AMERICAN SOCIETY of ANDROLOGY

SIXTH ANNUAL MEETING
and
POSTGRADUATE COURSE

March 11-14, 1981
Fairmont Hotel
New Orleans, Louisiana

THIS MEETING IS SUPPORTED IN PART BY THE FOLLOWING:
Tulane Medical Center: Delta Regional Primate
Research Center, Department of Urology,
Department of Obstetrics & Gynecology, and
Tulane Fertility Clinic

Serono Laboratories: Pharmaceutical, Diagnostic,
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1982 Meeting

Site: Hyatt Hotel, Hilton Head Island, S.C.

Date: February 22 - 25, 1982

Local Chairmen: Howard R. Nankin, M.D.
Tu Lin, M.D.

Contact Address: Dept. of Medicine - Bldg. T-28
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HISTORY

This Society was founded in 1975 to meet the needs and concerns of scientists and clinicians who are involved with research and medical practice associated with the male reproductive system. With a current membership of approximately 450 we find that slightly more than half are medical doctors and that the medical specialities of urology, gynecology, internal medicine, and endocrinology are well represented. Our basic scientists include animal science, physiology, anatomy, and related fields in their areas of expertise. Our Society welcomes interested scientists and if you are not a member we suggest you check at the A.S.A. Registration Desk for an application.

PAST PRESIDENTS

1975-1977 Emil Steinberger, M.D.
1977-1978 Don W. Fawcett, M.D.
1978-1979 C. Alvin Paulsen, M.D.
1979-1980 Nancy J. Alexander, Ph.D.

HONORARY MEMBERS AND DISTINGUISHED ANDROLOGISTS

1976 M.C. Chang, M.D.
1976 R.O. Greep, M.D.
1977 R.E. Mancini, M.D.
1978 T. Mann, M.D.,Sc.D.,Ph.D.,F.R.S.
1979 J. Macleod, M.D.
1981 A. Albert, M.D.

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Alan F. Taulor, M.D.,Organon Corp.
Samuel A. Paquale, M.D.,Ortho Pharmaceutical Corp.
Carole J. Kruppa, Searle Laboratories
Thomas Wiggans, Serono Laboratories
POSTGRADUATE COURSE

The Postgraduate Course will be presented on Wednesday, March 11. The emphasis of the morning session will be directed towards the diagnosis and treatment of male impotency. The afternoon session is more basic and concerns the use of animal models for andrological research. The entire course is recognized for continuing education credit by the A.M.A., the A.U.A., and the A.C.O.G. Only those enrolled in the course will be permitted to attend the sessions. Extra copies of the syllabus will be on sale for those who did not register for the course but would like copies.

RECEPTION

A reception will take place in the Grand Ballroom at 7:00 p.m. on Wednesday. There will be a cash bar and free hors d'oeuvres for the meeting participants.

EXHIBITS

There is a commercial exhibit hall in the Gold Room, across from the Grand Ballroom. These companies and organizations have helped to support our meeting and all participants are encouraged to visit them.

WORKSHOP

A workshop will be held in the Bayou I Room on Thursday from 5:15 to 7:15 p.m. The discussion on "Ethical Implications for Fertility Research" will be lead by a panel and everyone is invited to be there. There is no fee for this event. The panel members will be: Ronald W. Lewis, M.D., Pierre Soupart, M.D., Ph.D., H. Tristram Engelhardt, M.D., Ph.D., and John Schaefer, Ph.D.
POSTER SESSION

Approximately 34 abstracts will be presented in a poster session in the Grand Ballroom on Thurs. from 7:30 to 9:00 p.m. These abstracts include some of the highest rated abstracts submitted and cover a wide range of interests. For those who might be hungry by this time WINE and CHEESE will be served, without charge, during this time.

BUSINESS MEETING

The Annual Business Meeting of the A.S.A. will be held in the Bayou I Room on Friday at 5:30 until 6:30 p.m. All members are invited to attend. If possible all motions should be presented, in writing, to the Society secretary prior to the meeting.

BANQUET

The banquet will start with a cash bar cocktail party at 7:30 p.m. in the Grand Ballroom on Friday. You must have a ticket to attend. The cost of the banquet is $25 which includes the meal, wine, and the jazz band entertainment after the meal. The event will be over by 10:00 which leaves plenty of time to enjoy the French Quarter at night. The Fairmont Hotel in New Orleans is noted for its food so the banquet should be a culinary highlight.

TOURS

There is a tourist information booth in the lobby of the Fairmont Hotel that will assist you with tours. A tour of the Delta Primate Center can be arranged for Saturday afternoon. This tour would require prior signup. Transportation would be provided. More information can be obtained at the Society Registration Desk.
STATE-OF-THE-ARTS LECTURES

Thursday, March 12

Serono Lecture: "Mechanisms of Mammalian Fertilization". Pierre Soupart, M.D., Ph.D., Professor of Obstetrics and Gynecology, Vanderbilt University, Nashville, Tennessee.

Friday, March 13

"Natural Barriers to Sperm Transport: From the Cervix to the Site of Fertilization". Penelope Gaddum-Rosse, Ph.D., Assistant Professor, Department of Biological Structure, University of Washington, Seattle, Washington.

Saturday, March 14

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The following white pages provide the texts of the abstracts presented in the oral and poster scientific sessions. The abstracts are arranged in the following order:

Oral presentations are in the order of presentation. They are in two parts. Part I is pages 10 to 54; Part II is pages 87 to 97.

Poster presentations are in alphabetical order based on the first author's last name. These abstracts are on pages 98 to 131.

A complete author index appears on pages 132 to 134.

The program book is arranged so that the center yellow pages may be removed as a unit to provide a small program booklet. The membership list is on blue paper and can later be removed as a unit for future reference.
10. STATISTICAL COMPARISON OF TRADITIONAL AND PRECISE SPERM MOTILITY EVALUATIONS. 
H. Winet,* J.S. Walker,** and M. Freund.** *Department of OB/GYN, University of Southern California, Los Angeles, **Department of Physiology, Southern Illinois University, Carbondale.

A series of human semen samples recorded on motion picture film which had been previously evaluated using standard visual techniques by thirty well-established fertility clinics (Freund, 1969) were analyzed for sperm motility ratings frame-by-frame with the aid of a movie reader-computer image analysis system. Results of the latter analysis were considered an objective normal distribution. Statistical comparison of the subjective and objective sets of data utilized Chi-square, t-test and Pearson correlation measures of dispersion. The statistical analyses revealed that sperm progressive motility could be rated by the traditional method with reasonable accuracy when the percent of sperm swimming progressively at least $U = 30 \text{ um sec}^{-1}$ is outside the range $34 \leq U \leq 57$. Within this range, however, there is a better than 93% chance for an erroneous evaluation which will result in an invalid fertility workup, given the accepted threshold for fertility of 50% progressive motility. Results of additional studies with experienced semen evaluators show a high correlation between subjective and objective motility ratings throughout the critical percentage range when semen is diluted. It is concluded that dilution of semen with a medium which cancels the immobilizing dilution factor would improve the reliability of clinical semen workups significantly.

When results of the reader-computer analysis technique are compared with those obtained via the spectrophotometric method of Atherton (1975), again using film records of the same samples (in this case boar sperm) observed in the spectrophotometer, a poor correlation is obtained indicating that while the spectrophotometric method may be a precise measure of motility it does not discriminate progressive motility.

(Supported by NIH Grant HD 12102)
11. DETECTION OF ANTIBODY ATTACHED TO SPERM CELLS BY DIRECT OBSERVATION IN THE MIXED ANTIGLOBULIN REACTION. Sidney Shulman, Sperm Antibody Laboratory, New York Medical College, Metropolitan Hospital, New York 10029.

A method of mixed agglutination between sperm cells and sensitized red blood cells has been proposed. A drop of each of the following components is used: 1) the fresh semen (motile sperm), 2) group O Rh-positive erythrocytes coated with anti-D antibody, and 3) rabbit anti-human IgG. These are mixed and then observed microscopically after 10 or 15 minutes. A positive reaction, showing vibrating clumps of red cells with the motile sperm cells attached, indicate that the spermatozoa were coated with IgG antibody. Negative reactions show no vibrations in the red cells clumps, and many free swimming spermatozoa will be seen. Comparisons have been made with results of antibody tests on the serum from these men using the Kibrick method (Gelatin Agglutination Test) and the F-D method (Tube-Slide Agglutination Test). A high degree of correlation was found between the sperm and serum results. The incidence of sperm antibody in infertile men is about 9% of the group. The MAR (mixed antiglobulin reaction) can be used as a useful screening procedure.

(This study was supported by the Sperm Antibody Laboratory Fund).
Populations of human lymphocytes previously incubated with human sperm were a stimulant factor for the second population of human lymphocytes in allo- and autogenic systems. The spermatozoa induced-cells were blocked by Mitomycin C.

A positive response was observed after combination with lymphocytes versus allogenic sperm.

Reaction of restimulation was only positive in sperm-stimulated lymphocytes versus lymphocytes in autologous system.

The phenomenon seems to provide a new explanation for the still unclear nature of spermatozoa-lymphocyte interaction.
Seminal testosterone (T) and fructose (F) concentrations were measured in 78 men and compared with several standard parameters of the ejaculate as well as with sperm fertilizing capacity determined by in vitro penetration of zona-free hamster ova. This in vitro penetration test recently has been shown to be an accurate indicator of male fertility (Rogers et al., Fertil. Steril., 1979; Hall et al., Am. Fertil. Soc., 1980). Strong association of T or F with the fertilizing capacity of sperm could afford a substitute correlate of male fertility where the in vitro fertilization test-system is not available. T and F concentrations in semen were 42 ± 19 (ng/100ml ± SD) and 313 ± 151 (mg/100ml ± SD), respectively. No significant correlation was observed between T or F levels and ability of sperm to penetrate zona-free ova. Significant negative correlations, however, were found between T concentrations and ejaculate volume \( (r = -0.24, P < 0.05) \). When percent ova fertilized and the six semen parameters (sperm density, percent motile, motile sperm density, percent dead, were each tested for correlation with T and F together, no significant relationship was noted. These data support the premise that neither individually nor combined do seminal testosterone and fructose values serve as an index of human sperm fertilizing ability and have apparent predictive capacity for male fertility.
14. IN VITRO BOVINE CERVICAL MUCUS PENETRATION TEST TO ASSESS FERTILITY IN
MEN. N.J. Alexander and J.H. Sampson. Oregon Regional Primate Research Center,
505 N.W. 185th Avenue, Beaverton, Oregon, and Depts. of Ob/Gyn and Urology,
University of Oregon Health Sciences Center, 3181 S.W. Sam Jackson Park Road,
Portland, Oregon.

Presently, beyond evaluation of sperm count and motility, there are few
ways to assess potential fertility in men. We use an in vitro cervical mucus
penetration test to more fully predict male fertility. Bovine cervical mucus
(BCM) is collected at midcycle, drawn into capillary tubes, and frozen. Tubes
are thawed, placed in a small amount of the semen to be tested, and incubated
1.5 hours at room temperature. The extent of migration is then evaluated micro-
scopically. Samples with a migration of 15 mm or more are considered adequate.
We have tested the semen of 220 men attending our Infertility Laboratory. Of
particular interest are those with sperm counts and motility values considered
to be in the normal range. We have compared the subsequent pregnancy rates
(4 months or more after evaluation) of patients with adequate and inadequate
mucus penetration. For those with adequate penetration and subsequent conception
and those that did not conceive, respectively, the sperm counts were 83.5 +
19.0 and 100.5 + 17.0 million/cc, and the percentages of motility were 60 +
1.8 and 64.6 + 3.3. For those that conceived and had poor cervical mucus pene-
tration and those that did not conceive, respectively, the mean sperm counts
were 62.5 + 31.2 and 54 + 8.8 million/cc and the percentages of motility were
52.9 + 3.0 and 38.5 + 10.4. Even though there was no statistically significant
difference in the count and percent motility between those that conceived and
those that did not, significantly more men with good BCM penetration caused
a pregnancy (p < 0.05). We believe the BCM penetration is useful in the evalua-
tion of male fertility.
It has been reported that anti-HCG sera can be shown by immunofluorescence to react with dead, fixed, human sperm (Asch et al., 1977 Fert. Steril. 28, 1258-1262). Studies were carried out to determine whether such sera also showed reactions with living human sperm. Suspensions containing a high proportion of motile sperm were obtained by layering buffer over semen and removing this buffer after one hour when it contained motile sperm which had penetrated this layer (Hellema et al., 1978, Clin. Exp. Immunol. 31, 1-11). Anti-HCG sera showed reactions with living human sperm causing both sperm agglutination and complement dependent sperm immobilization. However, reactions with human red cells were also observed and in at least some antisera the anti-sperm activities could be largely removed by absorption with human red cells, but not by absorption with rabbit red cells. The nature of the possible association of an "HCG-like substance" with human sperm will be discussed in the light of the results of these investigations.
This study was designed to investigate whether the number of elongated spermatids in human testicular biopsies correlates with sperm output. Twelve men with sperm counts ranging from 0.1 to 54.4 million/ml (mean ± std. error: 15.0 ± 5.6 million/ml, at least 5 semen analyses over a minimum of 2 months per individual) underwent bilateral testicular biopsy for evaluation of infertility or in conjunction with varicocelectomy. The tissues were fixed in Cleland's and four-micron sections were stained with PAS-hematoxylin. Elongated spermatids were identified and counted using a phase-contrast microscopy (x 635 magnification) in 20 seminiferous tubule cross-sections (only round tubule sections were selected for counting). The number of elongated spermatids per tubule ranged from 2.0 to 29.6 (mean ± std. error: 16.5 ± 1.8) and significantly correlated with the sperm count (r = 0.75, p < 0.005) and the total sperm count (r = 0.7, p < 0.025). In patients with less than 20 elongated spermatids per tubule the sperm count (3.7 ± 0.9 million/ml) and the total sperm count (7.8 ± 1.9 million per ejaculate) were significantly lower than in patients with more than 20 elongated spermatids per tubule (37.6 ± 9.1 million/ml and 41.5 ± 9.6 million/ejaculate, respectively). All men with more than 20 spermatids per tubule had sperm counts above 10 million/ml and total sperm counts above 20 million/ejaculate. The results of this study suggest that quantitation of elongated spermatids in human testicular biopsies could aid in detection of cases of oligospermia not related to disturbances of spermatogenesis (e.g., partial obstructions of the excretory ducts of the testes, disturbed sperm transport in the epididymis or vas). In such cases a discrepant high number of elongated spermatids would be found in the face of low sperm output.

The purpose is to report that reduced zinc concentrations (<15 mg%), elevated pH (>7.7), and neutrophils (5-10 WBC/HPF or more) can suggest the presence of an asymptomatic infection. Forty patients with the above alterations underwent special urine and seminal cultures attempting to isolate Ureaplasma urealyticum. Twenty of the 37 cultures had positive Ureaplasma urealyticum isolated from the semen using special culture techniques.

This report will emphasize a clinical approach based on the above changes. Normal semen has up to 3 PMNs per high power field, based on a random survey of at least 506 semen samples. When PMN counts in excess of 5 per high power field are seen, then infection is a possibility. The finding of reduced zinc levels and elevated pH levels of the semen suggest the presence of protatitis. Antibiotic treatment is necessary. We will report on the bacterial sensitivity studies.

We will also discuss a separate category of patients who may have only a reduction in zinc without the presence of white cells. This type of patient is treated only with zinc sulfate for a minimum of 6 months. The patients with neutrophilia, altered zinc and elevated pH are treated with minocycline for a minimum of 4 weeks.
CHARACTERIZATION OF RADIO Labeled COMPONENTS OF BULL SPERM SURFACE AND SEMINAL PLASMA. L.G. Young and S.A. Goodman. Departments of Physiology and Microbiology, Emory University School of Medicine, Atlanta, GA 30322.

Externally oriented components on the surface of ejaculated bull sperm and components in bull seminal plasma were labeled by enzymatic iodination with lactoperoxidase and $^{125}$I-NaI. SDS-7.5% PAGE of labeled sperm surface resolved six components with approximate molecular weights of 77 K, 61 K, 44 K, 36 K, 24 K and 15 K daltons. SDS-7.5% PAGE of labeled seminal plasma resolved four components with approximate molecular weights of 74 K, 33 K, 24 K and 15 K daltons, each of which comigrated with a labeled sperm surface component. To identify the chemical composition of the radiolabeled components, labeled sperm surface and labeled seminal plasma were submitted to isopycnic density gradient centrifugation in cesium chloride, a procedure which separates proteins from lipids. With bull sperm surface, two areas of radioactivity were resolved, one having a density characteristic of protein and the other of lipid. Iodinated seminal plasma banded in one discrete area having a density characteristic of protein.

Electrophoretic analysis of each area of radioactivity recovered from the gradients demonstrated that five of the six sperm surface and all of the seminal plasma components were in the protein fractions. The 15 K dalton sperm surface component banded as a lipid, whereas the 15 K dalton seminal plasma component banded as a protein. However, the similarity in chemical composition of the other three seminal plasma components to sperm surface components supports the hypothesis that these proteins or glycoproteins are adsorbed to the surface of ejaculated bull sperm from seminal secretions.

(Supported by WHO-78082 and by RRSG-05264 to EUSM).
Boar sperm plasma membranes (PM) contain antigens capable of binding to the porcine zona pellucida (ZP) (Peterson et al., 1980). The antigens appear to be species and tissue specific. Approximately 9 antigen-antibody complexes can be identified by crossed immunoelectrophoresis using anti PM-IgG raised in rabbits (Spaulding et al., 1980). Two methods were used to assess the ability of PM polypeptides to bind to isolated ZP. Crossed immunoelectrophoresis patterns of detergent solubilized PM were compared in the presence and absence of ZP fragments added in the first dimension. Six PM antigens were compared: two antigens, which represented 20% of the total antigen response, were markedly changed in electrophoretic mobility and precipitin peak height. The major antigen complex (46% of the total response) reacted only weakly with the zonae. In the second experiment 1 mg of detergent (Triton X-100) solubilized PM, labelled with $^{125}$I, was absorbed to a column containing 350 mg purified ZP in tris-NaCl buffer. The column was washed with detergent-buffer and bound components were eluted with anti PM-Fab and finally with SDS-urea. Eluted proteins were enriched in several polypeptides which migrated in PAGE-SDS gels below 22 Kd. Comparison of PAGE profiles of PM originating primarily from the head (50 PSI gas cavitation) with those enriched in membranes from other areas of the sperm PM (950 PSI cavitation) and with fused PM-outer acrosomal membranes from acrosome reacted sperm, suggest that these low molecular weight polypeptides are concentrated in the sperm head. These experiments have identified several antigens and polypeptides that interact strongly with the porcine ZP. Attempts to isolate these polypeptides and determine their physiological significance are in progress.

(Supported by NIH Grant HD 13047).
DIVALENT METAL ION STIMULATION OF THE PROTEOLYTIC ACTIVITY OF BOAR SPERM ACROSIN. Richard F. Parrish and Kenneth L. Polakoski. Departments of Urology and Obstetrics/Gynecology, Washington University School of Medicine, St. Louis, Mo. 63110

Although acrosin is presently thought to be involved in sperm penetration of the outer investments of the ovum, little is known about the proteolytic properties of the enzyme, and almost nothing is known about the divalent metal ion effects on proteolytic activity. We now wish to report that divalent metal ions dramatically stimulate the proteolytic activity of porcine acrosin. A linear relationship was observed between calcium concentration and stimulatory effect on acrosin catalyzed proteolysis. When 0.002 M calcium was included in the reaction mixture, there was a 2-fold stimulation of the proteolysis of Azocoll, while in the presence of 0.05 M calcium, there was a 46-fold stimulation of the proteolysis, relative to proteolysis in the absence of calcium. Magnesium, barium, and strontium also produced dramatic stimulation of acrosin catalyzed proteolysis of Azocoll. However, when a series of monovalent cations (i.e. sodium) at a concentration of 0.05 M was tested, a slight (2-3-fold) stimulation of proteolysis of Azocoll was observed. Qualitatively similar results were observed when denatured hemoglobin was utilized as the substrate. However, no stimulation of trypsin catalyzed proteolysis was observed in the presence of calcium. Since the specificity of trypsin and acrosin are very similar, this indicates that the observed stimulation of acrosin catalyzed proteolysis of Azocoll resulted from an effect on acrosin and not from an effect on the substrate. The divalent metal ion stimulation of acrosin catalyzed proteolysis appears to correlate with the presence of a divalent metal ion binding site on acrosin. A slight additional stimulation results from increasing the ionic strength of the reaction medium.

(Supported by National Institutes of Health Grants HD 09422 and 00296).
Spermatozoan collective motility depends upon continuous supply of available energy produced either by fructolysis or mitochondrial oxidative phosphorylation. Collective motility is usually determined by microscopic observation - an approach with disadvantages such as subjectivity, lack of precision, and variability of results. In order to study the exact interrelation between collective motility and the energy metabolism of the sperm cell, a multichannel system analyzing collective motility by reflectospermography approach has been used (Mayevsky et al., Int. J. Andrology, 1980, in press). Using the same multichannel system, we also monitored collective motility and pH (using a pH-sensitive fluorescent indicator - umbelliferone) from four semen samples simultaneously. Collective motility was measured in semen, obtained by electrical stimulation with a bipolar electrode. By using a 2-Deoxy-D-glucose, fructolysis was inhibited and mitochondrial respiration was inhibited by Antimycin A.

The results show that when motility is driven by mitochondrial respiration alone (after inhibition of fructolysis), the optimal pH for motility was in the acidic range (6.0-6.5). This motility was sensitive to the level of oxygen in the semen sample: low PO₂ decreased motility due to the lack of ATP and high PO₂ (hyperbaric O₂) also inhibited motility due to the toxicity processes developed. When sperm cells used fructolysis as an energy source (after inhibiting mitochondrial respiration) a rapid decline in the pH was recorded (using the fluorescence probe), which was correlated to the intensity of motility.
Tetravalent reduction of oxygen \((O_2)\) to water with concomitant conservation of the chemical energy as ATP is the fundamental reaction of cellular metabolism. Under some conditions the reactive and deleterious partially reduced \(O_2\) species hydrogen peroxide \((H_2O_2)\) and superoxide anion \((O_2^-)\) can accumulate in cells (Chance, Sies and Boveris, 1979). Normally the enzymes catalase \((CAT)\) and superoxide dismutase \((SOD)\) protect but in highly specialized cells like spermatozoa certain enzyme functions may be absent. We sought answers to the questions: do sperm produce \(H_2O_2\) and \(O_2^-\) and have they the requisite protective enzymes?

Sperm collected from the excised epididymides of New Zealand white rabbits were used intact or HTRES were prepared (Keyhani and Storey, 1973). \(O_2\) was assayed by reduction of acetylated ferrocytochrome c while \(H_2O_2\) was assayed by the cytochrome c peroxidase mediated oxidation of acetylated ferrocytochrome c. Both intact and HTRES generate \(O_2\) and \(H_2O_2\). Oxidisable substrates, particularly lactate plus malate, increased production of both entities but not as much as ADP. Antimycin A, a respiratory chain inhibitor, gives maximal rates of production for both \(O_2\) and \(H_2O_2\) implying part of their production is of mitochondrial origin. Rotenone has no effect, hence a component of the chain between the Antimycin A and rotenone sensitive sites is implicated. Neither uncoupling or inhibition of phosphorylation affected \(O_2\) or \(H_2O_2\) production on HTRES. The inability of aminotriazole, a specific CAT inhibitor, to increase \(H_2O_2\) production and the time dependent accumulation of \(H_2O_2\) both imply sperm have little CAT activity. However, the SOD inhibitor cyanide causes a large increase in \(O_2\) production indicating the presence of SOD in HTRES. Rabbit sperm thus are ill equipped to deal with \(O_2\) and \(H_2O_2\) and we suggest that accumulated damage from these metabolites may in part determine the lifespan of the spermatozoa.

(Supported by NIH International Fellowship TW-2673 and NIH grant HD-06274).

Lactate dehydrogenase C₄ (LDH-C₄; LDH-X) is a sperm-specific antigen. Immunization with this protein reduces the fertility of female mice, rabbits, and baboons. To further exploit this phenomenon in the development of a immunological contraceptive, we have begun to identify peptide fragments of this protein which are amenable to chemical synthesis, which react with antibody to LDH-C₄, and which elicit an immune response to the native protein.

One such peptide has been isolated from a tryptic digest of LDH-C₄ by cation exchange chromatography. Its structure was determined by the Edman degradation. A synthetic preparation of this peptide was conjugated to bovine serum albumin via the amino-terminal of the peptide. Three rabbits immunized with this conjugate produced antibody strongly reactive and specific to the peptide. These antibodies also showed a weak reaction with native LDH-C₄. The success of this procedure prompted a search for more strongly antigenic peptides.

The tryptic peptides of LDH-C₄ were separated by high pressure liquid chromatography. Each pure peptide was tested for antibody binding, and its amino acid composition was determined. As judged by compositions, the peptides isolated to date account for at least 80% of the sequence. About 12 of these fragments, including 7 which could not be definitely assigned positions in the complete sequence, bind anti-LDH-C₄ antibody. The amino acid sequences of these active peptides are now being determined. This will provide a selection of material to test for antigenicity and ultimately for the immunosuppression of fertility.

(Supported by NIH Grant HD05863).
MICROPUNCTURE STUDIES OF THE BLOOD-TESTIS BARRIER TO METHOTREXATE (MTX) IN RATS. R. Vigersky, R. Riccardi, A. Bleyer, S. Barnes, and D. Poplack. Kyle Metabolic Unit, Clinical Investigation Div., Walter Reed Army Medical Center, Washington, D.C.; Children's Orthopedic Hospital Medical Ctr., Seattle, Wash.; and Pediatric Oncology Branch, N.C.I., N.I.H., Bethesda, MD.

The testis is the first and only site of relapse in up to 16% of patients with acute lymphocytic leukemia (ALL) (Cancer 38:2604). It has been suggested that the blood-testis barrier protects leukemic cells from the effects of chemotherapeutic agents. Using testicular micropuncture, we investigated the ability of MTX, an antimetabolite commonly used in the treatment of ALL, to pass from the blood into the interstitial space and seminiferous tubule (ST) of the rat.

MTX was administered to anesthetized adult Sprague-Dawley rats via a jugular vein cannula. Constant plasma levels of MTX were achieved by giving a priming dose followed by a constant infusion of 1 (n=2), 10 (n=7), or 100 (n=5) mg/kg/hour. Over the next 4 hours, blood (via a femoral vein cannula) and interstitial and ST fluids (via direct micropuncture) were periodically sampled. Plasma and testicular fluid levels of MTX were measured by a specific and sensitive competitive binding assay. The results (mean ± SE) are shown below:

<table>
<thead>
<tr>
<th>Infusion Rate (mg/kg/hr)</th>
<th>Plasma (x10^-6 M)</th>
<th>Interstit. (x10^-6 M)</th>
<th>Sem.Tub. (x10^-7 M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 ± 1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>16 ± 3</td>
<td>54 ± 39</td>
<td>59 ± 11</td>
</tr>
<tr>
<td>100</td>
<td>160 ± 30</td>
<td>-</td>
<td>220 ± 100</td>
</tr>
</tbody>
</table>

The data indicate that a blood-testis barrier to MTX exists. The linear increase in blood MTX levels with increasing infusion rates produces a parallel increase in ST fluid levels but at a 100-fold lower concentration. Interstitial levels are intermediate between those of plasma and ST. These findings justify the use of high dose MTX in an attempt to eliminate the testis as a sanctuary for leukemic cells.
Leydig cells contain high affinity, low capacity receptors for estrogen and estrogen can directly inhibit testicular steroidogenesis. However, there is little information regarding the effect of antiestrogen on testicular androgen formation. Present studies were undertaken to evaluate the in vitro effects of tamoxifen, a potent antiestrogen, on rat interstitial cell steroidogenesis. Dispersed interstitial cells from the testes of 60-90 day old Sprague-Dawley rats were prepared by collagenase digestion. Cells were incubated at 34°C in Medium 199 with 0.1% bovine serum albumin and 0.1 mM 3-isobutyl-1-methyl-xanthine. Varying amounts of LH, 8-bromo-adenosine-3', 5'-monophosphate (8-bromo-cyclic AMP), 17β-estradiol (E2) and/or tamoxifen were added; testosterone and cyclic AMP were measured after three-hour incubations. The results are expressed as means ±SE per 10^6 cells. LH, 5 mIU/ml, increased testosterone formation to 13.18 ± 0.62 ng. Tamoxifen significantly inhibited LH-stimulated testosterone formation in a dose-dependent manner. At tamoxifen concentrations of 10^-6, 10^-5 and 10^-4 M, testosterone levels were reduced to 87%, 61% and 6%, respectively of control levels. Tamoxifen also significantly inhibited LH-induced cyclic AMP formation. With the addition of 8-bromo-cyclic-AMP 10^-6 M, testosterone formation increased to 9.04 ± 0.35 ng. Tamoxifen (10^-6 to 10^-4 M) significantly blocked 8-bromo-cyclic AMP-induced testosterone formation. When equimolar concentrations of E2 and tamoxifen were added concomitantly to interstitial cells, the inhibition of testosterone synthesis was additive. The inhibitory effects of tamoxifen could not be reversed by estrogen. Present studies demonstrate that tamoxifen has direct inhibitory effects on testicular steroidogenesis: both at the plasma membrane level resulting in decreasing cyclic AMP formation and also at steps subsequent to cyclic AMP.
SPERMATOGENESIS FOLLOWING CANCER CHEMOTHERAPY WITH DOXORUBICIN (ADRIAMYCIN).
M.F. da Cunha, M.L. Meistrich, and H.L. Ried. M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Cytotoxicity to the testicular germinal epithelium is one of the many undesirable side effects of cancer chemotherapy. It has been well documented that some drugs, either used as single agents or in combination regimens, will induce temporary or permanent sperm depletion. Little data is available on the sterilizing effect of doxorubicin (Adriamycin, ADR) in humans.

The sperm production of 9 patients who received doxorubicin was investigated after cessation of therapy. ADR was used in various multiple-drug protocols for the treatment of several malignancies. ADR was administered in multiple courses and total cumulative doses ranged from 180 to 625 mg/m$^2$. Sperm concentration and motility were measured at periods of time that ranged from 7 to 79 months after discontinuation of ADR.

Two patients were azoospermic, 3 were oligozoospermic (range 1.6 to 5.4 million/ml), and 4 were normozoospermic (range 17 to 110 million/ml). Sperm motility at 30 min. (median: 70%) and volume ejaculate (median: 2.6ml) were within normal limits. Two of the normozoospermic patients who received a total of 625 and 375 mg/m$^2$ fathered children after treatment with ADR. All 4 resulting children appear to be clinically normal.

These data indicate that the damage to the testis produced by ADR is reversible, suggesting little or no toxicity to germinal stem cells. In contrast, relatively small doses (33 mg/m$^2$) administered to mice in single injections produce permanent sterility as a result of extensive stem cell killing. Quantitative interspecific differences in pharmacokinetics, drug penetration to the testes, and stem cell sensitivity may be responsible for the discrepancy between ADR effects on mice and man.

(Supported by grants CA-17364 and CA-06294 from the National Cancer Institute).
27. **EFFECT OF MEDROGESTRONE ON THE FERTILIZING ABILITY OF RABBIT CAUDA EPIDIDYMAL SPERM AND ON IN VITRO RELEASE ON ANDROGENS.** Saksena and In-Fai Lau. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

The present study was designed to test the effects of a progestational and anti-androgen 'Medrogestone' (6,17α-dimethyl-4,6-pregnadiene-3,28-dione), on the fertilizing capacity of the cauda epididymal sperm (CES) in the male rabbit. Medrogestone was injected into the cauda epididymis (500 μg/0.04 ml/epididymis). Fertility of CES was determined by artificially inseminating (AI) the estrous does which were induced to ovulate by hCG. Does inseminated with sperm from control males had fertilized eggs when the eggs were recovered from oviducts 40-48 h after AI. The proportions of females showing fertilized eggs after AI with CES exposed to Medrogestone for 12 days was significantly reduced (43%) whereas the percentage of eggs fertilized was also inhibited (90% controls vs 20% medrogestone treated). A shorter exposure (4 or 8 days) of CES to Medrogestone resulted in a reduced percentage of fertilized eggs (74 and 67%, respectively, as compared to 90% in controls). The release of testosterone (T) and 5α-dihydrotestosterone (5α-DHT) from the testicular mince into the Kreb-Ringer Buffer were stimulated in the presence of 50 mIU/ml hCG. Addition of 10 or 50 μM of Medrogestone significantly inhibited the release of T even in the presence of hCG. Likewise, the release of 5α-DHT was also inhibited. It is concluded that Medrogestone induced reduction of CES fertilizing ability may be due to its inhibitory effects on 5α-reductase activity and the lack of androgen metabolites might have altered the integrity of spermatozoa.

(Supported by HD-12216 from NICHD).
28. THE EFFECT OF ADRIAMYCIN ON THE REPRODUCTIVE SYSTEM OF THE MALE PREPUBERAL RAT. B.F. Giffin, P.K. Bajpai, M.J. McCully and B.G. University of Dayton, Dayton, OH and University of Michigan, Ann Arbor, MI

Adriamycin has been successfully used in the treatment of leukemias and lymphomas. Destruction of the germinal epithelium and subsequent infertility has been reported in both animals and humans receiving therapeutic doses of adriamycin. The following study was undertaken to investigate whether adriamycin therapy started in prepuberal animals would have any adverse effects on the onset of puberty. 192 male rats (21 day old) were assigned to control (80) and experimental (112) groups. Rats in the experimental group were injected intraperitoneally with 0.5 mg of adriamycin/kg of body weight twice a week for a total of 8 weeks. 10 rats from the control group and 14 rats from the experimental group were sacrificed at 2 week intervals for 16 weeks. The following parameters were examined: prolactin, testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), weight and histology of testes. Serum prolactin and LH were not significantly different between treated and control animals. Testosterone concentrations were within normal limits but significantly lower in the treated animals at 14 and 16 weeks. From the eighth week of treatment through the recovery period of subsequent eight weeks, levels of FSH were significantly higher in the treated animals. Testicular weights were lower in the treated group from the eighth week of treatment to the sixteenth week of recovery. This was due to loss of interstitial tissue rather than smaller tubule diameters. Leydig cell morphology and function were not influenced by adriamycin. The spermatogonial cell index was significantly lower in the treated animals at 8, 10, and 12 weeks. Loss of germinal epithelium was correlated with elevation of FSH. This study indicates that administration of therapeutic doses of adriamycin to prepuberal rats is not sufficient to disrupt the changes associated with onset of puberty.
29. EFFECT OF ESTROGEN ON STEROID LEVELS OF SPERMATIC VEIN BLOOD IN PATIENTS WITH PROSTATIC CANCER. Takumi Yamaihara*, Motomi Kanazawa*, Lu-Rung Chu*, Koichiro Isurugi** and Keiko Fukutani**. Department of Obstetrics and Gynecology*, Showa University School of Medicine* and Department of Urology, National Medical Center **, Tokyo, Japan

The levels of free and conjugated steroids in the spermatic vein blood in 20 patients with advanced prostatic cancer were measured by RIA. The hormone levels of the patients with (n=10) and without (n=10) previous estrogen therapy (diethylstilbestrol diphosphate, 300 mg for 3 months) were compared to evaluate the effect of estrogen on steroid secretion in the testes. The mean spermatic steroid levels found in the patients without estrogen therapy were as follows; testosterone (T) 180.4, androstenediol (AD) 43.6, DHA 35.9, T-sulfate (T-S) 12.8, AD-S 30.0, DHA-S 246.2, estradiol 0.83 ng/ml. The levels in the spermatic vein were statistically higher than that in the peripheral vein except DHA-S. A striking suppression of all free steroid levels measured in spermatic vein blood in the patients who were treated with estrogen was noted. The levels of conjugated steroids did not change significantly. LH and FSH levels in peripheral blood were also measured and it was found that the values were suppressed in the patients who had received estrogen. The peripheral levels of free T, AD were also significantly decreased in the patients with estrogen therapy. Significant correlations between peripheral and spermatic T and DHA-S levels was observed in all subject. Changes of steroid levels in spermatic and peripheral vein blood following the administration of estrogen are demonstrated.
30. TESTICULAR FUNCTION IN MEN WITH HODGKIN'S DISEASE (HD) PRIOR TO THERAPY. 

R. Vigersky, R. Chapman, A. Glass, and J. Berenberg. Kyle Metabolic Unit, 
Clinical Investigation Svc.; Hematology-Oncology Svc., Dept. of Medicine, 
Walter Reed Army Medical Center, Washington, D.C. and Dept. of Medicine, 
Uniformed Services University for the Health Sciences, Bethesda, Md.

Chemotherapy produces germinal aplasia and impairment of Leydig cell 
function in patients with HD and non-Hodgkin's lymphomas. To determine whether 
or not gonadal function in HD is normal prior to therapy, we studied 11 men, 
ages 19-38, with HD Stages 1 and 2 (n=5) and Stages 3 and 4 (n=6) and compared 
them to 20 normal men. Basal hormone levels are the mean of 3 consecutive 
samples obtained at 15 minute intervals.

Basal serum testosterone (T) levels (mean ± SE) were significantly lower 
in HD than in the control (444 ± 43 vs. 640 ± 53 ng/dl; p < 0.05). The acute 
(hour) increase of T after 4000 units of HCG i.m. was 24 ± 8% (normal 26 ± 5%). 
Basal serum 17-hydroxyprogesterone was normal (99 ± 12 ng/dl) and acutely 
increased 57 ± 25% after HCG (normal increase 75 ± 12%). Basal serum LH 
levels were 10.0 ± 1.6 mIU/ml (normal 12.5 ± 1.7 mIU/ml) and the LH/T ratio 
was 2.42 ± 0.4 (normal 1.7 ± 0.19). Basal serum FSH levels were 8.4 ± 1.6 
mIU/ml (normal 12.5 ± 1.7 mIU/ml). Semen analysis was performed 2 or more 
times in 7 of the 11 men (3 refused and 1 had a vasectomy). Sperm density 
was normal (20 x 10^6/ml) in 5 and borderline low in 2 of the men (18.5 
and 20.6 x 10^6/ml). Motility was "poor" (40%) in 4 and "equivocal" (41-60%) 
in 3 of the men. There were no differences in the results between men with 
Stages 1 and 2 compared to Stages 3 and 4, nor between those with or without 
systemic symptoms.

These preliminary data suggest that testicular function is impaired in 
some men with HD even before they receive therapy. The constellation of 
low basal serum T which responds normally to HCG and an inappropriately low 
LH suggests either a lowering of the set point for the feedback of T on LH 
or a combined pituitary and testicular abnormality in untreated men with 
HD.
Since hyperprolactinemia has an adverse effect on gonadal function, we have evaluated prolactin (PRL) secretion with physiological and pharmacological stimuli in 28 severely oligospermic or azoospermic (AZ) patients aged 20 - 42 years old and matched controls (CON). Mean ± SD (mIU/ml) basal LH (32.3 ± 23.7) and FSH (28.2 ± 19.4) were increased (p < 0.001) in AZ compared to CON (LH 11.1 ±3.0, FSH 6.0 ±2.7). Testosterone (ng/ml) was 4.9 ± 1.8 in AZ and decreased (p < 0.05) compared to CON (5.9 ±2.0). Estradiol (ng/ml) levels were similar in the 2 groups. The mean ± SD (ng/ml) peak PRL responses to 200 μg thyrotropin releasing hormone (TRH) IV were 69.7 ± 30.3 in 12 AZ compared to 37.7 ± 13.5 in 14 CON (p < 0.005). Corresponding peak PRL responses after 10 mg metoclopramide IV were 167.4 ± 53.2 in 11 AZ and 75.0 ± 15.7 in 12 CON (p < 0.001). Following 25 mg chlorpromazine IM, the PRL peaks were 54.0 ± 38.2 in 10 AZ and 25.3 ± 8.7 in 10 CON (p < 0.001). Mean basal PRL levels (representing a mean of 3 values prior to stimulation) were similar in the 2 groups (10.9 ± 3.8 in AZ and 9.7 ± 2.8 in CON). Plasma samples were taken at 20 minute intervals from 2100 hr - 0600 hr under EEG control following adaptation in 9 AZ and 12 CON. The number of PRL peaks during sleep ranged from 3 - 4 in all subjects. The average PRL concentration during all stages of sleep (mean of all values during this period) was 16.2 ± 11.1 in AZ and 12.4 ± 3.2 in CON. These values were not different from one another, but were increased (p < 0.05) compared to the corresponding mean waking PRL concentrations. It is concluded that AZ have increased PRL responses to pharmacological stimuli such as TRH, metoclopramide, and chlorpromazine. Although PRL concentrations increased during the physiological stimulus of sleep, levels were not greater than CON. The hyperprolactinemic responses following pharmacological stimuli in TF are presumably a consequence of the testicular disorder.
This report compares the perceived behavioral benefits of testosterone versus HCG/Pergonal therapy in the 4 gonadotropin (Gn) deficient hypopituitary males described in the abstract by MacGillivray and colleagues. The clinical and endocrine status as well as the treatment schedules are contained in that abstract (39 P).

Each man was individually interviewed using a standardized interview protocol during and after Gn treatment. All reported that the major benefits of testosterone treatment had been voice deepening, pubic hair growth, and some testes and scrotal enlargement. However, they were disappointed with the scantiness of beard, body, and axillary hair while on androgen therapy. After they were switched to HCG/Pergonal regimen, all males reported that the major somatic benefits of the Gn treatment were improved beard, body, and axillary hair growth and additional testicular enlargement and density of pubic hair.

While each patient reported improved erotosexual function while on androgen therapy, each stated that the most improvement resulted from Gn treatment. The frequency of erection, ejaculation, masturbation, dating, kissing, petting, intercourse, erotic sleep and daydreams were reported to occur at a higher frequency on Gn therapy versus androgen replacement alone. While androgen therapy was reported to improve interest in sex (libido) and self-confidence, each man reported the most improvement in these attitudes on Gn therapy. In all cases Gn therapy was preferred.

These data suggest that Gn therapy in these males provides relatively greater psychological benefits than those of androgen treatment. Whether the behavioral improvements noted on Gn therapy are a direct result of Gn replacement or an indirect effect of the improved virilization which resulted from the Gn regimen cannot be answered with these data.
33. ORIGIN OF ESTROGEN IN ADOLESCENTS WITH KLINEFELTER SYNDROME AND GYNECOMASTIA.

James Aiman, D.L. Hemseil, P.F. Brenner, C.R. Parker, and P.C. MacDonald. Department of Urology, Biochemistry, and OB-GYN. University of Texas Southwestern Medical School, Dallas, Texas.

Androgen and estrogen production rates were measured in 3 subjects, ages 16-18, with Klinefelter syndrome and gynecomastia. The mean production rates of estrone and estradiol were 83 µg/24h and 78 µg/24h, respectively. These values exceeded those of 4 normal adult men by 43% and those of 2 boys with pubertal gynecomastia by 77%. The increased estrone production in one subject was due to glandular secretion (41 µg/24h) and his plasma production rate of testosterone was low, 2374 µg/24h. Plasma production rates of testosterone in the other two subjects, 3583 µg/24h and 8626 µg/24h, were greater than the values of the boys with pubertal gynecomastia (2763 µg/24h and 1843 µg/24h) but were comparable to values of normal adult men (z=5700 µg/24h). Secretion rates of estradiol in these two Klinefelter subjects were 78 µg/24h and 70 µg/24h, values 12 times those of normal men. After 8 to 12 months of suppression of testicular function by testosterone or norethindrone acetate there was a reduction in the quantity of breast tissue and estradiol production rates 50% or less of pretreatment values. Dexamethasone had no effect on the plasma production rate of testosterone or on the production rate of estradiol.

There may be two mechanisms for the development of gynecomastia in adolescent subjects with gynecomastia. In one Klinefelter subject and two boys with pubertal gynecomastia estradiol production rates were comparable to values of normal men but plasma production rates of testosterone were low. In two subjects with Klinefelter syndrome, plasma production rates of testosterone were normal but secretion rates of estradiol, presumably from the testes, were increased. In either situation there is an excess in estradiol production relative to the quantity of testosterone produced.
Four male children and two teenage boys with bilateral high intra-peritoneal testes were studied for pre-operative localization, and for surgical transfer to the scrotum.

For localization EMI scan, testicular venography, and ultrasound were sometimes correct but usually unreliable, time-consuming, and misleading. Laparoscopy, which has not been previously utilized for this condition, was completely accurate in all six cases for precisely localizing the position of the testicle for surgical planning. Furthermore in unilateral cases of non-palpable testicle (where endocrinologic testing isn't possible), laparoscopy definitely determined whether or not a testicle was present without having to resort to open surgery.

In the six cases of bilateral high intraperitoneal testes, autotransplantation to the scrotum was carried out by dividing the spermatic vessel attachment. On one side they were microsurgically reconnected to the inferior epigastrics. On the other side reliance was placed solely on collateral circulation (as is presently recommended in the literature). Atrophy never occurred on the microsurgically revascularized side but did occur on the other side.

Thus laparoscopy should be utilized diagnostically in cases of non-palpable testes, unilateral and bilateral. Microsurgical revascularization of the autotransplanted testicle is safer than relying on collateral circulation via the vas and deferential vessels.
Bilateral scrotal exploration and testis biopsy performed in 100 male partners of barren marriage in which at least 3 semen samples revealed azoospermia or severe oligospermia, <10 million cells/cc. The serum FSH level was normal or < twice normal in all explored subjects.

Sixty-four patients had evidence of gross epididymal pathology which included cystic lesions (36), dysplasia (10), obstructed tubules (9), fatty infiltration of the caput (7), and miscellaneous lesions (2). Representative photographic documentation of each type of lesion will be presented.

The histological results of testes biopsy revealed a normal pattern (12), maturation arrest (48), hypopcellular tubules (9), disorganization with premature sloughing (19), germinal cell aplasai (9), and hyalinization (3).

The discovery of such a significant degree of gross epididymal pathology confirms our impression that evaluation of the male factor in patients with severe oligospermia should include formal scrotal exploration and not just a "window" testes biopsy. A correlation of subsequent results of medical and surgical therapy in these patients revealed only 2 of 64 couples in which epididymal pathology was evident that conception occurred.
Variation of sperm counts and sperm motility for oligozoospermic cases were studied before and after administration of methylcobalamin (CH$_3$-B$_{12}$). It is reported that CH$_3$-B$_{12}$ promotes protein metabolism in nerve cells, but action to germ cells has not been made clear. Fructose, zinc, prostatic acid phosphatase (PACP), and vitamin B$_{12}$ (V-B$_{12}$) in seminal plasma were measured, and testicular biopsies were performed. CH$_3$-B$_{12}$ was orally administered 1,500 micrograms/day for 3 months. A) Variation of total sperm counts: By administration of CH$_3$-B$_{12}$, cases whose total sperm counts had increased were 66/87 (71.2%), especially the cases whose total sperm counts had increased more than 100 million were 32/87 (36.8%). Previous value of fructose concentration in cases whose total sperm counts had increased more than 100 million was 280±18 (Mean ± SE) mg/100ml, while in the cases whose total sperm counts had not increased, the previous value was as high as 375±26 mg/100ml. Previous value of zinc concentration was low in the cases whose sperm counts had not increased, but previous value of PACP did not show appreciable difference in both groups. B) Variation of sperm motility: Cases whose sperm motility had increased more than 55% were 49/94 (52.1%). However, no remarkable difference in fructose, zinc, PACP, and V-B$_{12}$ in seminal plasma was found in the cases whose sperm motility had improved and those where it had not.

Spermatogenesis was improved in some cases after administration of CH$_3$-B$_{12}$, therefore histological findings of testicular tissue before and after administration of CH$_3$-B$_{12}$ will also be reported.
37. EFFECT OF SLEEP DEPRIVATION ON THE PITUITARY-TESTIS AXIS IN HEALTHY MEN.


Sleep disturbance is an extremely common complaint encountered in the medical field. However, there are no endocrine studies describing the association between sleep deprivation and gonadal physiology. This report represents an acute study on such association. Thirteen clinically healthy men of normal weight, aged 20 to 26 years, volunteered for the experiment; all had sleep pattern of 6 hrs minimum for the last six months. Phase I: single blood samples were obtained at 8:00 am in each subject to measure Testosterone (T), Androstenedione (A), Dihydrotestosterone (DHT) (pg/ml), Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Prolactin (PRL) (ng/ml). They were restless during the following 24 hrs working, reading, writing, etc., with free intake of meals and fluids. Phase II: a second blood sample was obtained the following morning at 8:00 am. Phase I & II were compared (X ± SD) T: 6680 ± 1765 vs 4646 ± 1434 (P < 0.01); A: 1411 ± 480 vs 950 ± 390 (P < 0.01); DHT: 511 ± 154 vs 376 ± 163 (P < 0.01); FSH: 121 ± 42 vs 136 ± 76 (P > 0.05); LH: 88 ± 24 (P > 0.05) and PRL: 4 ± 2 vs 4 ± 2 (P > 0.05). The results show a parallel decrease of each androgen and none of FSH, LH, or PRL. Perhaps the changes in testicular steroidogenesis consecutive to acute sleep deprivation are related to biogenic amine neurotransmitters in connection to higher cerebral structures.
38. STIMULATION OF SPERM MOTILITY INITIATION BY A SMALL PEPTIDE SECRETED BY THE RAT CAUDA EPIDIDYMIS IN VIVO. P.Y.D. Wong, A.Y.F. Tsang, W.M. Lee and C.M. Li. Department of Physiology, Faculty of Medicine, University of Hong Kong, Hong Kong.

Pure secretion of the rat cauda epididymidis has been collected by luminal perfusion of the rat epididymis in vivo. When subjected to gel filtration on Sephadex G-10, the secretion yielded two major fractions, one of which contained proteins. The other fraction separated out has molecular weight below 700. This small molecular fraction reacts weakly with ninhydrin but strongly with chlorine, indicating that it may contain peptides. Paper chromatography of this fraction sprayed with ninhydrin revealed one spot before and four spots after acid hydrolysis. Using the Micro Kjeldahl method to determine total nitrogen, it was calculated that the rat cauda epididymal fluid contains about 6 mg/ml and 0.54 mg/ml nitrogen derived from the proteins and small molecular weight fraction respectively.

The effect of the small molecular weight fraction on epididymal spermatozoa has been studied. Spermatozoa flushed out from the rat cauda epididymidis with a sodium free medium exhibited a transient motility. After 40 min., the forward motility was completely suppressed. However, if the "arrested" spermatozoa were resuspended in a sodium containing medium, their forward motility was completely restored within 15 min. This motility reinitiation required extracellular calcium (1.27 mM). It was found that the small molecular weight fraction could substitute calcium in sperm motility initiation. This effect was concentration dependent. Other biologically active peptides could not mimic the effect of the epididymal peptide in initiating sperm motility. It is concluded that the small molecular weight fraction (peptide) secreted by the rat cauda epididymidis in situ may play an important role in the initiation of sperm motility.

(Supported by the Committee of the Medical Faculty Research Grant Fund, University of Hong Kong).

Washed human spermatozoa were capacitated in vitro in medium (BWW) containing pyruvate (0.25mM) and lactate (21.6mM) as energy sources. Fertilizing ability was assayed after various incubation periods (0-22h) using zona-free hamster eggs. Differences among donors were found with regard to the minimum time required to achieve fertilization as well as changes over time in both the % fertilization and polyspermy. Most samples fertilized to a very low extent (0-10%) with 0-4h of incubation and showed maximal fertilizing ability with 10-22h of incubation. However, samples from some donors capacitated more rapidly and reached maximal fertilizing ability after 2-6h of incubation. Mean fertilization rates samples incubated in the presence of glucose were 17%, 66% and 71% when assayed after 6, 10 and 18-22h of incubation respectively. These rates were significantly higher ($P<0.01$, $n=5-7$) than those observed when the same samples were incubated without glucose (2%, 14% and 20% respectively) or when fructose was substituted for glucose (22% at 10h and 44% at 18-22h). While the % of motile sperm was not affected by the presence or absence of sugar, both the quality of motility and grade of forward progression were significantly higher in the presence of glucose than with fructose or in the absence of glycolysable substrate. Caffeine, which increases glycolysis in sperm, stimulated fertilization after 6h of exposure ($P<0.05$, $n=6$); fertilization averaged 41% in the presence of caffeine (7mM) compared with only 18% in the controls. The caffeine effect was not observed in the absence of glucose. These results suggest that exogenously supplied glucose enhances in vitro capacitation of human sperm and that fructose, the major sugar in seminal fluid, is less effective in this regard.
40. STEROIDOGENIC ENZYME ACTIVITY IN DIFFERENT POPULATIONS OF LEYDIG CELLS.  

Previous studies in this laboratory have revealed that there are two distinct populations (I and II) of Leydig cells present in the rat testis (Payne et al. Endocrinology 106, 1424). Although LH receptor concentrations (as measured by $^{125}$I-hCG binding) are identical in the two populations, testosterone (T) production in response to hCG is markedly higher in population II than population I. The purpose of this investigation was to determine if the activities of any of the enzymes that convert pregnenolone (Preg) to T differ between the two populations. Cells from collagenase-treated testes were centrifuged on a discontinuous metrizamide gradient (5 ml 27%, 10 ml 20% and 10 ml 11% metrizamide). Fractions (1 ml each) 10 to 12 from the top of the gradient were pooled as population I and fractions 20 to 24 were pooled as population II. $\Delta^5$-3$\beta$ hydroxysteroid dehydrogenase-isomerase ($3\beta$-HSD) activity was measured in cellular homogenates while 17-hydroxylase (17OHase) and C$_{17}$-C$_{20}$ lyase activities were measured in whole cells during a 1 hr incubation with saturating concentrations of $[^3H]$ labeled substrates. Products were separated by thin layer chromatography. Using 5 $\mu$M Preg, $3\beta$-HSD activity was significantly greater in population I than population II (16.7 pmol/10min/10$^3$ cpm $^{125}$I-hCG binding vs 7.96 pmol). In contrast, both 17OHase and C$_{17}$-C$_{20}$ lyase activities were greater in population II than population I. Using 3 $\mu$M progesterone as substrate, 17OH progesterone as substrate, C$_{17}$-C$_{20}$ lyase activity was 1.04 pmol/hr/10$^3$ Leydig cells in I and 3.76 pmol in II. These data indicate that 1) $3\beta$-HSD is not a limiting enzyme in the mature rat and 2) the markedly higher production of T in response to hCG in population II, compared to population I, may be at least partly the result of greater amounts of 17OHase and C$_{17}$-C$_{20}$ lyase in population II.
Acrosin, an acrosomal proteinase, has been proposed as the lysin responsible for spermatozoon penetration of the zona pellucida during fertilization. From morphological and biochemical evidence, it has been inferred that the zona lysin must remain adherent to the inner acrosomal membrane (IAM) during zona penetration. The objective of this research was to determine the ultrastructural location of acrosin using immunocytochemical techniques. Antibodies to highly purified porcine acrosin were produced in rabbits, and (Fab')₂ fragments of the purified IgG fraction were conjugated with horse radish peroxidase (HRP). Utilizing a direct labeled antibody technique, porcine sperm were washed, prefixed (0.1% formaldehyde), and resuspended in buffer containing HRP labeled antiporcine acrosin. The labeled antibody-antigen complexes were rendered electron dense by reaction with diaminobenzidine (DAB), and after washing, were secondarily fixed with the glutaraldehyde and osmium tetroxide. Electron microscopic examination of the stained sperm revealed HRP label associated with the outer acrosomal membrane (OAM) of intact sperm. In degenerate sperm displaying detachment of the superficial acrosomal structures, DAB reaction product was distributed along the IAM, which remained closely apposed to the underlying nuclear structure. In some cells, labeling was also observed throughout the acrosomal matrix. These results provide evidence that acrosin is associated with both the OAM and IAM. Localization of acrosin on the IAM is consistent with the hypothesis that acrosin is the spermatozoon lysin responsible for zona penetration. Furthermore, its presence on the OAM leaves open the possibility that acrosin participates in physiological events other than zona penetration.
THE IN VIVO TRANSFER OF VARIOUS MOLECULES ACROSS THE EPITHELIUM OF THE RAT CAPUT EPIDIDYMIS. B.T. Hinton and S.S. Howards. Depts. Urology & Physiology, University of Virginia School of Medicine, Charlottesville, Virginia 22908

Since the rat caput epididymidis appears important for sperm maturation and there have been no reported studies of transepithelial transport in this epididymal segment we investigated the in vivo transfer of $^3$H-H$_2$O, $^{14}$C-urea, $^3$H-polyethylene glycol (PEG), $^3$H-inulin, $^{125}$I-bovine serum albumin (BSA) and $^3$H-3-O-methylglucose ($^3$H-3OMG, a non-metabolisable analogue of glucose) across the epithelium of the rat caput epididymis using micropuncture techniques. Adult male rats were anaesthetised with urethane or inactin, cannulae inserted into the jugular vein and carotid artery, both kidneys tied-off and the animal prepared for micropuncture. 1mCi of isotope was injected into the jugular vein. At different times thereafter, a blood sample was collected via the carotid artery and a sample collected from the caput epididymidis. Samples were centrifuged and the radioactivity in the supernatant determined by liquid scintillation counting. Results were expressed as cpm in luminal fluid/cpm in blood x 100 = %. $^3$H-H$_2$O and $^{14}$C-urea equilibrated between blood plasma and luminal fluid within 20 min and 2 h respectively. $^3$H-PEG, $^3$H-inulin and $^{125}$I-BSA entered the lumen very slowly reaching less than 5% of blood value after 3 h; $^3$H-inulin remained less than 7% after 18 h. $^3$H-3OMG reached 25% after 3 h. We suspect that these compounds, except $^3$H-3OMG, entered the lumen by simple diffusion and $^3$H-3OMG via facilitated diffusion. The latter hypothesis was tested by rendering a group of rats hyperglycaemic (blood glucose 4-5x normal concentration) and observing the transport of $^3$H-3OMG. The results showed that the transport of $^3$H-3OMG was reduced by approximately 50% of the control. We suggest that glucose probably enters the caput epididymis by a facilitated diffusion mechanism.

(BTH is a recipient of a Rockefeller Foundation Fellowship).
Knowledge of Sertoli cell architecture has primarily come from silver staining techniques (Elftman, 1963) and interpretation of random thin sections. While the Sertoli cell is generally thought to be a columnar cell with numerous processes, there is no information relative to its dimensions, configuration, or relationships to other cells. Using the methodology previously described (Wong et al., 1981), the reconstructed Stage V Sertoli cell extended, with respect to the tubule, 89.8 \( \mu \text{m} \) in its centripetal axis, 41.9 \( \mu \text{m} \) in its longitudinal axis, and 29.5 \( \mu \text{m} \) in its circumferential axis. The volume of the cell, as measured by water displacement of the model, was 6,012 \( \mu \text{m}^3 \). The cell demonstrated a rounded base which rested on the basal lamina, a short supranuclear thickened region, numerous lateral and apical processes. Some lateral processes at the level of the blood-testis barrier were finger-like and stretched between several adjacent Sertoli cells. Other lateral processes at a higher level were short and primarily in the form of outwardly directed cups showing some deficiencies in their walls. They partially encompassed pachytene spermatocytes and the early generation of spermatids, although some of the latter were not related to the laterally directed cups. The apical processes were long (65 \( \mu \text{m} \)) and thin and related to elongated germ cells. These germ cells were completely enveloped by the apical processes with their heads embedded in the supranuclear thickening of the Sertoli cell. The apical processes extended as flasks with their widened ends ending luminally. Ten elongated step 17 spermatids were exclusively associated with the cell studied. Round adluminal compartment germ cells were shared between adjacent Sertoli cells. Twenty-six step 5 spermatids and eight pachytene spermatocytes were related to the reconstructed cell. Basally, two type B spermatogonia were in contact with the cell. This study confirms the non-syncytical nature of the Sertoli cell.
In the literature, there are many conflicting reports concerning the effects of hypoxia on the male gonad. Groups of three months old hamsters were submitted to a simulated altitude of 4400 m (pO₂ = 92 mm Hg) in an open-flow, hypobaric chamber kept at 22-26°C. After 9 (A) or 18 (B) days of exposure, animals were killed at 0, 9, 18 and 27 days thereafter. In (A), only hematological changes (hemoglobinemia and hematocrit increase) were recorded 9 days after exposure. In (B), body weight decreases up to 18 days post-exposure, with apparently no damage to the hypothalamic-hypophyseal-gonadal axis, since androgenic indicators do not change. An increase in the number of spermatozoa counted in the cauda epididymis 9 days after exposure led to the assumption that anticipated spermatization has occurred. This idea has been confirmed by radioautography which shows passage of step 14 to 17 spermatids into the epididymis. This is associated to epithelial vacuolization and adluminal cell exfoliation between 0 and 9 days after exposure. However, the general respiratory pattern of a mitochondrial-enriched testicular fraction does not show significant alterations of cytochrome oxidase activity. Essentially similar observations have been done in the mouse under the same experimental conditions. It is suggested that the hypoxic damage is mostly restricted to the Sertoli cells and hence, in our experimental set-up, the damage is basically reversible.

Cytoplasmic filaments are present in all eukaryotic cells; in non-muscle cells it is likely that these filaments provide the motive force for cell motility and active shape changes. Four types of intracellular filaments have been described: actin, 5-7 nm; myosin, varying thickness; intermediate filaments, 9 to 11 nm; microtubules, 24 nm. The Sertoli cell is exceptionally well endowed with cytoplasmic filaments. These are located in the following specific areas: 1) subjacent to the plasma membrane in the region of the tight junctions; 2) surrounding the head of acrosomal and maturation phase spermatids; 3) circumscribing the nucleus; 4) in the apical trunk portion and cytoplasmic extensions of the cell. These filaments are being characterized in cultured Sertoli cells and "in vivo". Sertoli cells "in vitro" maintain the general cytoskeletal organization observed "in vivo", but in addition, develop well defined stress fibers and cortical bundles of filaments. The stress fibers and cortical filaments contain myosin as demonstrated by the peroxidase-antiperoxidase immunocytochemical method using antiserum raised against platelet or smooth muscle myosin. Actin was demonstrated in these same regions using the S1 subfragment of rabbit skeletal muscle myosin. The S1 subfragment specifically binds to actin filaments to form characteristic arrowhead complexes. Filaments near the Sertoli tight junctions also decorate with the S1 subfragment and exhibit an arrowhead polarity directed away from the membrane. The perinuclear zone of filaments is of two types: 1) filaments that decorate with S1, and 2) ten nm filaments that do not decorate. The latter are believed to be intermediate filaments of the "vimentin" type. The 10 nm filaments (vimentin) are also located in the trunk portion of the cell. The presence of this elaborate filamentous network in Sertoli cells undoubtedly forms the structural basis for the dramatic changes in Sertoli cell shape and cytoplasmic organization that occur during spermatogenesis.

(Supported in part by NIH research grant HD 06969).
46. TESTOSTERONE CONCENTRATIONS IN THE INTRALUMINAL FLUIDS OF THE MALE RAT AND HUMAN REPRODUCTIVE TRACT. Terry I. Turner* and Charles S. Singhaus*, Departments of Urology* and Obstetrics and Gynecology*, School of Medicine, University of Virginia, Charlottesville, Virginia.

Testosterone (T) concentrations existing specifically in the intraluminal fluids of the testis and epididymis have not received comprehensive examination. Adult male Sprague-Dawley rats were assigned to one of four groups; a control group or a group receiving bilateral efferent duct ligation (EDL) either 4 hrs., 24 hrs., or 96 hrs. prior to micropuncture of the testicular and epididymal tubules. Micropuncture was performed to obtain seminiferous tubule fluid (SNF), rete testis fluid (RTF), caput epididymal fluid (CPF), cauda epididymal fluid (CDF), testicular arterial serum (TAS), and testicular venous serum (TVS). Cardiac blood was obtained by direct cardiac puncture. Cell-free fluids were obtained by centrifugation. All samples were analyzed for T by RIA. Inter- and intra-assay variability were 4% and 7%, respectively. Control group T concentrations (mean ± S.E., ng/ml) for cardiac blood (5.7 ± 1.0), SNF (52.1 ± 9.2), CPF (57.1 ± 12.0), were not significantly altered by EDL at any time period studied. T concentrations in control TVS (138.5 ±48.1) were significantly higher than in any other control fluid collected. RTF contained significantly less T than other control reproductive tract fluids. The persistence of high intraluminal T concentrations in the caput 4 days post EDL (thus, presumably in the absence of ABP) is indicative of either active transport of T from blood or of epididymal synthesis of testosterone. Micropuncture samples from human males ranging widely in physical condition yielded preliminary values of 44.5 ± 11.4 (n=4), 63.9 ± 16.4 (n=7), and 23.0 ± 5.05 (n=20) nanograms/ml for the lumen fluids of the caput, corpus, and cauda epididymidis respectively. One SNF sample and 6 RTF samples resulted in values of 327.8 and 238.4 ± 81.7 nanograms/ml. These data repeat the pattern seen in rats where RTF contained less T than SNF and caput T concentrations were higher than cauda T concentrations.
The ability of testosterone (T) to exert negative feedback action on luteinizing hormone (LH) secretion was examined in four long-term (chronic, 6 mo) castrate rams and in four rams that were not castrated until 2 wk after T replacement had begun. The 15 mo old animals were housed in a controlled environment and exposed to stimulatory short photoperiods (8L:16D). Each animal was subsequently implanted with five subdermal Silastic capsules containing T. As reported previously for castrate rams (Schanbacher, J. Androl 1980; 1:121), these implants provide physiological concentrations (4 ng/ml) of serum T. Serum LH was determined immediately before T replacement therapy began (0 wk) and 2, 4, 5, 6, and 7 wk later by averaging concentrations found during an intensive (30 min x 6 h) bleed. Values for individual chronically castrated (CC) rams and mean (+ SE) values for the four acutely castrated (AC) rams are shown below:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 wk</th>
<th>2 wk</th>
<th>4 wk</th>
<th>7 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC1</td>
<td>19.2</td>
<td>20.7</td>
<td>17.7</td>
<td>21.0</td>
</tr>
<tr>
<td>CC2</td>
<td>14.4</td>
<td>15.6</td>
<td>12.0</td>
<td>9.2</td>
</tr>
<tr>
<td>CC3</td>
<td>8.8</td>
<td>8.6</td>
<td>7.8</td>
<td>5.7</td>
</tr>
<tr>
<td>CC4</td>
<td>13.0</td>
<td>11.3</td>
<td>4.5</td>
<td>1.0</td>
</tr>
<tr>
<td>AC5-AC8</td>
<td>1.2 ±0.1</td>
<td>0.9 ±0.1</td>
<td>0.9 ±0.05</td>
<td>0.3 ±0.02</td>
</tr>
</tbody>
</table>

In addition, LH response to a 450 ng injection of luteinizing hormone releasing hormone was determined after 7 wk of T replacement therapy. LH response (maximum value minus preinjection value) was proportional to the preinjection concentration. CC rams 1 and 3 yielded an LH response of 43.8 and 17.6 ng/ml, respectively; whereas AC rams had low preinjection LH concentrations and yielded smaller LH responses (2.8 to 9.9 ng/ml). These data demonstrate 1) the efficacy by which T can inhibit LH secretion in acutely castrated males, and 2) a loss in sensitivity to T feedback in a proportion of chronically castrated males.

Two experiments were conducted with adult rams prior to the fall ovine-breeding season to investigate primarily seasonal changes in the relationship between LH and testosterone (T) secretion. Experiment 1 involved injecting (iv) 8 rams with either 10 µg NIH-LH-S18 or 1 ml vehicle every 80 min for 56 h (June); rams were bled by venipuncture from the jugular vein every 20 min during h 1-8 (day 1), 25-32 (day 2), and 49-56 (day 3). The serum LH profile for treated rams was characterized by 5.9 ± 0.1 peaks/8h averaging 4.3 ± 0.4 ng NIH-LH-S14/ml; in contrast, control rams exhibited 1.8 ± 0.3 peaks/8h averaging 8.2 ± 2.3 ng/ml. Increasing LH peaks to a breeding-season frequency resulted in a progressive 3-fold increase in circulating T with mean levels on day 3 (12.6 ± 1.2 ng/ml) reaching 50% of those observed for these rams in the fall (Oct.); increments in serum T in response to individual LH injections averaged 3.5 ± 0.8 and 6.7 ± 0.7 ng/ml on days 1 and 3, respectively. In Experiment 2, 8 rams were injected (im) with either 5 mg T or 1 ml vehicle every 4h for 4 days (Aug.). Rams were bled every 20 min for 12h following 36 (day 2) and 84 (day 4) of treatment; three 10ug doses GnRH were given (iv, one every 20 min) during h 9. Mean serum T averaged 9.8 ± 2.2 ng/ml for control rams and was elevated to 24.4 ± 1.4 ng/ml for treated rams, a concentration comparable to values obtained for these rams in Oct. Elevation of circulating T to peak breeding-season levels suppressed LH to undetectable levels by day 4 in 3 of the 4 rams. While LH-peak frequency was maintained at 2.5 ± 0.8/8h in control rams, frequency decreased progressively to nearly zero by day 4 in treated rams; however, this change was not associated with a significant decrease in the LH response to exogenous GnRH. Circulating levels of FSH and PRL were always comparable for both groups of rams in both experiments. Results indicate (1) the importance of seasonal increases in spontaneous LH release in promoting T secretion, and (2) a heightened responsiveness of the hypothalamic-pituitary axis to T during the non-breeding season.

(Supported by the MRC of Canada).
We have previously reported that prolactin (PRL) and PRL-producing ectopic pituitary grafts increase synthesis and release of FSH in male hamsters and mice. This effect of PRL is not mediated by the testes or the adrenals and presumably involves a direct effect of PRL on the hypothalamic-pituitary unit or on higher brain centers. In an attempt to distinguish between these possibilities we have examined the effects of a transplant of one pituitary from a female hamster placed under the kidney capsule on plasma LH and FSH levels in hamsters with anterolateral hypothalamic deafferentation and in sham-operated control animals. Deafferentation produced a significant decline in plasma LH levels ($P < 0.02$) and apparent decrease in the weight of the testes and the seminal vesicles and no change in plasma FSH levels. In each of seven control animals pituitary transplantation was followed by an increase in plasma FSH levels ($134 \pm 22 \text{ vs } 379 \pm 46 \text{ ng/ml}; P < 0.01$). In contrast in seven deafferented hamsters, plasma FSH levels were not affected by pituitary transplantation or by graft removal. Plasma LH levels were not altered by pituitary transplants in either control or deafferented animals. The hypothalamic-pituitary unit devoid of its normal connections with other parts of the brain fails to respond to PRL by increasing the release of FSH. This would seem to indicate that the ability of PRL to stimulate FSH release is due to its action at a suprathyroidal site. In support of this conclusion, we have observed that FSH release in response to exogenous LH in male hamsters is not affected by ectopic pituitary grafts.

Testes of several vertebrate species are known to secrete estrogens, yet the short-term regulation of estrogen secretion within the testis has not been well defined. We have used the in vitro perfused testis as a model to study estrogen secretion qualitatively and quantitatively in five common laboratory species. One of these species (rabbit) was then used to study the hormonal regulation of estradiol secretion by the in vitro perfused testis. Testes from mature rats (R), rabbits (B), hamsters (H), guinea pigs (G) and dogs (D) were perfused with added LH and FSH, and aliquots of the effluents were extracted and separated by high-performance liquid chromatography. Immunoreactive estrogens were quantified in 35 1-ml fractions by means of a "total-estrogen" antiserum (Abraham's S310-5). The major estrogen secreted by all five species was estradiol-17\(\beta\) (E\(_2\)); lesser amounts of estrone were also present. E\(_2\) was quantified in effluents of perfused testes (n=6 for each species) stimulated with LH and FSH. E\(_2\) secretion rates averaged 279\(\pm\)55, 835\(\pm\)46, 226\(\pm\)33, 473\(\pm\)70, and 565\(\pm\)149 pg/g testis/h for R, B, H, G, and D, respectively. Corresponding (T) secretion rates were 2.8\(\pm\)3, 6.4\(\pm\)1.1, 1.3\(\pm\)2, 5.4\(\pm\)4, and 2.4\(\pm\)3 \(\mu\)g/g/h. Rabbit testes perfused with 100 ng NIH-LH-S21/ml of medium secreted more E\(_2\) (p<.05) than unstimulated testes (926\(\pm\)93 vs 323\(\pm\)52 pg/g/h), and more (p<.05) T (6.76\(\pm\)53 vs 0.04 \(\pm\)0.01 pg/g/h). NIH-FSH-S10 at 100 ng/ml of medium had no significant effect on E\(_2\) secretion. The pattern of change in E\(_2\) secretion over time after stimulation with LH was coincident with that for T secretion. Testosterone perfused into rabbit testes mimicked the stimulatory effect of LH on E\(_2\) secretion and was dose-dependent up to 100 ng/ml of medium. E\(_2\) secretion at saturating T concentrations (10 \(\mu\)g/ml) was no greater than that observed for LH-stimulated testes. In the presence of saturating T concentrations, neither LH nor FSH stimulated E\(_2\) secretion further. We conclude that E\(_2\) secretion in the short-term is a function of substrate availability.
Opioid peptides have an inhibitory action on GnRH release in man. This observation is derived in our studies from the following findings: 1) a case of congenital indifference to pain was studied. Opioid levels were very high in the CSF. Gonadotropins were undetectable. Following administration of naloxone (a pure antagonist of opioids) the levels of LH returned to within the normal range. In basal conditions GnRH induces a mild response, whereas under naloxone infusion the LH response was normal; 2) in a group of controlled morphine addicts hypogonadotropinism was found with a slight response to GnRH test. Priming test with two subsequent doses of GnRH (respectively 25μg and 60 minutes later 100μg) normalizes the GnRH response; 3) in four normal subjects given Naloxone infusion iv (0.1 mg/kg/h) statistically significant increase in LH was found. Administration of GnRH during the infusion led to an exaggerated response in terms of LH.

We suggest that a "self priming action" by the endogenously produced GnRH occurs in these patients on account of the blocking of opioid peptide receptors induced by Naloxone.
We have previously shown that immunization of male rats with LHRH results in decreased serum gonadotropins. The purpose of the present work was to analyze the effect of anti-LHRH serum (ALS, 772) on LH release after exogenous LHRH in different male rat preparations. Immature rats (24 days old) and intact or castrate adult rats were injected (iv) at 9 AM with normal sheep serum (NSS) or with ALS. At 2 PM LHRH (150 or 400 ng) was injected sc and jugular blood collected up to four hrs later. In all NSS-treated animals LH peaked early after LHRH and then fell. ALS (0.5 ml) blocked LH release after 150 ng LHRH through three hrs in immature males but could neutralize the effect of 400 ng LHRH for only two hrs. Contrary to the response seen in immature animals, ALS (1 ml) did not completely block LH after 150 ng LHRH in intact rats. LH was higher in these animals at two hrs than in controls. In castrate males, LH release after 150 ng LHRH was similar to that in immature males. Injection of ALS-treated castrate animals with 400 ng LHRH produced an attenuated but broad peak of LH lasting through four hrs. In an attempt to reverse the effect of castration on LH release in ALS animals, castrated rats were pretreated for 48 hrs with testosterone propionate (1.5 mg) and 17β-estradiol (50 μg). At the time of serum injection, the animals were also injected sc with 0, 60 or 600 μg of testosterone in oil and the response to LHRH studied as before. In all NSS animals, LH was low at two hrs after 150 or 400 ng of LHRH. As in castrate animals not exposed to exogenous steroids, LH levels in ALS animals were not different from control rats after 150 ng LHRH. The injection of 400 ng LHRH caused a sustained rise in LH. Acute treatment with testosterone or pretreatment with steroids had little effect on LH levels. Therefore, the prolonged high levels of LH seen in castrate male rats treated with ALS and 400 ng LHRH were not prevented by steroid treatment.

(Supported by NIH HD-8-2819, AN 09094 and the VA).
A SIMPLE NON-CHROMATOGRAPHIC RADIOIMMUNOASSAY METHOD FOR QUANTITATION OF DIHYDROTESTOSTERONE. C.P. Puri*, V. Puri and T.C. Anand Kumar. Experimental Biology Unit, All India Institute of Medical Sciences, New Delhi-110029, India

Since the antiserum used to measure dihydrotestosterone (DHT) has considerable cross-reaction with testosterone (T), the chromatographic separation of DHT from T is a prerequisite to get precise estimate of DHT levels in biological fluids. To simplify this procedure a non-chromatographic method for the estimation of DHT is reported. The treatment of T with 0.5% aqueous solution of potassium permanganate (KMnO₄) for 30 min., at room temperature, selectively destroys its cross-reactivity with antiserum raised against T-3(carboxy-methyl) oxime-BSA. Whereas, thin layer chromatographic mobility of DHT in benzene:ethyl acetate (2:1) solvent system and its cross-reaction with T-antiserum remain unaffected (50%) following treatment with KMnO₄. Thus by treating the ether extracts of biological samples with KMnO₄ the endogenous T is inactivated and a simple non-chromatographic method is validated for quantitating DHT by radioimmun assay. In this assay procedure a linear dose-response curve (10-400 pg) is obtained on a logit-log transformation. The serial dilutions of pools of serum samples from adult rhesus monkeys show dose-response curves that are parallel to the standard curve. The with-in and between assay variation is invariably 10% and almost 100% of the added DHT is recovered from the serum sample. The correlation coefficient of DHT estimates obtained after chromatographic (sephadex LH-20) separation of DHT or following KMnO₄ treatment of serum samples is 0.99. Using this method, the mean serum levels of DHT measured in 20 adult male rhesus monkeys are significantly (p<0.05) higher at night (5.38 nmol/l) than during the day (3.22 nmol/l). This non-chromatographic method precisely and specifically measures DHT and thus should be useful to other investigators.

*Present address: Washington University School of Medicine, Dept. OB/GYN, St. Louis, MO 63110
54. VARIATION OF HUMAN FERTILIZATION USING THE IN VITRO FERTILIZATION ASSAY.

Barbara J. Bentwood, Jane Rogers and Cathy McCarville. University of Hawaii, Dept. of Obstetrics and Gynecology, Kapiolani Hospital, Honolulu, Hi. 96826

The in vitro fertilization of zona-free hamster eggs by human spermatozoa has been utilized both as a biological tool for the study of capacitation and as a clinical assay for infertility. With repeated testings we have observed large variations in the %fertilization (%F) in many men. The present study was initiated to define the possible sources of this variation. First, the internal consistency of the procedure of Rogers et al. (1979) was examined.

Semen samples were tested by two technicians using separately prepared eggs. In 24 experiments where each tech scored the same number of eggs \( \pm \), the standard error of the reported means ranged from 0-15% averaging ±4%. These results were good evidence for internal consistency in the assay. Next, possible differences in the specimens between repeat tests were considered. A group of randomly chosen men (n=27) were each tested on 5 occasions over a period of 1 year. For each man a mean %F ± SE was calculated. Eight men fertilized between 10 and 100% in all 5 tests with a group average mean of 30%. These 8 had the lowest values for their individual coefficients of variation (CV). Four men showed fertilization below 10% in all 5 tests, an average mean of 3% and high CV values (due to the small %F numbers). Fifteen men fertilized 0% in at least 1 test but varied as a group to maximums of 37-100%. The CV values were higher than those of the 10-100% group. These 15 men were also the most inconsistent subjects regarding abstinence prior to testing. This correlation was examined using selected donors with controlled abstinence times from 12 hours to 4 days. Critical times were shown within which each man's %F was significantly lowered below his usual maximum. When the abstinence was carefully controlled, the reproductibility for each man was within the reliability range of the assay. These results demonstrate the importance of repeated testing of apparently infertile patients. Reproductibility can be enhanced when attention is paid to a patient's abstinence time prior to each fertilization test.
Panayotis C. Apostolidis, M.D.
Suite 100
Landenau Medical Building
Philadelphia, PA 19151

Rodney A. Appell, M.D.
Dept. of Urology
L.S.U. Medical Center
New Orleans, LA 70112
Tel. (504) 568-4890

Ber Aron, M.D.
19241 Montgomery Village Ave
Gaithersburg, MD 20760

Ricardo H. Asch, M.D.
Dept. OB-GYN
University of Texas Health Science Center
7703 Floyd Curl Drive
San Antonio, TX 78284
Tel. (512) 691-7279

Dr. Baccia M. Baccetti
Institute of Zoology
University, Via Mattioli, 4
53100 Siena, ITALY
Tel. 0577/284173

Jerald Bain, M.D.
Mount Sinai Hospital
600 University Avenue
Toronto, Ontario M5G 1X5
CANADA
Tel. (416) 594-8436

Klaus Bandhauer, M.D.
Direktor der Urologische
Klinik Kantonspital
CH 9007 St. Gallen
SWITZERLAND
Tel 26-11-11

Wayne J. Barcellona, Ph.D.
Dept. of Biology
Texas Christian University
Fort Worth, TX 76129
Tel. (817) 921-1760

Raymon Bardia-Deu, M.D.
Apartado de Correos 276
Ciudad Bolivar, Bolivar
VENEZUELA
Tel. (085) 22979

Arnold B. Barr, M.D.
U.S. Public Health Service Hosp.
Staten Island, NY 10304
Tel. (212) 447-3010

Andrzej Bartke, Ph.D.
Department of Ob/Gyn
University of Texas
Health Science Center
7703 Floyd Curl Drive
San Antonio, TX 78284
Tel. (512) 691-6677

B. Norman Barwin, M.D.
Ottawa General Hospital
43 Bruyere Street
Ottawa, Ontario K1N 5C8
CANADA

Michael J. Bedford, D.V.M., Ph.D.
Dept. of Obstetrics/Gynecology
Cornell Medical College
515 East 71st Street
New York, NY 10021

Alain Belanger, Ph.D.
Molecular Endocrinology Laboratory
Le Centre Hospitalier de l'Universite Laval
2705 Laurier Boulevard
Quebec, G1V 4G2, CANADA
Tel. (418) 656-8253

Arnold M. Belker, M.D.
250 S. Liberty
Louisville, KY 40202
Tel. (502) 584-8051

Anthony A. Bellve, Ph.D.
Laboratory of Human Reproduction
and Reproductive Biology
Harvard Medical School
45 Shattuck Street
Boston, MA 02115
Tel. (617) 732-2070

Alan K. Bennett, M.D.
Head, Division of Urology
Albany Medical College
Albany, NY 12208

Barry B. Bercu, M.D.
4701 Willard Avenue, Apt. 518
Chevy Chase, MD 20015
Tel. (301) 496-1981

Cesar Bergada, M.D.
Talcahuano 1175 - 4. A
1013 Buenos Aires
ARGENTINA
Tel. 41-6248 or -1585

Gerald S. Bernstein, M.D., Ph.D.
Women's Hospital
L.A. County- USC Medical Center
1240 N. Mission Road
Los Angeles, CA 90033
Tel. (213) 226-3091
Lawrence Dubin, M.D.
137 East 36th Street
New York, NY 10016
Tel. (212) 532-0635

Martin Dym, Ph.D.
Department of Anatomy
Harvard Medical School
Boston, MA 02115
Tel. (617) 732-1762

Gabriel N. Egbunike, Ph.D.
Dept of Animal Science
University of Ibadan
Ibadan, NIGERIA

J. Charles Eldridge, Ph.D.
Bowman Gray School of Medicine
Dept. of Physiology and Pharmacology
Winston-Salem, NC 27103
Tel. (919) 748-1624

Rune B. Eliasson, M.D.
Reproductive Physiology Unit
Karolinska Institutet
Faculty of Medicine
S-104 01 Stockholm, SWEDEN
Tel. 08/34-05-60 #1508

LeGrand C. Ellis
Department of Biology
Utah State University
Logan, UT 84322
Tel. (801) 750-2563

Dr. Jean Claude Esperaire
40 Cours de Verdun
33000 Bordeaux, FRANCE
Tel. (56) 44-00-20

Richard F. Emslander, M.D.
Mayo Clinic
Rochester, MN 55901
Tel. (507) 284-2511

Ronald J. Ericsson, Ph.D.
Gametrics Limited
180 Harbor Drive
Sausalito, CA 94965
Tel. (415) 332-3141

T.M. Evans, M.D.
Director, C.S. Mott Center
Wayne State Univ. Medical School
275 East Hancock Avenue
Detroit, MI 48201

R. Donald Eward, M.D.
220 Cherry St., S.E.
Grand Rapida, MI 49503
Tel. (616) 942-5180
Larry Ewing, Ph.D.
Dept. of Population Dynamics
615 N. Wolfe Street
Baltimore, MD 21205
Tel. (301) 955-3106

G. Fabrizio Menchini Fabris, M.D.
via Ridolfi
27-56100
Pisa, ITALY

Mostafa S. Fahim, Ph.D.
Department of Ob/Gyn
University of Missouri
Medical Center
Columbia, MO 65201
Tel. (314) 882-6939

Fereshte Fahimi, M.D.
Department of Ob/Gyn
B316 Clinical Sciences
Michigan State University
East Lansing, MI 48824
Tel. (517) 353-4740

Wells E. Farnsworth, Ph.D.
Dept. of Biochemistry
Chicago Coll. of Osteopathic Med.
1122 East 53rd Street
Chicago, IL 60615

John F. Farrer, M.D.
307 Placentia
Newport Beach, CA 92663
Tel. (714) 646-5073

Don W. Fawcett, M.D.
c/o Deacon
171 Stow Street
Concord, MA 01742

Miguel Fischer, M.D.
1346 Washington Lane
Rybal, PA 19046
Tel. (215) 885-1114

W. Wesbit Fleming, Ph.D.
Curtiss Breeding Service
P.O. Box Y-9
Elburn, IL 60119
Tel. (312) 365-9241

Richard L. Fogel, M.D.
349 E. Northfield Road
Livingston, NJ 07039

Robin G. Foldesy, Ph.D.
Center for Population Research
Room C-2304
Vanderbilt University School of Med.
Nashville, TN 37232

Robert H. Foote, Ph.D.
201 Morrison Hall
Cornell University
Ithaca, NY 14853
(607) 256-2050

Jean L. Fourcroy, M.D., Ph.D.
6310 Swords Way
Bethesda, MD 20034
Tel. (202) 295-1241

Arthur L. Frankel, Ph.D.
Dept. of Biomedical Sciences
State University of New York
Bingham, NY 13901
Tel. (607) 798-2777

Michael J. Free, Ph.D.
Batelle Memorial Institute
Pacific NW Labs.
Battelle Blvd.
Richland, WA 99352
Tel. (509) 375-2211

Ruth Freeman, M.D.
Albert Einstein College of Medicine
1300 Morris Park Avenue
Bronx, NY 10461
Tel. (212) 430-3152

Frederick J. Frenzilli, M.D.
5530 Wisconsin Avenue, Suite 910
Chevy Chase, MD 20015
(301) 552-7872

Matthew Freund, Ph.D.
Department of Physiology
Southern Illinois University
Carbondale, IL 62901
Tel. (618) 453-2583

Jan Friberg, M.D.
5 High Elms Lane
Locust Valley, NY 11560
Tel. (516) 759-9196

Julian Frick, M.D.
Urological Department
Salzburg General Hospital
5020 Salzburg, AUSTRIA
Tel. 31581 #2980

Stanley Friedman, M.D.
921 Westwood Boulevard
Los Angeles, CA 90024
Tel. (213) 477-6765
Kallol Guha, Ph.D.
University of Kuopio
Dept of Anatomy
P.O. Box 140
70101 Kuopio 10, FINLAND

A.W. Gustafson, Ph.D.
Department of Anatomy
Tufts Univ. School of Medicine
136 Harrison Avenue
Boston, MA 02111
Tel. (617) 956-5697

Dr. E.S.E. Hafez
Dept. Obstetrics/Gynecology
Wayne State Univ. School of Med.
Medical Research Building
550 East Canfield
Detroit, MI 48201
Tel. (313) 577-1011

Mary Ann Handel, Ph.D.
Dept. of Zoology
University of Tennessee
Knoxville, TN 37916
Tel. (615) 974-2371

Jack E. Harclerode, Ph.D.
Dept. of Biology
Bucknell University
Lewisburg, PA 17837
Tel. (717) 824-1981

Richard M. Harrison, Ph.D.
Delta Reg. Primate Res. Center
Tulane University
Three Rivers Road
Covington, LA 70433
Tel. (504) 892-2040

M. Aly Hasson, M.D.
Suite 205
Phoenix Medical Centre
2155 Tenth Avenue
Prince George, B.C.
CANADA V2M 5J6

Norman B. Hecht, Ph.D.
Department of Biology
Tufts University
Medford, MA 02155

Jerrold J. Heindel, Ph.D.
Department of Reproductive Medicine and Biology
Univ. of Texas Medical School
P.O. Box 20706
Houston, TX 77025
Tel. (713) 792-5309

Wylie C. Hembree, M.D.
630 West 168th Street
New York, NY 10032
Tel. (212) 694-3684

Damon C. Herbert, Ph.D.
Dept. of Anatomy
University of Texas
San Antonio, TX 78284

Evelyn V. Hess
231 Bethesda Avenue
Cincinnati, OH 45267
Tel. (513) 872-4701

Barry T. Hinton, Ph.D.
Dept. of Urology
University of Virginia
School of Medicine
Charlottesville, VA 22908
Tel. (804) 924-5007

A.V. Hirsh, M.B.
Dept. of OB-GYN
Kings College Hospital
Denmark Hill
London SE5, ENGLAND
Tel. 01-289-6788

Anita P. Hoffer, Ph.D.
Department of Anatomy
Harvard Medical School
25 Shattuck Street
Boston, MA 02115
Tel. (617) 732-1724

Theresa A.M. Hoffman
184 Grove Terrace
Livingston, NJ 07039
Tel. (201) 992-4620

Louis R. Honore, M.B.
Provincial Lab. of Public Health
Pathology Department
University of Alberta
Edmonton, Alberta
CANADA T6G 2J2
Tel. (403) 432-8943

Richard Horton, M.D.
Chief of Endocrinology
LAC/USC Medical Center
2025 Zonal Avenue
Los Angeles, CA 90033

John Howard Hoskins, M.D., Ph.D.
1200 South Euclid Avenue
Sioux Falls, SD 57105
Tel. (605) 336-0635
Paul A. Kelly, Ph.D.
Medical Research Council Group
in Molecular Endocrinology
Centre Hospitalier
de l'Universite Laval
2705 Boul. Laurier
Quebec G1V 4G2, CANADA
Tel. (418) 656-8253

David M. Kelsey, M.D.
6 Gunning Lane
Gladwyne, PA 19035
Tel. (215) 649-6420

Edward J. Kengh, Ph.D.
Endocrinology Unit
Queen Elizabeth II Medical Centre
Medlands, Western Australia 6009

Jeffrey B. Kerr, Ph.D.
Dept. of Anatomy
Monash University
Clayton, Victoria
AUSTRALIA

Donald W. Killinger, M.D.
Wellesley Hospital
Rm. 122, E.K. Jones Building
160 Wellesley St. East
Toronto, Ontario
CANADA M4Y 1J3
Tel. (416) 978-6010

Rashad Y. Kirdani, Ph.D.
Roawell Park Memorial Institute
666 Elm Street
Buffalo, NY 14263

Stanley J. Kogan, M.D.
Division of Urology
1825 Eastchester Road
Bronx, NY 10461
Tel. (212) 430-3479

Robert C. Kolodny, M.D.
Reproductive Biology Research Fnd.
4910 Forest Park Boulevard
St. Louis, MO 63108
Tel. (314) 361-2377

Aarno I. Koskimies, M.D.
Univ. Central Hospital of Helsinki
Dept. of Ob/Gyn
Haartmaninkatu 3
00350 Helsinki 35, FINLAND
Tel. 90-4711

Howard S. Kulin, M.D.
Dept. of Pediatrics
The Milton S. Hershey Medical Center
Pennsylvania State University
Hershey, PA 17033

Fernand Labrie, M.D., Ph.D.
Groupe du Conseil de Recherches Medicale
en Endocrinologie Moleculaire
Center Hospitalier de l'Universite Laval
2705 Boul. Laurier
Quebec G1V 4G2, CANADA
Tel. (418) 656-8237

Roland O. Laferte, M.D.
906 Park Street
Stoughton, MA 02072

Michael F. Lalli, Ph.D.
McGill University
Dept. of Anatomy
Montreal, Quebec H3A 2K2
CANADA
Tel. (514) 392-4881

Joseph A. LaNasa, Jr., M.D.
Chairman, Dept. of Urology
LSU Medical Center
1542 Tulane Avenue
New Orleans, LA 70112

Juan Carlos Lavieri, M.D.
Santa Fe 1970 6 B
1123 Buenos Aires, ARGENTINA
Tel. 42-7805

Robert B. Leach, M.D.
3535 W. Thirteenth Mile
Suite 704
Royal Oak, MI 48072

Chung Lee, M.D.
Department of Urology
Northwestern Univ. Medical School
303 East Chicago Avenue
Chicago, IL 60611
Tel. (312) 649-8145

Hee Yong Lee, M.D.
Dept. Urology, College of Medicine
Seoul National Univ. Hospital
Seoul 110, KOREA

John M. Leonard, M.D.
USPHS Hospital
F.O. Box 3145
Seattle, WA 98114
Tel. (206) 324-7650 #491

Salvatore Leto, Ph.D.
Washington Fertility Study Center
2600 Virginia Ave., N.W.
Washington, DC 20037
Tel. (202) 333-3100
THE FOLLOWING YELLOW PAGES PROVIDE THE
PROGRAM OUTLINE, INCLUDING THE ABSTRACTS BY
TITLE AND AUTHOR FOR THOSE PRESENTED IN THE
ORAL SESSIONS AND BY TITLE ALONE FOR THOSE
PRESENTED IN THE POSTER SESSION. THIS SECTION
CAN BE REMOVED AS A UNIT TO PROVIDE A BRIEF
PROGRAM BOOKLET.
WEDNESDAY A.M.

8:00  Registration, Mezzanine Level, Front Escalator Foyer

8:30  Postgraduate Course, Clinical Sessions, Sexual Function and Dysfunction. (Emerald Ballroom, Second Level)

Male Erectile Impotence and Nocturnal Penile Erections. .. Ismet Karacan, M.D., D.Sc.

Sexual Dysfunction Associated with Medical Conditions. .. Max Ellenberg, M.D.

Drug Effects on the Reproductive System. Carol Grace Smith, Ph.D.

Surgical Treatment and Prostheses in Impotence. .. Ronald W. Lewis, M.D.

12:00  Lunch Break
WEDNESDAY P.M.

1:30 Postgraduate Course, Basic Science Sessions, Animal Models in Andrological Research and Clinical Correlation. (Emerald Ballroom, Second Level)

Small Animal Models for Andrological Research •• Ronald L. Urry, Ph.D.

Primate Model for Reproductive Pharmacology Research •• Carol Grace Smith, Ph.D.

Small Animals as Models for Andrological Research •• Luis J. Rodriguez-Rigau, M.D.

The Nonhuman Primate as Models for Andrological Research •• Ronald W. Lewis, M.D.

4:00 Panel Discussion

7:00 Reception in the Grand Ballroom

Cash Bar

Gratis Hors D'oeuvres
Thursday, March 12, 1981

8:00  Registration, Mezzanine Level, Front Escalator Foyer
8:30  Welcome Remarks, GRAND BALLROOM
8:45  Serono Lecture, "Mechanisms of Mammalian Fertilization,"  
     Pierre Soupart, M.D., Ph.D.
9:45  Break

10:00 SCIENTIFIC SESSION I - TESTING PROCEDURES  
     Moderator: Abraham T. K. Cockett, M.D.
10:00  Statistical Comparison of Traditional and Precise Sperm Motility  
     Evaluations. H. Winet, J. S. Walder, M. Freund
10:15  Detection of Antibody Attached to Sperm Cells by Direct Observation  
     in the Mixed Antiglobulin Reaction. S. Shulman
10:30  Restimulation of Lymphocytes by Spermatozoa-induced Cells.  
     M. Kurpisz, G. A. Szymczynski
10:45  Correlations of Seminal Testosterone and Fructose with Sperm Fertilizing  
11:00  In Vitro Bovine Cervical Mucus Penetration Test to Assess Fertility  
     in Men. N. J. Alexander, J. H. Sampson
11:15  Reactions of Anti-HCG with Living Human Sperm. R. J. T. Hancock,  
     A. T. K. Cockett
11:30  Correlation Between Sperm Output and Number of Elongated Spermatids  
     in Human Testicular Biopsies. L. J. Rodriguez-Rigau, K. D. Smith,  
     E. Steinberger
11:45  The Role of Neutrophils, Reduced Zinc Concentrations and Elevated pH  
     Levels in Seminal Plasma Suggesting the Presence of Asymptomatic Infections. J. R. Valvo, A. A. Caidamone, L. B. V. Emilson, S. Hipp,  
     A. T. K. Cockett
12:00  Lunch Break
1:30  SCIENTIFIC SESSION II - BIOCHEMISTRY  
     Moderators: William P. Kennedy, Ph.D. and Richard F. Parrish, Ph.D.
1:30  Characterization of Radiolabeled Components of Bull Sperm Surface and  
     Seminal Plasma. L. G. Young, S. A. Goodman
1:45  Interaction of Detergent Solubilized Boar Sperm Plasma Membranes with  
     Isolated Porcine Zonae Pellucidae. R. N. Peterson, L. D. Russell,  
     D. Bundman, M. Freund
2:00  Divalent Metal Ion Stimulation of the Proteolytic Activity of Boar Sperm  
     Acrosin. R. F. Parrish, K. L. Polakoski
Thursday, March 12, 1981

SCIENTIFIC SESSION II - Continued

2:15 The Interrelation Between Energy Metabolism and Spermatozoan Collective Motility in Fresh Undiluted Ram Sperm. A. Mayevsky, B. Bartoov, D. Barsagie

2:30 Oxygen Metabolism by Intact and Hypotonizally Treated Rabbit Epididymal Spermatozoa (HTRES). M. K. Holland, B. T. Storey

2:45 Isolation of Antigenic Peptides from LDH-C4. E. Goldberg, E. Wheat, V. Gonzales-Prevatt

3:00 Break

3:15 SCIENTIFIC SESSION III - EFFECTS OF DRUGS AND CANCER ON TESTICULAR FUNCTION
Moderator: Carol Grace Smith, Ph.D.

3:15 Micropuncture Studies of the Blood-testis Barrier to Methotrexate (MTX) in Rats. R. Vigersky, R. Riccardi, A. Bleyer, S. Barnes, D. Poplack

3:30 Direct Inhibition of Rat Leydig Cell Function by Tamoxifen. T. Lin, E. Murono, J. Osterman, H. R. Nankin

3:45 Spermatogenesis Following Cancer Chemotherapy with Doxorubicin (Adriamycin). M. F. da Cunha, M. L. Melstrich, H. L. Ried

4:00 Effect of Medrogestone on the Fertilizing Ability of Rabbit Cauda Epididymal Sperm and on In Vitro Release of Androgens. K. Saksena, I. Lau


4:30 Effect of Estrogen on Steroid Levels of Spermatic Vein Blood in Patients with Prostatic Cancer. T. Yanaihara, M. Kanazawa, L. Chu, K. Isrugii, K. Fukutani

4:45 Testicular Function in Men with Hodgkin's Disease (HD) Prior to Therapy. R. Vigersky, R. Chapman, A. Glass, J. Berenberg

5:00 Break

5:15 WORKSHOP - "ETHICAL IMPLICATIONS FOR FERTILITY RESEARCH"
BAYOU I ROOM

7:15 Break

7:30 POSTER SESSION, GRAND BALLROOM, WINE AND CHEESE WILL BE SERVED

9:00 END OF DAY'S ACTIVITIES
<table>
<thead>
<tr>
<th>Time</th>
<th>Event Description</th>
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<tr>
<td>8:30 - 12:00</td>
<td>Welcome 8:30</td>
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<td>State-of-the-Arts P. Soupart, M.D., Ph.D.</td>
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<td>10:00 - 12:00 Scientific Session I</td>
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<td>1:30 - 5:00</td>
<td>POSTGRADUATE COURSE</td>
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<td>&quot;Animal Models for Andrological Research and Their Clinical Significance&quot;</td>
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<td>Scientific Session II</td>
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<td>5:15 - 7:15</td>
<td>5:15 - 7:15 WORKSHOP</td>
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<td>&quot;Ethical Implications for Fertility Research&quot;</td>
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<td>7:00 - 9:00</td>
<td>7:00 - 9:00 RECEPTION</td>
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<td>Gratis Hor d'oeuvres</td>
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<td>7:30 - 9:00 POSTER SESSION</td>
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<td>Wine &amp; Cheese Served</td>
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<td>FRIDAY 3/13</td>
<td>SATURDAY 3/14</td>
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| State-of-the-Arts
P. Gaddum-Rosse, Ph.D. | State-of-the-Arts
R.H. Foote, Ph.D. |
| Presidential Address | Scientific Session VII |
| Scientific Session IV | Scientific Session VIII |
| Scientific Session V | |
| Scientific Session VI | |
| Business Meeting | |
| 7:30 - 10:00 | COCKTAILS (cash bar)
BANQUET
JAZZ ENTERTAINMENT |
Friday, March 13, 1981

8:00  Registration, Mezzanine Level, Front Escalator Foyer

8:30  State-of-the-arts Lecture, "Natural Barriers to Sperm Transport: From the Cervix to the Site of Fertilization," Penelope Gaddum-Rosse, Ph.D.

9:30  PRESIDENTIAL ADDRESS, PHILIP TROEN, M.D.

10:00  Break

10:15  SCIENTIFIC SESSION IV - CLINICAL STUDIES AND PROCEDURES
       Moderator: Joseph A. LaNasa, Jr., M.D.

10:15  Prolactin Secretion in Azoospermia. I. M. Spitz, P. Lavie, N. Kalderon, D. LeRoith


11:00  High Intra-abdominal Testes: Laparoscopy and Autotransplantation. J. Silber

11:15  Results of Bilateral Scrotal Exploration and Testis Biopsy in 100 Sub-fertile Males with Azoospermia and/or Severe Oligospermia. O. Romano, J. A. LaNasa, Jr.

11:30  Effects of Methylcobalamin on Sperm Counts and Sperm Motility in Oligospermic Cases. M. Kimura, S. Orikasa, S. Mitsukawa

11:45  Effect of Sleep Deprivation on the Pituitary-testis Axis in Healthy Men. V. Cortes-Gallegos, G. Castandea, R. Alonso, C. Cervantes, A. Parra

12:00  Lunch Break

1:30  SCIENTIFIC SESSION V - BIOCHEMISTRY
       Moderator: B. Jane Rogers, Ph.D.

1:30  Stimulation of Sperm Motility Initiation by a Small Peptide Secreted by the Rat Cauda Epididymis In Vivo. P. Y. D. Wong, A. Y. F. Tsang, W. M. Lee, C. M. Li

1:45  Effects of Various Sugars on the Time Course of Human Spermatozoal Capacitation In Vitro. S. D. Perreault, B. J. Rogers

2:00  Steroidogenic Enzyme Activity in Different Populations of Leydig Cells. P. J. O'Shaughnessy, A. H. Payne

Friday, March 13, 1981

SCIENTIFIC SESSION V - Continued

2:30 The In Vivo Transfer of Various Molecules Across the Epithelium of the Rat Caput Epididymidis. B. T. Hinton, S. S. Howards

2:45 Three-dimensional Reconstruction of a Stage V Rat Sertoli Cell: Size, Configuration and General Relationship to Germ Cells. L. Russell, V. Wong

3:00 Hypoxia and Testicular Function in the Hamster. E. Bustos-Obregon, R. Celis

3:15 Sertoli Cell Cytoskeleton: Monkey, Rat, Ground Squirrel. M. Dym, C. A. Suarez-Quian, A. W. Vogl

3:30 Break

3:45 SCIENTIFIC SESSION VI - HORMONAL REGULATION

Moderator: Mary V. Nekola, M.D.

3:45 Testosterone Concentrations in the Intraluminal Fluids of the Male Rat and Human Reproductive Tract. T. Turner, S. Singhas

4:00 Testosterone Regulations of Luteinizing Hormone Secretion: Effects of Time After Castration. B. D. Schanbacher

4:15 Pituitary-gonadal Endocrine Interrelationships in the Ram. L. M. Sanford, K. H. Ponzilis

4:30 Effects of Prolactin on Gonadotropin Release in Hamsters with Hypothalamic Deafferentation. A. Bartke, A. Carrillo

4:45 Estrogen Secretion by In Vitro Perfused Testes. D. L. Thompson, Jr., L. L. Ewing, B. L. Lasley

5:00 Suprahypothalamic Control of Gonadotropins. Role of Opioid Peptides. C. Santoro, A. Fabbri, L. Ferraris, R. Ferretti, F. Fraioli

5:15 Modification of LH Release in Castrate and Intact Male Rats Treated with Anti-LHRH Serum. M. V. Nekola, E. Pedroza, J. Vilchez-Martinez, A. Arimura, A. V. Schally

5:30 BUSINESS MEETING, BAYOU I ROOM

6:30 Break

7:30 COCKTAILS, BANQUET, JAZZ ENTERTAINMENT - GRAND BALLROOM

10:00 END OF DAY'S ACTIVITIES!
Saturday, March 14, 1981


9:30  SCIENTIFIC SESSION VII - TESTING PROCEDURES AND AGE-RELATED STUDIES
      Moderator: Howard R. Nankin, M.D.

9:30  A Simple Non-Chromatographic Radioimmunoassay Method for Quantitation of Dihydrotestosterone. C. P. Puri, V. Puri, T. C. Anand Kumar

9:45  Variation of Human Fertilization Using the In Vitro Fertilization Assay. B. J. Bentwood, B. J. Rogers, C. McCarville

10:00 Androgens and Behavior in Fetal Stallions. J. W. Turner, Jr., J. F. Kirkpatrick

10:15  Ultrastructural Investigation of Human Orchiectomy Tissue in the Older Age Group. S. Siew, P. Troen, H. R. Nankin

10:30 Effect of Cryptorchidism on the Age-dependent Loss in FSH Responsiveness of Rat Sertoli Cells. J. J. Heindel, S. J. Strada, A. Steinberger

11:00 SCIENTIFIC SESSION VIII - FERTILITY CONTROL
      Moderator: L. J. D. Zaneveld, D.V.M., Ph.D.

11:00 Spermicidal Effect of Gossypol in an In Vivo Animal Model for Vaginal Contraceptives. D. P. Waller, S. M. Cameron, L. J. D. Zaneveld

11:15 Inhibitors of Sperm Hyaluronidase as Vaginal Contraceptive Agents. C. L. Joyce, L. J. D. Zaneveld


11:45 Cholinomimetic Agents as Inhibitors of Mouse Fertilization In Vitro: Indications of a Different Approach to Male Fertility Regulations. H. M. Florman, B. T. Storey

12:00 Effect of Gonadotropin Releasing Hormone on the Secretion of Luteinizing Hormone and Testosterone in Aged Male Rhesus Monkeys. J. A. Robinson, B. T. Farbstein, W. E. Bridson

12:15 Effects of LHRH Agonist Treatment on the Metabolism of Tritiated Progestins and Androgens by Dispersed Leydig Cells and Testicular Homogenates. R. Carmichael, A. Belanger, S. Caron

12:30 Significance of Antisperm Antibodies Following Vasovasostomy. E. F. Fuchs, N. Alexander

12:45 Effects of Vasectomy on Testicular Tissue in Men - A Light and Electron Microscopic Study. J. Chakraborty, J. Jhunjhunwala
POSTER SESSION ABSTRACTS BY TITLE

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--- | ---
98 | Prednisone Treatment for Circulating Antisperm Antibodies.
99 | Effect of Genotype on the Testicular Gonadotropin Binding in the Mouse.
100 | Culture of Principal Cells from the Rat Epididymis.
101 | Effect of Sexual Maturation on Testicular LH Receptor Levels and on Basal as Well as O-LH-Induced Steroid Content in Rat Tissue.
102 | Pyospermia, Seminal Bacteriology and the Sperm Penetration Assay in Men from Infertile Couples.
103 | The Effect of Age on Sperm Concentration in Hamsters.
104 | Species Differences in TeBG Steroid Specificity.
105 | Degenerative Changes in the Testes of the Golden Hamster.
106 | Teaching First-Year Medical Students the Male Reproductive System Using an Organ Format with Clinical Problems.
107 | Comparison of the Acute Effects of Marijuana, Ethanol, and Morphine on Sex Hormone Levels in the Male Rhesus Monkey.
109 | Dihydrotestosterone Receptor in Human Thymic Tissue.
111 | An Anatomical and Histological Study of the Rat Prostate.
113 | Identification of an Intra-Molecular Mechanism for the Conversion of Proacrosin into the ma-Acrosin.
114 | A Comparative Study of Sperm cAMP-Dependent Protein Kinases by a Photoaffinity Analysis.
Advantages of Gonadotropin (GN) over Testosterone Therapy for Virilization of GN Deficient Hypopituitary Males.

Effects of Hypothyroidism and Hemicastration on Gonadal Growth and LH Levels in Immature Rats.

Does Immunization of Monkeys with Ovine Follicle Stimulating Hormones (o FSH) Break the Immunologic Tolerance to Endogenous FSH and Lead to Autoimmunity?

The Aging Human Leydig Cell: Response to HCG.

Involvement of Dolichol Phosphates as Intermediates in the Mannosyl and Galactosyl Transferases of Rat Testicular Germ Cell Golgi Apparatus Membranes.

Identification of an Androgen-Binding Protein in Human Seminal Plasma (SPASP) that is Distinct From Epididymal ABP and Serum TeBG.

Cytoplasmic and Nuclear Inclusions in Leydig Cells of the Chinese Hamster.

Effect of Prolactin (PRL) on Retention of Dihydrotestosterone (DHT) in the Rat Prostate.

Reduction of Sperm Concentration in a Population Exposed to Ethylene Dibromide (EDB).

Protein Kinases: Control by Ca\textsuperscript{2+} and cAMP in Rat Caudal Epididymal Sperm.

Study of 1,200 Pregnancies, Obtained by Artificial Donor Insemination with Both Fresh and Frozen Semen.

Effect of (D-Trp\textsuperscript{6})-LHRH on the Pituitary-Gonadal Function in Male Rhesus Monkeys.

Evaluation of Chlorinated Pesticides in Human Semen.

Testosterone, Seven of Its Precursors and Estradiol in the Testes of Elderly Men.

Effect of Prolonged Systemic Administration of an LHRH Agonist on Prostatic Size and Function in Geriatric Dogs.

Mechanisms of Surface Modifications in Ram Spermatozoa.

Reversible Hypogonadotropic Hypogonadism in an Obese Man.
John B. Nanninga, M.D.
Dept. of Urology
Northwestern University Medical School
Chicago, IL 60611
Tel. (312) 649-8415/743-8485

William B. Neaves, Ph.D.
Dept. of Cell Biology
The Univ. of Texas Southwestern Medical School at Dallas
5323 Harry Hines Blvd.
Dallas, TX 75235
Tel. (214) 688-2509

Maria T. New, M.D.
Professor of Pediatrics
Cornell Unv Medical College
1300 New York Avenue
New York, NY 10021

Robert A. Newton, M.D.
2000 Washington Street
Newton Lower Falls, MA 02162
Tel. (617) 332-7705

Robert C. Northcutt, M.D.
Mayo Clinic
Rochester, MN 55901
Tel. (507) 284-2511 #7427

Sally P. Nyquist
Biology Dept.
Bucknell University
Lewisburg, PA 17837
Tel. (717) 524-2912

Deborah C. O'Brien, Ph.D.
Harvard Medical School
LHRBB - 45 Shattuck Street
Boston, MA 02115
Tel. (617) 732-2074

William D. Odell, M.D., Ph.D.
Department of Medicine
School of Medicine
University of Utah
Salt Lake City, UT 84132
Tel. (801) 581-7605

Peter Ofner, Ph.D.
Steroid Biochemistry Lab.
Lemuel Shattuck Hospital
170 Morton Street
Jamaica Plain, MA 02130
Tel. (617) 522-8110 #336

Dr. Guy C. Oliver
Centre Hosp. de Univ. Laval
2705 Boul. Laurier
Quebec P.Q., CANADA G1V 4G2
Tel. (418) 656-8130

B.S. Orekondy, M.D.
P.O. Box 1111
1041 Troy-Schenectady Rd.
Latham, NY 12110
Tel. (518) 783-1111

Marie-Claire Oregebin-Crist, Ph.D.
Vanderbilt University
Dept. of Ob/Gyn
Nashville, TN 37232
Tel. (615) 322-6587

Yoshio Osawa, Ph.D.
Medical Foundation of Buffalo, Inc.
73 High Street
Buffalo, NY 14203
Tel. (716) 856-9500

Juraj Osterman, M.D.
Division of Endocrinology
University of So. Carolina V.A. Hospital Enclave
Columbia, SC 29201
Tel. (803) 776-6575

John W. Parrish, Ph.D.
Biology Department
Emporia State University
Emporia, KS 66801

Dolores J. Patanelli, Ph.D.
3324 Raymond Lane
Potomac, MD 20854
Tel. (301) 496-1601

C. Alvin Paulsen, M.D.
University of Washington
Box 3145
Seattle, WA 98103
Tel. (206) 322-2131

David F. Paulson, M.D.
P.O. Box 2977
Duke Univ. Medical Center
Durham, NC 27710

John D. Paulson, M.D.
4801 Kenmore Avenue
Alexandria, VA 22304
Tel. (703) 751-0400

Anita Hart Payne
Steroid Research Unit
Dept. Obstetrics and Gynecology
University of Michigan
Ann Arbor, MI 48109
Tel. (313) 764-6430
Bruce D. Schanbacher, Ph.D.
US Meat Animal Research Center
USDA
Box 166
Clay Center, NE 68933
Tel. (402) 762-3241

Isaac Schiff, M.D.
122 Abbott Road
Wellesley, MA 02181
Tel. (617) 235-3005

Wolf-Bernhard Schill, M.D.
Dept. of Dermatology
University of Munich
Frauenlobstrasse 9
D-8000 Munich 2
WEST GERMANY
Tel. 089 153 97 637

Cy Schoenfeld, Ph.D.
Fertility Laboratory, Inc.
137 East 36th Street
New York, NY 10016
Tel. (212) 532-0255

Gebhard F.B. Schumacher, M.D.
Dept OB/Gyn
The University of Chicago
5841 Maryland Avenue
Chicago, IL 60637
Tel. (312) 947-5342

Luis Schwarzstein, M.D.
Cordoba 1764
2000 Rosario
ARGENTINA

Arthur G. Seski, M.D.
1059 Fisher Bldg
Detroit, MI 48202
Tel. (313) 875-8329

Sydney A. Shain, Ph.D.
Southwest Foundation for Research and Education
P.O. Box 28147
San Antonio, TX 78284
Tel. (512) 674-1410 #352

John M. Shane, M.D.
6465 So. Yale
Suite 304
Tulsa, OK 74136

Hoobollah Sharifi, M.D.
4100 Harvey Avenue
Western Springs, IL 60558
Tel. (312) 248-0338

Ira Sharlip, M.D.
3838 California, Suite 408
San Francisco, CA 94118
Tel. (415) 241-9189

William T. Sheehy, M.D.
1187 Dundee Ave
Elgin, IL 60120

Richard J. Sherins, M.D.
10708 Deborah Drive
Potomac, MD 20854
Tel. (301) 496-4686

Leon J. Sholiton, M.D.
3200 Vine Street
Cincinnati, OH 45220
Tel. (513) 861-3100

Sidney Shulman, Ph.D.
Sperm Antibody Laboratory
New York Medical College
1249 5th Avenue
New York, NY 10029
Tel. (212) 580-8080

Barry S. Shultz, M.D.
301 South Seventh Avenue
West Reading, PA 19611
Tel. (215) 375-4579

Sherman J. Silber, M.D.
Urology and Microsurgery
Ballas Parkway Medical Center
456 N. New Ballas Road, Suite 108
St. Louis, MO 63141
Tel. (314) 569-2290

Norman Silverman, M.D.
4900 W. Oakland Park Blvd.
Lauderdale Lakes, FL 33313

C. Edward Skeeters, M.D.
6475 S.W. Borland Road
Tualatin, OR 97062

W. Ronald Skowsky, M.D.
Dept. of Endocrinology N-8
Long Beach Veteran's Administration Hospital
5901 East 7th Street
Long Beach, CA 90822
Tel. (213) 498-1313 #2637/2199

Charles F. Skripka, Jr., M.D.
P.O. Box 220
Union City, TN 38261
Tel. (901) 885-5621
Henry Wagner, M.D.
16902 El Camino Real
Suite 1-D
Houston, TX 77058

Patrick C. Walsh, M.D.
Brady Urological Institute
Johns Hopkins Hospital
Baltimore, MD 21205
Tel. (301) 955-6769

Nancy E. Warner, M.D.
1065 S. San Rafael Ave.
Pasadena, CA 91105
Tel. (213) 224-7123

Dwight W. Warren, Ph.D.
8330 N. Brook Lane
Bethesda, MD 20014

Frank J. Weakah, Ph.D.
Dept. of Anatomy
University of Texas Health Sciences Center
San Antonio, TX 78284

Paul S. Weatherbee, Ph.D.
Dept. of OB/GYN
University of California
Irvine Medical Center
101 City Drive S.
Orange, CA 92668

Alan J. Wein, M.D.
5-Silverstein
3400 Spruce Street
Philadelphia, PA 19104
Tel. (215) 662-2891

Dr. Wolf H. Weiske
Allmersbacherstr 8
7000 Stuttgart 50
WEST GERMANY

Wayne D. Weissman, M.D.
1203 Franklin Street
Iowa City, IA 52240
Tel. (319) 356-1166

Earl F. Wendel, M.D.
707 North Fairbank Court
Chicago, IL 60611
Tel. (312) 649-8146

Kevin Whaley, M.S.
Dept of Physiology
Medical College of Ohio
C.S. 10008
Toledo, OH 43699
Tel. (419) 381-4141

Julius H. Winer, M.D.
9915 Santa Monica Boulevard
Beverly Hills, CA 90212
Tel. (213) 553-7400

Howard Winet, Ph.D.
Bone & Connective Tissue Grp.
USC Orthopedic Hospital
2400 Flower Street
Los Angeles, CA 90007
Tel. (213) 742-1459

Stephen J. Winters, M.D.
Montefiore Hospital
3459 Fifth Avenue
Pittsburgh, PA 15213
Tel. (412) 683-1100 #628

Patrick Y. D. Wong, Ph.D.
Dept. of Physiology
Faculty of Medicine
University of Hong Kong
HONG KONG

Isamu Yanagisawa, M.D.
Department of Biochemistry
Tokyo Medical College
6-Chome, Shinjuku
Tokyo, JAPAN
Tel. (03) 351-6141 #244

Muazaz Younes, Ph.D.
Dept of OB/GYN
Baylor College of Medicine
Houston, TX 77030
Tel. (713) 791-4108

Leona G. Young, Ph.D.
Department of Physiology
Emory Univ. School of Medicine
Atlanta, GA 30322
Tel. (404) 329-7415

Yang-Dar Yuan, Ph.D.
The Upjohn Company
301 Henrietta Street
Kalamazoo, MI 49001
Tel. (616) 386-6732

L. J. D. Zaneveld, D.V.M., Ph.D.
Department of Physiology
College of Medicine
Univ. of Illinois Medical Center
Box 6998
901 South Wolcott Street
Chicago, IL 60680
Tel. (312) 996-7620
SUSTAINING MEMBERS

Fran K. Keshanian, M.D.
Asst. Director, Clinical Research
Ayerst Laboratories
685 Third Ave
New York, NY 10017

Battelle Memorial Institute
c/o H. Drucker, Ph.D.
Pacific Northwest Laboratories
Battelle Boulevard
Richland, WA 99352

Genetic Semen Bank
Univ. of Nebraska Medical Center
42nd and Dewey Avenue
Omaha, NE 68105

Dr. Alan F. Taulor
Vice President
Organon Corp.
West Orange, NJ 07052

Dr. Samuel A. Paquale
Executive Director of Medical Research
Ortho Pharmaceutical Corp.
Raritan, NJ 08869

Carole J. Kruppa
Professional Education Services
Searle Laboratories
Box 5110
Chicago, IL 60680

Thomas Wiggins
Serono Laboratories, Inc.
11 Brooks Drive
Braintree, MA 02184

EMERITUS MEMBERS

Alexander Albert, Ph.D.
Mayo Clinic
Rochester, MN 55901

Jessamine O. Hilliard, Ph.D.
1511 Clear View Lane
Santa Ana, CA 92705
ANDROGENS AND BEHAVIOR IN FERAL STALLIONS. J. W. Turner, Jr. and J. F. Kirkpatrick. Medical College of Ohio, Toledo, OH & Eastern Montana College, Billings, MT.

Although there have been numerous studies of animal behavior in the field, few have attempted to relate this to the endocrine state. This study correlates plasma androgen levels with specific aspects of social behavior in male feral horses of the Pryor Mountains of Montana. Behavioral endpoints measured include 1) stallion scent marking behavior in response to elimination by mares, 2) mounting behavior, 3) copulation, and 4) ejection of maturing males from the harem (family group). Plasma total 17-OH androgens and testosterone (T) were measured by CPB assay in samples obtained by jugular venipuncture from captured animals. 24 hourly samples from each of three stallions revealed diurnal variation in plasma T with a nadir (1.37+0.75 ng/ml) at 2300 and a peak (3.48+0.12 ng/ml) at 0800. Levels were plateaued during daylight hours (0800 to 1800), when all other samples in this study were taken. Two of these stallions, which were sexually active and maintained a harem, had 24 hr. mean plasma T of 3.0+0.6 ng/ml. The third stallion, a bachelor which had never attempted to acquire a harem in 5 years of adulthood and was never observed to be sexually active, had significantly lower 24 hr. mean plasma T (1.5+0.9 ng/ml). In samples from 34 sexually mature stallions taken in 6 different months during the year, definite seasonal patterns in plasma T and total androgens were present, with a nadir in December and a peak in May. Testosterone as percent of the total androgens also increased from 58% in December to 85% in May. The frequency of both stallion mounting behavior and stallion scent marking behavior showed a seasonal pattern similar to that for plasma androgens. Young males are ejected from the harem upon sexual maturation. Of 21 two-year old males studied during the breeding season only two were ejected. These had descended testes and average plasma T of 3.35ng/ml, which was significantly higher than the other 19 with undescended testes and plasma T of 1.14+0.23ng/ml. These data show a consistent correlation between plasma androgen levels and the display of various behaviors in feral stallions in the field.
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89. EFFECT OF CRYPTORCHIDISM ON THE AGE-DEPENDENT LOSS IN FSH RESPONSIVENESS OF RAT SERTOLI CELLS. J.J. Heindel, S.J. Strada, and A. Steinberger. The University of Texas Medical School at Houston. Departments of Reproductive Medicine & Biology and Pharmacology, Houston, Texas 77000

Sertoli cells cultured from 18 day old rats respond to FSH with a greater stimulation of cAMP accumulation than do cells cultured from animals 35 days of age or older (Steinberger et al., Biol. Reprod. 19: 566, 1978). This decline in normal responsiveness correlates in vivo with the onset of meioses of germ cells and the appearance of spermatids. Post-meiotic germ cells either disappear or fail to develop when adult or immature animals, respectively, are made bilaterally cryptorchid. This surgical procedure was used to examine the role of post-meiotic germ cells in the age-dependent loss of FSH responsiveness of Sertoli cells. Rats 34 or 69 days of age were made bilaterally cryptorchid and Sertoli cell cultures were prepared 16 or 29 days post-operatively. The cAMP response to FSH of all groups was similar to that of sham-treated control animals. In no case did cryptorchidism result in a restoration of the Sertoli cell responsiveness to FSH. Cryptorchidism of 20 day old rats, before meioses was complete, resulted in a delay of about 10 days in the loss of FSH responsiveness, but thereafter the cAMP response paralleled that seen in the normal rat. Sertoli cell cultures from 18 day old rats maintained at either 32°C or 34°C for 4 days showed an identical cAMP response to FSH. We conclude from these studies that the presence of post-meiotic germ cells does not account for the inability of Sertoli cells from adult rats to respond to FSH with a cAMP accumulation equivalent to that observed in the immature animal.

(Supported by USPHS HD-08338 and NSF PCM 7824561)
Oral doses of gossypol, a disocterpenoid aldehyde, from the cottonseed plant have been reported to cause infertility in males of several species including man. A decrease in sperm motility is one of the early effects observed during treatment. Recent studies in our laboratory demonstrated that a gossypol-polyvinylpyrrolidone (PVP) coprecipitate was spermicidal when tested in a modified Sander-Cramer test using human spermatozoa. A concentration of 40 mg/ml of gossypol-PVP completely immobilized human spermatozoa within 20 seconds while at 5 mg/ml all spermatozoa were immobilized within 3 minutes. Our current investigation used an in vivo primate model to evaluate the vaginal spermicidal efficacy of the gossypol-PVP coprecipitate. Test mixtures were prepared by dissolving gelatin in boiling distilled water, cooling until viscous and an appropriate amount of gossypol-PVP added. These test mixtures were inserted into the vagina of female macaca arctoides (stumptail Macaques). Immediately following insertion, the females were paired with males and observed for mating behavior. After the male exhibited signs of ejaculation, the female was removed, a sample of vaginal fluid obtained and the spermatozoa observed under a microscope for motility. Gossypol-PVP concentration of 10, 25, 50 and 100 mg/ml in a gelatin base were tested. A dose response relationship was observed, i.e. with increasing doses, a decrease in motility occurred. More than 80% of the spermatozoa were immotile at doses of 50 mg/ml or greater. These data confirm the in vitro observation that gossypol-PVP has spermicidal activity and demonstrate the efficacy of gossypol-PVP as a vaginal contraceptive.
Previous reports from other laboratories and ours indicate that inhibitors of sperm hyaluronidase can have antifertility activity. In order to determine if such inhibitors are useful as vaginal contraceptives and to obtain some that are non-toxic, 7 compounds claimed to be hyaluronidase inhibitors and 5 anti-inflammatory agents were screened for their hyaluronidase inhibitory activity. Three compounds in the first category were found to be active: PS₅₃ hydroquinone-sulfonic acid formaldehyde polymer (I), phosphorylated hesperidin (II), and penicillamine (III); and three in the second: myocrisin (IV), fenoprofen Ca²⁺ (V) and phenylbutazone (VI). Kinetic studies showed that compounds III, IV, and VI are reversible inhibitors of hyaluronidase and that I, II and V prevented the in vitro fertilization of mouse gametes, VI being the most active. On vaginal application to rabbits, compounds I, II, and VI possessed high contraceptive potency and IV was slightly active. These data indicate that no correlation exists between the reversible/irreversible nature of inhibitor-hyaluronidase interaction and their antifertility activity, and that an antifertility effect in vitro does not mean that conception will be prevented when the agents are applied vaginally. One of the compounds (PS₅₃) (I) that are not active in vitro, did have high vaginal contraceptive potency, indicating that it acts via a different mechanism than by hyaluronidase inhibition. Phenylbutazone (VI) was highly active in vitro and in vivo in µg quantities and is approved by the FDA for human use. Oxybutazone, a compound with structural similarities to phenylbutazone and also used clinically as an anti-inflammatory agent, proved to be a potent contraceptive when applied vaginally to rabbits. This class of compounds may be clinically applicable as vaginal contraceptives.

(Supported by PARFR 204 and NIH HD 09868)
SUPPRESSION OF SEXUAL FUNCTION IN MALE DOGS WITH A NEW AGONISTIC ANALOGUE OF LHRH: POTENTIAL FOR MALE CONTRACEPTION WITHOUT NEED FOR ANDROGEN REPLACEMENT?


Male beagle dogs (1-2 yrs old) were injected i.m. daily, with 10μg/kg of D-Nal(2)6 LHRH or vehicle alone. The increase in plasma testosterone (T), measured by RIA, observed at 2 and 4 hours after the 1st injection was substantially reduced by the 8th. Basal plasma T levels were reduced by 3 days and maximally suppressed by 6 days of treatment. After 10 and 38 days of treatment, groups of 2 treated and 2 control dogs were sacrificed for histological examination of reproductive organs. Partial suppression of spermatogenesis was evident at 10 days and exfoliated germinal cells were present in the caput epididymis. By 38 days the seminiferous tubules contained Sertoli cells and a few germinal elements. Testis volume was decreased by 3 weeks. Weekly ejaculates showed a decline in sperm count, motility and maturation, a decline in volume, and the appearance of germinal cells. After 30 days ejaculates could not be obtained. Injections were discontinued in 2 treated and 2 control dogs after 13 days. Basal T levels returned to normal 4 days later. Ejaculate volume increased after 10 days. However, sperm count continued to decline until 24 days after the last injection to a 98% suppression. All parameters returned to normal. Return of sperm count showed the longest lag time of 45 days from last injection. The difference between times of return to normal of plasma T levels and of spermatogenesis suggests that intermittent administration of LHRH agonists might lead to male contraception without the need for androgen replacement.
93. CHOLINOMIMETIC AGENTS AS INHIBITORS OF MOUSE FERTILIZATION IN VITRO: INDICATIONS OF A DIFFERENT APPROACH TO MALE FERTILITY REGULATION. H.M. Florman and B.T. Storey. Departments of Obstetrics & Gynecology and Physiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

The fertilization of zona-intact mouse eggs in vitro is inhibited in a dose-dependent fashion by 3'-quinuclidinyl benzilate (QNB), a specific antagonist of the muscarinic class of cholinergic receptors. The dose-response curve for inhibition follows the concentration dependence for binding of $^3H$-QNB to the sperm. The inhibitory effect of QNB on mouse fertilization occurs at the level of penetration of the zona pellucida: QNB has no effect on the in vitro fertilization of zona-free eggs, but does inhibit fertilization with zona-intact, cumulus-free eggs and with zona-intact, cumulus-intact eggs. In order to see if inhibition of fertilization by compounds such as QNB were related to their efficacy as muscarinic antagonists, we tested two more such compounds: n-propyl benzyllycholine mustard (PrBCM) which is an irreversible, specific muscarinic antagonist (Young, Hiley, & Burgen (1972), J. Pharm. Pharmac., 24, 950) and carbamyl choline (CbCh) which is a cholinergic agonist active with muscarinic receptors. PrBCM does not inhibit fertilization. CbCh is an effective inhibitor of fertilization, acting as does QNB. Further, the dose-response curve for CbCh inhibition follows that for its inhibition of the binding of $^3H$-QNB to sperm. These results suggest that mouse sperm have a binding site involved in the zona penetration reaction, which is blocked by QNB and related compounds. The fact that the related compounds do not follow muscarinic antagonist activity suggests that compounds may be synthesized which would be potent inhibitors of fertilization by acting on the sperm surface, but have no effect on neural design of fertility control agents.

(Supported by NIH HD-06774, NSF-PGM-77-15965 and the Andrew W. Mellon Foundation.)

Plasma levels of radioimmunoreactive luteinizing hormone (LH) and testosterone (T) were determined in adult male rhesus monkeys before and after the administration of synthetic gonadotropin-releasing hormone (GnRH). Five aged (>20 yrs) and 4 young adult monkeys, weighing 6.6-10.9 kg, were tranquilized with ketamine and anesthesia subsequently maintained with halothane for a 4-hr period. Blood samples were withdrawn through a venous catheter, the patency of which was maintained with heparinized saline. These samples were obtained every 15 min from 12:15 to 16:00 hr and the plasma analyzed for LH and T. GnRH (25 μg) was injected immediately following the 13:00 hr sample. Basal LH values were significantly higher in the aged animals (11.2 ± 0.85 vs 7.9 ± 0.88 ng/ml; p < 0.05) whereas T concentrations were significantly lower (1.8 ± 0.24 vs 6.0 ± 0.92 ng/ml; p < 0.01). Within 45 min after GnRH, LH levels in young animals reached peak concentrations (22.7 ± 6.70 ng/ml). Maximum LH levels for the old animals were 28.5 ± 11.11 ng/ml, but these levels were not obtained until 16:00 hr. Testosterone secretion lagged slightly behind the LH with peak concentrations (19.3 ± 8.23 ng/ml) being attained in the younger animals about 75 min after GnRH. Maximal T concentrations (10.1 ± 0.96 ng/ml) were attained in the older animals in approximately the same time period, but were significantly lower than in younger adults (p < 0.01). Since basal plasma LH concentrations were appropriately elevated in aged animals in which basal T concentrations in response to GnRH induced LH release were subnormal in aged animals, we conclude that primary gonadal dysfunction occurs in aging male rhesus monkeys which is similar to that reported in aging human males.

(Supported by NIH Grants RR00167 and AG01612).
95. EFFECTS OF LHRH AGONIST TREATMENT ON THE METABOLISM OF TRITIATED PROGESTINS AND ANDROGENS BY DISPERSED LEYDIG CELLS AND TESTICULAR HOMOGENATES. R. Carmichael, A. Belanger and S. Caron. Department of Molecular Endocrinology, CHUL, Quebec, Canada.

Our previous in vivo studies have shown that the loss of LH receptors induced by treatment with an LHRH agonist (LHRH-A) is accompanied by a marked inhibition of testicular Δ4-androgen formation, in the presence of unchanged or increased levels of progesterone (P) and pregnenolone, thus suggesting a blockage at the level of 17α-hydroxylase. However, we recently observed that testicular androstane-3α, 17β-diol formation was stimulated after LHRH-A treatment, thus indicating an increase of testicular 5α-reductase activity. We then investigated the effect of daily treatment with 1 μg of (D-Ser(TBu)6, des-Gly-NH210) LHRH ethylamide for 8 days on the metabolism of 3H-P and 3H-androstenedione (Δ4) in testis. In order to dissociate between Leydig cells and whole testis metabolism, tritiated steroids were incubated with a suspension of Leydig cells or with a testicular homogenate in the presence of the appropriate cofactors. During incubation of 3H-P with Leydig cells, no formation of 5α-reduced steroids was detected, while treatment with LHRH-A reduced the sum of Δ4-metabolites from 55 ± 3% to 8 ± 1%. In contrast, incubation of 3H-P with testicular homogenates (for 10 minutes) resulted in an increase of the 5α-reduced metabolite formation, from 11 ± 1% to 56 ± 3% following LHRH-A treatment as compared to controls, while the metabolism into Δ4-metabolites was inhibited from 20 ± 2% to 4 ± 1%. Incubation of 3H-Δ4 for 30 minutes with testicular homogenates also resulted in an increase from 6 ± 1% to 26 ± 2% of 5α-reduced steroid formation after LHRH-A treatment. These in vitro data clearly show that LHRH-A treatment causes two important changes in the metabolism of steroids in the testis: inhibition of 17α-hydroxylase activity in Leydig cells, and stimulation of non-Leydig cell 5α-reductase activity.
Microsurgical vasovasostomy has been performed on 85 patients. Forty-seven couples have been followed for more than six months; of those, 37 couples have been trying to achieve pregnancy and 21 have succeeded. Eleven couples have been followed for 18 months or more, 9 have achieved pregnancy. Serum sperm agglutinating antibody (SSAA) and immobilizing antibody (SSIA) has been tested in 29 patients pre-operatively and/or post-operatively. Pregnancy has been produced by 14 of those patients. SSAA positive to a titre of greater than or equal to 1:20 has been discovered in 18 patients. These men have produced 8 of 14 pregnancies. Six of 14 pregnancies have occurred in wives of men who were SSAA negative. The difference is not significant. SSIA of greater than 4 SIV has been detected in 10 men, three of whom have produced a pregnancy. Eleven pregnancies have been achieved by men found to be SSIA negative. The difference is not statistically significant. Seminal plasma sperm agglutinating antibody (SPSAA) has been measured in 22 men, eight of whom have achieved pregnancy. SPSAA titres of greater than or equal 1:10 have been discovered in four men, none of whom have produced a pregnancy. Men with a negative titre have produced eight pregnancies. The difference is not statistically significant. It would appear that the level of serum agglutinating or immobilizing antibody has little or no role in the ultimate success (pregnancy) of a vasovasostomy. The significance of SPSAA is less clear. Our numbers are small, but if the present trend of our data continues, statistical significance will be established and perhaps SPSAA determinations can be used as a preoperative screening test to discover those men who have little chance of successful vasovasostomy.
In recent years with an increasing demand for the reversal of the vasectomy to regain fertility (Amelar et al., J. Urol. 127:547-550, 1979), a greater need for understanding the effect of vasectomy on various factors controlling the male reproductive process became apparent. Although improved surgical methods can increase the rate of patency of vas, the rate of pregnancy may remain unaltered (Lee and McLoughlin, Fertil. Steril., 33:54-55, 1980). Therefore, it is also important to understand the factors regulating both sperm and semen quality, besides the improved surgical techniques for higher rate of vas patency. Testicular factors especially require further investigation. During the present project we studied testicular biopsies from five patients undergoing vas reanastomosis. They were 28 to 40 years old with 2-5 children prior to vasectomy. The intervals between vasectomy and vas reanastomosis ranged from 5 years to 11 years. Small pieces of testicular tissues, processed as usual for electron microscopic investigation were studied at both the electron and light microscopic levels. In most cases the lumen of the seminiferous tubules were either partially filled with spermatocytes and immature spermatids or had become completely occluded. Variable thickening of the basement membrane was accompanied by extensive protrusions of basal lamina inside the seminiferous tubule epithelium. Disorganization, vaculolization and abnormal development of germ cells were common features. There was a wide range of variation in fine structural morphology of testicular tissues obtained from different patients. Age and duration of vasectomy seemed to have no apparent effect. However, an abnormality in the peritubular area and basement membrane seemed to have considerable effect in inducing alteration of seminiferous tubular morphology and physiology.
It has become apparent that circulating antisperm antibodies in both males and females decrease fertility. Recently, there have been several reports that corticosteroids can suppress circulating antibody levels and thus enhance fertility in such subfertile individuals. We determined the effectiveness of steroid therapy on the pregnancy rate of 14 men and 6 women with antisperm antibodies. The Isojima sperm-immobilization assay and the Kibrick sperm-agglutination test were used to evaluate antibodies. The patients were treated with 60 mg of prednisone daily for 1 week. The number of couples who conceived in the next 4 months was compared with the pregnancy rate of 20 couples in which one partner had comparable antibody levels but did not receive treatment. The rates were 45% and 15%, respectively, for the treated and untreated groups. These values were not significantly different by chi-square analysis. All patients did not exhibit a total drop in antibody titer after treatment. In fact of 20 patients tested, 8 had total drops in circulating antisperm antibodies after treatment; 6 had partial drops; and 6 had no drop. Those that exhibited a total or partial drop in sperm-immobilizing antibodies had a significantly higher chance of pregnancy than those with no drop (p < 0.05). Of 14 men on whom we had pre- and post-treatment data concerning the presence or absence of sperm-agglutinating antibodies in the seminal plasma, 4 were positive before treatment and 3 after treatment; the 1 who became negative after treatment caused a pregnancy. Our data suggest that 60 mg of prednisone daily for 7 days may cause a reduction in circulating antisperm antibodies and may enhance the chance of conception.
99. EFFECT OF GENOTYPE ON THE TESTICULAR GONADOTROPIN BINDING IN THE MOUSE.

Armando Amador, Mary van Sickle, & Andrzej Bartke. Department of Obstetrics & Gynecology, University of Texas Health Science Center, San Antonio, Texas, 78284.

We have measured testicular gonadotropin receptors and down-regulation of LH receptors by hCG in mice of different genotypes, including mutants which are used as models of human endocrinological diseases. In animals with testicular feminization (Tfm/y), the concentration of LH receptors was elevated (24 vrs 160 fmols/mg protein) and their loss 24 hrs after injecting 0.3 IU hCG/g b.wt. was proportionally greater (68 vrs 90%) than in their littermates. In Dwarf mice (dw/dw), the number of LH receptors was reduced (27 ± 3 vrs 16 ± 2 fmols/mg protein) but their loss after hCG injection was similar (70 vrs 65%). Two strains, HIOV and LIOV which were selected for high and low ovulatory response to PMSG/hCG and differ in testicular responsiveness to gonadotropins in vitro, were shown to differ in testicular FSH binding (2.2 ± 0.4 vrs 3.2 ± 0.5 fmols/mg protein; P < 0.05) while LH binding was similar (32 ± 3 vrs 35 ± 3 fmols/mg protein). However, loss of LH receptors after hCG administration seems greater in HIOV, the strain with greater testosterone response to gonadotropin stimulation (46% vrs 59%). In Little mice (lit/lit), the concentration of LH receptors was similar (21 vrs 23 fmols/mg protein) to that of their littermates, but the loss of LH receptors after hCG administration is apparently greater than in their littermates (97 vrs 90%). The evidence for polygenic control of testicular gonadotropin binding and its regulation and for the involvement of sensor type genes will be discussed.
Studies of epididymal function would be facilitated if principal cells or basal cells could be cultured. Principal cells from B capita epididymidum were isolated by elutriation (Klinefelter & Amann, 1980) and suspended (4 x 10^6/ml) in leucine-free MEM (Earle's salts) supplemented with 10% ovine rete testis fluid, 0.2% BSA, 20 μM L-leucine, 1mM L-carnitine, 100 nM insulin, 100 nM cortisol, 200 nM testosterone and 15% bovine calf serum. Equal volumes (1.0 ml) of principal cell suspension and ice-cold collagen solution were pipetted simultaneously into 60-ml plastic tissue culture dishes and mixed. Within 20 min (34°C; 5% CO₂) gelation was completed. Each gel was detached from the dish and allowed to float on 3 ml of medium. After 20-24 hrs, serum-free medium was used. For morphological studies, segments of culture matrix were fixed and evaluated by SEM or TEM. Cultures contained both isolated cells and clusters of cells surrounded by collagen fibers. Cell division was not seen. Morphology of principal cells on days 1, 3 or 5 was similar to that of cells in situ. Metabolism of (3H)-testosterone (650 pmoles) was evaluated over days 1-3 and 3-5. (3H)steroids extracted from the medium were isolated by celite chromatography and HPLC. Virtually all of the 3H was recovered as 5α reduced metabolites (70% dihydrotestosterone). (3H)protein produced during 48 hr incubation with (3H)-L-leucine was isolated by gel filtration, dialysis and lyophilization. Lyophilized protein was evaluated by immunoelectrophoresis against anti-rat cauda epididymal plasma; one precipitin arc was seen consistently. Filtration of native lyophilized protein on Sephadex G-100, revealed > 3 (3H)proteins (107,000, 65,000 and 26,000 daltons). The middle peak was concentrated and on Sephadex G-75 gave a single peak (62,000 daltons). SDS electrophoresis revealed numerous (3H) polypeptides. Based on cell morphology, testosterone metabolism and protein secretion, principal cells cultured within a collagen matrix retained their integrity and function for at least 5 days.
101. EFFECT OF SEXUAL MATURATION ON TESTICULAR LH RECEPTOR LEVELS AND ON BASAL AS WELL AS o-LH-INDUCED STEROID CONTENT IN RAT TESTIS. A. Belanger, C. Sépinin, S. Caron, and F. Labrie. Department of Molecular Endocrinology, CHUL, Quebec, Canada

Previous studies have shown that testicular 5α-reductase and 3α-hydroxysteroid oxidoreductase activities are high in young rats and decline gradually with age. In agreement with in vitro studies, there are high amounts of 5α-androstan-3α, 17β-diol (3α-diol) in the peripheral plasma in immature rats while, in the adult, testosterone (T) appears as the major steroid. The present study was designed to correlate testicular LH receptor levels with testicular basal as well as o-LH-stimulated levels of progesterone (P), 17-hydroxyprogesterone (17-OH P), T and 3α-diol during maturation. Rats at the age of 10, 20, 35, 50 and 75 days were killed by decapitation two hours after injection of 10 μg of o-LH or the vehicle. Testicular LH receptor levels gradually increase from 125 ± 5 to 950 ± 100 fm/g testis between 10 to 50 days of age and remain constant until day 75. Basal testicular concentrations of P and 17-OH P in groups of rats aged 10-75 days are 5 ng/g testis or less. However, when LH is injected, only rats at 75 days of age respond with a stimulation to 62 ± 4 and 34 ± 2 ng/g testis for P and 17-OH P, respectively. Unlike progestins, basal concentrations of T which are the same at days 10 or 75 of age (~75 ng/g testis) are markedly decreased before puberty with the lowest concentration at day 35 (3.0 ± 0.5 ng/g testis). Moreover, while the T response to LH is maximal at days 10 or 75 of age (750 and 1400 ng/g testis, respectively) the increase at 35 days of age is low (12 ng/g testis). In contrast, the testicular content of 3α-diol is low at days 10 and 75 of age (25-30 ng/g testis) and becomes high (100-150 ng/g testis) during the period of 20-50 days of age. In addition, contrary to that of T, the maximal response of 3α-diol to LH is observed at 35 days of age (1575 ng/g testis). In conclusion, the basal secretion of total androgens remains approximately the same throughout maturation while the capacity of the testis to secrete androgens in response to LH is highest at days 35 and 75 of age. These changes are independant to those of LH receptor levels.

The Sperm Penetration Assay (SPA) utilizing human sperm and hamster ova was performed on semen samples from 217 men from infertile couples. The assay results were correlated with the number of white blood cells in the semen and the results of semen analysis (count, motility, speed, % normal sperm, % immature sperm). The % penetration of the SPA most closely correlated with the number of leukocytes in the semen (r = -0.42). Seventy-nine of 103 men with SPA ≥ 14 (normal) had < 1 wbc/100 sperm, whereas 77 of 114 men with SPA ≤ 12 (abnormal) had ≥ 1 wbc/100 sperm (p < 0.005). Forty-two of these men had simultaneous semen cultures for aerobic bacteria, C. trachomatis, U. urealyticum, M. hominis, C. genitalium, and C. vaginales. No difference was found in the seminal bacteriology of the 24 SPA positive (≥14%) and the 18 SPA negative (≤12%) patients. Nineteen patients with negative SPAs underwent empirical antibiotic treatment and 11 tested positive post-treatment. Whereas, none of 59 men with negative SPAs who were retested without treatment became positive (p < 0.005).
The Effect of Age on Sperm Concentration in Hamsters. F. Cisneros and L.E. Franklin. Univ. of Houston, Houston, Texas 77004

In developing a quick method for determination of sperm concentration, it was noted that sperm taken from different aged males had their own turbidometric properties. Sperm from males aged 2-4 mo., 12 mo. and 24 mo. were serially diluted in 0.006M EDTA to prevent head-to-head agglutination. Data points, optical density vs sperm counts, were computer fit for each age group. Each data group had a high correlation coefficient indicating that each relationship was linear. The difference in sperm concentration is highly significant between the 2-4 mo. males and the aged males (12 and 24 mo.). At the 99% confidence level the standard curves of sperm concentrations from the 12 and 24 mo. old males were the same line. The results show that as the males age the optical density of the sperm sample decreases while the sperm numbers increase. To eliminate the possibility that frequency of mating was influencing the sperm counts, all males were mated 9 days before sacrifice. The matings did not change the differences in sperm numbers between young and aged males. It was suspected that an increase in separated heads and tails might be responsible for the differences in optical density readings but there was no significant difference between sperm treated with 1% trypsin (92% separated heads) and the controls (0.5% separation). To eliminate the possibility that some epididymal fluid factor was influencing the O.D. readings, the sperm samples were washed in Tyrode's buffer before turbidometric analysis. Washing the sperm did not influence the O.D. readings of sperm from either young or aged males. The data indicate that male hamsters have reached a peak in sperm production by 12 mo. Thereafter there is no decline in the peak in fertile males throughout the lifespan of 24 mo.
SPECIES DIFFERENCES IN TeBG STEROID SPECIFICITY. G. R. Cunningham, D. J. Tindall, T. J. Lobl, and J. A. Campbell. Departments of Medicine and Cell Biology, Baylor College of Medicine and the Veterans Administration Medical Center, Houston, Texas, and The Upjohn Company, Kalamazoo, Michigan.

The affinity of an androgen for testosterone estrogen binding globulin (TeBG) is thought to play an important role in determining its metabolic clearance rate and its biological activity. To assess species differences and steroid structural modifications, we have utilized steady state polyacrylamide gel electrophoresis (Tindall et al., J. Biol. Chem. 253:166, 1978) to study the affinity of unique analogues of dihydrotestosterone (DHT) and testosterone (T) for human, baboon, dog and rabbit TeBG. Competition studies were conducted using an 0.2nM concentration of \(^3\)H-DHT and a 10 nM concentration of each of 25 analogues. These data indicated that the steroid specificity of baboon TeBG is most similar to the human and that the specificity of dog TeBG is least similar. In order to assess apparent differences between these steroids, complete competition curves were determined. The following table indicates the concentration of unlabeled steroid required to displace 50% of the \(^3\)H-DHT (K1):

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Human</th>
<th>Baboon</th>
<th>Dog</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>17(\beta)-OH-5(\alpha)-androstan-3-one (DHT)</td>
<td>1.46</td>
<td>1.43</td>
<td>5-10</td>
<td>1.75</td>
</tr>
<tr>
<td>17(\beta)-OH-4-androst en-3-one (T)</td>
<td>1.9</td>
<td>2.1</td>
<td>56</td>
<td>1.9</td>
</tr>
<tr>
<td>17(\beta)-OH-5(\alpha)-estran-3-one (19 nor-DHT)</td>
<td>8.2</td>
<td>7.2</td>
<td>46</td>
<td>18</td>
</tr>
<tr>
<td>5(\alpha)-androstan-3(\beta),17(\beta)-dial</td>
<td>0.04</td>
<td>1.1</td>
<td>&gt;25</td>
<td>&gt;25</td>
</tr>
<tr>
<td>17(\beta)-OH-7(\alpha)-CH(_3),1(\beta)-estradien-3-one</td>
<td>1.0</td>
<td>1.6</td>
<td>&gt;50</td>
<td>12</td>
</tr>
</tbody>
</table>

We conclude that in vitro studies of this type are important in selecting synthetic androgens and animal models for pharmacodynamic studies. Of the three animal models examined, the baboon is most similar to man and the dog is the least similar.
The testes of fertile male hamsters, 4 to 25 months of age, were excised, fixed, and prepared for light and electron microscopy. The degenerative changes, normally associated with advancing age, were observed in all age groups studied. These changes involved a cessation of spermatogenesis, a progressive loss of the germinal epithelium and an intratubular accumulation of hyaline material. In advanced stages only the Sertoli cells remained, embedded in a hyaline matrix, at the periphery of the tubule, or the collapsed tubules were completely occluded and atrophied. Detached germ cells, singly or in groups, were often observed in areas of apparently normal spermatogenesis.

Tubular degeneration was not uniform throughout the testes. Abnormal tubules could be found alone, surrounded by normal tissue, or in groups. They were most frequently encountered in close proximity to the tunica albuginea.
Reproductive Biology is taught to first year medical students during the Endocrine/Reproductive Block of our curriculum. A teaching team composed of practicing physicians, anatomists, embryologists, physiologists, pharmacologists and psychologists integrate material of the Endocrine/Reproductive System. Using that portion of the curriculum dealing with male reproduction, we illustrate how the student makes use of real clinical cases of Cryptorchidism and Retarded Sexual Development to learn and integrate the anatomy, physiology, and embryology of the system, with an appropriate introduction to pathophysiology. Basic science modules which contain behavioral objectives, evaluation criteria, learning resources, and study guides deal with neuroendocrine regulation, androgen biosynthesis and metabolism, sperm transport, semen analysis, contraception, and hypogonadism. Basic science lectures, "help" sessions, laboratory demonstrations, and clinical discussions are also illustrate our mastery (criterion-referenced) grading system by describing a simple method in which students engage in directed relearning as a consequence of incorrect answers to test questions.
Studies in our laboratory have attempted to define the effects of certain drugs of abuse on the reproductive system using the nonhuman primate as a model. We have attempted to classify the effects of these drugs at the level of the hypothalamic-pituitary axis, the gonad, and the tissues of the reproductive tract. The present study consists of a comparison of the acute effects of 3 commonly abused classes of drugs and definition of the mechanisms by which these effects are produced. Sexually mature male rhesus monkeys were administered intoxicating doses of delta-9-tetrahydrocannabinol (THC), morphine sulfate (MS), and ethanol (EtOH). Blood was drawn at hourly intervals after drug or vehicle administration for measurement of hormone and drug levels. Comparisons of the effects of the drugs on hormone levels were made on the basis of relevant dose levels of the drugs. THC (0.5-2.5 mg/kg; im) administration produced a significant depression in testosterone levels that lasted as long as 24 hours. Morphine sulfate administration (1.0 mg/kg; im) produced a significant depression in testosterone levels that was present only at 3 and 6 hours after drug treatment. Acute intravenous administration of ethanol (maximum blood level of 200 mg/100mls after a 20 min infusion) produced no consistent effect on testosterone levels in these monkeys.

These results indicate that single doses of either THC or morphine can decrease testosterone levels in male primates (probably by a hypothalamic-pituitary mechanism). The depressions in testosterone levels usually associated with chronic alcohol use were not evident following the acute alcohol administration in these studies.

(Supported by NIDA ROI-2063).
THE RELATIONSHIPS BETWEEN SEMEN PRODUCTION, ENVIRONMENTAL TEMPERATURE, AND CARBONIC ANHYDRASE ACTIVITY IN THE REPRODUCTIVE SYSTEM OF THE MALE CHICKEN. K. Goto and G. C. Harris, Department of Animal Sciences, University of Arkansas, Fayetteville, AR.

Forty adult male chickens that had been exposed for 30 weeks to controlled diurnal temperatures of either 18.3-29.4°C or 23.9-35.0°C were studied. Males of both groups showed significant positive correlation coefficients between volume of semen, sperm, or seminal plasma and carbonic anhydrase (CA) activity of testis. No relationship was found between CA activity of epididymis and ductus deferens with semen production in the moderate temperature group. In contrast, males of the high temperature group showed significant positive correlations between CA activity of ductus deferens and volume of semen, sperm or seminal plasma, and also between CA activity of epididymis and volume of semen or seminal plasma. The volume of spermatozoa that were produced by males of either temperature group was not related to body weight, testis weight or epididymis weight. Males of the high temperature group had significantly lower CA activity of erythrocytes than males of moderate temperature group. No significant differences in CA activity of testis, epididymis or ductus deferens were observed between males of the two environments.
We have demonstrated that rat thymic tissue contains specific, high affinity receptors for both dihydrotestosterone (DHT) and estradiol (E2). We currently are investigating human thymic tissue obtained as a by-product of cardiac surgery from children of both sexes, ages 6 mos to 15 yrs. Tissue was homogenized in 0.1 M Tris, 1 mM EDTA, 0.012 M Thioglycerol, 10% Glycerol to study DHT receptor (DHT-R) or in 0.1 M Tris, 1 mM EDTA to study E2 receptor (E2-R) and then centrifuged at 120,000g for 30 mins to prepare a cytosol fraction. Utilizing Scatchard plot analysis the cytosol fraction was shown to contain DHT-R (K_a: \(8.5 \times 10^{9} \text{M}^{-1}\)) at a concentration of 3.7 \pm 0.3 pmol/g tissue (n=26) and E2-R (K_a: \(8.6 \times 10^{9} \text{M}^{-1}\)) at a concentration of 0.2 \pm 0.02 pmol/g tissue. Employing dextran-charcoal competition assay the results for DHT-R were as follows using DHT as 100%: Testosterone, 43%; E2, 7.2%; Progesterone, 13%; Cortisol, 0%. For E2-R using E2 as 100%: Diethylstilbestrol, 8.9%; DHT, 0.4%; Cortisol, 0%. Sucrose gradient centrifugation studies reveal that both human thymic DHT-R and E2-R sediment in the 4.8s region of 5-20% sucrose gradients. It appears that human thymus contains a DHT-R of high affinity and limited specificity similar to the androgen receptor reported for other tissues but having a sedimentation coefficient of 4.8s rather than 8s. These results also suggest that the properties of the E2 binding protein in human thymic cytosol are similar to sex steroid binding globulin. However, since E2 levels unlike DHT levels in children are relatively high, the E2-R may not be present in the cytoplasmic fraction.

Fresh, cumulus-free eggs of the golden hamster were used to secure live sperm at appropriate intervals from suspensions in capacitating (complement-inactivated human blood serum: Tyrode's solution, 1:1) and control (Tyrode's solution) media. The eggs were examined to ensure that all adherent sperm were motile, then fixed for electron microscopy. In the control medium, maximum amounts of spermatozoa were bound to the zona within 15 minutes. Sections of eggs and sperm collected at 15 min., 1, 3, and 5 hr. post-insemination revealed only unreacted sperm (i.e., with intact acrosomes) and acrosomal remnants or "ghosts" representing products of the degenerative acrosome reaction. In the capacitating medium, 30 or more minutes were required for maximal sperm-zona binding, though far less sperm were bound per egg than in the control medium. Sections of eggs and sperm collected at 15 min. and 2½ hr. post-insemination revealed only unreacted sperm and acrosomal ghosts bound to the zona. At later time intervals (3½ hr., and 5 hr.) a growing number of reacted spermatozoa (i.e., lacking an acrosome) were also seen tightly bound to the zona. Hybrid vesiculation characteristic of intermediate stages of the normal acrosome reaction could be best observed at 3½ hr. None of the discarded acrosomes observed in the control (non-capacitation) medium exhibited hybrid vesicles. Instead, the plasma membrane was absent and a scalloping of the outer acrosomal membrane was observed.
The rat is often used as a model to study conditions affecting the human prostate, such as neoplasia and hyperplasia. We have characterized the various morphological features of the rat prostate complex by anatomical dissection and light microscopy, viewing the accessory complex as a whole rather than as isolated structures. The vascular supply of the prostate and surrounding structures which include the seminal vesicles, coagulating glands, vasa deferentia and ampullary glands, urinary bladder, and ureters is reviewed. The three lobes of the prostate are designated as ventral, lateral, and dorsal according to their relation to the urethra. These lobes are connected to the urethra by fascia and by a series of ducts, which are traced to their origin. The ventral lobe is characterized by mainly ovoid acini lined with columnar epithelium and filled with pale staining secretion. Each ventral lobe is drained by 4 or 5 ducts. The dorsal and lateral lobes are composed of larger, more irregularly shaped acini with many infoldings, lined mainly with cuboidal to columnar epithelium and filled with strongly eosinophilic secretions. There are 7 or 8 ducts for each lateral lobe and 10 to 14 ducts for each dorsal lobe. Each duct in the dorsal and lateral lobes drains from 1 to 4 acini; these connective tissue wrapped ducts can be dissected free from their neighbors. Regardless of point of origin and length, all ducts from glands enter the prostatic urethra at the same level. While the acinar pattern of each lobe is unique, the dorsal and lateral lobes resemble each other more closely than they do the ventral lobes.

(Supported by the Grainger Foundation and by NIH grant HD-01611).
A COMPARISON OF METHODS FOR ESTIMATING DAILY SPERM PRODUCTION IN FIXED VERSUS FRESH TESTICULAR PARENCHYMA. L. Johnson and W.B. Neaves, Department of Cell Biology, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

Daily sperm production per gram of testicular parenchyma (DSP/g) was estimated for ten men by a) histometric analysis of round spermatid nuclei in glutaraldehyde-perfused testes, b) enumeration of both round and c) maturation-phase spermatid nuclei in homogenates of fixed testicular parenchyma, and d) enumeration of maturation-phase spermatid nuclei in unfixed contralateral testes. Calculations relied on data from Heller and Clermont (1964) indicating a time divisor for round spermatids of 8.9 days (duration of Stages I through III) and for maturation-phase spermatids of 7.9 days (Stages I and II). Mean DSP/g for these ten men by histometric analysis (method a = 5.89 ± 0.87 x 10^6) was not different (p>0.05) from that estimated in homogenates of fixed parenchyma (method b = 5.52 ± 0.83 x 10^6 and method c = 5.46 x 10^6). The average difference in values between methods a and b (r = +0.93) and between methods a and c (r = +0.86) was not significantly different from zero (p>0.05). Mean DSP/g based on homogenates of unfixed contralateral testes (method d = 2.06 ± 0.38 x 10^6) was significantly lower (p<0.01) than values obtained from fixed testes; however, individual values were significantly correlated (p<0.01) with values obtained by methods a (r = 0.79), b (r = 0.83), and c (r = 0.79). The systematically lower values obtained with method d probably reflect a failure of unfixed nuclei from younger maturation-phase spermatids to survive the homogenization procedure and strongly suggest that an inappropriately large time divisor was used in method d. Indeed, if a time divisor of 3.1 days (Stage II only) is used, method d yields a mean value of 5.26 ± 0.96 x 10^6, which is similar (p>0.05) to values obtained by the other methods.
113. IDENTIFICATION OF AN INTRA-MOLECULAR MECHANISM FOR THE CONVERSION OF PROACROSIN INTO mα-ACROSIN. William P. Kennedy and Kenneth L. Polakoski. Department of OB/GYN, Washington University School of Medicine, St. Louis, MO 63110

An alternative to the acrosin-catalyzed mechanism for the conversion of proacrosin into acrosin in vitro has been identified and characterized. In the presence of excess leupeptin, a potent acrosin inhibitor, highly purified boar proacrosin spontaneously and completely converted into active enzyme, but only into its initial enzyme form, mα-acrosin. Kinetically, the rates of proacrosin conversion into mα-acrosin appeared first order with a half-life (t½=1.4 hr) that did not vary over a 10-fold range of leupeptin or initial proacrosin concentration nor was it affected by the exogenous addition of mα-acrosin. These results demonstrate that the conversion of proacrosin into active enzyme does not require acrosin activity since it was completely eliminated in these experiments by excess leupeptin. Nor does the conversion require the activity of some other undetectable protease that may have been present in the proacrosin preparation. Rather, these data show that proacrosin is capable of catalyzing its own conversion into active acrosin by an intra-molecular mechanism. These in vitro data suggest that the in vivo conversion of proacrosin into mα-acrosin does not necessarily require auxiliary enzymatic activity. Accordingly, regulation of this intra-molecular conversion mechanism may be a significant feature in the physiological regulation of the proacrosin-acrosin system.

(Supported by NIH Grants 09422, 00296 and 12863).
The epididymal maturation and phylogenetic distribution of the cAMP-dependent protein kinase regulatory subunits were studied by use of the photoaffinity probe \((\text{32P})-8\text{N}\text{J}_{\text{AMP}}\). Autoradiographic analysis of SDS-polyacrylamide gels indicates that both Type I (49,000 M\(_r\)) and Type II (55,000 M\(_r\)) regulatory subunits are present in rat sperm from the caput and caudal regions of the epididymis. Incorporation of the photoprobe was quantified by liquid scintillation, and Type I subunit incorporated substantially higher amounts than the Type II subunits in sperms from both regions of the epididymis. In caput to caudal sperm both regulatory subunits were saturated with 125 nm to 150 nm BN\(_3\) cAMP. Analog binding to these proteins could be blocked by excesses of cAMP but not cGMP, ATP, GTP, or NADH, indicating the cAMP specificity of the subunits. Protein kinase regulatory subunits appear to be qualitatively similar in epididymal sperms of rat, hamsters, guinea pigs, and bulls, and in ejaculated sperms from rabbits, bulls and human (Schoff, et al. 1979). But sperms from species lacking an epididymis, i.e., sea urchins *Lytechinus pictus* and *Strongylocentrotus purpuratus*, photoincorporated BN\(_3\)-cAMP only into Type I regulatory subunits. These studies suggest that both Type I and Type II cAMP-dependent protein kinases are widely distributed in both ejaculated and epididymal sperm of mammals. But only Type I cAMP-dependent protein kinase can be detected in two species lacking an epididymis.

(Supported in part by Grants GM 21998 (NIH:EBH) and HD 11991 (NIH:RWA).)
ADVANTAGES OF GONADOTROPIN (GN) OVER TESTOSTERONE THERAPY FOR VIRILIZATION OF GN DEFICIENT HYPOPITUITARY MALES. M.H. MacGillivray, R.E. Peterson, M.L. Voorhees, R.R. Clopper and T. Mazur. Dept. Pediatrics and Behavioral Science, SUNY at Buffalo, School of Medicine and Dept. Medicine, New York Hospital-Cornell Medical School.

Aim: Experience has shown that testosterone (T) Rx fails to virilize Gn deficient males with childhood onset hypopituitarism. This study compares the benefits of HCG/Pergonal vs. testosterone therapy.

Study: 4 males, 17-31 yrs. with idiopathic (2) or organic (2) hypopituitarism who lacked beard growth, axillary and body hair, psychosexual maturation and libido despite high dose T Rx (>6 yrs. in 3). T therapy had stimulated pubic hair, phallic enlargement and voice deepening. All were GH, TSH, ACTH, FSH, LH deficient with normal prolactin levels and all had received prior GH Rx. Current meds.: thyroxine (4) and cortisone (2). T was stopped 1 month before starting HCG (2000 U.) and Pergonal (1 vial), I.M., 3 times weekly for 12-24 mos.

Results: Marked improvements noted in facial, body and pubic hair growth; testes volume; muscle strength and libido. Increased plasma dihydrotestosterone (DHT), androstenedione (A4), dihydroepiandrosterone (DHEAS) and 17-hydroxyprogesterone (17-OHP) did not correlate with the degree of virilization. Sperm counts in 3 varied between 6 to 24 x 10^6. Behavioral changes with Gn therapy are given in the abstract by Clopper, et. al.

Baseline (A) and Treatment (B) Plasma Steroids (ng/dl)

<table>
<thead>
<tr>
<th>Age</th>
<th>T</th>
<th>DHT</th>
<th>A4</th>
<th>DHEAS</th>
<th>17-OHP</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>446</td>
<td>80</td>
<td>125</td>
<td>48</td>
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<td>25</td>
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<td>31</td>
<td>360</td>
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<td>300-1000</td>
<td>30-80</td>
<td>50-150</td>
<td>100-350</td>
<td>100-200</td>
</tr>
</tbody>
</table>

Conclusion: Gn Rx is superior to T Rx. Gn Rx may be essential for androgen stimulation of the hair follicle and psychosexual maturation. Alternatively, Gn Rx increases the output of gonadal steroids which directly or indirectly contribute to virilization and erososexual function.

Hemicastration in both sexes results in compensatory hypertrophy of the remaining gonad. Since hypothyroidism slows metabolic and maturation rates, it was of interest to determine the interactive effect of these conditions on gonadal growth and plasma LH. Two week old Sprague-Dawley male (40) and female (40) rats were sham-operated or right side hemicastrated and two days later half of each group received i.p. saline or 0.5 mCi $^{131}$I in saline. At four weeks of age animals were sacrificed, organs were weighed and collected blood was immediately centrifuged and plasma was stored at -70°C. No difference in body weights among the experimental groups was observed in either sex (mean 80.9 ± 2.0 for males, 74.8 ± 2.7 for females). In both sham and hemicastrated males, $^{131}$I treatment resulted in significantly lower testicular weights (25% and 28% lower, respectively). In females $^{131}$I treatment resulted in 27% lower ovarian weights in hemicastrates, but $^{131}$I treatment produced no difference in ovarian weights in sham-operated groups. In both males and females hemicastration resulted in significant compensatory hypertrophy (25% and 41%, respectively) when compared to sham controls, and $^{131}$I did not prevent this compensatory hypertrophy. Plasma LH levels in males were lower only in the hemicastrate group (20.5 ± 2.9 ng/ml) compared to controls (34.8 ± 5.9 ng/ml). In females, there were no differences in plasma LH among any groups studied. In conclusion, hypothyroidism retards gonadal growth but doesn't prevent gonadal compensatory hypertrophy and does not influence plasma LH levels. There appears to be a sex difference in the response to hemicastration since plasma LH is only decreased in male hemicastrates.
DOES IMMUNIZATION OF MONKEYS WITH OVINE FOLLICLE STIMULATING HORMONES (o FSH) BREAK THE IMMUNOLOGIC TOLERANCE TO ENDOGENOUS FSH AND LEAD TO AUTOIMMUNITY? G.S.R.C. Murty and H.G. Madhwa Raj. Depts. of Ob/Gyn and Pharmacology, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514.

We have previously demonstrated that active immunization of monkeys (M. fascicularis) with ovine FSH or its \( \beta \) subunit suppresses spermatogenesis without reducing plasma testosterone (T) levels. (G.S.R.C. Murty, H.G. Madhwa Raj and M.R. Sairam, Biol-Reprod. 22, Suppl. 1, P. 54A, 1980). The present study was undertaken to examine whether there is break of tolerance to endogenous FSH in these chronically immunized monkeys, leading to autoimmunity. For this purpose control (n=3) and immunized (n=10) monkeys were administered 100 \( \mu \)g of LHRH intravenously to release endogenous FSH. Blood samples were collected from femoral vein at various time intervals and analysed for FSH antibody titers, FSH and T levels using suitable radioimmunoassays. Control monkeys exhibited a 2-3 fold rise in plasma FSH levels beginning 1 hour after LHRH challenge. These elevated FSH levels were sustained up to 6 hours. Such a rise could not be assessed in immunized monkeys due to interference from antibodies to ovine FSH present. Measurements of serum T revealed a 3-10 fold elevation over basal levels at 1 hr. after LHRH challenge in both groups. These high levels were sustained even at 6 hrs., and returned to basal levels, when examined at 7 days. This data indicates a rise in LH and FSH levels in the monkeys as a result of LHRH challenge. However, the FSH antibody titers did not rise in the immunized monkeys at 7 days after such an LHRH challenge.

From the above data we conclude that active immunization with oFSH does not break immunologic tolerance for endogenous FSH.
THE AGING HUMAN LEYDIG CELL: RESPONSE TO HCG. Nankin, T. Lin, E.P. Murone, and J. Osterman. Medical Service, Wm. Jennings Bryan Dorn Veterans' Hospital and Department of Medicine, University of South Carolina School of Medicine, Columbia, South Carolina.

To assess the capacity of the aging human Leydig cell to respond to in hCG, six healthy men 68 to 86 years old, and eight healthy younger subjects 25 to 42 years of age were studied. After two control specimens each subject was given hCG 40 IU/kg of body weight im at 0900h. Blood specimens were then obtained hourly for 6h and at 24, 48 and 72h.

The younger men revealed higher control concentrations of testosterone and 170H-progesterone, and lower basal levels of estradiol. The early (1-6h) and late (24, 48 and 72h) testosterone and 170H-progesterone responses to hCG were significantly lower in the older men. The older men demonstrated significant estradiol increases at 3 and 6h, while the younger group had no changes during the first 6h. However, the late estradiol rises were significantly greater in the younger men.

The elderly Leydig cell demonstrates altered early (1-6h) and late (24, 48 and 72h) responses to in hCG, as assessed by circulating concentrations of testosterone, 170H-progesterone and estradiol.
INVOLVEMENT OF DOLICHOL PHOSPHATES AS INTERMEDIATES IN THE MANNOSEY AND GALACTOSE TRANSFERASES OF RAT TESTICULAR GERM CELL GOLGI APPARATUS MEMBRANES.

S.E. Nyquist, K. Flanagan, and W.H. Scouen. Departments of Biology and Chemistry, Bucknell University, Lewisburg, PA 17837

Golgi apparatus membranes from the spermatocyte and early spermatids of rat testis were isolated by density gradient centrifugation and examined for their ability to incorporate [14C]-mannose and [14C]-galactose into glycolipid and glycoprotein fractions. Transfer of mannose from GDP-[14C]-mannose into a Lipid I fraction, identified as mannosyl phosphoryl dolichol, showed optimal activity at 1.5 mM manganous and at pH 7.5. Low concentrations of Triton X-100 (0.1%) stimulated transferase activity in the presence of exogenous dolichol phosphate (Dol-P). The enzyme, however, was inhibited by detergent concentrations greater than 0.1%. Maximal activity of this enzyme (GDP:MPP mannosyl transferase) occurred at 25 pM Dol-P. GDP:MPP mannosyl transferase activity using endogenous acceptor was 2.34 picomoles/mg/min; whereas in the presence of 25 mM Dol-P the specific activity was 284 picomoles/mg/min, a stimulation of 125-fold. Incorporation of mannose into a Lipid II (oligosaccharide phosporyl dolichol) and a glycoprotein fraction, was also examined. Time course studies in the absence of exogenous Dol-P showed rapid incorporation into Lipid I with a subsequent rise in Lipid II and a glycoprotein fraction, suggesting precursor product relationships. Addition of exogenous Dol-P to galactosyl transferase assays showed only a minor stimulation, less than 2-fold, in all fractions. Over the concentration range of 9.4 to 62.5 µg/ml Dol-P, only 1% of radioactive product accumulated in the combined lipid fractions. This study suggests that the mannosyl transfer involves Dol-P intermediates and also that these Golgi membranes may be involved in formation of oligosaccharide core as well as terminal glycosylations. Further work needs to be done to conclusively rule out the involvement of lipid intermediates in galactose transfer; however, from these and other data it seems clear that mannosyl and galactosyl transferases operate through distinctly different mechanisms.
IDENTIFICATION OF AN ANDROGEN-BINDING PROTEIN IN HUMAN SEMINAL PLASMA (SPABP) THAT IS DISTINCT FROM EPIDIDYMAL ABP AND SERUM TEBG. T.J. O'Brien, T. Oda, R. Smith, G.S. Bernstein, and R.M. Nakamura. Department of Obstetrics and Gynecology, University of Southern California School of Medicine, Los Angeles, California 90033 and Department of Surgery, University of Texas Medical School, Houston, Texas 77030

A high affinity-low capacity binding protein for dihydrotestosterone (DHT) has been identified in human seminal plasma. Scatchard analysis of the DHT-binding showed a range of 35 f mol to 9500 f mol per ejaculate with a dissociation constant of $K_d(app) = 1.6 \times 10^{-9} \text{M}$. No correlation was found between the concentration of SPABP and sperm count or motility but there was a significant correlation ($p = .025$) with the volume of seminal plasma.

In order to determine the source of this binding protein we analyzed seminal plasma from vasectomized men, studied split ejaculates from normal volunteers, and compared SPABP from serum by isoelectric analysis. There was no significant difference in the amount of SPABP in semen from vasectomized and non-vasectomized men. The isoelectric analyses showed that SPABP was distinctly different from epididymal ABP and serum TEBG.

Analysis of split ejaculates showed the following: 1) the sperm density was significantly greater in the first fraction; 2) the volume of the second fraction was significantly greater; 3) the concentration of fructose was greater in the second fraction; and 4) the concentration of binding protein was the same in both fractions.

Thus, we have identified a binding protein in seminal plasma which is similar in affinity and specificity to both epididymal ABP and serum TEBG, but differs in its isoelectric point. It apparently originates from both the prostate and seminal vesicles.
The human Leydig cell contains crystalloids of Reinke and several types of paracrystalline inclusions. These inclusions are not membrane bound, are found commonly in the cytoplasm and are occasionally located in the nucleus. While they are known to be composed of protein, their function has not been determined. Crystalloids of a similar nature have not previously been reported in rodents. Recent EM studies in our laboratory have revealed a type of non-membrane bound inclusion in the Chinese hamster Leydig cell which was not visible in lum plastic sections stained with toluidine blue.

The inclusions vary in size and do not appear to be specifically related to any particular cytoplasmic organelle. In longitudinal sections they consist of a parallel series of serrated, electron dense lines equidistant apart, whereas in cross-section they are composed of equally spaced dot-like densities. These morphological features are indicative of paracrystalline structures. The inclusions were not found in the interstitial cells of one day old newborn, but were occasionally present in the cytoplasm of 19 day old males. They were also found in the cytoplasm and an occasional nucleus of 33 day old animals, but were most common in the cytoplasm and nucleus of Leydig cells from sexually mature males (5-18 months of age). These studies indicate that similarities exist between the inclusions in Leydig cells of the human and Chinese hamster suggesting that the latter species may be a good animal model for determining the role of crystalloids in the human testis.
PRL is known to stimulate the prostate gland, an effect which requires the presence of testosterone (T). The mechanism of this synergism is not known. Research in this laboratory has shown that in the rat, only the lateral lobe exhibits increased growth in response to elevated PRL produced by pituitary grafts (Biol. Reprod. 22, 351, 1980). In order to more closely examine the interaction of PRL with T, we used the same model to analyze the influence of PRL on the half-life (T_1/2) of DHT in the 3 prostatic lobes. Male Sprague-Dawley rats were castrated and given subcutaneous implants of silastic tubes containing T. Two female anterior pituitaries were then grafted under the renal capsule of 25 rats while 25 controls received muscle. Three weeks later, 100 μCi were injected via the femoral vein and rats were decapitated 5', 10', 30', 60', 90', 2', 4', 6', and 18' afterwards. The ventral, dorsal, and lateral lobes were dissected, weighed, and snap frozen. Steroids were extracted from tissue homogenates and separated via TLC. Serum PRL levels were significantly (P<.001) greater in the grafted (114.5 ± 15.0 ng/ml) vs control (23.9 ± 4.7) groups. There were no differences in the ventral and dorsal weights and protein content between the two groups. However, the lateral lobe was markedly (P<.001) greater in weight and protein content in the grafted rats. Disappearance of DHT/mg protein after the pulse followed first-order kinetics. The T_1/2 was the same in the ventral (5.08 hrs.), dorsal (5.20), and lateral (5.58) lobes in the control group (P>0.10). Pituitary grafts did not markedly alter the T_1/2 in the ventral (5.15) or dorsal (4.69) lobes. However, there was a significant (P<.005) decreased T_1/2 in the lateral lobe (3.98) of the rats with pituitary grafts when compared to controls. These data show that the high circulating levels of PRL which stimulate the growth of the lateral lobe also decrease the time of retention of DHT in that lobe. This may explain how PRL synergizes with T to augment the growth of the rat lateral prostate.

(Supported by NIH grant HD 11611).
REDUCTION OF SPERM CONCENTRATION IN A POPULATION EXPOSED TO ETHYLENE DIBROMIDE (EDB). B.J. Rogers, J.S. Fujita, L. Najita and R.W. Hale. University of Hawaii, Department of Obstetrics and Gynecology, Kapiolani Hospital, Honolulu, Hawaii 96826.

The discovery of well water contaminated with high levels of ethylene dibromide (300 ppb), a fumigant used in controlling nematodes in the pineapple fields in Hawaii, prompted an investigation into the health risk of exposure to contaminated water. The objective of this study was to assess potential fertility impairment by routine semen analysis (including sperm count, percentage motile and morphology), by in vitro fertilization tests and by monitoring hormone levels (LH, FSH and testosterone) in serum. Semen analyses were done on 83 individuals, 46 from Kunia and 37 from a neighboring plantation, Poamoho (control group). Occupationally exposed workers were removed from each group for analysis and placed in a separate group. Results were compared for three groups: (1) Kunia (n=39), (2) Poamoho (n=28) and (3) Workers (n=16).

The mean sperm counts in millions/ml were (1) 103.7, (2) 102.1, (3) 108. The % motile values were (1) 53.5, (2) 55.9, (3) 56.3. The values for morphology expressed as % normal forms were (1) 62.4, (2) 62.1, (3) 61.7. Further analysis of distribution of counts, motility and morphology demonstrated that 34.2% in group 1 had abnormally low counts (<40 million/ml) compared to 17.8% in group 2 and 43.8% in group 3. An increased proportion of Kunia individuals and workers having abnormalities was not reflected in motility or morphology. Testosterone measurements by RIA showed 6 of the 39 Kunia men with abnormal levels (<3ng/ml). The LH and FSH distributions were not significantly different among the three groups. Of the 41 individuals that had fertilization tests done, group 1 had 8/22 (36.3%) that tested in the infertile range (<10%), group 2 had 6/15 (40%) and group 3 had 4/4 (100%). These results suggest that some individuals exposed to EDB occupationally or through contaminated water had reduced sperm counts. Whether this represents a cause and effect relationship cannot be definitively concluded.
Protein kinases of rat caudal epididymal sperm exist in three populations, an intracellular set, an extracellular set, and a second extracellular set which requires Ca\(^{2+}\) for topological stability. Catalytic and some regulatory subunits were washed off the cells by gentle centrifugation in Ca\(^{2+}\) titers below 2.5mM. Enzymes were released by the addition of endogenous Ca\(^{2+}\) and this effect was enhanced by the addition of 1mM EGTA. The released enzyme is cellular as catalytic activity was not present in epididymal fluid. The unstable kinase is a discrete set since there was a limit to the activity which could be solubilized. The first EGTA wash released 70% of the unstable enzyme and 100% was released by 3 washes. Comparison of solubilized to retained activity gives ratios from 0.5 to 1.2. Additional catalytic activity can be released by adding cAMP to EGTA-washed cells. Activity in these cells increased only 10% when disrupted by sonication; however, subsequent ultracentrifugation produced a membrane fraction containing 75% of the total activity and a soluble fraction with 25%. Analysis by \(\gamma^{32}\)p ATP labeling and SDS-PAGE indicated that qualitatively distinguishable phosphorylation of endogenous substrates occurred in intact and disrupted cells. The primary phosphorylated substrate in intact sperm with no added cAMP or Ca\(^{2+}\) was a 35,000 Mr protein. The addition of Ca\(^{2+}\) decreased incorporation into this protein by 50% but stimulated phosphorylation in proteins of 56,000 and 42,000 Mr. cAMP had no effect, but with Ca\(^{2+}\) stimulated phosphorylation in 3 additional proteins and enhanced Ca\(^{2+}\)-mediated incorporation. Sonicated cells with no added cAMP or Ca\(^{2+}\) phosphorylated proteins at 35,000 and 44,000 Mr. Ca\(^{2+}\) decreased incorporation in both proteins but stimulated phosphorylation in 5 more proteins. cAMP acted as in intact cells, having no effect alone, but with Ca\(^{2+}\), stimulated new phosphorylation and enhanced Ca\(^{2+}\)-mediated reactions.

(NIH HD-43-8339-02, GM 21998-00, CA 00563-01)
125. STUDY OF 1,200 PREGNANCIES, OBTAINED BY ARTIFICIAL DONOR INSEMINATION WITH BOTH FRESH AND FROZEN SEMEN. A. Schuyssman-Du Bouck, R. Schuyssman, and P. Devroye, Az-Vul, Brussels.

The authors present a large group of successful donor inseminations leading to 1,200 obtained pregnancies.

The overall success-rate has been studied with regard to the quality of semen used, either fresh or frozen, with regard to the number of inseminations per cycle and also with the necessity for inducing ovulation. The overall success-rate is 85%, the reasons for failure are analysed and the fairly constant number of successes obtained at each cycle is stressed.

Attention is drawn on the percentage of abortions and malformations with regard to both fresh and frozen semen.
Chronic administration of superactive analogs of LH-RH decreases plasma LH and testosterone (T) levels and disrupts spermatogenesis in rats providing a basis for their investigation as possible contraceptive agents. We studied the effects of (D-Trp⁶)-LHRH (A) on pituitary-testicular function in rhesus monkeys. Acute administration of 5-500 μg sc of A caused a rapid increase in serum LH levels which reached peak values (50-100 fold) at 2-4 h and then declined gradually to pretreatment levels by 24-48 h. Serum T levels showed a gradual increase (10-15 fold) and peaked at 24 h before declining to control values by 72 h. Based upon the regimen found to disrupt spermatogenesis in rats, 500 μg of A was administered to monkeys twice weekly for 12 weeks. During the course of treatment serum LH and T levels were measured at 2,4 and 24 h after A administration. Repeated administration of A led to a decline in the pituitary responsiveness. Following the last injection serum LH increased to only 20-30% of that obtained by the first injection. In contrast the T response to the endogenous LH remained unchanged as did the sperm count and their motility in the electroejaculated semen. Since the administration of A twice weekly did not inhibit testicular function, the effect of daily administration was next investigated. Daily treatment for 3 months led to a drastic decrease of LH response in all the animals. In 2 of 4 animals the T response was also abolished. After the loss of T response these 2 animals also failed to ejaculate on electrical stimulation. After cessation of A treatment electroejaculatory response was restored in 4-6 weeks and sperm counts were restored in 12-14 weeks. Conclusions: 1. Chronic treatment of monkeys with A leads to desensitization of the pituitary and the testes. 2. Rhesus monkeys are much more resistant to the antifertility action of LHRH analogs than rats.
EVALUATION OF CHLORINATED PESTICIDES IN HUMAN SEMEN. G. A. Szymczyński and S. Waliszewski. Department of Andrology, Medical School of Poznań, Poland.

Studies were undertaken to determine the residues of chlorinated pesticides, BHC isomers, DDT isomers, and HCB, in human semen. Samples of semen were collected from a random population of fertile and infertile men. The contents of 50 samples of human semen were analysed individually for residues of chlorinated pesticides and their metabolites, by gas-liquid chromatography.

A relatively high level of alpha-, delta-, and epsilon- BHC isomers was found with respect to gamma-BHC/Lindane/ used in agriculture.

The high proportions of alpha-, delta-, epsilon- BHC can only be explained by specific biotransformation in human metabolism or specific storage by genital glands.

Many authors suggest, however, a low percentage of such transformation /1%/ or of absence of transformation.

DDE - being one of the metabolites of DDT - was found in samples from 40% of the individuals.

An attempt is made to correlate the results of analysis of semen with the values of residues of chlorinated pesticides in the same sample of semen.
TESTOSTERONE, SEVEN OF ITS PRECURSORS AND ESTRADIOL IN THE TESTES OF ELDERLY MEN. J. Takahashi, Y. Higashi, T. Yanaihara, S. J. Winters, H. Oshima, and P. Troen. Dept. of Medicine, Montefiore Hospital and the University of Pittsburgh School of Medicine, Pittsburgh, PA.

To explore the intratesticular milieu, nine unconjugated steroids, including testosterone (T), seven of its precursors (pregnenolone, (Ps); progesterone, (P4); 17α-hydroxyprogrenolone, (17Ps); 17α-hydroxyprogesterone, (17P4); dehydroepiandrosterone, (DHA); androstenediol, (Diol); and androstenedione, (Dione) and estradiol, (E2) were measured simultaneously by specific radioimmunoassays in the testes of 20 untreated elderly men undergoing orchiectomy as treatment for prostatic carcinoma. Testicular homogenates, equivalent to 30 mg of testicular tissue were extracted with methanol (1ml/mg) and divided into 3 portions which were applied to Sephadex LH-20 microcolumns. The solvent systems used were 1) hexane:benzene:methanol (85:10:5) for the separation of (P4), (Ps), and (T); 2) benzene:methanol (95:5) for the separation of (Dione), (17P4), and (17Ps) and 3) benzene:methanol (90:10) for the separation of (DHA), (Diol), and (E2). Recoveries of all steroids were consistently greater than 80%. The fractions were then assayed in specific radioimmunoassays. Blank values (0.25 M sucrose) for all steroids were negligible (≤ 10 pg/tube). Intra-assay and inter-assay coefficients of variation ranged from 5 to 12% and 12 to 16%, respectively. Steroid concentrations (ng/g wet tissue) (Mean ± SE) were as follows: (Ps), 255.1 ± 49.4; (P4), 70.5 ± 13.6; (17Ps), 73.2 ± 13.7; (17P4), 393.6 ± 62.2; (DHA), 313.8 ± 44.5; (Dione), 148.0 ± 24.3; (Diol), 155.1 ± 18.1; (T), 1008.4 ± 173.4; (E2), 13.5 ± 3.2. Linear regression analysis revealed positive correlations between Ps and 17Ps; 17Ps and DHA, DHA and Dione, DHA and Diol, Diol and T, and Dione and T; Ps also correlated with P4. These relationships support the contention that the Δ5 pathway for testosterone biosynthesis is predominant in the testes of elderly men. This method is applied to the study of testis biopsy tissue from infertile men.
Four geriatric male dogs of mixed breed with subclinical, palpable prostatic hyperplasia were studied. Plasma samples, collected three times weekly, were assayed by RIA for testosterone (T) levels. Lateral pneumocystograms taken once weekly, were examined to determine prostate size. Two dogs were implanted subcutaneously with 125μg/kg of pelleted Dtrp6, LHRH ethylamide (LHRH-T) and two were sham implanted. Pellets were removed at seventy-one days. Three months later, one previously treated dog was sham implanted and one previously control dog was implanted with 170μg/kg of pelleted LHRH-T. Semen samples were collected once weekly from these two dogs. In each case T levels rose for ~7 days following LHRH-T implantation and then declined precipitously. T levels returned to normal at 44 and 58 days after implantation in two treated animals but remained low through 60 days in the third. The radiographs of one treated animal were of poor quality and not analyzed. In the remaining two treated animals prostate size declined about two weeks following the decline in T. In one animal the prostate dimensions reached a nadir (40% of pretreatment values) at 8 weeks after implantation. In the other, the prostate regressed to a position within the pelvic brim, a state resembling that seen in young dogs. After T levels rose, prostate dimensions increased slowly, again lagging behind the changes in T. A decline in ejaculate volume paralleled the decline in T, although sperm count remained normal for 3 weeks. After the 5th week no ejaculate was obtained. The data suggest that LHRH agonists may have a utility for treatment of hormone-responsive prostatic syndromes in man.

Ram spermatozoa undergo profound surface modifications during their period of maturation in the epididymis. To elucidate mechanisms involved in these changes, we studied: a) internalization of endogenous 125I-labeled surface proteins of testicular spermatozoa (TS), and b) adsorption and internalization of extracellular proteins from 125I-labeled rete testis fluid (RTF) or cauda epididymal fluid (CEF) collected through surgically implanted catheters in the rete testis and vas deferens. The criterion for internalization was inaccessibility of the 125I-labeled proteins to externally applied trypsin. One- and two-dimensional SDS-polyacrylamide gel electrophoretic analysis revealed that internalization of surface components of TS was minimal, except that accessibility of an 89,000 mol wt component increased with time of incubation. Adsorption of labeled RTF proteins was nonspecific and all remained accessible to trypsin during prolonged incubation, indicating negligible internalization. When TS were incubated in 125I-labeled CEF, they exhibited time-dependent uptake of a single major component with a mol wt 24,000. Between 21% and 30% of the adsorbed CEF component was inaccessible to trypsin throughout incubation. To determine the distribution of adsorbed proteins over the sperm cell body, TS were subjected to ultrasonification after incubation in 125I-labeled RTF or CEF and then fractionated on sucrose gradient. Adsorbed RTF components were fairly evenly distributed over the sperm cell body, except a 70,000 mol wt component which was more concentrated in the membrane vesicle (MV) fraction than in the head- or tail fractions. The 24,000 mol wt component adsorbed from 125I-labeled CEF was also more concentrated in the MV fraction. These results suggest that adsorption and internalization may be important mechanisms whereby spermatozoa modify their surface characteristics during their sojourn in the epididymis.

(NIH HD 09356 and NCI P30 12708)
A 57 year old man presented with complaints of impotence and breast enlargement. After fathering 3 children in his twenties, he gained 80 lbs. in weight over several years. Breast enlargement occurred, remaining constant until the past 8-10 years, when it progressed and was accompanied by impotence and a decrease in libido. When evaluated he was taking no medications and was otherwise healthy. He was obese (101 kg) and appeared hypogonadal with scant body hair. Breasts were massively enlarged with 8 cm areolae; galactorrhea was absent. The phallus was normal; the testis measured 25 ml on the right, 20 ml on the left. Serum testosterone (T) was low (165 ng%) and serum LH (57 ng/ml) and FSH (80 ng/ml) concentrations were normal, (LH adult male, 40-145; FSH adult male 45-290). Serum estradiol (E2) was elevated (59 pg/ml, normal less than 45) as was the TeBG binding capacity (5.2 μg%, normal less than 1.5). Testosterone, LH and FSH concentrations rose appropriately after 7 days of clomiphene citrate. Adrenal, thyroid, growth hormone and prolactin testing was normal, as were skull films and a CT head scan. Weight reduction was recommended and over the following fourteen months his weight decreased to 73 kg, concomitantly his serum T level rose to 750 ng%. Reduction mammoplasty was performed. Sexual activity improved and a semen analysis revealed a sperm density of 90 million/ml. Serum E2 and TeBG levels fell to 35 pg/ml and 2.6 μg%, respectively. In this patient impaired gonadotropin secretion occurred in the setting of obesity, perhaps because of the gonadotropin suppressive effects of increased estrogen production, and resulted in deficient testosterone secretion and clinical hypogonadism; this process was reversible with weight loss.
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