 5TDG twice daily, intraperitoneally, for periods from 1 to 9 weeks. Testicular weight was not altered following 4 weeks of treatment; from 5 to 9 weeks there was a gradual weight decrease to 60% of control. Epididymal weight was normal throughout the 9 weeks. Serum testosterone remained unaltered during the 9 weeks of treatment. The average ABP in control testis was 0.62 pm/mg cytosol protein. In treated testis, ABP levels were significantly increased from 3 to 9 weeks (2.80 - 3.70 pm/mg) of treatment and they reached a plateau of 4.60 pm/mg at 4 weeks. The average ABP in control epididymides was 2.70 pm/mg cytosol protein; after 5TDG treatment, ABP levels were much higher than controls during the entire 9 weeks, reaching a peak of 15.20 pm/mg at 4 weeks. In the treated groups, 79% of males mated with females after 2 weeks of treatment and then the mating rate decreased significantly to 69, 50, 41 and 27% at 3, 4, 5 and 9 weeks of treatment, respectively. No implantation sites were found in any of the females. Treatment was stopped after 9 weeks and the rats were permitted to recover. The mating rate stabilized at 50% and infertility continued until 13 weeks after the cessation of treatment. However, between 14 and 21 weeks after treatment ended 40% of mated females became pregnant. Thus, our results show that infertility in 5TDG treated rats is partially reversible after 5 months of recovery. If the ABP in the seminiferous epithelium resides predominantly within Sertoli cells, germ cell depletion would result in an increase in testicular ABP/mg cytosol protein. However, this would not account for the observed increase in epididymal ABP. The observations, therefore, encourage the speculation that Sertoli cells respond nonspecifically to chemical injury of the epithelium by a compensatory increase in ABP production. (Supported by NICHD grants Nos. HD 02344 and HD 06969).
MODULATION OF TESTICULAR, EPIDIDYMAL AND SERUM ANDROGEN BINDING PROTEIN (ABP) LEVELS BY THREE HETEROCYCLIC EXFOLIATING ANTISPERMATOGENIC AGENTS. T.Lobl, *G.Gunsalus, *N.Musto, *C.W.Bardin. The Upjohn Co., Kalamazoo, MI


The study of agents which influence the seminiferous tubules has been hampered by lack of quantifiable products other than germ cells. The observation that a Sertoli cell product, ABP, is secreted into both epididymis and blood suggested this protein could be used to investigate the mechanism-of-action of drugs affecting the testis directly. Availability of a radioimmunoassay for ABP permitted detailed investigation of the exfoliating spermatogenic compound, 1-(2,4-dichlorobenzyl)-1H-Indazole-3-carboxylic acid (DICA) in the present study. This agent was administered in a single oral dose (100-500 mg/Kg) to adult rats. All doses produced a decrease in testicular weight and an increase in serum FSH concentrations. Increased serum ABP levels (40-210% above controls) evident by 9 hours were uniformly elevated (194-318% of controls) on days 1 and 5. In contrast, testicular and epididymal ABP contents were decreased on days 1 and 5. The relationship between blood ABP levels and those observed in testis and epididymis suggests that DICA compromises the blood-testis barrier as well as disrupting ABP flow into the epididymis. In a long term study, serum FSH and epididymal ABP content remained abnormal for up to 71 days following a single oral DICA administration (50 mg/kg), while blood ABP returned to normal by 32 days. These findings indicated that a single exposure to this agent produces prolonged effects on testis function. Administration of two other antispermatogenic compounds, 2,3-dihydro-2-(1-naphthyl)-4(1H)-quinazolinone (DNQ) and 1,1-dimethyl-3-(5-methyl-2-benzimidazolyl) urea (DMBU) produced effects on testis weight and FSH levels similar to those observed with DICA. DNQ and DMBU elevated testicular as well as blood ABP. CONCLUSION: (1) Three exfoliating antispermatogenic agents elevate blood ABP levels. (2) Based on a model of bi-directional ABP flow (into both blood and epididymis) DICA affects the testis differently than do DNQ and DMBU.

Androgen binding protein (ABP), synthesized in Sertoli cells, is transported from the seminiferous epithelium of the testis to the epididymis via the rete testis and ductuli efferentes. Its concentration in the epididymis decreases after passing the caput region (French et al., In: Hormone binding and target cell activation in the testis, p. 265, M.L. Dufau and A.R. Means, eds., Plenum Press, 1974). We examined the fate of ABP in the epididymis of young adult rats. The peroxidase-antiperoxidase method (L.A. Sternberger, Immunocytochemistry, Prentice-Hall, 1974) was used to localize ABP in cryostat sections of rat epididymis. The antibody bound to vesicles in the apical and supranuclear cytoplasm of the principal cells in the proximal portion of the caput epididymis (zones 1 and 2 of Reid and Cleland, Austral. J. Zool. 5:223, 1957). This region is characterized by columnar cells and a relatively narrow lumen and it is separated from the distal portion of the caput by a transverse connective tissue septum visible as a groove on the epididymal surface. The distal caput (zone 3 of Reid and Cleland), as well as the corpus and cauda, were devoid of antibody binding. The specificity of the immunological reaction was tested by substituting normal serum IgG for the antibody, selective omission of components in the reaction sequence, dose response to serial dilution of antibody, and reacting the sections with antibodies to myosins as a nonrelated specific antibody control. The binding was inhibited in all control studies. The present findings suggest that ABP is taken up by endocytosis from the epididymal fluid by the principal epithelial cells in the proximal caput region. The biochemical demonstration of high ABP concentration in the caput and low concentration in the rest of the epididymis is in accord with the present morphological localization.
ANDROGEN RESPONSES AND BINDING IN SEMINAL VESICLE EPITHELIUM  M. J. Weinberger and C. M. Veneziale, Dept. Molecular Medicine, Mayo Clinic, Rochester, MN

Various androgens may subserve different functions in a specific target tissue. We have studied this problem in the cellularly homogeneous epithelium from the seminal vesicle of the adult guinea pig by observing changes in wet weight, cytoplasmic protein content and incorporation of labelled amino acids into four immunoprecipitable soluble secretory proteins in vitro following multiple injections (2 mg) of testosterone propionate (TP), dihydrotestosterone acetate (DHT-acetate) or 3α-androstanediol acetate (3αA-acetate) to castrate animals. The in vitro binding of ³H-DHT and ³H-3αA to cytosolic fractions and intracellular binding to nuclei have also been examined. TP was the only androgen tested which restored to normal all three parameters of tissue function. DHT-acetate effectively restored tissue wet weight and cytoplasmic protein content. Under the experimental conditions 3αA-acetate was only minimally effective in promoting restoration of tissue growth parameters but was as effective as DHT-acetate, if not more so, with respect to stimulation of soluble secretory protein synthesis. Injection of estradiol or diethylstilbestrol to intact animals markedly impaired tissue function.

Intracellular binding of radioactivity to nuclei was observed after incubation of epithelium with both ³H-DHT and ³H-3αA at 5 x 10⁻⁵M. Binding of both to cytosolic fractions in vitro was also observed. Nuclear radioactivity was partially extracted by 0.4M KCl, was resistant to treatment with dextran-charcoal and migrated to the 4S region of sucrose density gradients. Androgens and estrogens (5 x 10⁻⁷M) decreased the intracellular binding of ³H-DHT and ³H-3αA to nuclei.

These studies strongly support the concept that different androgens may support distinct cell functions (growth vs. secretion). Understanding the mechanisms whereby different androgens elicit specific biological responses must await a detailed analysis of the nature of the nuclear acceptor sites in this cellularly homogenous tissue. Supported by NIH grant HD 9140.
THE EFFECT OF IONIC STRENGTH ON THE INTERACTION OF THE ANDROGEN-RECEPTOR COMPLEX WITH SERTOLI CELL NUCLEI.


We have previously shown that cultured Sertoli cells from testes of 26-30 d rats contain cytoplasmic androgen receptors and, when exposed to \(^3\)H-testosterone at 34\(^\circ\)C, accumulate label in the nuclear fraction which can be partially extracted with 0.4M KCl (Steroids 26: 493, 1977). In subsequent experiments a gradual increase in extractability of specifically bound label was noted as a function of ionic strength: 34\% (0.1M) to 60\% (0.3-0.6M). A similar degree of extraction was obtained using \(^3\)H-R1881 as ligand. Since the degree of extractability and extent of dependence on ionic strength differed from that reported for other steroid target tissues, a systematic study was undertaken. A progressive and significant difference (P<0.05) in the \% extracted label was seen as a function of ionic strength: 0.1M KCl, 29±0.3\%; 0.2M KCl, 42±3\%; 0.4M KCl, 50±2\% (1hr, 4\(^\circ\)C, n = 3). Furthermore, a statistically (P<0.05) higher percentage of the label extracted with 0.4M KCl was associated with macromolecules on Sephadex G-25: 0.1M KCl, 39±2\% extracted, 21±2\% bound; 0.4M KCl, 58±4\% extracted, 50±2\% bound. Bound label did not dissociate when rechromatographed. In 2 sequential 1 hr incubations, ethidium bromide (100 \(\mu\)M) or actinomycin D (50 \(\mu\)M) extracted 40\% of the label whereas 0.4M KCl extracted 77\%. Sequential treatment with KCl (1hr) and either one of these reagents (1hr) did not release more label than KCl alone. In conclusion: (1) The labeled steroid accumulated in Sertoli cell nuclei is only partially extracted by salt. (2) Prolonged exposure to 0.4M KCl is required to release > 60\% of the label. (3) The degree of extractability of the macromolecular-bound species increases with increasing ionic strength. (4) The "salt-resistant" (1-2hr) fraction is not preferentially released by the intercalating agents ethidium bromide and actinomycin D. These points must be taken into consideration when making quantitative estimates of nuclear-bound receptor in Sertoli cells. (NICHHD 5-P50-HD08338).
CHARACTERIZATION OF ANDROGEN RECEPTORS IN FEMALE RAT TISSUES. C.H. Chang and D.J. Tindall. Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas

Androgen receptors (AR) have been described in several female tissues. In order to determine if these AR are similar to those found in male accessory sex organs we have examined the physicochemical properties of AR from a variety of female organs and compared them to AR from testis Sertoli cells.

105,000 x g cytosols were prepared from female brain, submandibular gland, heart, lung, liver, kidney, uterus and Sertoli cell enriched testis from hypophysectomized rats in 20 mM Tris-HCl buffer containing 1.5 mM EDTA, 1.5 mM dithiothreitol and 20% glycerol. Preparations were then incubated for 3 h at 0°C with [3H]-testosterone (T), [3H]-dihydrotestosterone (DHT) or [3H]-methyltrienolone (R1881) ± 100-fold excess unlabeled steroids and then precipitated with protamine sulfate.

The greatest specific binding of all three steroids was found in uterine cytosol (18 fmol/mg protein) as compared to that found in other female organs, (0-7 fmol/mg protein) and in Sertoli cells (9 fmol/mg protein). Scatchard analysis revealed two binding components in both uterus and submandibular gland. The high affinity component bound R1881 with a Kd of 1-2 nM whereas the Kd of the low affinity component was 14-18 nM. Steroid specificity studies revealed R1881 > T >> DES > Epi-testosterone > cortisol. AR in both female tissues and Sertoli cell precipitated at less than 40% ammonium sulfate (AmSO4) whereas the Sertoli cell androgen binding protein (ABP) precipitated at 50% and albumin at 80%. The dissociation constant (t½) of AR from all tissues was greater than 16 h. These studies demonstrate that the rat uterus contains high concentrations of AR with similar physicochemical properties to the AR found in male target organs.
ANDROGEN RECEPTORS OF THE NORMAL AND NEOPLASTIC HUMAN PROSTATE AND LYMPH NODE METASTASES OF PROSTATE ADENOCARCINOMA. Sydney A. Shain¹,²,³, Robert W. Boesel¹, Donald L. Lamm²,³, and Howard M. Radwin²,³.

¹Southwest Foundation for Research and Education, ²University of Texas Health Science Center, ³Audie L. Murphy Memorial Veterans Administration Hospital, San Antonio, TX.

Saturation protocols were developed for quantitation of steroid occupied (RA) and unoccupied (R) nuclear and cytoplasmic androgen receptors in human prostate. Incubation of the synthetic androgen R1881 (17β-hydroxy-17α-methyl-estra-4,9,11-trien-3-one) with cytoplasmic or nuclear extracts (0.6 M KCl) of prostate tissue for 20 - 24 hr at 15°C permitted quantitation of cytoplasmic and nuclear R plus RA under conditions which we have demonstrated do not cause a loss of rat and canine prostate androgen receptors by inactivation. Steroid specificity studies showed that only the potent androgens testosterone, 5α-dihydrotestosterone, and 19-nortestosterone were effective inhibitors of R1881 binding; progesterone and the synthetic progestin R5020 (17α,21-dimethyl-19-norpregna-4,9-diene-3,20-dione) were ineffective. These data show that R1881 bound to prostate androgen receptors and failed to bind to putative progesterone receptors.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Receptor Content fmoles/100 µg DNA</th>
<th>Affinity Constant (x 10⁻⁷)</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Cytoplasmic</td>
<td>Nuclear</td>
<td>Cytoplasmic</td>
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<tr>
<td>Benign Hyperplasia</td>
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<tr>
<td>Adenocarcinoma</td>
<td>58</td>
<td>11</td>
<td>27</td>
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</tbody>
</table>

Androgen receptor concentration is highest in benign hyperplastic prostate. Although the remaining data are for a limited number of specimens, receptor concentration of "normal" prostate from aged subjects is not markedly different from that of primary prostate adenocarcinoma. Significantly, normal lymph node did not contain demonstrable androgen receptors. It remains to be determined whether quantitation of androgen receptors will be a useful prognostic aid in selection of therapy for prostate adenocarcinoma patients. Supported in part by NCI NO1-CP-33379.

15 adult rhesus monkeys were given a local irradiation to the testis. Divided in three groups of 5, they received 50, 100 or 200 rad of X-rays. The effect on testicular histology was evaluated in testicular biopsies taken at a number of intervals of time after irradiation.

50 rad did not, but 100 and 200 rad did cause a significant depletion of the seminiferous epithelium. Using the progression of the depletion after 200 rad, I estimated the duration of the cycle of the seminiferous epithelium to be 11.0 days.

The effect of the irradiation in the monkey closely resembled that in the human. Apale and Adark spermatagonia decreased slowly in number. Soon after depletion differentiating spermatogenic cells reappeared. However, cellular associations with one or more generations of spermatogenic cells missing and isolated clones of developing spermatocytes or spermatids were observed. This phenomenon gradually disappeared and after 5 months normal cell associations prevailed. Recovery was slow and varied considerably between individual monkeys.

Blood samples were drawn 0, 7, 18, 25, 34 and 124 days after irradiation. No significant changes were found in testosterone levels (Dr. F.H. de Jong, Rotterdam). However FSH and LH levels (Dr. R.M. Lequin, Nijmegen) did change significantly. FSH levels were found to be about 60% higher 7, 18, 25 and 34 days after irradiation, while at 124 days no significant difference with control values were found. LH levels were higher 25, 34 and 124 days, after irradiation. At 25 and 34 days they were about 150% above control level and at 124 days about 90%.
RECOVERY OF SPERMATOGENESIS FOLLOWING IRRADIATION AND CYCLOPHOSPHAMIDE. John M. Leonard and Jean E. Sanders. Department of Medicine, USPHS Hospital and University of Washington, Seattle, Washington.

Radiation and alkylating agents are known to affect reproductive function by direct injury to the testes. Whether these effects are reversible is unclear. The present study was undertaken to evaluate testicular function following exposure to 1000 rad total body irradiation (TBI) or a radiomimetic agent, cyclophosphamide (CP), employed in conditioning patients for bone marrow transplantation (BMT). Serum follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone (T) were measured and seminal fluid analyses performed one to six years post BMT. Two groups of patients were studied. Group I: 7 men, ages 17-28 years, with leukemia who received 1000 rad TBI and variable amounts of radiomimetic agents prior to BMT. Group II: 7 men, ages 13-35 years, with aplastic anemia who received CP (50 mg/kg/day X 4 days).

Results: Group I: serum T and LH levels were normal in 6/7; in one patient serum T was low and LH elevated. For the first 36 months post TBI 7/7 were azoospermic and/or FSH levels were elevated. Between 36 and 72 months germinal cells were identified in the ejaculate in 3/6 and FSH fell to normal in 3/7. Group II: serum T and LH levels were normal in 6/7; in one patient serum T was at the lower limit of normal and LH was elevated. Recovery of spermatogenesis appeared complete in 2/7, 55 and 61 months post CP respectively; in 2/7 germinal cells were present in the ejaculate, 31 and 44 months post CP; 3/7 remained azoospermic.

Conclusions: 1000 rad TBI and cyclophosphamide cause severe injury to the germinal epithelium. In some men sequential measurements revealed this effect was reversible but the recovery process is slow. Leydig cell function appears relatively resistant to injury from radiation and cyclophosphamide.
TIME COURSE OF THE EFFECTS OF TETRAHYDROCANNABINOL ON TESTOSTERONE LEVELS IN THE MALE Rhesus MONKEY. C.G. Smith*, N.F. Besch**, and N.J. Makela* *Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, Md. and **Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, Texas.

Controversy exists regarding the effects of marihuana and tetrahydrocannabinol (THC) on male sex hormones. Because of normal fluctuations in male sex hormones and difficulties in designing experiments to study the effects of cannabis derivatives on these hormones, the interpretation of the conflicting reports in the literature is difficult.

The present study was designed to observe the effects of a single dose of THC during the first 24 hours after drug administration. Sexually mature male rhesus monkeys were used in the study. The dose of the drug (2.5mg/kg of THC) was chosen from previous studies because it produces effects on testosterone that last 24 hours. The THC was prepared in a 3% solution of Tween 80 in saline and administered by an intramuscular injection. Blood was drawn immediately before drug administration, at 30 minute intervals for the first 3 hours, and at 6 hour intervals for the first 24 hours after drug administration. Testosterone was measured by radioimmunoassay.

Within the first three hours after THC administration, testosterone levels had decreased to approximately 50% of initial hormone levels. This decline continued over the next 24 hours until the testosterone levels were 10 to 20% of the control level. These results show that THC administration can decrease the blood levels of testosterone. The time course of the decrease indicates a prompt effect on testosterone production and/or secretion with a gradual decrease in blood levels of the hormone.

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TESTICULAR MORPHOLOGY OF RATS WITH PROTEIN - CALORIE MALNUTRITION. D.C. Herbert and F.J. Weaker. Department of Anatomy, The University of Texas Health Science Center at San Antonio, San Antonio, TX.

Thirty-three 20 day-old Sprague-Dawley male rats were placed into three groups and studied to determine the effect of dietary protein deficiency on the morphological development of the gonads. Group I contained three animals that were sacrificed immediately upon arrival. Group II was composed of 15 animals which were fed a normal laboratory diet containing 27% protein, while Group III contained an equal number of rats which were fed an 8% protein diet. The body weight of each animal and the amount of food consumed were monitored on a daily basis. Three animals from each of the latter two groups were sacrificed at 27, 34, 41, 48 and 55 days of age. The testes were fixed by whole body vascular perfusion with a mixture of 4% glutaraldehyde and 2% paraformaldehyde. The tissues were post-fixed with 1% osmium tetroxide, en bloc stained with 0.5% uranyl acetate and embedded in Spurr's plastic. The diameters of the seminiferous tubules and the nuclei of the Leydig cells were measured from 1µm thick plastic sections. The animals fed the control 27% protein diet weighed significantly more and consumed a greater amount of food than the rats fed the low protein diet. The daily calorie and protein intake of the controls were, therefore, greater than the values for the experimental rats. The diameter of the seminiferous tubules of these animals was significantly larger at each time period studied. Mature spermatozoa were observed at 41 days of age and beyond. In the experimental animals, the Leydig cells were smaller and less numerous. The apparent decrease in activity of these cells may have in part contributed to the large number of degenerating germ cells observed in the seminiferous tubules as well as the failure of the spermatids to fully mature. These data indicate a functional alteration in the pituitary-gonadal axis in the rats fed the low protein diet which led to a delay in the onset of puberty. Supported by NIH Grant HD 10914.
INHIBITION OF SPERMATOGENESIS IN THE RAT BY TREATMENT WITH HUMAN CHORIONIC GONADOTROPIN (hCG). G. Pelletier, L. Cusan and F. Labrie, MRC Group in Molecular Endocrinology, CHUL, Quebec, Canada.

Treatment with human chorionic gonadotropin (hCG) is well known to decrease testicular LH receptor levels. More recently, this treatment has been shown to be accompanied by a decrease in testis weight (Auclair et al., Biochem. Biophys. Res. Commun. 76: 855, 1977). Since this loss in testicular weight was likely to reflect some defect in spermatogenesis, it seemed important to investigate the morphological changes occurring in adult rat testis following administration of hCG. We thus studied the effect of hCG (100 I.U., twice a week for one to four weeks) on testicular morphology at both light and electron microscope levels. After one week of treatment, significant degenerative changes could be observed in some seminiferous tubules. The degenerative changes became more dramatic after two and four weeks of treatment, almost all tubules exhibiting signs of regression. At the ultrastructural level, the Leydig cells showed signs of hyperactivity at all time intervals. In all treated groups, the plasma levels of testosterone were elevated, whereas the plasma levels of LH were undetectable. On the other hand, plasma FSH levels were not significantly altered. Although the mechanisms by which administration of hCG induces inhibition of spermatogenesis is still unclear, the profound changes observed in the rats suggest that more detailed fundamental and clinical studies are required to obtain optimal response to treatment with hCG.
THE ROLE OF THE CHROMATOID BODY IN SPERMATOGENESIS.
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The function of a specific cytoplasmic organelle typical for spermatogenic cells, the chromatoid body, is far from being clearly understood. It arises in mid-pachytene spermatocytes at stage VII during rat spermatogenesis and has its most prominent structure in early round-nucleated spermatids.

We have been able to demonstrate that the chromatoid body in the young spermatids of the rat incorporates radioactivity derived from $^3$H-uridine during the haploid gene activity, thus participating to the RNA metabolism of the spermatids. Analyses of living spermatids in accurately defined stages of spermiogenesis by time-lapse cinemicrography have revealed that the chromatoid body actively and rather rapidly moves in relation to the nuclear surface. Predominantly in steps 1-2, the chromatoid body moves parallel to the nuclear envelope and has frequent contacts with the Golgi complex, and predominantly during step 3, the chromatoid body moves perpendicular to the nuclear envelope. Comparative electron microscopic analyses revealed that the chromatoid body is occasionally found in the proximity of prominent outpocketings of the nuclear envelope with material continuities through nuclear pore complexes to intranuclear 20 nm particles, nuclear informofers.

These observations together with earlier data give support to the idea that the chromatoid body participates to the RNA metabolism of the spermatogenic cells during the activity of the haploid chromosomes. The chromatoid body may be a storing organelle for the long-lived meiotic RNA, suggested to play a role in direction of the protein synthesis during late spermiogenesis, when the haploid genome is condensed to inactive form.
CYTOGENETICS OF MOUSE TESTIS CELLS BY FLOW MICROFLUOROMETRY. M.L. Meistrich, U. Hacker, W. Göhde, R.A. White, and J. Schumann. M.D. Anderson Hospital and Tumor Institute, Houston, Texas and Universität Münster, Münster, W. Germany.

The DNA content of testis cells may be measured with very high precision by flow microfluorometry (FMF). The precision of the measurement, as indicated by the coefficient of variation (CV) of the DNA histogram, can be about 1%. Thus, single chromosome changes, such as the difference in DNA content between X- and Y-bearing spermatids, could be measured. Cell suspensions were treated with pepsin, stained with the fluorescent dyes, ethidium bromide and mithramycin, and treated with RNase. The distribution of DNA contents of the cells was determined with a Phywe ICP 11 pulse cytophotometer. Peaks in the DNA histograms were observed corresponding to cells with DNA contents of 0.6C, 0.982C, 1.018C, 2.0C, and 4.0C where C is the haploid DNA content. These peaks correspond to elongated spermatids, Y-bearing round spermatids, X-bearing round spermatids, various diploid cells, and pachytene spermatocytes, respectively. The 1C and 4C peaks routinely had coefficients of variation of about 1%. The relative difference between the DNA content of the X- and Y-bearing spermatids was 3.6% which is consistent with the expected value of 3.5% based on chromosome length measurements. The sharpness of the peaks indicated that less than 1% of the cells had 1 extra chromosome. Mice carrying Cattanach's translocation, which involves an insertion of extra DNA into the X chromosome, showed a 5.2% relative difference in DNA content between X- and Y-bearing spermatids as analyzed by FMF. In addition, there appears to be some broadening of the haploid peaks (CV=1.7%) indicating that a slight amount of nondisjunction occurs in some of these animals. More dramatic broadening of these peaks is observed after treatment with mutagenic agents. For example, the CV is broadened to 6% after x-irradiation and to 8% after injection of adriamycin. The detection of chromosomal aberrations in spermatids can therefore be achieved by FMF.
PRELEPTOTENE DNA SYNTHESIS IN GERM CELLS OF THE NEONATAL RABBIT TESTIS. B. Gondos, A.G. Byskov, J. Larsen and J. Grinsted. Department of Pathology, University of Connecticut Health Center, Farmington, CT, and Finsen Laboratory, Finsen Institute, Copenhagen, Denmark.

Gametogenesis in the rabbit is characterized by initiation and completion of oogenesis in the neonatal period (Peters et al., J. Exp. Zool. 158: 169, 1963), while in the testis germ cell maturation is apparently arrested during this time, spermatogenesis beginning only at 7-8 weeks of age (Gondos et al., Am. J. Anat. 136: 427, 1973). We undertook an investigation to determine the behavior of testicular germ cells in the prepubertal prespermatogenic period, using ultrastructural, autoradiographic and cytofluorometric techniques. By light and electron microscopy, preleptotene condensation figures could be seen in testicular germ cells throughout the prespermatogenic period. Meiotic figures were not evident. Incorporation of 3H-thymidine by germ cells was found in 3-12% of cells at 2, 4, 7, 21 and 42 days of age. Since mitotic figures are not evident, it is assumed that the thymidine-labeled germ cells are those which enter the preleptotene condensation stages.

Flow-cytofluorometric measurements on nuclear suspensions of ovaries and testes showed that the DNA distribution was almost identical in the two sexes in the period from before birth to 1-2 days after birth. The fraction of cells with 4c DNA content increased to 12-13% at the time of birth, and then decreased to 9% at 2 days after birth. From this age the two sexes deviated. The 4c fraction of the male decreased further to 4-6%, but in the female the 4c fraction increased to 23% at 8 days after birth. A similar difference in the fraction of S-cells was evident from 2 days after birth, when the S-fraction became 14% in the female and 7% in the male.

It is concluded that the male germ cells can respond to a meiosis-inducing triggering as early as the female germ cells, but are prevented from entering meiosis by some environmental factors.
GAMMA-GLUTAMYL TRANSPEPTIDASE ACTIVITY IN TRANSSEXUAL, VARICOCELE, PROSTATIC CARCINOMA AND CRYPTORCHID CONDITIONS IN MAN. C.C. Lu & A. Steinberger. Dept. Reproductive Medicine & Biology, The University of Texas Medical School at Houston, Houston, Texas.

Gamma-Glutamyl transpeptidase (γ-GTP) has been considered a marker of Sertoli cell function (Hodgen & Sherins, 1973, Krueger et al, 1974). Studies by Lu & Steinberger (1977) indicated that most, if not all, γ-GTP activity in the rat testis is associated with the Sertoli cells. To assess the relationship of γ-GTP activity to various clinical conditions, we have measured γ-GTP activity in the testes and serum of varicocele, transsexual, prostatic carcinoma and cryptorchid patients. Homogenates of testicular biopsies and serum samples were assayed by the method of Lu & Steinberger (1977) using γ-Glutamyl-B-naphthylamide as substrate. The results were expressed as enzyme units (EU) per gram (g) testicular tissue, mg testicular protein (P) or ml serum. Serum pool from normal men, used as control, had 11.83 EU/ml γ-GTP activity. Nine prostatic carcinoma patients with normal testicular histology, ranging 56-76 yrs of age, had 1400.3 ± 174.6 EU/g tissue or 30.0 ± 10.0 EU/mgP. In four varicocele patients 24-28 yrs of age, testicular γ-GTP was 696.5 ± 137.0 EU/g tissue or 29.6 ± 11.6 EU/mgP. In five transsexuals aged 26-45 yrs and one prostatic carcinoma patient age 74, all following ≤ 1 yr estrogen treatment, testicular γ-GTP was 6.4 ± 1.0 EU/mgP, 379.5 ± 65.3 EU/g tissue and 5.5 ± 2.5 EU/ml serum. In an 17 yr child with unilateral (left) cryptorchidism, the testicular γ-GTP was 1.6 EU/mgP or 101.0 EU/g tissue. Similar levels in cases of cryptorchidism were reported by Sherins and Hodgen (1976).

Our study demonstrated that prior to sexual maturaty and in cases of germ cell depletion following prolonged estrogen treatment, the γ-GTP activity in the testes and serum is reduced. The findings suggest that γ-GTP is not affected by age but may be regulated by androgens.

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ULTRASTRUCTURAL CYTODIFFERENTIATION OF THE RAT SERTOLI CELLS. A.S. Ramos, Jr. Department of Veterinary Science, Electron Microscope Laboratory, University of Nebraska, Lincoln, NE

Fine structural cytodifferentiation of the Sertoli cells of the rat testis were examined at 2-week intervals from day 7 postnatally to sexual maturity. The cells undergo rapid morphological differentiation during the first 5 weeks after birth. These changes include a large increase in cell size and in the extent of cytoplasmic processes between germinal cells, a change in the pattern of nuclear chromatin condensation and membrane infoldings, and in the development of cellular organelles particularly the smooth endoplasmic recticulum, the Golgi apparatus, and the mitochondria. Differentiation of the basal cytoplasm comprises primarily a regional accumulation of microtubules and microfilaments, while the apical surface specialization consists of numerous finger-like cytoplasmic projections. An apparent sequence of events were observed in the development of the junctional complexes between neighboring cells and the formation of the tri-partite nucleus. This structural cytodifferentiation and specialization of the Sertoli cells occurring during the prepuberal period correlates with the appearance of the various physiologic and metabolic changes in the function of the developing testis at this stage of maturation.
IMMUNOCYTOCHEMICAL LOCALIZATION OF TESTOSTERONE IN THE RAT TESTIS: THE EFFECTIVENESS OF FIXATION. M. P. Leuschen, B. A. Hultman, and P. J. Gardner, Department of Anatomy, University of Nebraska Medical Center, Omaha, NE.

Subcellular localization of testosterone in microscopic preparations has been impeded by the tendency of steroids to diffuse during processing of tissues. In a preliminary study, Gardner (Anat. Rec., 181:359, 1975) demonstrated by the peroxidase-antiperoxidase complex technique that antitestosterone bound to sections of tissue fixed in glutaraldehyde followed by osmium tetroxide. The objective of the present study was to measure the effectiveness of a variety of primary fixatives in preserving ultrastructural integrity without loss of steroids prior to secondary fixation and immobilization with osmium tetroxide. The fixatives tested included: 1% glutaraldehyde, 3% glutaraldehyde, 1% paraformaldehyde and 3% paraformaldehyde. Male Sprague-Dawley rats were decapitated and perfused after the technique of Forssman et al. (Anat. Rec., 188:307, 1977). Equal amounts of each fixative were used for perfusion and subsequent immersion. The tissue was then rinsed twice in buffer for 15 minutes. After extraction with ether, and separation on LH-20 columns, the testosterone content of all fixatives and buffer washes was measured by radioimmunoassay. The data was expressed as pg. testosterone/ml/mg dry weight. The least diffusion of testosterone occurred in fixation with 3% glutaraldehyde (17 ± 3) and 3% paraformaldehyde (18 ± 7). Diffusion of testosterone was greatest (total loss 65 ± 55) in fixation with 1% glutaraldehyde. When total testosterone loss in both fixation and wash steps was calculated, the minimum loss was again found to occur in fixation with 3% glutaraldehyde (44 ± 6). Appreciable amounts of testosterone were lost during buffer wash steps after fixation with 1% paraformaldehyde (92 ± 29) and 3% paraformaldehyde (73 ± 12). Immunocytochemical studies verified that fixation with 3% glutaraldehyde followed by osmium tetroxide adequately preserved both ultrastructure and binding sites for antitestosterone. (Supported by NIH Grant HD 10872).

Method: Testicular biopsies were obtained from 20 men having surgical reversal of their vasectomy after an interval of 1-10 years. Part of the biopsy was fixed in Bouin's solution and the histological appearances assessed by the Johnson technique. The remainder of the biopsy was snap frozen and stored in liquid nitrogen. The biopsy was incubated with fluorescein isothiocyanate conjugated antisera. The following rabbit anti-human antisera was used: IgA, IgE, IgG, IgM, C3, C4, Clq, fibrinogen and albumin. Testicular biopsies were also taken from 4 men undergoing varicocele surgery to form a small control group. It was not felt necessary to enlarge on this group.

Results: Histological examination of 36 testicular biopsies following vasectomy showed good spermatogenesis in all specimens and the mean Johnson score was 8.4 ± 0.8. No persistent abnormalities were detected in the Leydig cell or interstitial tissues. None of the testicular biopsies following vasectomy showed the deposition of any immune complex but the biopsy of one patient with a varicocele contained immunoglobulin and complement components.

Conclusion: The histological appearances of the human testis are well preserved following a vasectomy and in a series of 20 men there was no evidence of deposition of any immune complex.

The Doppler stethoscope has been evaluated for the detection of venous reflux in infertile men. A pilot study in varicocele patients enabled the audible characteristics of spermatic cord venous reflux to be correlated with bidirectional ultrasonograms.

As a preliminary study the incidence of valsalva induced venous reflux was compared qualitatively in the spermatic cords of 44 fertile and 107 subfertile men examined in erect and supine postures. The incidence of varicocele was 23% in both groups. There was a high incidence of venous reflux in the spermatic cords of both varicocele (left 100%, right 70%) and non-varicocele (left 82%, right 60%) groups, with no significant difference between fertile and subfertile sub-groups.

Utilizing a directional Doppler velocimeter it is possible to compare venous waveform characteristics recorded at the internal inguinal ring, spermatic cord and lower pole of the testis. 28 infertile men have been examined in the erect posture breathing normally and under valsalva.

It is considered that the different waveform characteristics demonstrated in varicocele patients may be utilized to differentiate between competent and refluxing internal spermatic veins. The technique may also be used to detect subclinical internal spermatic vein reflux in patients without clinical varicoceles.

These findings may be of value in the assessment of the infertile male.
DIHYDROTESTOSTERONE (DHT), ANDROSTENEDIONE (A) AND TESTOSTERONE (T) VALUES OF HEALTHY MEN AND MEN EXPOSED TO 1,2-Dibromo-3-chloropropane (DBCP). G. Castañeda, H. Arellano, R. Alonso, N. Bedolla, and V. Cortés-Gallegos.

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Androgens are closely related to normal spermatogenesis and particularly DHT may, in part, be the mediator of androgen action. The present work compares DHT, A and T values in blood of healthy men (group I, N=5, ages: 21-30 yrs) with those of men exposed to DBCP from 1 to 7 yrs with complaint of infertility (N=5, ages: 25, 26, 34, 42 and 50 yrs). Blood samples (3 to 6) were obtained on different days at 8:00 hrs, to perform androgens RIA (groups I & II), and a testicular biopsy in each subject of group II. The results (x ± SD pg/ml) show in group I: DHT= 694 ± 229, A=1237 ± 320 and T= 6916 ± 1712; while for group II, 25 yrs subject: DHT= 508 ± 139, A=84 ± 38, T=569 ± 473 and aplastic germinal epithelium; 26 yrs subject: DHT=674 ± 148, A=652 ± 140, T= 7947 ± 1037, and hypoplastic germinal epithelium; 34 yrs subject: DHT= 333 ± 9, A=490 ± 34, T=4439 ± 182, and aplastic germinal epithelium; 42 yrs subject: DHT= 801 ± 71, A= 2169 ± 356, T=4003 ± 672, and hypoplastic germinal epithelium; 50 yrs subject: DHT=655 ± 184, A=875 ± 182, T= 5793 ± 475, and no testicular damage. These data suggest: a) The association of aplastic germinal epithelium, azoospermia, A and T lower than DHT after 7 yrs of DBCP exposure, b) The association of hypoplastic germinal epithelium, oligospermia, decrease in A and T, no change in DHT after 4 yrs of DBCP exposure, and finally, c) Neither hormonal change nor histological damage at 1 yr exposure of DBCP.
ESTRADIOL-INDUCED INHIBITION OF TESTOSTERONE PRODUCTION BY TESTICULAR INTERSTITIAL CELLS INCUBATED IN VITRO.

H.E. Grotjahn, Jr. and E. Steinberger. Department of Reproductive Medicine & Biology, University of Texas Medical School, Houston, Tx.

It has been suggested that estradiol (E2) inhibits testosterone (T) production (Tp) by a direct action on the testis. Demonstration of reduced Tp by isolated testicular interstitial cells (ICs) in the presence of E2 concentrations found in vivo would support such a conclusion. ICs from adult male rats were incubated in vitro with various concentrations of E2 (10^{-8} to 10^{-6}M).

These concentrations were chosen because E2 benzoate (EB) treatment (50 µg/d) yields testicular E2 concentrations of approximately 10^{-6}M. ICs were preincubated for 0, 2, 4 or 6h with E2. After preincubation, buffer with or without hCG (2nd IS, 10 ng/ml final concentration) was added and the incubation continued an additional 3h. T in the media was measured by double antibody radioimmunoassay. Under these conditions, basal Tp increased with time but was not affected by E2. E2 slightly inhibited (19%) hCG-induced Tp but only at 10^{-6}M and only when hCG and E2 were added at the beginning of the incubation. E2 did not alter hCG-induced Tp when E2 was preincubated with ICs for 2-6h. EB or benzoate (10^{-8} to 10^{-6}M) did not alter basal or hCG-induced Tp when used in the same experimental design. E2 inhibition of Tp was further characterized by examining complete hCG dose response curves after 0 or 4h preincubation; without preincubation 10^{-6}M E2 decreased the maximal T response but did not shift the curve. With a 4h preincubation, 10^{-6}M E did not affect the hCG dose response curve. These results demonstrate that E2 can inhibit Tp by ICs but only at concentrations ~100x those found after chronic EB injections. Thus, an acute action on ICs by E2 after EB injection in vivo is unlikely. Supported by NIH 5 P50 HD 08338 and Institutional Funds from The University of Texas Medical School at Houston.
EFFECT OF ESTROGENS AND OF PROLACTIN ON TESTOSTERONE METABOLISM IN THE BRAIN AND IN THE ANTERIOR PITUITARY. L. Martini, F. Celotti, and R. Massa. Department of Endocrinology, University of Milano, Italy.

In the brain and in the anterior pituitary (AP) testosterone (T) is transformed into 5α-androstan-17β-ol-3-one (dihydrotestosterone, DHT) and into 5α-androstan-3α, 17β-diol (3α-diol) by a 5α-reductase-3α-hydroxysteroid dehydrogenase system. Experiments have been performed in order to study whether estrogen and homologous prolactin might modify the activity of these enzymes in the hypothalamus and in the AP of normal or castrated male and female rats. Castration increases the 5α-reductase activity of the anterior pituitary but not that of the hypothalamus. Estradiol benzoate (EB), in a dose of 50 ng/rat/day (for either 7 or 14 days) does not change the enzymatic activities of the AP of castrated male and female animals and of the hypothalamus of castrated male rats. On the contrary, this dose of EB significantly enhances the conversion of T in the hypothalamus of castrated female rats. Higher doses of EB (5 µg/rat/day) proved able to bring back to precastration levels the 5α-reductase activity of the AP (7-day administration) and to decrease it below normal controls (14-day) in both sexes. The same dose was ineffective on the enzymatic activities of the hypothalamus of castrated females but decreased their activities in males.

A 5-day treatment with 800 µg/rat of rat prolactin did not modify the conversion of T in the AP and in the hypothalamus of normal male rats and in the AP of castrated males, but significantly inhibited such a conversion in the hypothalamus of castrated males.

The data suggest that EB and prolactin may modulate the effects of T on gonadotropin secretion by modifying the conversion of T into DHT and 3α-diol at AP and hypothalamic level.

Two-day-old cultures of Sertoli cells from testes of 18-day-old Long Evans rats were incubated with: 1) 0.25 µCi (4-¹⁴C)-testosterone (57 mCi/mmol); 2) 0.25 µCi (4-¹⁴C)-testosterone plus 5 µg/ml FSH (NIH-FSH-S11); 3) 1.25 µCi (¹α,2α-³H)-testosterone (1 mCi/4.9 µg); 4) 1.25 µCi (¹α,2α-³H)-testosterone (1 mCi/4.9 µg) plus 5 µg/ml FSH (NIH-FSH-S11). The cultures were incubated in 2 ml Eagle's minimum essential medium at 4°C for 6h.

The following steroids were isolated and identified by crystallization to constant specific activity from control and FSH-treated cultures: Testosterone (unconverted substrate); androstenedione; dihydrotestosterone; 5α-androstane-3α-ol-17-one; and 5α-androstane-3α-17β-diol.

The radioactivity tentatively identified as estradiol-17β after sequential chromatography systems did not crystallize to constant specific activity while the radiolabeled estradiol-17β used as recovery tracers did. The production of estradiol-17β from testosterone (5 x 10⁻⁷M) was investigated by radioimmunoassay. Radioimmunoassay results indicate that an estradiol-like compound reacted with the estradiol-17β antiserum and that FSH stimulated this by 333% by 96h.

The data demonstrate that: 1) cultured Sertoli cells from immature (18d) rats contain 5α-reductase; 3α- and 17β-hydroxysteroid dehydrogenase activities; 2) these enzymes are stimulated by FSH; and 3) estradiol-like steroids produced by the Sertoli cell cultures are not estradiol-17β.
EFFECTS OF HCG-TREATMENT ON STEROIDOGENESIS IN THE STALLION TESTIS. V.K. Ganjam and R. P. Amann.
Section of Clinical Reproduction, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA and Animal Reproduction Laboratory, Colorado State University, Fort Collins, CO.

Production of testosterone but not estrogens is markedly elevated 12 days after hemicastration of stallions. To determine if estrogen production by the testes of intact stallions was independent of testosterone production, stallions (8/group) received 1500 IU of HCG 1 or 2 hr before castration or a control injection of NaCl. Jugular blood was taken hourly before and after HCG injection and testicular vein blood was taken at castration. The total 17β-OH androgen content of jugular blood was elevated 1 or 2 hr after HCG (3.3 ± 0.5 vs 1.5 ± 0.2 ng/ml), but concentrations of total estrogens remained unaltered (36.8 ± 4.1 vs 31.6 ± 3.5 ng/ml). The concentration of total 17β-OH androgens (70% testosterone and 9% dihydrotestosterone) in testicular venous blood was markedly elevated 1 hr after HCG injection (0.040 ± 0.160 vs 120 ± 31 ng/ml) while concentrations of total estrogens tended to be lower after 1 or 2 hr in the HCG-treated stallions than in control stallions (15 ± 2 vs 21 ± 4 ng/ml). The 2-hr interval should be adequate for transfer of steroid to and from Sertoli cells and aromatization of androgens to estrogens. Thus, concentration of total estrogens in testicular venous blood was independent of testosterone production rate or peripheral concentration of either total 17β-OH androgens or total estrogens. Possibly the aromatise system regularly functions at maximum capacity. Alternatively, the availability of substrate androstenedione or testosterone to the aromatizing enzymes in the Sertoli cells does not fluctuate markedly despite pulsatile or exogenously induced discharge of testosterone from Leydig cells. The authors presently are at Auburn University and The Pennsylvania State University.
EFFECTS OF BILATERAL CRYPTORCHIDISM ON TESTOSTERONE SECRETION IN BULLS. B.D. Schanbacher. Agricultural Research, Science and Education Administration, USDA, Clay Center, NE.

Surgically induced cryptorchidism in bulls results in azospermia which is accompanied by elevated serum concentrations of FSH and LH but near-normal circulating concentrations of testosterone (T). Because cryptorchid bull testes appear relatively insensitive to elevated serum gonadotropins, the in vivo and in vitro T secretory capacity of both cryptorchid and scrotal testes was determined. The ability to secrete T in vivo was tested by releasing endogenous gonadotropins with gonadotropin releasing hormone (GnRH) and by administering 2 mg of exogenous gonadotropin (NIH-LH-S18). LH and FSH were released by 100 µg of GnRH in both intact and cryptorchid bulls. Subsequently, serum T in intact bulls increased from 2 ng/ml to peak concentrations of 15 ng/ml. Serum T in cryptorchid bulls, on the other hand, was not increased (P>0.10) above baseline concentrations of 4 ng/ml. Similarly, purified LH caused T concentrations to triple in intact bulls and yet concentrations of T in cryptorchid bulls remained unchanged (P>0.10).

Testosterone secretion was determined in vitro from testes collected at the time of slaughter. Testes from cryptorchid bulls weighed significantly less than testes from intact bulls (68±6 vs 65±36 gm). The ability to secrete T was determined by incubating minced pieces of testes at 36 C in Krebs-Ringer bicarbonate buffer (pH 7.2) containing 1 mg/ml of glucose and 100 mIU/ml of hCG. Cryptorchid testes secreted slightly more T than scrotal testes on a unit weight basis; however, when expressed on a paired testes weight basis, T secretion by cryptorchid testes was minor (8±2 vs 60±10 µg). Although hCG binding sites within the testes of intact and cryptorchid bulls have not been quantitated, autoradiographic localization techniques which utilized radioiodinated hCG verified their existence. These studies demonstrate that cryptorchid bull testes have a reduced capacity to secrete T and that normal circulating levels of T can be maintained only in the presence of increased serum gonadotropins.
ACTION OF GONADAL STEROIDS IN AFFECTING LIPOGENESIS IN CULTURED LIVER CELLS. B. R. Tulloch and S. Lee. Endocrinology, University of Texas Medical School, Houston, TX.

A lower cardiovascular mortality is apparent in females during the premenopausal years, suggesting that female hormones may be in some way protective. Yet, by comparison, women taking synthetic estrogen-containing oral contraceptives show a five-fold increment in cardiovascular risk\(^1\) together with raised hepatic production of fibrinogen and very low density lipoproteins (VLDL). To study the longer-term effect of natural and synthetic gonadal steroids on hepatic metabolism we have established a long-term culture of epithelial cells from rat liver. The cells retain the hepatocyte-specific antigen and synthesize and secrete albumin, fibrinogen, and lipoproteins into the medium. In cells grown in the presence of charcoal-extracted fetal calf serum, 24 hrs prior exposure to beta-estradiol (10\(^{-8}\)M) or ethinyl estradiol (10\(^{-11}\)M) augments both \(^{14}\)C acetate and \(^{3}\)H\(_2\)O incorporation into hepatocyte lipids. Under similar conditions of cell culture, progesterone (10\(^{-11}\) to 10\(^{-9}\)M) and nor-ethisterone (10\(^{-11}\) to 10\(^{-8}\)M) are without consistent effect, while the androgens oxandrolone (10\(^{-11}\) to 10\(^{-7}\)M), testosterone acetate (10\(^{-11}\) to 10\(^{-7}\)M), methandienone (10\(^{-11}\) to 10\(^{-7}\)M) and oxymethalone (10\(^{-11}\) to 10\(^{-7}\)M) decrease fatty acid and sterol labeling from \(^{14}\)C acetate.

These studies demonstrate that a significant component of the in vivo action of gonadal steroids on lipoprotein metabolism may be secondary to alterations in hepatic metabolism. These are in part reproducible under cell culture conditions.

DEVELOPMENT OF TESTICULAR 3α-HYDROXYSTEROID DEHYDROGENASE (3α-HSD) ACTIVITY IN THE MALE RAT.

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In the testes, 17β-hydroxy-5α-androstan-3-one (dihydrotestosterone, DHT) is converted to 5α-androstan-3α,17β-diol (3α-diol) by the enzyme 3α-HSD. This steroid possesses biological activity in the male rat and plasma and testicular concentrations are greater in the prepubertal than the adult rat. We designed this study to localize, characterize, and quantitate the activity of 3α-HSD throughout development to gain insight into its possible significance in terms of the onset of puberty in the male rat.

Subcellular components of whole testis homogenates, fractionated by differential centrifugation, were incubated in the presence of 3H-DHT and either NADPH or NADH, at pH 7.4 and 34°C for varying lengths of time. Activity of testicular 3α-HSD was highest in the cytosol fraction. The preferred cofactor for optimum 3α-HSD activity was NADPH. A single Km value of 1.25 µM was determined for 3α-HSD in the cytosol fraction. Specific activity of 3α-HSD, expressed as pmol of 3α-diol converted from DHT per minute per mg cytosol protein, was high in young rats from 10 to 22 days of age, and was followed by a decline between day 22 and 37, with activity remaining low through adulthood. Total testicular activity of 3α-HSD, expressed as nmol of 3α-diol converted from DHT per minute per pair of testes, gradually increased from day 10 to day 60 and remained high in the adult. In the post-pubertal period, the lack of available substrate, DHT, or possible endogenous testicular regulatory mechanisms acting on 3α-HSD activity might account for the actual decrease in 3α-diol concentration in the blood and testes of mature rats.
CHANGES IN TESTICULAR VENOUS BLOOD TESTOSTERONE CONCENTRATION WITH TIME IN ANESTHETIZED RATS. M.J.Free, R.A. Jaffe, Battelle, Pacific Northwest Laboratories, Richland, WA, and Hsien-Chen Cheng, Endocrine Research Laboratory, University of Kansas Medical Center, Kansas City, KA.

Mature albino rats (340-480g) were anesthetized with either pentobarbital (6mg/100g), urethane (100mg/100g), halothane/N₂O/O₂ or ethrane/N₂O/O₂. Catheters were placed in the left femoral artery and the left subcapsular testicular vein exposed through a small scrotal incision. The scrotal incision was repaired and blood samples collected at 15 min intervals over 3-4 hr while body temperature was maintained at 37.5°C. Plasma was assayed for testosterone by radioimmunoassay. Mean testicular vein testosterone did not differ significantly between pentobarbital, ethrane and halothane anesthetized animals but declined significantly with time. Combined means (±SE ng/ml) for these three anesthetics were as follows: 0.25-1 hr, 112±16; 1-2 hr, 72±18; 2-3 hr, 45±7. Most animals exhibited a steady declining pattern of testosterone concentration although in a few cases some cyclic variations were superimposed upon the downward trend. In the case of urethane anesthetized animals, large cyclic variations were observed in the first 1.5 hr post-induction resulting in a mean level of 224±29 SE ng/ml for the first hour. These cyclic variations damped out subsequently to yield mean levels of 114±32 SE ng/ml for the second hour and 67±5 SE ng/ml for the third hour. In 6 rats of similar age (body wt 350-380g) and background, testicular blood flow was measured by the reference flow method using Cr-51 labeled microspheres (15µ) injected through a left ventricular catheter. Mean testicular flow measured at 20-60 min post-induction with ethrane/N₂O/O₂ was 19.2±0.7 SE ml/min/100g. Based on 15-60 min testicular vein plasma testosterone levels for ethrane anesthetized animals (110±28 SE ng/ml), average testis weight (1.6±0.02 SE g) and testis vein blood hematocrit (52±2 SE%), the mean secretion rate of testosterone in undisturbed conscious rats under our conditions probably exceeds 10 ng/min/g testis.

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THE RELATIONSHIP OF AVERAGE TESTOSTERONE (T) LEVELS AND THE PATTERN OF T IN PLASMA TO MATING BEHAVIOR IN MICE. S. Dalterio and A. Bartke. Department of Obstetrics and Gynecology, University of Texas Health Science Center at San Antonio, San Antonio, Texas.

Testosterone (T) is considered to exert a permissive effect on sexual responses in the adult male, with minimal amounts required for the expression of these behaviors and additional T failing to enhance activity. In these studies adult male mice were castrated and maintained on T replacement therapy, using Silastic implants or injection, to provide a range of plasma T levels. In castrate males in which plasma T levels were maintained at about 25 ng/ml for prolonged periods, the level of copulatory activity, as assessed by mount and intromission latencies and number of mounts and intromissions during a one hour test session, was significantly enhanced (p > .05) as compared to intact animals, as well as to males maintained at lower T levels. In those animals receiving one short-lasting spike of T/day, there was a complete absence of copulatory activity (p > .01) at a time in which T levels were high and declining toward the lower end of the normal range. This finding may suggest a possible explanation for our previous observations that a moderate drug-induced reduction in plasma T levels can be associated with a suppression of copulatory behavior in male mice (Dalterio et al, Pharm. Bioch. Behav. 8:673, 1978). Male mice normally exhibit an episodic or pulsatile pattern of T release with increased testicular T production reflected almost immediately by increased peripheral T concentrations (Bartke & Dalterio Steroids 26:749, 1975). However, the naturally occurring female-induced spike of T observed in male mice are of unknown function (Macrides et al, Science 189:1104, 1975). These spikes may have a priming effect since in an additional group of males receiving one spike/day, an additional spike resulted in normal levels of copulatory behavior even though animals given one spike/day did not mate. These results suggest that the presence of sustained or multiple peak levels of plasma T are facilitative, and even essential for the expression of normal levels of copulatory activity in adult male mice.
CHANGES OF TESTICULAR STEROIDOGENESIS INDUCED BY TREATMENT WITH [D-ALA^6, DES-GLY-NH_2^10]LHRH ETHYLAMIDE. A. Belanger, C. Auclair, P.A. Kelly and F. Labrie, Laboratory of Molecular Endocrinology, Le Centre Hospitalier de l'Université Laval, Quebec, Canada.

We have recently found that treatment of adult male rats with LHRH or its agonistic analogues leads to a marked loss of testicular LH and prolactin receptors accompanied by decreased testis and secondary sex organ weight as well as circulating androgen levels. It was then of interest to study the site(s) of alteration of steroidogenesis induced by treatment with LHRH agonists through measurement of testicular and plasma levels of the main steroid intermediates. For this purpose, adult male rats were injected daily for 1, 2, 4 or 8 days with 1 µg of [D-Ala^6, des-Gly-NH_2^10]LHRH ethylamide and killed 24 h after the last injection. LH receptor levels were 90% decreased after 4 days of treatment while maximal inhibition (70%) of prolactin receptor levels occurred 24 h after the last injection. While there was little change of the levels of testicular pregnenolone (7.8±1.7 ng/g) and progesterone (P, 8.8±1.8 ng/g), there was a marked reduction of 17α-OH-P (from 3.1±0.7 to 0.9±0.1) 4 days after beginning treatment, thus leading to a 80% decrease of the ratio of 17α-OH-P and P. A progressive inhibition of testicular androstenedione (Δ^4) and testosterone (T) levels was found, a maximal effect (from 4.2±0.1 to 0.24±0.03 and 116±29 to 9.0±1.0 ng/g for Δ^4 and T, respectively) being seen at 4 days. The testicular levels of 5α-dihydrotestosterone (DHT) decreased more rapidly, a maximal effect being found 24 h after the first injection (1.4±0.3 vs 5.0±0.7 ng/g). The concentrations of estradiol and estrone (14±2.0 and 7.9±0.6 pg/g, respectively) were not affected during the period of treatment. Changes of plasma steroid levels were parallel to those measured in the testis. The present data show that changes of endogenous LH release induced by treatment with an LHRH agonist lead to a marked reduction of T, Δ^4 and DHT biosynthesis secondary to blockage of the activity of 17α-hydroxylase and 17-20 desmolase activities.
CLINICAL TRIAL ON REVERSIBLE MALE CONTRACEPTIVE WITH LONG-ACTING SEX HORMONES. H. Y. Lee and S. I. Kim, Departments of Urology and Laboratory Medicine, Seoul National University Hospital, Seoul 110, KOREA

In order to develop a reversible male contraceptive method by monthly use of combined medroxyprogesterone acetate (Depo-Provera, D-P) with testosterone cypionate (Depo-Testosterone, D-T), 30 fertile healthy volunteers were selected and divided into group I, 10 cases, receiving D-P 200mg plus D-T 200mg, group II, 10 cases, D-P 400mg plus D-T 200mg, and group III, 10 cases, D-P 200mg plus D-T 400mg for 15 months (control phase, 3 months; drug phase, 6 months; and recovery phase, 6 months). Parameters investigated in this trial are semen analyses with fructose, hormonal assays (serum FSH, LH and testosterone), blood biochemical and hematological analyses, urinalyses, clinical evaluations of general health and sexual activity, and psychiatric interviews (MMPI).

The results from the trial revealed that azoospermia or severe oligospermia induced in all but 3 subjects who failed to respond the compounds 5 months (3-6 months) after drug administration. Full recovery to control sperm counts occurred 6 months (3-10 months) after drug discontinuation. No significant adverse side effects except transient loss of libido in some men who received D-P 400mg with D-T 200mg throughout the trial period. The results also suggest that lower dose of D-P 200mg with D-T 200mg was proved to be an adequate combination for the suppression of spermatogenesis and for the avoidance of impotence. The control phase might be cut from 3 months to 2 months but the recovery phase should be extended from 6 months to 10 months because of delay in full recovery of sperm production. In view of these findings, the combined administration of long-acting progestin and androgen is proposed as a male fertility regulation method when these compounds produce azoospermia in virtually all subjects who are treated.

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FSH LEVELS IN ADULT MALE RATS FOLLOWING PASSIVE IMMUNIZATION WITH LH ANTISERUM. M. Dym, Y.C. Lin, and *H.G. Madhwa Raj. Department of Anatomy, Harvard Medical School, Boston, MA. *Department of Obstetrics and Gynecology, University of North Carolina, Chapel Hill, NC.

To examine the feedback control mechanism for FSH production, antiserum to ovine LH (LH A/S), produced in rabbits, or normal rabbit serum (NRS) was injected into groups of adult male rats, and serum FSH, LH and testosterone (T) levels were measured by radioimmunoassay (RIA). The first group was bled 6 hours after a single injection of either 1 ml of NRS or LH A/S; blood was collected from the second group after 3 daily injections (i.e., 72 hours after the start of treatment). In a third experiment, a single injection of either 1 ml of NRS, 0.25 ml LH A/S, 0.5 ml LH A/S, or 1.0 ml LH A/S was given to a group of rats; all these rats were bled 6 hours later. In order to carry out RIA for gonadotropins, the excess circulating LH antibody was removed from the serum by incubation with a two-fold excess, by volume, of sheep antirabbit gamma globulin (SARGG) at 4°C for 2 days. Saturated ammonium sulfate (pH 7.8, 36%) was used to precipitate the excess SARGG. After centrifugation, the supernatant was dialyzed against the gonadotropin RIA buffer at 4°C for 2 days. To correct for loss of free gonadotropin during these procedures, iodinated LH and FSH were used as tracers in parallel serum samples. The average recovery of the labeled LH and FSH was 75% and 63%, respectively, and the values obtained from RIA were corrected accordingly. Serum LH and testosterone levels were lowered to about 10% of controls in the groups treated with 1 ml or 0.5 ml LH A/S. However, the FSH levels in all treated groups (6 hours: 710±170 ng/ml; 72 hours: 730±240 ng/ml) were not different from the controls (800±110 ng/ml). These results are in accord with a recent report by Raj et al (1978) where polyethylene glycol was used to separate antibody bound hormone from free hormone. The unchanged FSH levels in the LH and T suppressed rats imply that a factor, other than testosterone, may be involved in the regulation of FSH secretion. (Supported by NICHD Grant No. HD 10004).
EFFECTS OF GROWTH HORMONE AND THYROXIN IN TWO STRAINS OF DWARF MICE: DIFFERENCES FROM PROLACTIN. P.C. Doherty, S. Dalterio, and A. Bartke. Departments of Anatomy and Obstetrics and Gynecology, The University of Texas Health Science Center, San Antonio, Texas.

Treatment of male dwarf mice with prolactin (PRL) leads to the induction of fertility and general body growth. To assess if these effects of PRL could be the result of its somatotropic activity, we have examined the effects of growth hormone and thyroxin on the pituitary-testicular axis in these animals. The results of these experiments show that: 1.) daily injection of male dwarf (dw) mice with 1 µg D,L-thyroxin-Na (T₄), or 38 or 150 µg ovine growth hormone (GH) for 28 days leads to significant increases in body weight, testes and seminal vesicle weights, and increases spermatogenesis as determined by counts of different cell types in histological cross sections of the testes; 2.) treatment of male dwarf (dw) mice with T₄ 3x weekly for six weeks (4 µg/injection weeks 1-3, 6 µg/injection weeks 4-6) results in significant increases in somatic growth and growth of the testes and seminal vesicles but no significant effects on the levels of plasma LH, FSH, or testosterone (T); 3.) daily injection of male dwarf mice of the dwarf (dw) and Ames dwarf (df) strains with 1 µg T₄ or 125 µg GH for 14 days significantly increases body weight and testes and seminal vesicle weights. In addition, plasma levels of LH, FSH and T are increased in GH treated Ames dwarf mice. Neither treatment affects the ability of the testes to produce T in vitro under stimulation with hCG. These results indicate that the previously reported ability of PRL to increase spermatogenesis, induce fertility, and increase the sensitivity of the testes of the dwarf mouse to LH (Bartke et al., Endocrinology 101:1760, 1977) may be a specific action of PRL on the pituitary-testicular axis and not simply a result of its inherent somatotropic activity.
Adult male rats were exposed to Mh smoke delivered by a Walton smoking machine. Five groups were studied: laboratory control (I), machine control (II), 16 puff placebo exposure (III), 4 puff Mh exposure (0.4 mg/kg Δ⁹-THC) (IV) and 16 puff Mh exposure (3 mg/kg Δ⁹-THC) (V). A significant decrease in epididymal sperm count (ESC) (x10⁶) was noted after 30 daily exposures to 16 puffs of both placebo smoke (36 ± 2.3) and Mh smoke (29 ± 2.8), as compared to 4 puff Mh (62 ± 3.7) and controls (I - 49 ± 6.3 and II - 63 ± 7.9). After 75 daily exposures, ESC remained abnormal only in the 16 puff Mh smoking rats (V - 50 ± 3.7 vs. I - 69 ± 6.9; II - 73 ± 8.2; III - 64 ± 1.9 and IV - 62 ± 4.7). In spite of a significant reduction in ESC, ³H-thymidine incorporation into testicular DNA did not change significantly, suggesting that spermatogonial (Sg) proliferation was not affected by Mh. However, when Vitamin A deficient (VAD) rats were exposed to Mh smoke after Vitamin A (PVA) re-initiation of spermatogenesis, DNA synthesis and early Sg divisions were markedly reduced. Neither LH nor testosterone levels were altered by Mh smoking. However, a significant elevation of FSH was observed after 30 days of 16 puff Mh (V - 646 ± 40 vs. I,II,III - 543 ± 29 ng/ml) and after 75 exposures of both 4 puff (IV - 644 ± 38 ng/ml) and 16 puff Mh exposure (V - 631 ± 52 vs. I,II,III - 534 ± 16 ng/ml); similarly, the FSH elevation normally observed PVA in VAD rats (796 ± 20 ng/ml) was significantly greater in Mh smoked rats (1256 ± 103 mg/ml).

Conclusions: 1) High dose Mh smoking caused a sustained reduction in ESC after 30 and 75 days; 2) Mh smoking did not reduce germ cell DNA synthesis in normal rats, although in VAD rats, the Sg response PVA was inhibited; 3) ESC reduction was not hormonally mediated; and 4) the monotropic FSH elevation associated with Mh exposure suggests a primary effect upon Sertoli cell function.

Short-term administration of THC to rats has been reported to increase PRL secretion (Daley et al, J Endocrinol 63:415, 1974); however, a decrease in PRL was noted after acute injections (Kramer and Ben David, Proc Soc Exp Biol Med 147:482, 1974). Furthermore, in humans no effect on the PRL secretion was observed (Lemberger et al, Life Sciences 16:1339, 1975).

The present experiments were designed to study the immediate effects of a single dose of THC on PRL levels in both male and female rhesus monkeys.

Sexually mature males and ovariectomized females received a single IM injection of THC (2.5 mg/kg, 3% Tween in saline). Blood was drawn before and at +30, +60, +90, +120 and +180 minutes after injection of THC.

Following THC administration there was a prompt and significant decrease in serum PRL levels within 60 to 90 minutes in both male and female monkeys. The maximum decrease was an average of 85% in the females and 74% in the males. The time interval at which the maximal decrease occurred and the duration of the response varied among animals regardless of sex.

This acute decrease in PRL levels is quite interesting since we have shown that THC also produces a decrease in FSH and LH levels by 60 minutes after its injection. The decrease in FSH and LH levels lasted for 18 to 24 hours, while PRL generally returned to control levels by 3 hours after drug administration.

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Excessive daily urinary secretion of serotonin and 5-HIAA is found in infertile men with oligospermia, azoospermia and impaired sperm motility. The detrimental effect of serotonin can be prevented, in animals, by a serotonin antagonist. A group of 154 patients who attended our male infertility clinic, had clinical and laboratory examinations with measurement of urinary serotonin and 5-HIAA. All patients were treated with an antagonist of serotonin-cyproheptadine (Periactin®, Oractine®), 4 t.i.d. for 2 courses of 3 months each with 2 week intervals. The only side effects were weight gain and drowsiness. Excessive serotonin levels were detected in 37 patients, the others having only mildly elevated to normal levels. The results of treatment of patients with high serotonin levels showed a significant improvement of sperm concentration and motility of 59% of patients compared to only 18% of the others. Normal sperm count was achieved by 35% of patients with high serotonin and 30% of their wives conceived. Among the rest of the patients, only 17% reached normospermia with 4% pregnancy rates. When the "high serotonin group" was divided according to sperm concentration and motility, best results were achieved by moderate oligospermic patients and by men with impaired sperm motility.

The presence of a varicocele has been shown to be related to certain abnormalities in the semen. Various theories have been proposed to explain the detrimental actions of a varicocele; a popular concept suggests the retrograde flow of a toxic substance, such as serotonin, into the testicular milieu. In a series of dog studies, experimental varicoceles were created by partial ligation of the left spermatic vein between the inferior vena cava and testicular vein. This resulted in a significant decrease in sperm count, motility score, and an increased percentage of immature forms. Spermatic vein serotonin levels were determined, the mean spermatic vein serotonin level was significantly higher than that from peripheral blood (924 ng/ml vs. 620 ng/ml). Based on this finding, we obtained simultaneous spermatic vein and peripheral blood samples for serotonin levels in 28 infertility patients undergoing high spermatic vein ligation for varicoceles causing stress pattern on semen analysis. Blood was taken from the inferior direction after proximal spermatic vein ligation was performed. Sixteen patients demonstrated serotonin values higher peripherally than that in the spermatic vein (Group I) while 12 patients demonstrated serotonin values higher in the spermatic vein than the peripheral blood (Group II). The mean preoperative semen parameters for each group were determined on at least 3 specimens for each patient. There were no significant differences between the two groups in any of the semen parameters. Peripheral serotonin levels of five normal males were found to be statistically similar to groups I and II. Our results indicate that although there are patients with varicoceles who have spermatic vein serotonin values greater than peripheral levels, this probably does not play an important role in the deleterious effect of a varicocele on semen quality.
ALTERATIONS IN THE FEEDBACK REGULATION OF GONADOTROPIN SECRETION BY NONAROMATIZABLE ANDROGEN IN MEN WITH PRIMARY HYPOGONADISM. Stephen J. Winters, Richard J. Sherins and D. Lynn Loriaux, Montefiore Hospital, University of Pittsburgh and ERRB, NICHD, Bethesda, MD.

The feedback regulation of gonadotropin secretion by sex steroids was studied in men with primary gonadal failure. Using a four day continuous infusion we have evaluated the effects of testosterone (15 mg/day), estradiol (90 µg/day) and 5α dihydrotestosterone (7.5 mg/day) on mean serum gonadotropin concentrations, the pattern of pulsatile release of LH and the response of LH and FSH to LHRH administration.

Testosterone infusion resulted in a fall in serum LH of 36±5.5% (M±SE) and a 30±3.0% decrease in serum FSH levels. These responses were similar to those of normal men (LH, 45±4.8%; FSH, 37±5.1%). The relative suppressive potency of estradiol was also similar in the hypogonadal (LH, 29±10%; FSH, 43±6.6%) and the normal subjects (LH, 22±4.4%; FSH 34±3.8%). By contrast, the LH suppressive potency of DHT in hypogonadal men (11±2.7%) was significantly less than that observed in normal men (35±10%) (p< 0.05). DHT failed to suppress FSH levels in both normal (16±14%) and hypogonadal men (10±4.0%) (NS). Neither T, E nor DHT infusion produced a decrease in spontaneous LH pulse frequency or pulse amplitude in hypogonadal men. DHT infusion augmented the amplitude of the LHRH induced LH pulse in normal men (83±10 vs 50±6.9 mIU/ml, p< 0.05) but failed to influence the LHRH induced LH pulse in hypogonadal men (201±32 vs 182±32 mIU/ml, NS), each patient as his own control.

The data indicate that the modulation of LH by sex steroids in men with primary hypogonadism differs from normal in that there is a resistance to the effects of pure androgen. The mechanism for this resistance remains to be determined.
TESTICULAR RESPONSE TO EXOGENOUS GONADOTROPINS FOLLOWING PROLONGED ANDROGEN THERAPY IN HYPOGONADOTROPIC MEN.

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The effectiveness of gonadotropin therapy in the induction and restoration of testicular function in patients with gonadotropin deficiency has been established. However, whether prolonged androgen replacement treatment influences the subsequent testicular response to human chorionic gonadotropin (hCG) and human menopausal gonadotropin (hMG) is unknown. We examined the response to hCG and hMG in 6 patients (ages 23-42 years) with gonadotropin deficiency who had received androgen therapy for 3.5-8 years (x=6.5). Four to 8 weeks after discontinuing exogenous androgens, 3 serum samples were obtained for measurement of FSH, LH, and testosterone (T) and a minimum of 6 seminal fluid specimens examined. Basal serum FSH and LH levels were undetectable in 5 and below our normal adult male range (N) in 1; serum T ranged from 10-70 ng/dl (vs 280-1440 ng/dl for N). All were azoospermic. HCG 1500-2000 I.U. TIW was administered for 6 months followed by 8 months of hMG 150 I.U. TIW and hCG at the same dosage. During hCG serum T rose to 750-1490 ng/dl (x=1170 ng/dl) and testicular volume (V) increased progressively (x=4.3 ml pre to 5.5 ml post). With the addition of hMG serum T levels remained unchanged, but a continued increase in V was observed (x=5.5 ml pre to 7.4 ml post). Sperm were identified in the ejaculate within 5 months of beginning hCG in 1 patient in whom basal serum FSH and LH levels were undetectable and in the other 5 patients at 1-8 months during combined therapy with hCG and hMG. Conclusions: (1) Testicular response to hCG does not appear to be affected by prior androgen replacement therapy. (2) Seminiferous tubular function as assessed by testicular volume and semen analysis remains responsive to hCG and hMG. (3) Although the duration of hCG and hMG treatment was relatively brief, these data suggest that the initiation of spermatogenesis in patients with gonadotropin deficiency is not compromised by prolonged androgen replacement therapy.
POSSIBLE RELATION BETWEEN FSH LEVELS AND LEYDIG CELL FUNCTION IN AZOOSPERMIC AND OLIGOSPERMIC MEN. L.J. Rodriguez-Rigau, K.D. Smith and E. Steinberger. Dept. of Reproductive Medicine & Biology, Univ. of Texas Medical School at Houston, Texas.

Of 59 males with mean total sperm count (TSC) below 25 million/ejaculate, 17 were excluded: 4 with Klinefelter's syndrome, 2 with hypogonadotropic hypogonadism and 1 with bilateral testicular atrophy secondary to torsion, orchitis or radiation and 8 with bilateral epididymal obstruction. Plasma testosterone (T), FSH and LH levels in the remaining 42 men (12 with azoospermia and 30 with oligospermia) were compared to those of a control group of 27 men with TSC above 25 million/ejaculate. All levels were within normal range in the control subjects. Although T levels were within normal range in all patients, mean levels in the azoospermic group were significantly lower than in either the oligospermic or control groups. In the 12 azoospermic patients, FSH levels were elevated in all and LH in 8. The T/LH ratio in azoospermic men was significantly lower than in oligospermic or control subjects. In the 30 oligospermic men, FSH levels were elevated in 14 and LH in 5. A statistically significant negative correlation was demonstrated between FSH levels and TSC. Nevertheless, the mean TSC of the 14 men with elevated FSH was not significantly different from the mean TSC of those with normal FSH. The mean T/LH ratio was significantly lower in the oligospermic group than in controls, and the T/LH ratio was significantly correlated with FSH levels.

In summary: 1) Normal FSH levels, in association with normal LH and T levels, were found in a significant number of oligospermic men. 2) Leydig cell dysfunction was suggested in a significant number of patients by high LH levels or low T/LH ratio. 3) FSH levels were highest in men with lowest T/LH ratio. These results suggest that FSH elevation in azoospermic or oligospermic men may not be caused by decreased spermatogenesis alone. Leydig cell dysfunction, resulting in Sertoli cell disturbance, could be responsible for elevated FSH levels in some patients.
EFFECT OF SUBCUTANEOUS IMPLANTS OF TESTOSTERONE AND/OR MELATONIN ON REPRODUCTION IN MALE HAMSTERS. L.J. Petterborg and R.J. Reiter. Department of Anatomy, University of Texas Health Science Center at San Antonio, San Antonio, TX.

The effects of subcutaneous implants of testosterone propionate on serum and anterior pituitary levels of LH, FSH, and prolactin in male hamsters were significantly altered by concurrent treatment with subcutaneous implants of melatonin. Testosterone implants were prepared by packing testosterone propionate into 100 mm Silastic capsules while melatonin implants were composed of 1 mg crystalline melatonin in 24 mg beeswax. After 7 weeks, blood samples and anterior pituitary glands were collected and body, testicular, and accessory organ weights recorded. Serum and pituitary levels of LH, FSH, and prolactin were determined using established radioimmunoassays. Four treatment groups were devised: 1) animals which received subcutaneous testosterone implants, 2) animals which received testosterone and melatonin, 3) animals which received melatonin, and 4) untreated animals. Mean accessory organ weight was higher in both groups which received testosterone implants. Serum LH was significantly depressed in all three groups receiving implants. Mean LH level for the testosterone only group was approximately 1/2 that of the 2 melatonin treated groups. Mean anterior pituitary concentrations of LH and FSH were significantly lower in the group which received testosterone when compared to those not receiving the steroid. But, in the group which received both melatonin and testosterone, mean LH values were 2X and mean FSH levels were 5X as high as in the group treated with testosterone only. Mean pituitary concentrations of prolactin were 3X as high in the testosterone group as in the melatonin and untreated groups. In hamsters treated with both melatonin and testosterone mean pituitary prolactin concentrations were approximately 2/3 that of animals given testosterone only. These data suggest that the pineal hormone, melatonin, may exert an effect on reproduction at the level of steroid feedback. (Supported by NSF grant #PCM 77-05734)
EFFECTS OF CHRONIC HYPERPROLACTINEMIA ON THE PITUITARY-TESTICULAR AXIS AND ON MATING BEHAVIOR IN THE GOLDEN HAMSTER. A. Bartke, P.C. Doherty, and B.D. Goldman. Department of Obstetrics and Gynecology, University of Texas Health Science Center at San Antonio, San Antonio, TX, and Department of Biobehavioral Sciences, University of Connecticut, Storrs, CT.

In the human male, hyperprolactinemia of various etiologies can be associated with impotence and hypogonadism. It has been reported from this and other laboratories that experimentally-induced chronic hyperprolactinemia in male rats and mice produces severe deficits in copulatory behavior and a reduction in plasma LH and FSH levels, while testicular function appears not affected. We have examined the effects of hyperprolactinemia in the golden hamster because prolactin (PRL) normally plays an important role in the regulation of testicular function in this species (Bartke et al., Int. J. Andrology Suppl. 2:345, 1978).

To induce hyperprolactinemia, four pituitary glands from female donors were transplanted under the kidney capsule of adult male hamsters. This procedure elevated plasma PRL levels to 400% of values observed in sham operated controls (P<0.01). However, plasma levels of LH and FSH were not affected (LH: 159 ± 40 vs 121 ± 16 ng/ml; FSH: 392 ± 36 vs 515 ± 48 ng/ml). Copulatory behavior, as assessed from mounting latency, intromission latency, ejaculation latency and number of mounts, intromissions and ejaculations did not differ in pituitary-grafted and sham-operated males. The weight of the testes and the seminal vesicles was significantly increased in grafted animals (testes 3417 ± 155 vs 4259 ± 202 mg, P<0.01; seminal vesicles: 550 ± 40 vs 786 ± 63 mg, P<0.01). In contrast to the situation described in rats and mice, chronic hyperprolactinemia in the golden hamster does not interfere with mating behavior or gonadotropin release and produces a significant elevation in testicular weight. (Supported by NICHD.)
CLOMIPHENE TREATMENT OF IDIOPATHIC MALE INFERTILITY

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Centre de Physiopathologie de la Reproduction Humaine
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33064 BORDEAUX-PESSAC

Various degrees of oligo-, nécro-, astheno- and/or terratospermia were considered as idiopathic in 133 males. There were no anatomical abnormalities such as testicular atrophy, infectious disease or varicocele. Plasma FSH, LH, Testosterone and Prolactin were determined prior to treatment. When performed, testicular biopsy showed various degrees of spermatogenetic arrest. The patients were given Clomiphene Citrate 25 or 50 mg daily for 100 days to four months. Plasma radioimmunoassay were repeated between day 15 and 20 of treatment. The mean of at least three spermograms at monthly intervals before treatment was compared with another spermogram within 20 days after completion of therapy.

Overall therapeutic results were poor, as 26 patients only improved their sperm quality. There was no amelioration in patients with azoospermia, nor in patients with elevated hormonal FSH levels. A study of correlations between hormonal and semen responses to clomiphene indicates that there was no improvement of sperm characteristics in the absence of a significant endocrine response, mainly testosterone, on the 15-20th day of treatment, nor in patients with exaggerated FSH response to clomiphene.

These hormonal data, in agreement with other reports, must help in the screening of hypofertile male patients for Clomiphene therapy, especially when long-term treatment is considered.
PROTECTIVE EFFECT OF FSH ON TESTICULAR DESENSITIZATION INDUCED BY LH IN INTACT IMMATURE RATS. S.I. Fox, J.R.D. Stalvey, and J.R. Wisner, Jr. Department of Physiology and Biophysics, University of Southern California School of Medicine, Los Angeles, CA.

Administration of LH to intact adult or immature rats results in a fall in testicular LH receptor numbers and a decrease in testosterone (T) production in response to a second administration of LH. Because FSH may be involved in regulation of testicular LH receptor numbers and the steroidogenic responsiveness of testes to LH, we have examined possible effects of FSH in modulating testicular desensitization induced in intact immature male rats treated with LH.

Immature (28 days old) male rats were injected subcutaneously once daily x 14 days with either saline, LH (NIH-LH-B10, 30 μg/day), FSH (NIH-FSH-S12, 30 μg/day), or FSH + LH (30 μg each/day) and killed 24 hrs. after the last injection. Ventral prostates (VP) and seminal vesicles (SV) were excised and weighed and decapsulated testes were incubated for 4 hrs. at 33C under a 95% O₂:5% CO₂ atmosphere in media containing either 20 ng of NIH-LH-B10 or 5 mM dibutyryl-cAMP. Incubation media T was estimated by radioimmunoassay.

In LH-injected animals, wet weights of VP and SV were significantly reduced to <60% of saline injected control animals and in vitro T production in response to either LH or dibutyryl-cAMP in incubation media was <40% of values obtained with testes from saline injected animals. In contrast to animals injected with LH alone, FSH or FSH + LH injections resulted in sex accessory weights and in vitro T production by testes incubated with LH or dibutyryl-cAMP which were statistically indistinguishable from saline treated control rats.

These results suggest FSH can exert a protective effect on LH-induced testicular desensitization. Additionally, data obtained on in vitro T production in response to dibutyryl-cAMP suggest this protective effect of FSH may lie beyond maintenance of testicular LH receptor numbers. (Supported by a Biomed. Research Support Grant to JRW from the U.S.C. Medical School).
TESTICULAR TRANSPLANTATION IN THE HUMAN: EFFECTS OF ISCHEMIA AND DENERVATION. S.J. Silber, St. Louis, Mo.

Two patients underwent successful microvascular transplantation of a testicle from identical twin donor. One of the recipients was azoospermic and anorchid prior to the transplant. The other recipient had bilateral testicular atrophy and sperm counts below 2,000,000 per cc with poor motility. The operative procedures were identical except that in the first patient, the testicular ischemia time was 90 minutes, and in the second patient, 32 minutes. Each recipient obviously had a totally denervated donor vas deferens until sympathetic nerve regeneration had time to occur. Comparing the time course of recovery of sperm count and hormone levels in these two patients, and their donors, we attempted to determine the relative effects of ischemia and denervation.
VALIDATION OF CONTACT SCROTAL THERMOGRAPHY FOR DIAGNOSIS OF VARICOCELE IN HUMANS AND MONKEYS. R. W. Lewis, T. S. Rusca and R. M. Harrison. Department of Urology, Delta Primate Center, Tulane University, Covington, LA.

Blind studies were conducted to compare scrotal temperatures determined by contact thermography with intratesticular temperatures determined by thermister probe readings. Male monkeys were anesthetized and tied to a surgical table. The table was inclined so that the monkeys were head-up at a 60° angle. Fifteen minutes were allowed for equilibration and then scrotal thermograms were made using the Clark Contact Thermography unit. Five minutes later the intratesticular temperatures were determined using a YSI Model 43 Tele-Thermometer and a 24 gauge needle probe. The thermography and thermister probe readings were recorded separately and then compared. In one series with monkeys before and after surgical induction of varicocele thermographic readings averaged 34.52°C and intratesticular probe readings averaged 34.48°C. Three monkeys with varicoceles of 1 to 2 years' duration were examined. Thermography found one with elevated left hemiscrotal temperature, one with elevated right temperature, and one with equivalent temperature. In all monkeys where a difference was found between left and right hemiscrotal areas using contact thermography the same direction of difference was found using thermister probes.

This study indicates that contact scrotal thermography is a reliable, nontraumatic means to diagnose thermal patterns in the scrotum of individuals with varicocele. The thermography procedure also provides a permanent record that can become part of the patient's clinical record.
MALE GENITAL MATERIAL AS POSSIBLE IMMUNE RESPONSE REGULATOR. Z.H. Marcus, J.H. Herman and E.V. Hess.
Department of Medicine, Division of Immunology, Division of Urology, Department OB/GYN, University of Cincinnati Medical Center, Cincinnati, Ohio 45267.

Several investigators found that male genital materials (MGM) from human and animals are causing suppression of lymphocyte activation. Concomitant with these observations a number of investigators claimed to demonstrate cell mediated immunity (CMI) to MGM in infertile female, male and vasectomized patients. It is the purpose of this presentation to clarify some of these contradictions.

Three in vitro methods were used for determining the effect of MGM on human cells: a) mitogen induced lymphocyte transformation (MIBT), b) E-rosette formation (E-RF) and c) histamine release test.

Human spermatozoa (HuSp), seminal plasma (HuSePl) and high molecular weight fractions prepared from extracts of HuSp or HuSePl were suppressive to MIBT, E-RF as well as platelet histamine release. The concentrations used in the above tests were between 5 ug/ml to 100 ug/ml protein. Tests done with lymphocytes obtained from 10 normal and 10 vasectomized donors did not reveal that the latter group is sensitive to MGM. The mechanism of MGM on CMI was investigated also through biochemical and electron microscopy studies. It was determined that CMI inhibition by HuSp results from interference with a terminal event in the response. MGM were shown to be suppressive to in vitro reactions and cannot be used in the studies of CMI in development of infertility.
COMPARISON OF SPERM MORPHOLOGY USING FRESH AND STAINED PREPARATIONS. R. M. Harrison, T. S. Rusca and R. W. Lewis. Department of Urology, Delta Primate Center, Tulane University, Covington, Louisiana.

A study was undertaken to see if techniques involved with the preparation of sperm smears on slides could cause changes in sperm morphology. Human semen samples were examined by two techniques: (1) fresh semen was diluted and examined using phase contrast microscopy, (2) semen was smeared on slides, air-dried, stained, and then examined using normal light microscopy. At least 100 sperm were classified using both procedures. Significant differences were found, most noticeably in the lower number of cytoplasmic droplets and the increased number of amorphous head forms on stained slides. Differences were also significant concerning sperm forms with abnormal midpieces and tapered heads; these changes naturally resulted in a significant difference in the percent of sperm classified as normal. No differences were found in sperm having coiled tails, small heads, large heads, double heads, or immature forms. This study suggests that smearing and staining sperm prior to morphologic evaluation may cause results significantly different from the natural state. The use of phase contrast microscopy for the evaluation of sperm morphology appears to be a faster and more reliable technique and only suffers from the lack of permanence. The authors suggest that both procedures be used in the clinical evaluation of males.

Glucuronic excretion is a useful index of oestrogen and progesterone metabolism in the assessment of follicular and luteal function.

In men one of the accepted parameters of androgen metabolism is the concentration of testosterone in peripheral plasma, but this does not reveal significant differences between fertile and infertile patients. The estimation of Testosterone 17β-Glucuronide (TGL) excretion may provide an additional index of androgen metabolism.

Simultaneous 24 hour, early morning (EMU) and mid-morning (MMU) specimens of urine were collected from 23 men of proven fertility for TGL and creatinine estimations, Peripheral venous plasma taken at the time of the MMU was assayed for testosterone.

RESULTS: n = 23

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<tr>
<td>Plasma Testosterone</td>
<td>23.10 ± 6.4 n mol/L</td>
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<tr>
<td>TGL Excretion</td>
<td>210 ± 151 n mol/24 hours</td>
</tr>
<tr>
<td>TGL content in EMU</td>
<td>33.3 ± 13.9 n mol/m mol creatinine</td>
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<tr>
<td>TGL content in MMU</td>
<td>34.6 ± 16.3 n mol/m mol creatinine</td>
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CONCLUSION: There was no correlation between TGL excretion and plasma testosterone. The wide variation in TGL excretion may preclude it's use as an indicator of androgen metabolism in men.
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